

## Annual Report 2001/2002



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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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# *Foreword to Annual Report 2001/2002*

### James E. Godfrey, Chairman of the Governing Body

I am pleased to report on another successful year for The Scottish Crop Research Institute (SCRI), Bio-Mathematics and Statistics Scotland (BioSS) and Mylnefield Research Services Ltd (MRS). All three organisations have produced excellent work and met their financial targets again, a major achievement.

Research has again been operating in a difficult environment. We have seen further dis-investment of the agricultural biotechnology companies from the UK to countries more committed to this technology, partly due to low profitability of agriculture, high costs of UK research and the political environment. UK agriculture is now 0.9% of Gross Domestic Product (GDP), but this is misleading because agriculture is part of a much larger food, drink and non-food chain accounting for a substantial portion of GDP. With margins squeezed in main-stream agriculture, many are looking at niche markets such as industrial oils, bio-fuel and energy crops, but these are not the panacea for the foreseeable future. Not all commodities are in oversupply, indeed, the EU is currently considering ways to increase the production of protein crops to reduce dependence on imported supplies.

Many of the publications and comments about the future of agriculture in the UK and the EU are introspective, and lack long-term vision. In contrast, "A Science Roadmap for Agriculture" prepared by the National Association of State Universities and Land Grant Colleges of the USA (see Report of the Director) provides a constructive way forward for US agriculture. An analysis adopting a similar approach in the UK would give a refreshing positive vision, and would complement the Curry Report and aid its implementation.

We need more stability in funding; for example, more core funding with additional flexibility for programmes to be determined by Institutes working within their mission statements. The insecurity of three-year contracts and problems associated with recruiting short-term staff mean much time is spent on administration and project writing resulting in less quality time spent on research, which in turn inevitably means innovation is stifled. Innovation is the prerequisite for research. It requires teams of people and freedom to operate; it is enhanced by collaboration and competition with other centres. This latter point can be seen by some as duplication, but this is rarely if ever the case. Great innovations are not driven from the paymasters down to teams but rather come up from the base, often from unexpected team members looking at a problem with a different perspective or carrying out a procedure in an unusual way.

If innovation is the prerequisite for research, then uptake is the goal. We must, however, use the technologies appropriate to the different markets; for example, in Japan and the USA their agricultural outputs have risen at similar rates over the past four decades. Nevertheless the technologies used to deliver the outputs have been different: Japan has used landsaving technologies, whilst the USA has used laboursaving technologies. Similarly Sub-Saharan Africa requires labour saving whilst India requires land-saving technology; in other words, it is using technology to maximise scarce resources, and it is research and development that delivers the technology chosen.

Concern must be expressed over the loss of areas of science in the UK. Nematology and virology are key areas under threat, both are important to the UK and globally, here at SCRI we are an international centre of excellence in both these disciplines.

During the year, the Institute, senior staff and the Governing Body through its Science Committee have reviewed the science programme and set a new science strategy based on three themes giving clearer focus to the research programmes. This will give us the platform to go forward. In the past few months we have reviewed our Knowledge Transfer and Exploitation (KTE) of our research culminating in a presentation to an expert panel as a prelude to the Visiting Group review of our science in May 2003. This KTE exercise demonstrated the enormous output of our science over the past few years with many examples of good returns on investment whether by Government or industry.

I thank my Governing Body for their support during the year, the staff at SCRI, BioSS and MRS for all their hard work and dedication in achieving another successful year of science and delivery of that science. Finally my special thanks to the Director, Professor John Hillman, for his tremendous energy, vision and dedication to the success and reputation of SCRI, BioSS and MRS.

### Introduction by the Director

John R. Hillman

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

Institute operates are administered by the Biotechnology and Biological Sciences Research Council (BBSRC).

The Institute is committed to the implementation of Corporate Governance, which requires the highest standards in the three key areas of openness, integrity, and accountability. The Governing Body has a Code of Practice to guide the conduct of its members and has established the appropriate procedures and remits to ensure adherence to these standards. Our specific strategic objectives to achieve this Mission are to:

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

### The Mission of SCRI is:

"To be Europe's leading centre for strategic and applied research into plant and crop-based bioscience, and related environmental sciences, creating knowledge, added-value and new products to benefit the food, drink, agriculture and related industries, the bioindustries, and the environment".

### Our specific strategic objectives to achieve this Mission are to:

Science

- Be an internationally recognized centre of excellence in plant and crop bioscience and products.
- Establish partnerships in key strategic research areas that are fundamental to the long-term vision for the Institute, which will include developing our links with universities and other related bodies.

Knowledge and Technology Transfer & Exploitation

Be an internationally successful model for knowledge transfer and for the spin-out and exploitation of scientific research at the Institute.

Finance and Physical Resources

- Develop new funding and commercialization relationships facilitated by an effective and responsive system of financial control.
- Provide a scientific, administrative and physical infrastructure that enables and supports high-quality, innovative, basic, strategic, and applied research.

Impact and Image

Raise our profile and promote public awareness and understanding of relevant bioscience and environmental issues to assist informed public debate.

Human Resources

Promote the recruitment and development of staff to the highest international standards to deliver the strategic science and commercialisation programmes.

Craigs, Edinburgh in 1921). In 1987, the Institute assumed managerial responsibility for Biomathematics & Statistics Scotland (BioSS), formerly the Scottish Agricultural Statistics Service.

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

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

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

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

The Mid-Term Implementation Report, (November 2000), showed that all 25 recommendations of the VG Report had been implemented. Many of the recommendations related to the need to formulate a more focused and integrated science strategy based on reviews involving a corporate approach, and this has now been achieved.

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

The breadth and depth of knowledge, technical expertise, and infrastructural resources available at SCRI attract extensive contracts and consultancies from, and foster collaborations with, numerous academic and corporate organisations around the world. Synergistic liaisons with other institutes, universities, and colleges in the UK and overseas are also integral to the scientific growth, development, and validation of the Institute's research activities. New links are being forged continuously, as well as existing contacts being developed and strengthened.

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



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

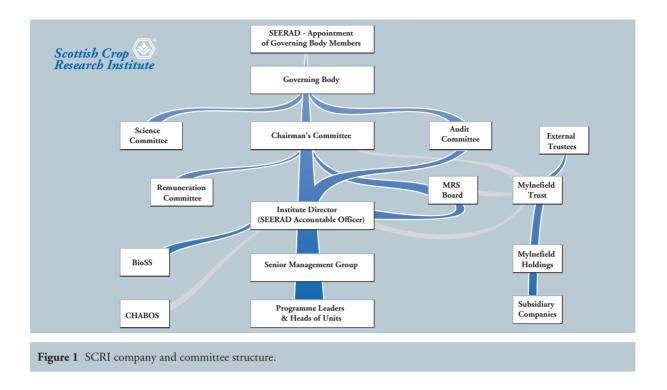
to promote research and scientific work in the life, environmental and related sciences, in particular production of agricultural, horticultural, and forestry crops, methods of limiting or eradicating pests and diseases, wood sciences and biomathematics, methods of increasing production or growth, improving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

to promote the dissemination of such research.

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

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

SCRI provides the base and secretariat for The Scottish Society for Crop Research (SSCR), a regis-



tered Friendly Society formed in 1981 by the amalgamation of The Scottish Society for Research in Plant Breeding and The Scottish Horticultural Research Association.

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

The Society:

organises interactive field walks and end-user/researcher discussion sessions;

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

stimulates crop-based sub-committees to support targeted research projects;

reinforces SCRI representation with trade associations, Levy Boards, and other user-groups;

administers the biennial Peter Massalski Prize to the most promising young scientist at SCRI.

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

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

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

Details of the annual accounts, Corporate Plan, health and safety provisions, and the SCRI/MRS

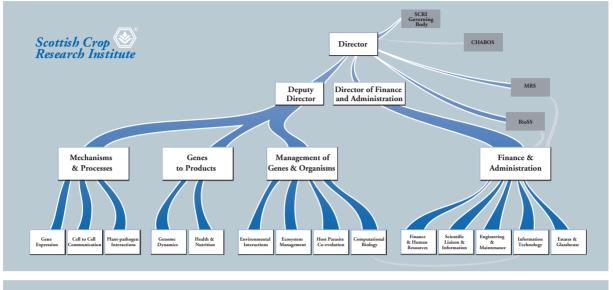


Figure 2 Institute management structure.

quality-assurance arrangements are available on request. (Figures 1 and 2 illustrate the SCRI company and committee structure, and the management structure).

On behalf of the staff and Governing Body, it is a pleasure once again for me to acknowledge with gratitude the staff of SEERAD for their continuing support of, and demonstrable commitment to, our research programme and to our development. Grants, contracts, donations, advice, and joint participation in our activities from the SSCR, other government departments and their agen-

cies, non-governmental agencies, our sister CHABOS institutions and BBSRC institutes with whom we coordinate our research, grower levy boards, local and regional authorities, commercial companies, farmers and other individuals, and learned societies, are also warmly appreciated.

SCRI continues to remain buoyant in generally difficult times for science and agriculture in the UK, justifying its existence in every respect. We are confident that we shall continue to develop and thrive.

# **Report of the Director**

John R. Hillman

Global perspectives of factors influencing agricultural, biological, and environmental sciences, and their associated industries : 2001-2002\*

### Preamble

9-11 The world became politically and economically unstable in 2001. At 08.48h local time on September 11 2001, American Airlines Flight 11 from Logan International Airport in Boston, USA, flew into the north tower of the twin-towered World Trade Center (WTC) in New York City, followed at 09.03h by United Airlines Flight 175 also from Logan International Airport, crashing into the south tower. From images captured on film and magnetic media, the world witnessed the attack on the WTC. At 09.40h, American Airlines Flight 77 from Washington Dulles International Airport, crashed into the Pentagon building, the military nerve centre. At 10.10h, United Airlines Flight 93 from Newark International Airport crashed near Shanksville, Pennsylvania, apparently aiming at the White House or the Capitol Hill building in Washington. The following day, Osama bin Laden was identified by US officials as the chief suspect behind these acts of terrorism, by which time the North Atlantic Treaty Organisation (NATO) invoked Articles of its founding treaty, declaring that the actions constituted an attack against all NATO members. Australia invoked the ANZUS Pact, placing sections of its armed forces on a higher state of readiness to provide assistance to the USA. The four Boeing airliners were hijacked by 19 young Arab Muslim terrorists linked to the al-Qaeda terrorist network led by the Saudi-born Osama bin Laden, and linked in turn to the ruling Taliban in Afghanistan. Over 3000 people were murdered.

**Democratic Modernity** Arising from the "9-11" event were a series of precautionary actions, social developments, and a growing political and economic fragility. On October 7, military strikes led by the USA were launched against targets in Afghanistan. Hitherto-open but vulnerable democratic societies, politically if not intrinsically favourable to multiculturalism, questioned the accommodation of those violently opposed to secular modernity. Indeed, terrorism of all kinds was more rigorously opposed. Several member countries of the United Nations (UN) have yet to experience societies akin to those that arose from the enlightenment of the 17<sup>th</sup> and 18th centuries in the Christian world. G. Kerevan, the Scottish journalist, pointed out that the highly pragmatic Scottish Enlightenment associated with David Hume and Adam Smith onwards, rejected tradition, superstition, and subservience to religious authority, in favour of rational scientific thought,

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

learning, law, and property ownership, which in turn would lead to a rational, wholesome, and civilised society capable of economic progress. Sceptical of arbitrary top-down, utopian rules, these philosophers saw benefit in heterodoxy and reasoned that rules should arise from rational human conduct, tempered by social experience and education. Before that, Europe had become more or less unified through the Christian church, aided by the movement throughout the continent of scholars, communicating in Latin. P. Watson in *The Times* (June 13, 2002) singled out the



roles of three scholars: the Bolognese monk Gratian who upgraded ecclesiastical law; Robert Grosseteste (1186-1253) the inventor of the experimental method; and Thomas Aquinas (1225-1274) who made possible objective study of the natural order, and therefore the idea of the secular state. There was evidence of an increase in individuality, starting between AD1050 and AD1200, inside and outside the Church. Global exploration, international commercialisation, and agricultural technology improvements including horses replacing oxen and a change from the two-field to the three-field system, together began to transform Europe from a backwater compared with the Arab world and the Far East, into the condition of modernity, whereas, for example, intellectual innovation and mercantilism in China became suppressed by its bureaucratic feudalism (mandarinate) and in the Arab world by its Mukhabarat. No autocratic country anywhere has participated fully in the scientific, technological, economic, and social openness of the more-developed countries (MDCs). Modern communications, recreational travel, education, a wider diffusion of prosperity, and entertainment will continue to challenge all forms of fundamentalism throughout the world, inevitably creating an endogenous backlash against imposed inflexibility and intolerance. Whether or not the 9-11 event, which led to a declared war on terrorism by US President G. W. Bush, will cause the isolation and collapse of those regimes and organisations actively supporting terrorism, much changed on the global political and economic landscapes. Posterity will judge harshly those purportedly democratic and sophisticated governments that fail to address the consequences of appeasement and cowardice in the face of terrorists and terrorism, including ignoring situations which do not directly affect them on home territory. The twin counterbalancing dangers of racism and xenophobia will also have to be confronted.

Anti-Enlightenment P. Gross and N. Levitt, in Higher Superstition : the Academic Left and its Quarrels with Science, refer to an "anti-enlightenment", detecting the shift in contemporary non-science academic culture that now extends to a widespread suspicion of science. See also The One Culture? A Conversation about Science edited by J. A. Labinger and H. Collins. This trend in open free democracies has spawned not only a suite of social theories but stimulated radical movements that are hostile to scientific logic and sceptical of Western values and institutions. The UK is a rich source of environmental, animal-rights, anti-globalisation, anti-science, and anti-business activists. Fear of science and its products, pessimism about the future, interest in the occult, but total dependency on science, characterise a dumbing down of many societies, obsessed with entertainment of various kinds and worship of vacuous celebrity. Now, insecurity is added to this mix. Parenthetically, S. Jenkins, the journalist and broadcaster, and others suggested a post-democratic future of sovereignty by the mediasavvy articulate, one in which politicians do not lead but simply represent media interests and pressure groups. Onora O'Neil, in the excellent and thoughtful Reith Lectures 2002 (http://www.bbc.co.uk /radio4/reith2002/onora.shtml) A Question of Trust, pointed out massive evidence of a pervasive culture of suspicion that afflicts professionals and people in public life. She analysed (a) the human rights movement, reasoning that the promulgation of rights also requires the counterbalancing acknowledgement of responsibilities; (b) new conceptions of accountability that superimpose overburdening managerial targets on bureaucratic processes; (c) the drive towards 'transparency' processes, insodoing sidelining the fundamental obligation not to deceive; and (d) a public culture that is only credulous of its own standards of communication. The emphasis on a culture of accountability is leading to the destruction rather than support of trust, especially where the media have acquired unaccountable powers that others cannot match. She referred to that well-known botanical metaphor: "Plants don't flourish when we pull them up too often to check how their roots are growing".

Europabarometer, the polling organisation of the European Commission (EC), conducted a poll of 16,000 people in all 15 European Union (EU) member states in 2001, noting that Europeans have become distinctly sceptical about the contributions science and technology make towards European society. More than 45% believed that too much reliance is placed on science and too little on faith, but only 36% disagreed with this view. Over 61% of people believed science and technology changed life too quickly. More than 56% thought genetically modified (GM) food is dangerous, over 94% want the right to choose whether to eat it, and 52% doubt whether scientists can help solve world hunger. The report revealed that youth in the EU is being turned off science, perhaps an unsurprising observation given the rapidly deteriorating career prospects and working environment for scientists.

Engineering Prodigious engineering feats were completed in 2001-2002. These include the Birecik Dam on the Euphrates River in Turkey, the Mohale Dam (a phase of the Lesotho Highland Waters Project) on the Sengunyane River, and the Algueva Dam on the Guadiana River, Portugal; all three dams will modify agriculture in their regions. Similarly, completion of phase 2 of the Great Man-Made River, an aquaduct for fossil waters linking the interior of Libya to the Tripoli region, and the completion of the Sheikh Zayad canal in Egypt providing irrigation for oases in central Egypt will aid local agricultural activity. Transport-related projects completed included in Incheon International Airport, South Korea; the Guangzhou International Airport, China; the Athens International Airport, Greece; the JFK International Airport, New York City; the Beijing-Shanghai Expressway, China; the Guangdong-Hainan heavy railway, China; the TGV Méditerranée high-speed railway, France; and the German High Speed railway linking Frankfurt and Cologne.

Libraries Traditional library functions of organising and classifying knowledge were advanced by improved networked computing, compact-discs, and bioinformatics, raising new challenges of storage and accessing text, genetic material, sounds, and the cultural heritage contained in museum collections. Important libraries were opened in 2001, including the extensive public library in Beirut, Lebanon, and the new Library of Alexandria in Egypt - the dramatically designed Bibliotheca Alexandrina. Both magazine and book publishing experienced difficulties in 2001, especially the electronic-book (e-book) market, and copyright issues were to the fore.

Scholarly communication through the market place has been subject to critical analysis, with concerns over the transfer of copyright to the publishers, the principle of charging for freely given – often, but not exclusively, publicly funded – material. Electronically published journals have yet to attain high citation impact values – a direct measure of popularity but indirect of quality, yet beloved of scientific assessment systems.

Internet Another form of communication and vehicle for globalisation, the Internet has become a crucially important tool for scientists, expanding both physically and in importance. In some respects, it has lived up to the term 'information superhighway' but in other respects - the equivalents of traffic jams, road closures, rush hours, and road rage - there are still technological improvements to be made. Text, graphics, data bases, music, video, games, and telephone conversations now co-exist on the Internet, utilising domain names (the set of words, numbers, and letters separated by dots used to identify an Internet server or group of servers), routers to select the most efficient route for information packets based on current system demands, and the Transmission Control Protocol/Internet Protocol (TCP/IP) which defines how information and requests generated by other protocols are transmitted and received over the Internet. Fibre-optic cable networks will assist in the transmission of telephone calls and video, and there is likely to be expansion of specific-user networks linking on to the Internet. In such an increasingly highly regulated world, it is remarkable that a system such as the

Internet on which much of modern society and science depends, has no central governing body, no legal jurisdiction, no formal censorship system, and thereby little opportunity for redress, no overarching planning, and is tax-free. It remains vulnerable to the malevolent, can be the source of misinformation or information for terrorist purposes (including bioterrorism), and especially in respect of e-mail, is insecure unless encrypted and comes with a digital signature. It also poses challenges to governments and organisations that wish to control information and apply taxes or charges. Both the British Computer Society and the Association for Computing Machinery oblige their members to operate codes of conduct and ethical behaviour.

**Grids** Grid computing initiatives are regarded widely as the next phase of Internet computing, linking highperformance computers capable of accessing and processing terabytes of data stored in global databases, to provide a single virtual source of processing capability. There are likely to be different kinds of grids for specific purposes, with interlinked consortia. In the UK, preparations were put in hand to develop the eDiamond grid in early 2003 to create an interactive national digital mammography archive for a group of specialist hospitals in Edinburgh, Glasgow, London, and Oxford.

Higher Education Higher education issues in 2001 included the use of educational and psychological tests for determining competencies of students and thereby the teaching performance of institutions. Very often the tests were standardised and cognitively related. Many of the measures of institutional performance were positively influenced by high levels of student intakes and the availability of short courses. Other issues were the trend towards international linkages, Internet-based learning, funding levels, value-formoney, the vocational utility of coursework, external audits, redress sought by students malcontent with the standards of tuition, and academic freedom. Against the tide of greater freedom of information, Cuba, Egypt, and Russia imposed restrictions on freedom of enquiry. In Cuba, four leaders of the independent library network were arrested because the libraries promoted parties hostile to the non-democratic government. The formerly prestigious Islamic Al-Azhar University in Cairo forbad any publications that "lacked respect" for God. Instructions were issued by the Russian Academy of Sciences requiring the nation's 53,000 researchers to report attempts by scholars to apply for foreign grants, to submit for

inspection articles intended for publication outwith Russia, and to report visits by foreigners. Elsewhere, the implications of affirmative action programmes that favour ethnic, cultural, and social groups, rather than academic merit were debated in several countries. There was a growing demand for English as the preferred language of tuition and communication in science- and technology-based subjects, and management studies.

Many in the UK public-sector universities were unaware of the implications of the developing market in higher education arising from the General Agreement on Trade and Services (GATS), and were also unaware of the bilateral agreement between the European Commission and the USA on this topic. There is every likelihood that higher education will form part of the negotiations following the GATS talks scheduled in January 2005 unless prior agreements are put into place. Acquisition of formal accreditation roles and associated recognition, access to public funding, and greater competition for students and staff will undoubtedly open up opportunities for profit-making organisations. Presumably, non-degree-awarding research institutions such as SCRI will have a greater choice of affiliation partners, and new entrants to the market will be in a position to establish research capabilities themselves to compete for research and development (R&D) funding. Debate will ensue on the definition and involvement of 'public good' and reducing or eliminating privileged access to public funding by essentially centrally controlled, formula-funded quasi-nationalised universities and further-education bodies. In 1998, 3% (equivalent to \$30 billion) of the total trade in services of the 30 countries of the Organisation for Economic Co-operation and Development (OECD) was the training and education of overseas students. Over 70% of these students were trained in Australia, France, Germany, UK, and the USA, the latter accounting for 28% of the total. When the data are analysed as a proportion of total enrolments, however, overseas students accounted for 17% of the enrolment in Switzerland, 11% in the UK, and only 4% in the USA. In the UK, about 1.1% of GDP is spent on higher education compared with 2.3% in the USA. A further problem for UK universities followed a sweeping ruling in June 2002 in the European Court of Justice that judged legislation in Germany exempting scientific research in universities from Value-Added Tax (VAT), did not comply with European Union (EU) law. It would appear that although grant

income from the higher-education funding councils and the research councils will remain exempt, research-contract funding from government departments, the National Health Services, and charities (which rarely pay overhead costs) will attract VAT.

### **Economics and Politics**

**Global Economy** Contrasting with 2000, when the global economy grew by 4.7%, its fastest rate for 15 years, economic growth declined in 2001, beginning with a slowdown in the first six months that was more severe than projected. The terrorist attacks on the USA in September undermined confidence throughout the world's financial markets and echoed through the banking sector, international trade, business, insurance, and the political world. Growth in the economies of the 30 MDCs in the OECD was projected to have been around 1%. Deflation threatened several economies, raising prospects of the 'liquidity gap' when real investment is impossible as a progressive reduction takes place in the price level, in line with a collapse in confidence by consumers and investors.

Various factors contributed to the initial slowdown. For most Western European countries, a contribution of tight monetary policies, higher oil prices, weakening pension schemes, and falling corporate profits depressed growth. Falling demands for information technology products adversely affected Asian producer countries, with the exception of China which sustained a buoyant economy. After the September terrorist attack, the USA - engine of the world's economy by virtue of its domestic consumption - faltered with declines in output for the first time in 20 years. Redundancies and lay-offs, company failures, and a curtailment of world travel and tourism affecting airlines and hotels were all products of the year's events. Early in 2001, the US economy experienced an ongoing slump in demand for information and communications equipment leading to an 80% drop in telecommunications, media, and technology (TMT) shares. Nonetheless, strong household spending and an easing of monetary policies, coupled to relatively high employment levels, helped ameliorate the slowdown being experienced elsewhere. Following the 9-11 attacks, it was necessary for the Federal Reserve to cut the federal funds rate, bringing the rate down to 1.75% in December. Unemployment in the USA rose to 5.8% in December, the highest level in more than six years. A return to a federal deficit was projected in 2002 as the President, G. W. Bush, was allocated \$40 billion to respond to the terrorist attacks. An additional \$15 billion was granted to help US airlines, already in a financially precarious position before September. A package of \$75 billion was planned to stimulate the economy.

**UK** The UK had one of the most resilient economies of the major advanced countries, with a forecast growth in output of 2.0-2.5%, making it the fastest growing of the industrialised Group of Seven (G-7) countries (Canada, France, Germany, Italy, Japan, UK, and USA). Declines in overseas demand severely reduced exports of goods and services. Agriculture and tourism were badly affected by the foot and mouth disease (FMD) epidemic (see sections on Agriculture & Food and UK Agriculture) and poor weather. The TMT sector showed signs of collapse. The competitiveness of UK goods and services was eroded by the relative strength of sterling against the euro. Even so, the UK economy benefited from strong domestic demand. Household spending and retail sales rose at rates similar to those in 2000. On a claimant-count basis, unemployment was the lowest for 26 years, and was estimated to be about 5.1% of the total labour force (declining to a seasonally adjusted 3.1% in 2002), compared with 8.5% in the EU, 8.1% in Germany, 8.9% in France, 10% in Italy, 5% in Japan, and 4.8% in the USA. Wage pressures were strong, and as a result of the priority given by the Government to the public sector, public-sector earnings rose faster than those in the private sector. Consumer debt stood at around £800 billion in 2002, including mortgages, and house-price inflation reached 24% in many areas of the UK.

**Euro Zone** The euro zone ("Euroland") showed economic weakness during 2001, as trade and business investment stagnated. According to the International Monetary Fund (IMF), *World Economic Outlook* October 2001, the estimated percentage annual change in real gross domestic product (GDP) was 1.8% in the EU. Unemployment remained high in most Euroland countries except Luxembourg and The Netherlands. Food and commodity price rises led to pressure on the rate of inflation for most of the year.

**Countries in Transition** In the former centrally planned economies – also referred to as the countries in transition – there was a 2% increase in output, down from 6.3% in 2000. Exports fell largely as a result of a drop in demand from Western Europe which takes about 50% of the region's exports. The Commonwealth of Independent States, in particular, suffered the greatest fall in regional economic growth, although domestic demand helped avert the worst

effects of the downturn. For most countries in the region, inflation rates declined to an average of 15%, although they were lower (*circa* 9%) in Central and Eastern Europe, and higher in Russia (*circa* 20%).

LDCs Growth in most of the less-developed countries (LDCs) was affected by economic weakness and reduced demand in and from the MDCs. The IMF projected that the annual change in real growth domestic product in all the LDCs was 4.3%, down from 5.8% in 2000. Most buoyant of the regional groups of the LDCs was Asia, where excluding the newly industrialised countries (NICs e.g. Singapore, South Korea, Taiwan) growth was around 5.6%. Public debt started to be regarded as a problem. Conflict in Afghanistan affected all its neighbouring countries, drastically disrupting trade and increasing external debt in Pakistan. By virtue of its relatively closed economy, India was not substantially affected by the global economic slowdown during the year, but was expected to be affected during 2002. Bangladesh benefited from an upturn in agricultural output. Growth in output from sub-Saharan Africa fell from 3% to 2.7%, but improved agricultural output in Ethiopia, Kenya, and Mozambique improved their domestic situations. Unstable regimes in Angola, The Sudan, and Zimbabwe impeded growth. In Latin America, there was negligible growth as demand from the USA weakened. Argentina, Brazil, and Mexico the three largest economies - were markedly affected, and developing political and financial perturbations in Argentina started to undermine the economy. Lower oil prices, global downturn in the demand for goods and services, and the conflict between Israel and the Palestinians reduced growth in the Middle East from 5.5% in 2000 to no more than 2.3% by the end of the year, with projections into 2002 distinctly bleak.

**Markets** The September 11 event seemed to trigger worldwide loss of confidence in the financial markets, the most significant, and heralding a string of collapses in 2002, being the filing for Chapter 11 protection from bankruptcy in November 2001 of one of the world's largest conglomerates, the US energy trader Enron Corporation. An unprecedented analysis was undertaken of the rôles and practices of external auditors, and the veracity of company accounts, focusing on real earnings and profits. Large-scale job losses were announced such that over 97,000 workers were laid off in the EU during October alone. Markets fell heavily after the Spring of 2002 as further corporate accounting irregularities and excesses in the USA involved companies such as Xerox and WorldCom. **WTO** Despite the worsening economic situation, or perhaps because of it, new agreements were reached at the World Trade Organisation (WTO) Conference in Doha, Qatar, in November 2001, enabling globalisation to continue and foreign direct investments to flow. It has been referred to as the 'development round', and the 'Doha development agenda'.

China became the 143<sup>rd</sup> member of the WTO in December, a decade and a half after first applying to join the General Agreement on Tariffs and Trade. Taiwan, a democratic country proper, was also approved for membership of the WTO, but to accede to the sensitivities of the leadership of China, it was designated as a separate customs territory together with its three offshore islands of Matsu, P'eng-hu, and Quemoy.

Globalisation Globalisation in respect of economic integration continued notwithstanding set-backs from the 9-11 event. Internationally integrated multinational companies have assisted in breaking down national differences; their employees usually have global interests and perspectives. Collaboration with trade unions to force through protectionist demands has been weakened, especially as the political influence of the unskilled and semi-skilled workforce has declined in relation to their proportion of the total workforce. Governments have become committed to multilateral and bilateral agreements. For Europe, the loss of large swathes of manufacturing, a steady increase in the numbers of retired people, and a willingness to purchase and use imported products, lessened resistance to globalisation in many sectors of the economy, even in the culturally relatively resilient areas of agriculture and food.

The previous year was notable for nearly 150 regulatory changes to be made by 69 countries in investment conditions to foster global trade. Although overall foreign direct investment (FDI) figures were expected to decline in 2001, in line with a sharp fall in merger and acquisition activity, in 2000 FDI had grown to an unprecedented level of \$4,270 billion. Allied to this was around \$15,680 billion in sales by the 800,000plus affiliates of transnational companies. An investment boom into Hong Kong helped FDI into the LDCs of Asia rise, undoubtedly linked to the final stages of China joining the WTO, but there were a string of disinvestments in Indonesia. China, Hong Kong, and India were mainly responsible for an outflow of FDI from Asia amounting to \$85 billion. Only Latin America and the Caribbean recorded declines in regional FDI levels in 2000 compared with 1999.

For the first time in recent history, the growth in world output during 2001 exceeded the volume in world trade. During the 1990s, the annual rise in the volume of merchandise exports had exceeded by three to one the growth of GDP, domestic growth was growing, and trade in services accounted for about 25% of all cross-border trade. Led by the recession in the TMT sectors, the economic slowdown in Europe, Japan, and the USA affected adversely the East Asia-Pacific region (principally Malaysia, the Philippines, Singapore, South Korea, and Taiwan), Latin America, and the countries in transition.

Overall, the current account of the balance of payments in the MDCs remained in deficit for the third year, falling to around \$223 billion. Both the UK (\$23 billion) and USA (\$407 billion) had substantial deficits, as did Australia (\$11 billion) and Portugal (\$10 billion), but the euro zone progressed into surpluses, led by Germany and Spain with \$14 billion surpluses. The four NICs had a total surplus of \$48 billion. A surplus of \$20 billion was sustained by the LDCs, falling from a level of \$60 billion in 2000 as the deficit in Latin America grew to \$58 billion and the surplus in the Asian LDCs reduced to \$22 billion. There was only a slight easing of LDC indebtedness to £2,155 billion.

One manifestation of globalisation was the synchronised downturn in the economies of Europe, Japan, and the USA. There was no evidence that trade liberalisation adversely affected national economies, or that imports led in general to a widening of the gap between rich and poor, although there were many specific examples of inefficient industries and activities undermined by open markets.

Central Banks With the stark exception of the euro zone, central banks in the MDCs started to cut interest rates early in 2001 and fiscal policy was modified essentially to reinforce confidence in the household, corporate, and market sectors as the main strategy to prevent recession and an economic "hard landing". Eleven cuts to interest rates were made in the USA, reducing the Fed rate to 1.75%. In the UK, the Bank of England steadily brought down the rate to 4%. Japan had few options and room to manoeuvre; nominal interest rates had been below 1% since the mid-1990s, and the financial markets were already depressed. The Bank of Japan eased monetary policy, increasing liquidity and reinstated zero rates. In April, it also announced an emergency package that included a proposal to force its under-pressure banking sector to face up to its long-standing bad-debt problem. Later in the year, it engaged in extensive selling of the yen in an attempt to steady exchange rates. The European Central Bank (ECB) admitted Greece at the beginning of the year as the twelfth EU country to join the euro system. This was a time that the euro appreciated against both the dollar and sterling. By April, market sentiment turned against the euro, as interest rates remained unchanged reflecting the view of the ECB that it was constrained by high inflation and there were prospects of healthy economic growth. Soon thereafter, however, the ECB was forced into three cuts leading to an interest rate of 3.75% by December.

€ The euro zone is dependent on fixing interest rates on a continental scale, and reliant on the assumed authority of European institutions acting under a remit given by governments, rather than allowing its various citizens to act in a truly free market. These citizens currently live under differing political, educational, and legal systems; speak a wide variety of languages; and have differing attitudes to social and economic developments, influencing thereby the processes leading to wealth creation and the quality of life. Some of the nation states have undergone several currency changes as well as political traumas since the end of World War II in 1945. Political integration will be aided specifically by the adoption of the single currency, the euro, in turn further weakening national governments and their control of monetary policy, and lengthening the chain of contact between citizen and bureaucrat. Even more constraint to national governments comes from the Stability and Growth Pact that limits governmental borrowing and budget deficits, amplifying the effects of economic downturns. With extremely limited geographic and wage flexibility of its workforce, even though a one-size-fitsall economic policy has been adopted, there is also the undesirable possibility of protectionist anti-free-market policies coming to the fore, seen most clearly in agriculture, financial services, air travel, and the utilities. Under the guise of 'harmonisation', further anticompetitive stances could be taken as politicians try to curry favour with a voting public disillusioned with full-time politicians generating a flow of enterprisequashing legislation on a massive scale. At this juncture, the European Central Bank and the relevant member states do not have large enough central budgets to offset economic shocks, a basic requirement of an optimal currency area. Regardless of intrinsic difficulties, a remarkable event took place in the euro zone

in that within a space of two months at the beginning of the year, the national currencies of the 12 euro zone countries were efficiently replaced by new euro notes and coins, with minimal disruption despite the logistic and security challenges.

Stock Exchanges Nearly all the stock-exchange indices in the MDCs declined in both dollar and local-currency terms. According to the Financial Times, declines of over 5% were recorded in the Brussels BEL20 (-8%), Brazil Bovesta (-11%), Toronto Composite (-14%), Denmark KFX (-13%), Finland HEX General (-32%), Paris CAC 40 (-22%), Frankfurt Xextra DAX (-20%), Hong Kong Hang Seng (-25%), Milan Banca Commerciale Italiana (-25%), Japan Nikkei Average (-24%), The Netherlands CBS AU Share (-21%), the Philippines Manila Composite (-22%), SES All-Singapore (-15%), Switzerland SBC General (-22%), UK FT-SE 100 (-16.2%), and the USA Dow Jones Industrials (-7%). The global Morgan Stanley Capital International (MSCI) was calculated to have reduced by -16.9%. In contrast, stock market performances were up in Austria, Mexico, Russia, and Taiwan. On a global scale, investor and business confidence fell, and greater focus brought to bear on the veracity of externally audited accounts, share earnings, and executive reward packages.

Agricultural Commodities With the exception of sugar to some extent, and cocoa which was influenced by disease and agronomic problems in Côte d'Ivoire the main producing country - and market speculation, agricultural commodity prices remained largely depressed as supply more or less matched, or was capable of exceeding, demand. Newer technologies meant that there were greater guarantees of switching on high-quality production, and competition remained intense. Few producers had the market muscle of food processors and particularly the major international or dominant national retailers. The forest-products industry suffered from market volatility, falling prices, and collapsing markets. In contrast, the tobacco industry was resilient. Non-agricultural commodity prices were generally depressed, although oil prices were volatile, yet ended the year below \$20 a barrel as non-OPEC producers did not cut back production in line with a decline in demand from the main importers. Gold retained its attractiveness as a precautionary investment irrespective of the large-scale sale of gold reserves by certain central banks. Most other commodities were depressed, even copper, the bell-weather metal which entered a so-called "absorbing state" well below its long-term average, the state which tends to persist until events lead to a reversion back to the long-term average price.

**Surveillance** Another outcome of the September 11 events was the rapid introduction of broad-spectrum investigative and surveillance powers primarily by the US Government which, in turn, brought international banking and financial services to the fore in the war against terrorists and terrorism. The relatively low profile of the OECD's aim to combat money laundering, the Financial Action Task Force, was raised as it identified jurisdictions that were regulated inadequately. Remedial action and improved reporting were introduced in Argentina, Bermuda, Canada, The Cayman Islands, Israel, Italy, Luxembourg, and Panama.

Banking National and international banking systems, and the legal and regulation framework in which they operate, continued to adjust to the reality of a globalised and integrated financial system encompassing banking, investments, securities, insurance including deposit insurance schemes, mortgages and similar types of lending, and other financial services. The World Bank released a report describing 112 episodes of systematic banking crises in 93 countries since the late 1970s. Reform of its domestic financial regulatory arrangements was considered by South Africa with consideration given to the Australian and UK model of establishing a single financial regulator outwith the central bank, yet there was no international consensus on structures. Changes to the Basel Capital Accord were proposed at the beginning of the year by the Basel Committee on Banking Supervision although there was no resolution of the key issues of (a) using an internal ratings-based approach to selling riskbased capital standards; (b) the possibility of incorporating measurements of operational risk into the standards; and (c) disclosure standards under designated market-discipline principles. Corporate governance was generally agreed to be a primary level of control, and best-practice codes were directly and indirectly supported by governments. Electronic banking and commerce, the associated legal framework for electronic signatures, Internet payment systems, virtual banking, and electronic funds transfers were all under legal and regulatory review in nearly all countries. In the UK, there was emphasis on riskmanagement processes (e.g. risk registers and risk management committees) in companies and organisations, public- and private-sector alike.

IT Compounding the retrenchment caused by the massive contraction in the so-called dot-com companies in 2000, the computer and information systems sector suffered badly from falling demand and investor sentiment in the 2001 recession, extending well into 2002. The 9-11 event triggered a sharp downturn in the whole technology sector. Massive downsizing of workforces took place worldwide. Although the Microsoft Corporation had been found guilty of violating US federal antitrust laws by actions designed to sustain a monopoly on the operating systems (OS) of personal computers, the US Department of Justice decided not to force a breakup of Microsoft, nor pursue the issue of bundling its Internet Explorer browser with the Windows OS. Rival companies and several States, however, contested the federal government's settlement.

Shipments of personal computers were estimated to have declined for the first time since 1986, yet the Gartner Group, a technology-market research company, reported that by 2002, a billion personal computers had been sold over 25 years. The marketplace was made more competitive by price wars, especially in the consumer sector, lowering revenues for the industry by around 10%, and leading to company closures, mergers, acquisitions, and modified supply agreements. Dell displaced Compaq at the world's largest manufacturer. Technological developments and releases included Windows XP with its controversial electronic fingerprint; Mac OS X and its upgrade OS X version 10.1; faster microchips; and improved hand-held personal digital assistants.

**E-commerce** At a time when electronic-commerce (ecommerce) expanded, e-commerce companies and ecommerce arms of traditional companies declined in both profitability and valuation, alternative highspeed digital subscriber line (DSL) Internet-access providers collapsed, and even the competing cable modem services and wireless high-speed networking companies were also under pressure. Internet advertising revenues declined, but there was a growing awareness that the Internet was being used for product research by prospective purchasers from 'brick-andmortar' retailers. There was a rise in the number of business-process patents and lawsuits related to infringement of these patents.

Credit-card fraud, the sale of listings of potential customers that have accessed some e-commerce sites, malicious attacks by Internet viruses and worms, and Internet security and spying technology were genuine issues of concern. Governmental actions following the 11 September terrorist attacks included the tracking of Internet users, *e.g.* the Carnivore technology deployed in the USA; the development of improved security systems, especially for remotely controlled factories, power grids, and telecommunications, as well as for transport systems; and encouragement of computerised disaster-recovery strategies. Examples of potential large-scale fraud by company executives and investment analysts defrauding investors by falsifying accounts and earnings and valuation projections were becoming manifest throughout the year.

Semiconductors Evidence of the economic downturn in 2001 was seen in the dramatic 31% fall in sales of semiconductors, but the Semiconductor Industry Association optimistically projected a rise in sales through to 2004. A parallel decline in the sales of flash memory was also thought to be capable of rapid reversal, driven by demands of digital photography, and automotive applications. Digital signal processors, dynamic random access memory devices, microcontrollers, programmable logic devices, and the optical storage markets were all though to have excellent prospects for growth despite declining sales in 2001. The Americas were displaced by the Asia-Pacific area as the world's largest semiconductor market. As in 2000, the top three semiconductor suppliers on the basis of sales were the Intel Corporation, Toshiba Corporation, and NEC Corporation.

Bribes Transparency International surveyed 835 business experts in 15 LDCs that trade most with multinational companies and constructed the Transparency Bribe-Payers index for 2002. This survey is thought to give an indication of the bribery of public officials in LDCs by companies in MDCs and aspirant MDCs. Scores were awarded to countries indicating the level of corruption involving multinational companies from that country. The ranking from least to most corrupt was Australia (1), Sweden and Switzerland (2), Austria (4), Canada (5), Netherlands and Belgium (6), UK (8), Singapore and Germany (9), Spain (11), France (12), US and Japan (13), Malaysia and Hong Kong (15), Italy (17), South Korea (18), Taiwan (19), China (20), and Russia (21). All were far less corrupt than domestic companies in the 15 LDCs. A free press coupled to active anti-corruption investigations by government were regarded as the most effective ways of combating bribery, in line with the convention adopted two years ago by the OECD. The LDCs perceived as most corrupt in

increasing degrees of corruption were Moldova, Uganda, Azerbaijan, Indonesia, Kenya, Angola, Madagascar, Paraguay, Nigeria, and finally, Bangladesh.

**IP** Country-by-country translation and registration fees, annual renewal fees, the absence of a proper central court of patents (European Patent Judiciary), delays in processing applications and inconsistent decisions dog entrepreneurial and commercial initiatives in the EU. Patenting within the EU remained a protracted and expensive process compared with the USA. The 2001 statistics from the European Patent Office for 2001 are given in http://www.europeanpatent-office.org/epo/an\_rep/2001/pdf/8\_e.pdf . In order to safeguard public access to research innovation and ensure full exploitation of intellectual property (IP), the strategy of 'defensive publication' has arisen in which scientists disclose details about their innovation to the public, thereby preventing others from gaining patent protection. This strategy is not confined to the public sector; commercial companies use it to prevent or forestall competitors from gaining advantage. In Defensive Publishing : A Strategy for Maintaining Intellectual Property as Public Goods, by S. Adams and V. Henson-Apollonio, International Service for National Agricultural Research (ISNAR), Briefing Paper 53, 2002, the strategy is outlined (see also On the defensive about invention, by R. Poynder, Financial Times, September 19, 2001). Guidelines on how to provide a robust defensive publication include (a) a complete and comprehensive description of the entire innovation or concept, (b) description of the use of the research product or innovation, (c) the publication must be made available to the public especially accessible by patent office examiners, (d) the essence of the work must be brought to the public quickly and/or predictably, (e) it must be possible to prove the date on which publication was disclosed to the public, (f) it may be possible to defer surrender of all or part of the property rights. There are two main routes to defensive publication: (a) self-publishing through company publicity materials, company report series including websites, occasional publications, and possibly ephemeral literature, or (b) more to be preferred, third-party publishing such as commercial public disclosure (e.g. Research Disclosure (www.researchdisclosure.com), the peer-reviewed literature, national publications (e.g. Statutory Invention Registrations of the US Patent and Trademark Office), and other IP titles, such as the utility model system (e.g. Gebrauchsmuster in Germany).

2003 IMF expectations of global growth during 2003 in the order of 3.7% appeared to some observers to be rather high, given the trend for growth of around 4% in recent times, and an estimate of 2.8% for 2002. Weak growth was expected, especially in France, Germany, and Italy, as well as but to a lesser extent in the USA and UK, recognising the acknowledged risks of (a) an overdependence on the USA as the locomotive of the global economy by virtue of its domestic expenditure and willingness to import goods and services; (b) pronounced rises and turbulence in oil prices reflecting political and security concerns and actions in the Middle East, especially in Iraq; (c) volatile equity markets during a period of rapidly declining company valuations and earnings; (d) risky emerging markets in countries with weak economies; and (e) growing imbalances in some of the major economies, most notably the USA's widening current-account deficit.

### **Conflicts and Populations**

Afghanistan The most important global conflict in 2001 was the aftermath of the terrorist attacks in the USA on September 11. Allied air strikes in Afghanistan, led by the USA, were launched on October 7 to rid that drought-afflicted country of its al-Qaeda group and the Taliban that ruled most of the country. Using the anti-Taliban Northern Alliance as an ally to provide the bulk of the ground troops, the USA and allies forced the Taliban and al-Qaeda groups to abandon the major population centres and suffer massive losses. Neither Osama bin Laden nor the Taliban leader, Mohammed Omar, were located or captured. By December 2001, an interim administration led by Hamid Karzai was installed following a UN-sponsored conference in Bonn in late November. The Afghanistan crop of opium poppies had almost disappeared from the Taliban-controlled areas, welcome to an international community trying to control the drug trade, but economically devastating to many subsistence-level farmers. When the Taliban control was removed, poppy-growing resumed.

**Elsewhere** India and Pakistan, two antagonistic nuclear-armed nations, were close to war footing in their dispute over Kashmir. By the end of the year, there were signs that the government of Sri Lanka and the Liberation Tigers of Tamil Eelam would renew their cease fire that was broken in April 2001. As a country with one of the most culturally, ethnically, and religiously diverse populations, Indonesia suffered from communal violence and threats of successions: Aceh, Irian Jaya, Kalimantan, and the Maluku archipelago have witnessed war-like conditions. War was declared by the Philippine government on Muslim extremists in Jolo. In the Balkans, there were armed clashes between the Macedonian security forces and ethnic Albanians. Chechen successionists continued to harass Russian forces in Chechnya. In Africa, there was fighting in Burundi, the Democratic Republic of the Congo, and the Sudan, but both Ethiopia and Eritrea respected the 2000 Algiers agreement which led in 2001 to the creation of a security zone. The Middle East was one of the most politically fragile areas with ominous global overtones. Iraq had continued to reconstruct production facilities for weapons of mass destruction, but had been territorially reined in by patrolling war planes from the USA and UK over the northern and southern no-fly zones. Israel and the poverty-struck Palestinian Autonomous Areas were engaged in an unequal war, pitting suicide bombers and small arms of the Palestinian militants against Israeli civilian targets, and unleashing retaliatory attacks by the Israeli armed forces which protected Israeli settlements in Palestinian lands. Peace in the Middle East would boost the global economy, and remove one of the major stated 'justifications' for terrorist acts.

Agreements After 13 years, the final inspection was carried out under the terms of the 1987 Intermediate-Range Nuclear Forces Treaty originally agreed between the former Soviet Union and the USA, thereby eliminating an entire class of nuclear missiles. In contrast, at the end of the year, the USA announced its intention to withdraw from the 1972 Anti-Ballistic Missile Treaty with Russia, regarding the Treaty as an impedance to the development of its National Missile Defense System. The USA opposed a treaty to control the illicit trade in small arms and light weapons as it failed to distinguish between firearms used for traditional and cultural purposes rather than for wars. In addition, the USA withdrew support for a protocol aimed at including verification processes based on international site inspections in the 1972 Biological and Toxin Weapons Convention.

**Refugees** According to the United Nations High Commissioner for Refugees (UNHCR), at the beginning of 2001, there was a decrease in the number of refugees and persons of concern, from 22,300,000 in 1999 to 21,800,000 in 2000. Thus, 12,000,000 were officially recognised refugees; 914,000 asylum seekers; 786,000 returnees; and about 8,000,000 persons requiring protection or assistance. R. Lubbers, the former Prime Minister of The Netherlands, succeeded S. Ogata of Japan as the UN High Commissioner for Refugees in January 2001.

Coinciding with the 50<sup>th</sup> anniversary of the 1951 Convention Relating to the Status of Refugees, the Global Consultations on International Protection initiative was launched to reinvigorate international protection mechanisms for refugees and persons of concern. The 'gold-standard' solution has always been regarded as repatriation followed by resettlement and integration at the local and national levels, but although this has been achieved (e.g. East Timor, parts of the Balkans), this is not the case in Guinea, Liberia, and much of Sierra Leone. There were expectations that 1,800,000 internally displaced people and 350,000 refugees from the Democratic Republic of the Congo would return following the installation of the new President and implementation of the Lusaka cease-fire agreement. Likewise, it was expected that there would be progress towards repatriating 567,000 Burundian refugees. Around 4 million people were displaced or directly affected by war and other problems in Angola, including 350,000 refugees mainly in the Democratic Republic of the Congo and Zambia. In Asia, the long-standing Afghanistan situation meant that the Afghans formed the world's largest refugee population. By late October 2001, around 1.2 million people were displaced within the country, and circa 7.5 million required humanitarian assistance. Well before the launch of the international effort to banish the Taliban, substantial numbers of Afghan refugees and asylum seekers were recorded by most of the best-known recipient countries. The severe drought, internal warfare, persistent humanrights violations, poor education, hostility to modernisation, low prices for agricultural produce and minerals, and relatively low levels of donor assistance all contributed to the large-scale crisis.

As in Afghanistan, resolution of the Balkans situation seemed elusive. By mid-2001, the Macedonian conflict contributed to a total of 140,000 displaced people in the adjacent Kosovo province of Serbia, and 12,000 others to southern Serbia; over 50,000 were internally displaced in Macedonia. By the end of the year, around 57,000 refugees had returned from Kosovo and southern Serbia under the auspices of a NATO-enforced cease-fire agreement. Elsewhere in the Balkans, there were signs of improving relationships that would lead to more settled conditions in Yugoslavia, Croatia, and in Bosnia and Herzegovina. In Kosovo, however, there was little evidence of accord between ethnic Albanians and non-Albanians, especially with respect to the small remaining Serb community.

The two major guerrilla forces in Colombia – the Revolutionary Armed Forces of Colombia (FARC, apparently aided by the terrorist Irish Republican Army) and the National Liberation Army (ELN) contributed to a refugee problem that overspilled into many other countries, such that Colombians comprise a major portion of those seeking asylum throughout the world. Besides terrorist actions, the country was beset by problems of corruption, the drug trade, and a weak economy.

Many MDCs, especially the UK, experienced substantial increases in the numbers of asylum seekers and attempts by illegal immigrants to circumvent the proper entry procedures, sometimes using desperate measures. Post September 11, and the introduction of antiterrorist measures, there were widespread clampdowns on the asylum process, and a rising risk of both xenophobia and racism affecting the innocent and vulnerable. In the year that the term of office of the UN Secretary-General, Kofi Annan was extended by five years, and both he and the UN had been awarded the Nobel Prize for Peace, the General Assembly was able to approve by the end of the year an increase of nearly 4% for the next two years, essentially to support peace-keeping operations. The budget authorised \$2,625 million for regular operations in 2003. Coincidentally, the UN was also able to collect \$4.2 billion in current and overdue payments by the end of the year. Earlier in 2001, China ratified the International Convention on Economic, Social and Cultural Rights but resisted the obligation to allow workers to form and join free trades unions. LDCs were responsible for the USA losing its seat on the UN Commission on Human Rights, a seat it has held since the foundation of the UN in 1945. Illtempered debate took place in the UN World Conference Against Racism, held in Durban, South Africa from August 31 to September 7.

**Populations** According to *Britannica World Data*, in 2001, the world's population was estimated to be 6,130,169,000, with a population density of 45.1 *per* square km. The population was estimated to increase to 7,477,335,000 in 2020, by which time the urban population is expected to double, giving rise to an increasing demand for convenience foods and meat products. Around 40% of the demand for meat products in the LDCs, which is expected to reach 213 mil-

lion metric tonnes (compared with 114 million metric tonnes in MDCs) will come from China. Life expectancy in 2000, the latest date for which data are available, was estimated to be 64.7 years for males and 68.9 years for females. Infant mortality in 2000 was estimated to be 53.6 *per* 1,000 births. Only 76% of the global population had access to safe water in the period 1989-1998. In 1998, there were 4,270 people *per* 1,000 hectares of arable land. During the period 1995-2000, the fertility rate declined from 5.4% to 3.1% annually, and the population growth rate declined from 1.9% to 1.4% annually, according to the United Nations Development Programme (2002).

The populations of Africa (816,524,000): Anglo-America (317,195,000-USA, Canada, Greenland, Bermuda, St Pierre and Miguelou); Latin America (524,099,000 - Caribbean, Central America, Mexico); South America (350,514,000 - Andean group, Brazil and rest of South America); Eastern Asia (1,503,611,000 - China, Japan, South Korea, other Eastern Asia); South Asia (1,378,341,000 - India, Pakistan, other South Asia); Southeast Asia (1,378,341,000 - Indonesia etc.); Southwest Asia (312,679,000 - Central Asia, Gulf Cooperation Council, Iran, other Southwest Asia); Eastern Europe (375,196,000 - Russia, Ukraine, other Eastern Europe); Western Europe (391,637,000 – the EU countries and non-EU countries); and Oceania (31,377,000 - Australia, Pacific Ocean Islands) vary greatly in their growth rates, densities, life expectancies, infant mortality, access to safe water, and Gross National Product per capita.

Africa's population is expected to grow to 1,163,522,000 by 2020; its current population density is 26.9 persons per square km, life expectancy is 51.1 for males and 53.2 for females, infant mortality was 86.9 per 1,000 births in 2000, and in 1989-1998 only 57% of the population had access to safe water. The population per 1,000 hectares of arable land was 4,300, and the GNP per capita was only \$700. The AIDS epidemic and other endemic diseases pose enormous threats to Africa's population and the quality of life. The recent experience of sub-Saharan Africa has illustrated the Malthusian (after Thomas Malthus 1766-1834) problem of per capita incomes being driven down to subsistence level by the tendency of the population to grow faster than output. Elsewhere, the combination of technical advancements in agriculture, falling birthrates, and exploitation of new natural resources has averted the problem. Anglo-America's population is expected to reach 369,868,000 by 2020;

its current population density is 14.8 persons per square km; life expectancy is 74.4 for males and 80.2 for females, with infant mortality of just 6.7 per 1,000 births in 2000, and 91% of the population having access to safe water (1989-1998). The population per 1,000 hectares of arable land was 1,350, and the GNP per capita was \$30,750. Comparative data for Latin America are a projected population in 2020 of 645,387,000; population density of 25.5 per square km; life expectancy of 65.2 for males and 72.2 for females; infant mortality of 32.6 per 1,000 births; 78% of the population had access to safe water; the population per 1000 hectares of arable land was 3,720, and the GNP per capita was \$3,850. For South America, the 2020 projected population is expected to expand to 424,569,000; current population density is 19.6; life expectancy is 63.8 (male) and 71.5 (female); infant mortality 33.0; 76% had access to safe water; there were 3,450 persons per 1,000 hectares of arable land, and the GNP per capita was \$4,030. In all, the current population of the Americas is estimated to be 841,294,000, and projected to grow to 1,015,255,000 by 2020.

The population of Eastern Asia is expected to grow to 1,673,386,000 by 2020; the current population density is 127.7 per square km; life expectancy is estimated at 70.3 (male) and 74.6 (female) years; infant mortality was 26.5 per 1,000 births in 2000, and 71% of the population had access to safe water; there were 10,930 persons per 1,000 hectares of arable land, and the GNP per capita was \$3,970. South Asia's population is expected to reach 1,783,298,000 by 2020; the current population density is 274.4 per square km; life expectancy is 61.3 (male) and 62.4 (female) years; infant mortality was 70.3 per 1,000 births in 2000, and 80% of the population had access to safe water; there were 6,520 persons per 1,000 hectares of arable land, and the GNP per capita was a lowly \$440. Southeast Asia's population is also expected to increase sharply, to reach 651,001,000 by 2020; the population is presently 115.1 per square km; life expectancy 64.5 (male) and 69.5 (female) years, infant mortality was 40.3 per 1,000 births, and 70% of the population had access to safe water; there were 8,050 persons per 1,000 hectares of arable land; and the GNP per capita was \$1,160. Southwest Asia's population is expected to reach 431,929,000 by 2020, increasing the current population density of 29.6 persons per square km; life expectancy is 65.8 (males) and 70.3 (female) years; infant mortality is 52.3% per 1000 births, and 79% of the population had access to safe water; there were 3,010 persons *per* 1,000 hectares of arable land; and the GNP *per capita* was \$2,740.

Europe shares many characteristics with the Americas with the exception of population growth. The population of Eastern Europe is expected to decline to 322,138,000 by 2020, thereby leading to a decrease in the current population density of 17.4 persons per square km; life expectancy is 64.1 (male) and 73.9 (female) years; infant mortality was 18.1 per 1,000 births, and 95% of the population had access to fresh water (1989-1998); there were 1,570 persons per 1,000 hectares of arable land in 1998; and the GNP per capita was just \$2,380. These data contrast with Western Europe, including the EU, where the population is expected to increase slightly to 398,507,000 in 2020, such that there will be only a small effect on the current population density of 105.6 persons per square km; the life expectancy in 2000 was 75.0 (male) and 81.5 (female) years, with just 5.1 infant mortalities per 1,000 births in the same year; all the population have access to safe water; there were 5,040 persons per 1,000 hectares of arable land in 1998; and the GNP per capita was \$22,990. Scotland's population stood at 5,064,200 in June 2001, down from its peak of 5.24 million in 1974. By 2010, its population is predicted to fall below 5 million. At present there are fewer people aged under one than in any other age group up to the age 60, and life expectancies were 73.4 (male) and 78.8 (female), according to the Registrar-General. Oceania's population is projected to increase to 38,299,000 by 2020, and with a current population density of only 37 persons per square km there is clearly little relative pressure on land, other than in the Pacific Ocean Islands were the population per 1,000 hectares of arable land is already 5,530. Life expectancy in 2000 was 73.2 (male) and 78.8 (female) years; infant mortality was 24 per 1,000 births (just 5.0 in Australia), and 86% of the population has access to safe water. The population per 1,000 hectares of arable land in 1998 was 540, and the GNP per capita was \$15,510 (\$20,950 in Australia and \$6,370 in the Pacific Ocean Islands).

Clearly, within each of the regions or blocs there is wide diversity in economic and social indicators, perhaps best illustrated by a comparison of GNP data. Thus, in Africa, the GNP *per capita* varied between \$3,040 in Southern Africa and \$280 in East Africa. In the Americas, the GNP *per capita* of the USA was \$31,910, contrasting with \$1,690 in Central America. In Asia, the GNP *per capita* of Japan was \$32,030 whereas that of India was \$440, similar to that of Pakistan, and that of China \$780. In Europe, the GNP per capita of Germany at \$26,620 (\$23,500 for the UK), contrasts with \$14,800 for Spain, and just \$2,250 for Russia, and just \$840 for the Ukraine. The GNP refers to the total value of the final goods and services produced both from within the country, or averaged for a region, and from relevant foreign transactions in a given accounting period, usually a financial year. Thus, GNP is equal to the Gross Domestic Product (GDP) adjusted by net factor income from abroad (i.e. income residents receive from abroad for factor services - labour, investments, interest - less similar payments to those non-residents who contribute to the domestic economy). Unsurprisingly, GNP data closely align with other economic and social indicators in a country or region. It so happens that the 25 worst-nourished countries are the worst-governed countries.

### **Agriculture and Food**

Production According to the United Nations (UN) Food and Agriculture Organisation (FAO, http://apps.fao.org), global agricultural and food production in 2001 increased slightly above the level in 2000, although per capita food production declined by 1.1%. Of the major MDCs, only Japan, South Africa, and some of the so-called transitional countries in the former Soviet Union and Eastern Europe experienced increases in agricultural production, although the countries in transition and Japan have yet to restore production to the levels of 1989-1991. Total food as opposed to agricultural production in Canada, Japan, South Africa, and the USA declined, and declines in per capita food production were recorded in Australia, Canada, the European Union (EU), Japan, South Africa, and the USA.

In contrast, agricultural production increased in many LDCs, such as Argentina, Brazil, China, Indonesia, Malaysia, and Mexico; but declines were noted in Bangladesh, India, Turkey, and Vietnam. Total food production in the LDCs increased marginally, and principally in Argentina, Brazil, China, Indonesia, Malaysia, and Mexico. *Per capita* food production in the LDCs declined on average, reflecting rising populations although increases were posted in Argentina, Brazil, China, Malaysia, and Mexico. In the Democratic Republic of the Congo, Ethiopia, Turkey, and Venezuela, *per capita* food production was significantly lower than in 1989-1991.

The low-income food-deficit countries (LIFDCs), particularly in sub-Sarahan Africa, received the bulk of

food aid in 2000. A total of 8,464 million metric tonnes (mmt) of cereals were shipped between July 2000 to June 2001 to LIFDCs and other countries such as Afghanistan and North Korea, a marked decline from 11,168 mmt the previous year (1999-2000), but more than the 6,241 mmt in the period 1997-1998. Most of the reduction in food aid was attributable to a 35% decline in shipments from the USA to 4,697 mmt, a reduction from 1,387 to 707 mmt from the EU, and a reduction from 421 mmt to 192 mmt from Canada. Japan increased its shipments from 331 mmt to 720 mmt, however. Both currency devaluations and food aid can distort agriculture and agricultural trade.

**WTO** Attempts were made at the World Trade Organisation (WTO) meeting in Doha, Qatar in November to open a new round of negotiation on trade liberalisation, recognising particular difficulties in agricultural trade and associated export and production subsidies. Some argued that negotiations were less about trade liberalisation than justifying trade distortions. Agriculturally related trade disputes heard by the WTO included barriers against the importation of bananas, hormone-treated beef, bovine hides, wheat gluten, and lamb meat.

USA Despite its criticism of countries that distort trade by subsidies, tariffs, and other barriers, the USA consistently made supplementary payments to its agricultural producers since 1998, culminating in the early summer of 2002 with a new farm aid bill, the most expensive farm support programme in US history. Later in the year, an additional \$6 billion was voted by the Senate in aid for farmers affected by the worst drought since the 1930s. Unsurprisingly, the level of support was heavily criticised by a raft of other agricultural exporting countries, justifiably by the Cairns Group of free-trading nations (Argentina, Australia, Bolivia, Brazil, Canada, Chile, Colombia, Costa Rica, Fiji, Guatemala, Indonesia, Malaysia, New Zealand, Paraguay, the Philippines, South Africa, Thailand, and Uruguay).

**Subsidies** Farm subsidies in the period 1998-2000, according to the European Commission and the OECD, were complex. One measure is the producer support estimate *per* farmer, which strictly refers to total transfers to total transfers including subsidies to producers, ranged from over \$30,000 in Iceland, Norway, and Switzerland; over \$20,000 in Japan, South Korea, and USA; over \$15,000 in the EU; over \$10,000 in the countries of the OECD; but less than

\$5,000 in the Czech Republic, Hungary, Australia, Poland, Mexico, and New Zealand. The ministerially controlled European Agricultural Guidance and Guarantee Fund in 2000 ranged from around  $\in$ 9 billion in France; around  $\in$ 5 billion in Germany, Italy, and Spain;  $\in$ 4 billion in the UK; around  $\in$ 2.5 billion in Greece; and less than  $\in$ 2 billion in Ireland, Netherlands, Denmark, Austria, Belgium, Sweden, Finland, and Portugal; with a relatively small amount of spend on the Community Initiative Programme.

Livestock diseases Livestock diseases dramatically depressed rural economies in several countries, particularly the UK where after the expensive episode of bovine spongiform encaphalopathy (BSE), the clovenhoofed livestock sector was afflicted with foot and mouth disease (FMD). Five years after the public declaration of a link between BSE and new variant Creutzfeldt-Jakob disease (CJD), continental Europe suffered more cases of BSE than the UK. Early in 2001, however, an outbreak of FMD spread over large areas of the UK, devastating an already weakened industry. The implementation of a large-scale slaughter policy that received an international profile of ghastly images of burning pyres of dead livestock; closure of rural footpaths; a ban on livestock movements and meat exports; and massive public-sector expenditure on veterinary services and compensation, served to highlight the largely unrecognised rôle of agriculture in underpinning several key areas of the economy such as tourism. Field research of all kinds was badly affected. Over 100 farmers committed suicide. The potential of other kinds of disease control, such as ring vaccination, was debated furiously, and a variety of enquiries were launched. By mid-January 2002, the UK was declared officially free of FMD. Without proper import controls on unsterilised meat and animal products, and in the absence of enforced related biosecurity protocols, the UK remains vulnerable to future epidemics (see UK Agriculture section). Meat consumption in Europe fell. FMD in Argentina, BSE in Japan, and poultry influenza in Hong Kong disrupted the international meat trade.

**Trade** Spreading global recession was rapidly expressed in shrinking values of agricultural trade, but not volumes. A relatively strong US dollar rendered many US agricultural products uncompetitive, other than the technologically advanced genetically modified (GM) soybean and maize commodities. World grain production increased from 1,836 mmt to 1,843 mmt. Coarse grain production increased by 2% but trade declined by 4%. For the second year, global rice

production declined but its trade remained at 24 mmt; wheat production fell but its trade rose 4%. Grain prices remained at depressed levels even though consumption rose and reserve stock levels fell to levels last experienced in the mid-1990s.

Record levels of oilseeds were recorded in 2001, continuing the trend since 1991, particularly in soybeans from Argentina, Brazil, and the USA. With trade growing by nearly 6%, ending stocks declined by nearly 2%. As in the cereals sector, prices were depressed.

A 2% decline in global sugar production to 34.2 mmt largely reflected a remarkable decline of 12.5% in EU production, and with growing consumption there was a modest firming in prices. Enhanced coffee production and increasing exports from countries outside the Association of Coffee Producing Countries led to low prices for already hard-pressed growers.

**Roadmap** Agriculture is the single most important activity for human existence. The efficient production of crops and livestock for food supplies and industrial feedstocks has released humanity from the hunter-gatherer treadmill. Scientific, engineering, and technological advances have removed the drudgery in many countries. World food production has quadrupled since 1950, using just 1% more cultivated land; the world's population has grown and has the capability to expand further; less than one in ten people do not have enough to eat whereas 50 years ago, that figure was one in four. Civilisation has been allowed to proceed. Food-supply security is no longer a political priority in the UK and many other countries. The global economy, human health, and societal development have been, for the most part, positively influenced by agriculture, but there are debates about the environmental costs of certain types of agriculture, although large portions of the terrestrial environment have been shaped by mankind. Drawing on A Science Roadmap for Agriculture (cited as Task Force on Building a Science Roadmap for Agriculture, National Association of State Universities and Land-Grant Colleges, Experiment Station Committee on Organization and Policy, "A Science Roadmap for the Future". November 2001 (www.nasulgc.org/ comm\_food.htm)) there are huge challenges in defining the needs of agriculture and the future direction of the various strands of agriculturally relevant science. The Science Roadmap was designed for the USA, but it has resonance for the EU and the UK. It followed a conceptual framework of needs to (a) be competitive

in a global economy; (b) add value in future harvests; (c) adjust agriculture to a changing climate; (d) be good stewards of the environment and natural resources; (e) make agricultural enterprises profitable; (f) make families and communities strong; and (g) modify foods for improved health and safety. From these needs arose seven challenges which align closely with European agricultural perspectives, addressing common points in the background information and rationale, the consequences of failing to address these needs, the specific objectives of the research programmes, and potential impacts of the research. These challenges should take precedence over the negativity ingrained in the recommendations of the recent Curry Commission report on the Future of Farming and Food, not least because of the extensive and expert analyses devoted to the US study.

Challenge 1 refers to the development of new and more competitive crop products and new uses for diverse crops and novel plant species - logical in the light of the fact that the US farm community is responsible for record harvests but suffers economic losses despite its relative efficiency, and fails to capture value-added benefits. By a combination of gaining social acceptance of continued biotechnological R&D, increased investments in plant and microbial genomics that are combined with plant breeding and bioinformatics, crop biomass production, and precision and low-tillage farming – efforts that may extend over 20 years – US agriculture would generate great profitability, offer more options, have reduced reliance on fossil fuels, and release new products for consumers.

Challenge 2 is the development of new products from and new uses for animals. Interestingly, the focus is on production efficiency and lessening the impact of livestock intensification on animal and employee welfare, and on lessening the undesirable impacts of manure and its disposal. Consumer issues are relevant in respect of coaxing society to accept the practices and products of modern livestock farming. Specific objectives include expanded R&D programmes in functional genomics, proteomics, bioinformatics, utilisation and preservation of germplasm, value-added products, animal behaviour, animal handling systems, and systems to increase food safety. From these areas would arise a sustainable food-animal industry with reduced environmental impacts. Within the UK, disposal of animals and their byproducts is an urgent policy matter brought to a head by the FMD and BSE debacles, and new environmental regulations.

Challenge 3 - lessening the risks of local and global climatic change on food, fibre, and fuel production mixes medium- to very long-term R&D objectives. It was noted that "crop and livestock production in the USA has shifted to states where climate conditions are more favourable than in others. For example, the four major US crops (corn, wheat, soybeans and cotton) now are grown in just four or five states. Similarly, the livestock industry, including beef cattle, dairy, swine, poultry, and fish, is moving toward concentration in just a few states. Further shifts in climate conditions may accelerate this pattern and affect both food and fibre production". This pattern has, is, and will occur in the UK and the EU, regardless of sociopolitical manipulations. Soil conditions, availability of water, climate, transport, skill base, and access to profitable markets will ultimately determine location of agricultural and horticultural activities. The Roadmap expects rain-fed crop acreage to decline dramatically in the face of climate change, while irrigated-land acreage should increase modestly in areas where water will become more readily available and affordable. It also refers to research that suggests the economic impacts of climate change could exceed those due to population growth. Vindicating a part of SCRI's research portfolio, Challenge 3's needs highlight the development of accurate mathematical models, simulation models, expert systems, and decision-support systems that will help assess and adapt to short- and long-term effects of climate change in crop and livestock systems. Interactions between plants and soil microbes, and the regulation of nutrient and water uptake were prioritised, as were the development of stress-resistant crops and livestock, and strategies to prevent the loss of biodiversity. New crops, conservation tillage, soil amendments, and land reclamation should be investigated. Most telling was the stated need to develop new institutions that allow scientists and policy makers to work together to design and implement flexible agricultural and environmental policies for land use and crop production.

Integral to Challenge 3 is Challenge 4 – provision of the information and knowledge needed to improve environmental stewardship, which recognises the critical role of R&D on ecosystem goods and services. This means the conceptual shift from considering only fields or farms to the management of complete ecosystems (*e.g.* whole watersheds), sustaining the natural resources base essential for its existence. Seven topics were listed. (i) More environmentally friendly crop and livestock health protection programmes building on new opportunities from genomics research leading to a replacement of chemically based plant and animal health strategies with biotechnological solutions. (ii) More scientifically sound natural resource preservation strategies, noting the threat to a sustained agricultural production posed by urban developments. (iii) Fertilizer management, reining back nitrogen- and phosphorus-based fertilizers. (iv) Better environmental pollution prevention and management schemes, mitigating the effects of agriculture, and considering GMOs for bioremediation. (v) A greater dependence on science-based environmental regulations, so that understanding is gained of the trade-offs involved and alternatives can be developed as well as providing the necessary freedom to farm. (vi) Technology-based waste management solutions, helping to handle and dispose of the vast volumes of waste generated in food production and processing. It was noted that ignoring this latter topic could result in serious public-health hazards and environmental pollution, and increased public opposition to modern food production and processing practices. New understanding is also required of pathogenesis and resistance mechanisms.

Challenge 5 deals with improvement of the economic return to agricultural producers, noting that the focus may either be on the global market place or local or regional markets, and that there are several factors that lead to a competitive advantage. One of the primary objectives of conventional agriculture is to produce as much food and fibre for the least cost, capitalising so much on industrialisation to shift from subsistence requirements to meeting the needs of commerce. Inevitably, farm sizes have increased, the numbers employed have fallen leading often to rural depopulation, yields increased, capital investments increased, and the processing and retailing companies aggregated. As in Europe, small farms, usually family farms, barely make ends meet, collectively occupy a large land area, but account for a disproportionately small part of the total value of agricultural production, having less market muscle and little access to technology. The range of farm types, scale of operation, ability or willingness to take risks, markets addressed, and management capability vary such that there is not a single research thrust that will meet the needs of the marketplace. Diversity of operation will diminish unless there are improved decision-support systems for riskbased management in farming. Market analyses, novel and value-added products, strategies for community-supported food-production systems, and focusing on local and regional markets and consumers, were deemed essential components of a national strategy, leading to food and fibre security and economic viability.

Challenge 6 - strengthening communities and families - is an extension of Challenge 5, and in the light of national and international competition, the situation in the USA is affected by the mobility and diversity of the population at large, inadequate community structures, and inadequate workforce competencies. Europe is somewhat different, with relatively little workforce mobility, relatively static communities, and a markedly ageing workforce. Relatively few young people wish to enter agriculture with its poor rewards. The Roadmap document noted the need for community vitality which is affected by both globalisation and technological change. Entrepreneurship reinforces communities, and thus support of the selfemployed was recommended. Unintended consequences of Federal policies adversely affecting rural communities translate all too readily to EU policies. Mechanisms to buffer against the adverse effects of globalisation seem to rely on a mixture of a supportive infrastructure, sustaining a farmland base, and giving access to technology. Proposed research in Challenge 6 is less tangible than in the other sectors, relying on documenting, surveying, and defining communities undergoing change.

Finally, Challenge 7 concerns food safety and health through agricultural and food systems, with the intention of lengthening the period of healthy life and eliminating health disparities (e.g. cardiovascular disease, obesity, diabetes). Specific foods are known to influence certain health conditions, and new experimental tools are beginning to unravel the mechanisms involved. Although much of the research is in its infancy, the public has "enthusiastically embraced some health claims that science has yet to prove, while neglecting others that have been proven for years". Throughout the world, food safety has improved markedly over time, and more than 200 known pathogens are transmitted by food. The R&D needs include improved understanding of the components of human health and wellness; how nutrients relate to physiological function; interactions between food components; and new methods to deliver foods to people to meet individual needs. Biomarkers for nutrients and phytochemicals; creation of healthier foods by using conventional and molecular methods; pathogen detection, identification, and characterisation; toxin levels in foods; food treatments to control

pathogens; and modification of behaviour in food handlers, preparers, and consumers were noted, all of which figure in the UK's Food Standards Agency operations. All in all, the Roadmap has a buoyancy of purpose and positivity missing from most European exercises looking at the future of agriculture.

CAP Reform Reform of the CAP is long overdue. Its origins lie in the original common market that reduced tariffs on industrial products from Germany in exchange for financial support for French smallscale farmers. CAP was largely designed to protect German small-scale farmers from French competition. Expansion of the system to incorporate other European countries with their own agricultural systems gave rise to bureaucratic complexities, opportunities for large-scale fraud and corruption, surpluses, waste, trade distortion to the detriment of agriculture in LDCs, the idiocies of unstructured set-aside, and a massive downturn in the wellbeing of UK agriculture. From a position of strength in 1973 when the UK joined the European Community, UK agriculture lost its competitive edge, disadvantaged by its support system relative to its European partners, the strength of the pound, political pressures to suppress consumer prices, and the effects of being swathed in regulations. Pressures for change are manifold: the sheer cost of CAP for the European Union, problems with the WTO and other countries, and trading blocs, the likelihood of bankruptcy for the EU if enlargement of the EU takes place without major changes to the CAP, the low profitability of agriculture and poor competitiveness of European agriculture in world markets as supply exceeds demand, and lack of political sympathy. Amidst discussion on the future direction of the CAP, notwithstanding the proposals by Franz Fischler, the EU Agriculture Commissioner, there is still the need for politicians properly to understand the original essence of support mechanisms for agriculture and horticulture - the provision of mechanisms to improve efficiency and access to low-cost, high-quality food supplies, cushioning the vagaries of the weather, pests and diseases, and volatility in world markets. This surely represents a food-access protection mechanism for poorer members of society. Trade distortion is a consequence of this underpinning support, thus exceptional care is needed to lessen its impact. There is little documented evidence of profound thinking about the impacts of the main proposals: linkage of payments to animal welfare, health and safety, and environmental laws and regulations; increasing support for rural development, however

defined; compulsory long-term set-asides on arable land; mandatory inspections; capping of direct subsidies at €300,000 per year. In essence, the European Commission aims to complete the process of decoupling CAP aid from farm production, a process started in 1992. If the proposals are agreed, there would be a single integrated payment for each holding, but that would be conditional on meeting certain animalhealth and welfare, environmental, and food-safety standards. These conditions would operate under a common framework with basic implementation criteria enforced through an as-yet-to-be-clarified auditing system for farms receiving over €5,000 per annum. Starting in 2004, it is proposed that there would be "dynamic modulation" - a compulsory cumulative annual levy of 3% on direct payments. Together with capping payments, the savings will go to the "Second Pillar" rural development budget to be distributed from intensive cereal- and livestock-producing countries to poorer and more extensively farmed and mountainous countries, with the expectation of bringing about "positive environmental effects". Support will be given for assurance and certification schemes, producer groups, the farm audit scheme, and for livestock farmers using politically acceptable animal-welfare systems. Long-term set-aside (10 years) will be compulsory on arable land and will be subject to the same standards required of land in production. A 'carbon credit' of €45 per hectare will be given for non-food crops produced with a view to carbon-dioxide management, with a maximum guaranteed area of 1.5 million hectares. (see http://europa.eu.int/comm/ agriculture/mtr/comdec\_en.pdf). It is a moot point as to whether tax payers will want to pay for many environmental goods. Large-scale efficient farming units, especially those in the UK, will be penalised. Assumptions that small-scale "organic" farmers are needed in greater numbers will be tested on the rack of the market place. The UK would be a substantial loser in respect of the return from its contributions to the EU; food imports will increase. Agriculture would become even more centralised through regulation and monitoring even though direct payment support for product would decline. The overall cost of the CAP would not decline, however. Research and development to improve agricultural efficiency and product diversification would diminish, and the competitive position of EU agriculture would be weakened.

Market manipulation comes at a high price in the medium-to-long term. The opportunities for fraud and corruption will still exist. Applicant countries rightly perceive that they will not receive the benefits of the CAP they originally understood would come their way. Nevertheless, there is resistance to change, principally from France, the Republic of Ireland, and the Mediterranean EU countries. France had 679,000 farmers in 1998, occupying 30 million hectares; its farmers and farmworkers represented 3.9% of the workforce, and agriculture accounted for 2% of French GDP. Possible advantages of the proposals would be a better matching of supply and demand food production would pay due regard to market conditions and environmental impacts. Export subsidies would be scaled back. Encouragement to form cooperative groupings may assist in negotiation with the small number of powerful supermarket retailers, and develop confidence in investing in the future.

In 2000, 5% of farmers in the EU received over 50% of the CAP funding. With the exception of Greece where data were not available, 4,450,000 claimants received  $\leq 22.37$  billion in direct payments but only 1,880 farmers received more than  $\leq 300,000$ , the proposed capping level for payments in the mid-term review. Germany had 1,260 farms of this size, the UK 380, and Spain 130. Half of the EU's farmers received less than  $\leq 2,000$  on an individual holding basis, and 70% less than  $\leq 5,000$ , reflecting the small size of the individual farming operations. In the UK, there were 166,500 claimants that each received an average of  $\leq 19,256$ , but 47% received less than  $\leq 5,000$ , and 24% less than  $\leq 1,250$ .

**EU Enlargement** Enlargement of the EU by accession is dependent on applicant countries being stable European democracies with free market economies. Any Accession Treaty must be approved by the Governments and parliaments of all the member states, as well as by the European Parliament as well as the government and parliament of the applicant state. Agreements have been signed with 10 countries; Bulgaria, the Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Slovakia, and Slovenia. Those agreements commit both the EU and the applicant countries to long-term political and economic integration and thereby EU membership. Chiefly in response to the intended enlargement of the EU, a 105-member European Convention was established on December 2000, and headed by V. Giscard d'Estaing. Its remit is to propose "a framework and structures for the EU which are geared to changes in the world situation, the needs of the citizens of Europe and the future development of the EU."

So-called association agreements which initially offer EU financial assistance have been signed with Cyprus, Malta, and Turkey. Partnership and co-operation agreements that foster political and economic relations but exclude the possibility of membership have been implemented with Georgia, Kazakhstan, Kyrgyzstan, Moldova, Russia, Ukraine, and Uzbekistan; those agreements with Belarus and Turkmenistan are not in force. The European Economic Area (EEA) which came into effect in January 1994 from the members of the European Free Trade Area negotiated preferential access for their goods, services, labour, and capital to the European Commission Single Market. After Austria, Finland, and Sweden joined the EU in January 1995, only Iceland, Liechtenstein, and Norway remain as the non-EU EEA members, but they agreed to adopt the EU's acquis communautaire, apart from the sections dealing with agriculture, fisheries, coal, and steel.

CAP Budget Agriculture plays a major rôle in the EU and its budget. The EC is limited to a percentage (currently 1.27%) of gross national product (GNP) it can raise from its member states, and budget revenue and expenditure must balance. Only four sources of funding are permitted: customs duties on imports from non-EU states; levies charged on agricultural imports from non-EU states; contributions based on shares of a notional Community harmonised Value-Added-Tax (VAT) base; and contributions based on shares of Community GNP, a budget-balancing item meant to cover the difference between total expenditure and the revenue from the other three sources. The "Mrs Thatcher's Rebate" initiated in 1984 (comprising 66% of the difference between the UK contributions to the budget and what it receives), represents compensation for disproportionate contributions by the UK caused by its high levels of agricultural and other imports from non-EU countries and its relatively small receipts from the CAP component of the budget. One impact of the rebate is that the UK Treasury is resistant to spending on EU schemes in the UK as this would reduce entitlement to the rebate, and agriculture in particular is disadvantaged.

In the General Budget of the EU for the Financial Year 2001, Agriculture represented 45% ( $\in$ 43.3 billion) of the total ( $\in$ 96.2 billion), Regional and Social 34% ( $\in$ 32.7 billion), External Action 5.1% ( $\in$ 4.9 billion), Administration 5.1% ( $\in$ 4.9 billion), Research and Technology 4.1% ( $\in$ 3.9 billion), and Pre-accession Aid 3.3% ( $\in$ 3.2 billion). In the Official Journal of the European Communities C series 1.12.2000, the major

contributors to the EC budget in 1999 were Germany (€21.07 billion; 25.5%), France (€13.99 billion; 17%), UK (€11.08 billion; 13.4%), Italy (€10.77 billion; 13.0%), Spain (€6.23 billion; 7.5%) and the Netherlands (€5.09 billion; 6.2%). The net recipients were Spain (+€6.66 billion), Greece (+€3.64 billion), Portugal (+€2.67 billion), and the Republic of Ireland (+€1.82 billion). The main "losers" were Germany (-€11.36 billion), UK (-€5.29 billion), the Netherlands (-€3.36 billion), Italy (-€1.76 billion), Belgium (-€1.28 billion), Sweden (-€1.23 billion), and France (-€1.16 billion); the remaining countries had "losses" less than €1 billion.

As agricultural markets, political imperatives, and production technologies evolved, reform of the CAP was inevitable, but as in most walks of life retarded by vested interests. A particular series of reforms to the CAP took place in 1984, 1988, 1992, 1997, and 1999. Co-responsibility levels and national quotas for certain products were established in 1984. The glorious set-aside grants to take land out of production started in 1988. The 1999 reforms were designed to reduce surpluses of cereals, beef, and milk by cutting intervention prices and compensating producers through area payments. Agenda 2000 was introduced to prepare the EU for the accession of new member states but has increased the cost of the CAP by around €1 billion per annum in compensation payments. The CAP is a major factor in the development of the concept of the Single Market, as codified in the EC 1985 White Paper on completing the internal market and the Single European Act which came into force in January 1993. The Act has not yet been fully implemented in respect of eliminating frontier controls and harmonisation of taxes. Budgetary and WTO pressures, improving transparency of process, and public debate will drive more reform of the CAP.

**Influences** Overarching influences on European agriculture include not only the CAP, decisions of the WTO, global weather systems, and pests and diseases, but population and social changes. According to the 1994 UN report *The Sex and Age Distribution of the World Population*, the population of Europe will shrink from 729 million in 2000 to 677 million in 2050, whereas there will be increases in Africa (831 million to 2.14 billion), and Oceania (30,651 to 46,070) (see also Conflicts and Populations section). Urbanisation and suburbanisation, an increase in single-person households, increased demands for convenience foods and recreation, demands of food processors and retailers, and development of non-food

agricultural and forestry outputs including industrial feedstocks, will shape the quantity and nature of supplies and various types of demand. Ownership of relevant intellectual property will become a major issue.

**Nutraceuticals** Datamonitor recorded 204 launches of neutraceutical-type products in the world's major markets a period of 18 months up to October 2001. Most products were "probiotic" types, followed by products claimed to reduce the risk of heart disease, including those that lowered blood cholesterol levels. The Datamonitor report predicted a weakening demand for simple vitamin dietary supplements as consumers switch to food products that target specific ailments.

Pesticides Pesticide usage will be severely curtailed in the EU in 2003. The EC will withdraw 320 pesticides, including fungicides, herbicides, and insecticides, in the light of manufacturers declining to defend their products for economic reasons, a process arising from notification procedures introduced during 2000-2001. There is the possibility that a further 150 substances could be withdrawn in July 2003. Thus, more than 60% of the substances on the market in 1993 will have been withdrawn; safety assessments on all the remaining defended substances should be completed by 2008. The current state of plant breeding, and the known propensity of pests and diseases to circumvent resistance mechanisms, ought to engender a search for new-generation crop-protection agents, but the European agricultural scene is generally not favourable to widespread investments in this area.

In January 2001, the EC adopted the Communication on the Sixth Environmental Action Programme together with a proposal for a Decision of the European Parliament and the Council of Ministers for its adoption in June 2002. This stated in Article 7 that the impact of pesticides on human health and the environment must be reduced, and that there is a need to achieve a more sustainable use of pesticides as well as a significant overall reduction in risks and of the use of pesticides consistent with the necessary crop protection. Moreover, there was a need to ratify both the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade, and the Stockholm Convention on Persistent Organic Pollutants.

The generic term 'pesticides' includes all substances or products that kill pests including but not exclusively those deployed in agriculture, horticulture, and forestry. Related to those products are plant-protection products (PPPs) used specifically to protect plants or plant products against organisms deemed to be pests, or to prevent the effects of these harmful organisms; they act in diverse ways, such as by killing (i.e. true pesticidal action), repulsion, physical barriers, providing alternative attraction sites, modifying reproduction of the pests, or modifying the growth or composition of the host plant, etc. The term 'biocide' is widely regarded as substances that kill or control pests in the non-agricultural sectors e.g. wood preservation, domestic uses; see Directives 98/8/EC and 91/414/EEC. Most concerns in the EU are directed towards PPPs, according to Eurostat and the European Crop Protection Association. In 1999, the EU represented around 25% of the world market in PPPs, amounting to circa 320,000 tonnes of active substances sold per year, with fungicides (43%), herbicides (36%), insecticides (12%), and other pesticides (9%) as the main products. Non-agricultural deployment of pesticides amounted to only 2% of total use, according to Environmental Pressure Indicators for the EU, June 2001, Eurostat. Data on the volumes of pesticides used offer little related information on their environmental impacts. Applications vary according to the type of crop (vines, cereals, vegetables, and potatoes receive the largest quantities); pest, weed and disease frequency; seasonal factors; and agronomic practice. Data are scant on costs of pesticides and their application; in-field variation; and country or region where applied. The highest application rates are apparently noted in The Netherlands. The PPP industry in Europe employs around 35,000 people, and the crop protection market in the EU is estimated to be in excess of  $\in 6$  billion *p.a.* 

A particularly contentious area – bedevilled by strong opinions unfounded on fact, intolerant opinions expressed by certain farming and environmental pressure groups, unprofessional behaviour, and lack of understanding of agriculture – is that of a risk-benefit analysis of the use of PPPs. R. Bates, former head of Pesticide Registration and Surveillance Department in the former Ministry of Agriculture, Food and Fisheries noted that the public perception of the nature and magnitude of the health risks associated with pesticides is so at odds with available facts that it has become increasingly difficult to distinguish fact from fiction. Many have noted the disconnection between market demands for zero or low usage of pesticides with the maintenance of food supplies all-year

round, with healthy and safe produce. Pesticides are used in organic and conventional agriculture with the aim of preventing yield losses from weeds, pests, and diseases; to protect or improve the visual appearance, quality and safety of harvested agricultural produce; to protect plant products in storage; and to improve agronomic efficiency and competitive position. Efficiency gains in agriculture have led to the availability of relatively low-priced, high-quality plant products throughout the EU as well as lessen the demand for cultivated land, reduce energy-demanding erosion-inducing tillage, reduce waste, and sustain profitability. The consumer-led marketplace decides on the acceptability and value of plant material. For very good reasons - the presence of anti-nutritional compounds and the depletion of vitamin C levels consumers avoid diseased and blemished produce. Some of the attacking microorganisms themselves produce toxins. Losses due to pests and diseases, both in the field and the store, can amount to 40% of total food production. The level of safety testing of modern pesticides if applied to many staple foods would cause them to fail. Some of the chemicals permitted for use in organic-farming systems would also fail, and the various plant extracts that are allowed (e.g. neem, pyrelthrins, rotenone, tobacco), do not have established maximum residue levels in crops. B. Ames and colleagues have noted that 99.99% by weight of the crop protection products in the American diet have been synthesised by the plants themselves. Only 52 of these naturally occurring compounds have been tested in high-dose cancer tests and about half are rodent carcinogens. They estimate that Americans eat 1.5g of natural crop protection compounds per person per day, some 10,000 times more than any synthetic crop protection compounds they might consume. Syngenta Crop Protection UK Ltd. have produced a useful booklet on this topic, Gaining Consumer Confidence : Residues of Crop Protection Products in Food, by N. A. Atreya, C. Turner, and P. Parsons, May 2002.

Nonetheless, most PPPs are intrinsically dangerous (see Directive 67/548/EEC), Health and Safety Executive (HSE) Agriculture and Wood Sector. Pesticide Incidents Report 2000/2001) but it has proved difficult to quantify in monetary terms the actual adverse effects of PPPs. Human health can be affected acutely or chronically by direct exposure of those producing, applying, or consuming pesticides, or advertently coming into contact with them. According to the UK HSE, in the year 2000-2001

there were 170 pesticide incidents, 71 of which were alleged to have caused ill-health. Particular emphasis has been placed on those categories of the population regarded as sensitive, namely the elderly, the sick, and children, as well as those likely to encounter prolonged exposures. Drinking, surface, ground, and irrigation waters are subject to monitoring procedures. The most commonly identified pesticides in groundwater are the broad-spectrum herbicide atrazine and simazine. Persistence of active compounds and their bioaccumulation on the food chain, leading to possible adverse effects on human health (e.g. carcinogenicity, mutagenicity, genotoxicity, endocrine disruption) and indirect effects on the environment (principally loss of biodiversity) are active areas of research and monitoring, but there are remarkably few reports globally on pesticide-induced ill-health in consumers of agricultural products. Bad practice in the production and selection of pesticides, applications that lead to diffuse pollution or excessive doses, contamination after applications often through the cleaning of equipment, and disposal of surplus pesticides, their containers, and protective clothing pose risks. These are exacerbated in LDCs where there may be less strict monitoring systems, reliance on old-fashioned, broadspectrum toxic compounds, imprecise application technologies, lack of protective equipment, and poor training, all manifestations reflecting financially stressed circumstances.

Reduction of risks associated with PPPs over their life cycle hinge on several factors, not least regulation, monitoring, packaging, and labelling, continual R&D, phasing out of pesticides with unacceptable effects, proper disposal systems, modified farming practices, and plant breeding to introduce new forms of resistance to pests and diseases. Risk assessments and their limitations are discussed in the *Communication from the Commission on the Precautionary Principle* (Com (2001)1 final). Complexity arises, however, from the formulation of active ingredients with other active ingredients, various adjustments, carriers, diluents, and supplementary fertilisers, leading to poorly understood additive or synergistic effects.

The Council Directive 91/414/EEC over-optimistically initiated a 12-year programme to review all bioactive substances in the EU marketplace, but the *Report from the Commission to the European Parliament and the Council on the evaluation of the active substances of PPPs* (Com(2001)444 final of 25.7.2001) recognised the enormity of the task and requested a postponement of the deadline to July 2008. The request was approved.

Maximum Residue Levels (MRLs) in foodstuffs are often cited in terms of toxicologically acceptable. Acceptable Daily Intake (ADI) measures using Good Agricultural Practices (GAP). Directives 76/895/EEC, 86/362/EEC, 86/363/EEC, and 90/642/EC relate to the fixing of MRLs derived from the standpoint of application. These Directives are connected to Good Farming Practice (GFP), which refers to the standard of farming adopted by a reasonable farmer in the region concerned. Member States are required to set out the standards which entail compliance with general mandatory requirements. Thus, GFP is mentioned in Council Regulation (EC) 1257/1999 in respect of support for rural development from the European Agricultural Guidance and Guarantee Fund, and the related Commission Regulation 1257/2002/ The potential cumulative effects of different PPPs have yet to be considered in the setting of MRLs. Most important, there is strong evidence from national monitoring exercises that residue levels in plant products have decreased over recent years. The overall picture is given in Monitoring of Pesticide Residues in Products of Plant Origin in the European Union, Norway and Iceland, 1999 Report (SANCO/397/01-final), in which 64% of fruit, vegetables, and cereals in 1999 were without detectable residues, compared with 60% in 1994. MRLs were exceeded in 4.3% of samples in 1999, but actual consumer exposure to pesticide residues remained well below ADI levels.

Among the various international initiatives and regional co-operation initiatives, on pesticides, the Codex Alimentarius has an influential role, with its recommendations having benchmark status upon which the WTO evaluates national food measures and regulations. Within the EU, there is a drive to set EU MRLs more rigorous than those of the Codex MRLs, an action that could be seen by importers as a contrivance to distort world trade. Discussions will be needed with other trading blocs, the European and Mediterranean Plant Protection Organisation (EPPO), and FAO, especially with regard to the FAO International Code of Conduct for the distribution and use of pesticides.

Water quality in the EU is controlled under the aegis of the Water Framework Directive (WFD); Directive 2000/60/EC, October 2000, establishing a framework for community action in the field of water policy; OJ L 327 of 22.12.2000), based on Directives 91/414/EEC, 75/440/EEC, 76/464/EEC and 80/68/EEC encompassing surface water, discharges of dangerous substances, and groundwater. Member States are expected to prepare comprehensive river basin management plans by 2009, including measures to tackle pesticide pollution.

CAP and the Environment Fundamental to the future of agriculture in the EU is the way in which the CAP shapes land use, intensification, crop choice, the economic viability of farming, and the direction of rural development. Bureaucratic systems have been introduced to lessen the environmental impact of CAP-influenced agriculture e.g. Council Regulation (EEC) No 2078/92, Agenda 2000, Council Regulations (EC) 1257/1999 and 1259/1999. The latter Regulation links environmental protection requirements and direct support to producers from the CAP, giving flexibility to Member States to take the environmental measures they consider appropriate, if necessary to cut direct payments as a sanction to support the enforcement of environmental requirements. Less-favoured areas receive special attention. At present, though, less than 3.5% of total CAP spending is devoted to agri-environmental measures, but affect more than 20% of the total EU agricultural area. Policy makers favour various "environmentally friendly" (i.e. no-pesticide) systems. Thus, integrated agriculture, integrated production, integrated crop management (ICM), and integrated pest management (IPM) systems include different minimum requirements for the protection of the environment or for pest control, and the various measures permitted to bring about control. In essence, these systems aim to balance finances, pest and disease control, product quality, public health and food safety, working conditions, and environmental impact. Often, they are conducted in tandem with certified production schemes to provide a transparent, audited, due-diligence-related assurance to food processors, retailers, and consumers. Many producers do not receive adequate recompense for the cost of the assurance schemes.

"Organic" production is defined and regulated in Council Regulation (EEC) 2092/91 of June 1991, and operates under several bodies characterised by the use of relatively few pesticides, some of which, however, are controversial. Overall, it is a moot point as to whether there will be a downward trend in the use of PPPs in agriculture in the EU. Directive 2001/18/EC of the European Parliament and of the Council of Ministers on the deliberate release into the environment of genetically modified organisms (GMO) repealing Council Directive 90/220/EEC (Commission Declaration. OJ L 106 of 17.4.2001) takes into account possible adverse effects of the widespread cultivation of GM crops. With regard to PPPs, several EU countries are conducting field trials on GM crop releases, and the EC has set up a Working Group on Herbicide-Tolerant Crops based on Directives 90/220/EEC and 2001/18/EC, considering also the use of herbicides. Gene flow and potential long-term effects of GMOs are also under review.

In COM (2002)17 final, the EC adopted a proposal in January 2002 for a Directive on environmental liability with the intention of establishing an environmental liability regime to assist in the prevention and remediation of environmental damage to waters, biodiversity, and land. This would cover the manufacture, use, storage, transport, and disposal of PPPs.

The UK has already stated its intention to follow Belgium, Denmark, and Sweden in introducing taxes on PPPs as a method of rationalising their use. Even so, statistics on the utilisation of PPPs throughout the EU are incomplete, but members of the European Crop Protection Association have provided Eurostat with their data on the use of pesticides for the major crop groups in the EU. In absolute terms, the highest consumption of pesticides takes place in those countries with the largest areas under crops (France, Germany, Italy, and Spain), but in terms of pesticide use *per* hectare, Belgium, Italy, France, and the Netherlands are the most intense users.

The EC proposes to introduce a thematic strategy to achieve sustainable use of pesticides, involving consultations, harmonisation of existing statutory instruments and initiatives, and the development of new instruments and initiatives to address the risks associated with the use of PPPs. This would involve the establishment within two years of national plans to reduce hazards, risks, and dependence on chemical controls (including a ban on aerial spraying); improved controls on the use and distribution of pesticides; substituting the most dangerous active substances with safer, possibly non-chemical, alternatives; encouragement of low-input or pesticide-free farming systems but not punitively by imposing an EU-wide scheme of levies on PPPs; and finally improved datagathering for reporting and monitoring progress in achieving strategy and policy objectives. Of course,

much hinges on funding replacement strategies for the existing PPPs in an expeditious way, lest EU agriculture is sacrificed. Conventional plant breeding is too slow and the European plant breeding industry too unprofitable to provide resistant cultivars within the anticipated deadlines. Unreasonable demands will either simply switch off production, possibly increasing dependence on imports but satisfying the whims of certain pressure or retail groups, or provide the necessary stimulus to introduce high-speed modern breeding systems capable of matching the challenges arising from the fascinating adaptability of pests and diseases. Analysis of human life expectancies show an interesting correlation between the introduction of agrochemicals into farming systems and increasing life span. Better data are required on the biological effects, persistence, and degradation of the PPPs, and crucially, on their alternatives; altogether this represents a massive task. Crop-spraying specialists, farm advisory services, UK Levy Boards, research institutes, colleges, and government agencies provide informative briefings on current best practice and likely trends. It is axiomatic that the present level of R&D is wholly inadequate to address the scale of the exercise to review all PPPs, not least because data sets are discontinuous, science albeit advancing worldwide at a phenomenal rate is under-resourced in Europe, practices differ from country to country, agriculture is an economically frail industry, and politicians continually fall prey to food scares.

Non-Food Crops Non-food uses of crops would seem to be an attractive area of agricultural activity, creating new types of investment, income, and employment mainly in rural areas. Strategies to encourage the use of alternative fuels to lessen the dependence on oil-based and fossil fuels have been proposed over the past four decades. Biofuels, predominantly bio-diesel and bio-ethanol, but including bio-methanol, bio-oils, and bio-gas could displace fossil fuels but will not be able to establish a proper market foothold unless there are changes to fuel taxation, introduction of more emission controls, and encouragement to meet Kyoto targets. Major scientific, engineering, and technological advances are possible, focusing on the generation of improved cultivars, agronomy, harvesting and processing, storage, combustion, and energy conversion and storage. The topic is a potentially rewarding area for innovation and creation of intellectual property (IP), but requires multidisciplinary effort. To date, most effort in the UK has been expended by the public sector with little focus on encouraging the creation of IP. A pertinent source of reference in this topic is *The Technology Roadmap for Plant/Crop-Based Renewable Resources* 2020 : A Vision to Enhance U.S. Economic Security *Through Renewable Plant/Crop-Based Resource Use* (http://www.oit.doe.gov/agriculture/), prepared by Inverizon International Inc. on behalf of the Renewables Vision 2020 Executive Steering Group, published in January 1998.

According to the Department of Trade and Industry (DTI), biofuels represented 82.3% of the UK's renewable energy sources in 2000, hydroelectricity 14.6%, windpower 2.7%, and active solar heating 0.4%. Biofuels comprised landfill gas (24.4%), refuse combustion (21.2%), wood combustion (16.8%), other biofuels (12.1%), sewage gas (5.4%), and straw combination (2.4%). Total renewable sources accounted for 3 million tonnes of oil equivalent of primary energy use, with about 2.2 million tonnes used to generate electricity, and about 0.8 million tonnes to generate heat. With a stated intention of achieving 10% of electricity needs from renewable sources by 2010, the UK government introduced a renewables policy in February 2000 imposing an obligation on electricity suppliers (including nuclear power) to supply a specific proportion derived from renewables, but exempting renewable electricity sources from the Climate Change Levy. Coupled to this policy were expanded support programmes for new and renewable energy, including capital grants and an expanded R&D programmes, as well as the development of a regional strategic approach to planning and targets for renewables. The Non-Fossil Fuel Obligation (NFFO) Renewables Orders are the main UK mechanism for developing renewable energy sources; the fifth such Order was made in September 1998. A separate system of Scottish Renewables Orders operates north of the border. (See section on UK Agriculture).

**CGIAR** Since its inception in 1971, the Consultative Group on International Agricultural Research (CGIAR) has conscientiously addressed its aim to deploy modern science to promote sustainable development by reducing hunger and poverty, improving human nutrition and health, and protecting the environment. It now has a membership of 62, including 23 developing countries, 23 industrialised countries, and four foundations. A network of 16 'Future Harvest' Centers are supported (Table 1), and there are more than 8,500 CGIAR scientists and staff working in more than 100 countries. All of the research is in the public domain and subject to regular, rigorous

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CIAT	International Center for Tropical Agriculture (Centro Internacional de Agricultura Tropical)
CIFOR	Center for International Forestry Research
CIMMYT	International Maize and Wheat Improvement Center (Centro Internacional de Mejoramiento de Maiz y Trigo)
CIP	International Potato Center (Centro Internacional de la Papa)
ICARDA	International Center for Agricultural Research in the Dry Areas
ICLARM	World Fish Center (International Center for Living Aquatic Resources Management)
ICRAF	World Agroforestry Center (International Center for Research in Agroforestry)
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IFPRI	International Food Policy Research Institute
IITA	International Institute of Tropical Agriculture
ILRI	International Livestock Research Institute
IWMI	International Water Management Institute
IPGRI	International Plant Genetic Resources Institute
IRRI	International Rice Research Institute
ISNAR	International Service for National Agriculture Research
WARDA	West Africa Rice Development Association

### Table 1 'Future Harvest' Centers of the CGIAR.

scrutiny. In 2001, its budget was \$337 million. See also *Food Systems for Improved Human Nutrition : Linking Agriculture, Nutrition, and Productivity,* edited by P. K. Kataki and S. C. Babu, *Journal of Crop Production,* **6**, Numbers 1/ 2, 2002.

Genome Mapping Following the publishing at the end of 2000 of the DNA blueprint (the genome map) of Arabidopsis thaliana (thale cress), an achievement representing the first published genome sequence of a plant, the genome sequence of Oryza sativa (rice) was published in early 2001. The larger rice genome (430 million basepairs) was unravelled by Syngenta International AG and Myriad Genetics Inc., whereas the smaller Arabidopsis genome (115 million basepairs - about 28,000 genes, 17,000 of which have evidence of expression) involved around 300 scientists of various affiliations worldwide. Complete DNA sequences have been determined for over 60 microbial species, most recently the plaque bacillus, Yersinia pestis (4.65 million basepairs); about 4% of the genome comprises pseudogenes whose open reading frames (ORFs hence the term 'ORFeome') are incomplete and therefore silent in respect of protein expression. Great interest was shown in the 13.8 million basepair DNA sequence of fission yeast, Schizosaccharomyces pombe, a eukaryote with 4,824 ORFs, 43% of which contain introns, non-coding regions that have to be spliced out when the DNA is transcribed to RNA prior to protein synthesis. SCRI is involved in unravelling the Erwinia and Phytophthora genomes, amongst others.

Patents The US Supreme Court ruled in December 2001 that new plants developed by genetic engineering or other breeding techniques could claim protection under a section of federal patent law. The law incorporates newly developed plants reproduced from seeds, and reaffirmed the 1980 Supreme Court ruling that cited the principle that "anything made under the sun made by man" - including living things - could be patented. In previous Reviews in this series, I have described the Union Internationale pour la Protection des Obtensions Végétales (International Union for the Protection of New Varieties of Plants) (UPOV) convention which is widely regarded as a reasonable mechanism of bringing conventionally bred cultivars to the marketplace, protecting the rights of breeders, researchers, and farmers.

**Transgenic Crops** Much cited, the annual review series *Global Review of Commercialized Transgenic Crops*, published as Briefs by the International Service for the Acquisition of Agri-Biotech Applications, and authored by Clive James, provides a valuable and authoritative oversight on the global status of commercialised transgenic (genetically modified or GM) crops. Brief 24 presents data for 2001. Despite the unprecedented level of anti-GM activity stemming largely from activists of various kinds in Europe, the estimated global area of GM crops for 2001 was 52.6 million hectares grown by 5.5 million farmers, three quarters of whom were resource-poor farmers planting Bt cotton in China and South Africa. This represents

an increase of 19% over the 2000 level, equivalent to 8.4 million hectares. Over 25% of the global transgenic crop hectarage was in LDCs.

Four transgenic crops dominated production : soybean (63% of global area); maize (corn, 19%); cotton (13%); oilseed rape (canola, 5%). Four countries grew virtually all these crops: USA (68%), Argentina

(22%), Canada (6%), and the country with the fastest expansion in GM cropping, China (3%). Bulgaria, Germany, Indonesia, Mexico, Romania, Spain, and Uruguay also grew GM crops. The principal traits deployed were herbicide tolerance and insect resistance, with herbicide tolerance, deployed in soybean, maize, and cotton, accounting for 77% of the global area. Of the remainder, 15% were planted with insect-resistant GM crops, and 8% with crops engineered with stacked genes for both herbicide tolerance and insect resistance. The competitive advantage conferred by the technology was manifest by the portion of the global area of the major crops down to GM cultivars: 46% of the global 72 million hectares of soybean; 20% of the 34 million hectares of cotton; 11% of the 25 million hectares of oilseed rape; and 7% of the 140 million hectares of maize. C. James states that the adoption rates

"Governments, supported by the global scientific and international development community, must ensure continued safe and effective testing and introduction of transgenic crops and implement regulatory programs that inspire public confidence. Leadership at the international level must be exerted by the international scientific community and development institutions to stimulate discussion and to share knowledge on transgenic crops with society that must be well informed and engaged in a dialogue about the impact of the technology on the environment, food safety, health of producers and consumers, sustainability and global food security. Societies in food surplus countries must ensure that access to biotechnology is not denied or delayed to developing countries seeking to access the new technologies in their quest for food security, because the

most compelling case for biotechnology, more specifically transgenic crops, is their potential vital contribution to global food security and the alleviation of hunger in the Third World. In summary, we must ensure that society will continue to benefit from the vital contribution that plant breeding offers, using both conventional and biotechnology tools, because improved crop varieties are, and will continue to be the most cost effective, environmentally safe, and sustainable way to ensure global food security in the future."

Clive James : Global Review of Commercialized Transgenic Crops: 2001

for transgenic crops are unprecedented, and are the highest for any new agricultural technology. Cautious optimism was expressed that the global area and the number of farmers planting GM crops will continue to increase in 2002. It was estimated that the global direct and indirect economic benefit of GM crops in 1999 alone was in the order of \$1 billion or more. Analysis of the literature shows that by early 2001, more than 187 crop events involving nine basic phenotypic characteristics have been deregulated or approved for planting, feed, or food use in at least one of 13 individual countries plus the EU (M. C. Marra, Agricultural Biotechnology : A Critical Review of the Impact Evidence to Date. In The Future of Food Biotechnology Markets and Policies in an International Setting, edited by P. G. Pardey, International Food Policy Research Institute, 2001).

Food Crops Plant breeding is central to the success and efficiency of agriculture, horticulture, and

> forestry. In a succinct and timely analysis of the rationale for exploiting novel germplasm in plant-breeding programmes, J.S. Heslop-Harrison (Exploiting novel germplasm; Aust. J. Agric. Res., 2002, 53, 873-879), pointed out that of the 250,000 species of flowering plants, 12 species provide 75% of the food eaten, and only four species account for half of all the food eaten (Table 2). All the listed crops are capable of being biotechnologically modified.

> Cartagena Protocol The Cartagena Protocol on Biosafety : Reconciling Trade Biotechnology in with Environment and Development edited by C. Bail, R. Falkner, and H. Marquant, Royal Institute of International Affairs, 2002, details the background and outcome of the adoption of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD) in January 2000 after

nearly four years of international negotiations that were themselves founded in biosafety meetings in the early 1970s. Although the nub of the Protocol was how to protect the environment and human health from the potential danger of GMOs or Living Modified Organisms (LMOs), the negotiations were conducted without firm evidence of any environmental damage caused by the release or LMOs, and sharp divisions over the potential risks involved. The Protocol is essentially a precautionary instrument, and emerged during intensifying politicisation of the GMO debate. Unfortunately, scientists did not play a

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Сгор	2001 production (Mt)
Sugar cane	1254
Maize	605
Rice	593
Wheat	579
Potato	308
Sugar beet	234
Cassava	179
Soybean	177
Barley	139
Sweet potato	136
Oil palm fruit	119
Tomato	100
Banana & plantain	98
Watermelon	77
Grape	62
Orange	61
Apple	60
Sorghum	58
Cabbage	55
Coconut	51
Onion	47
Yam	39
Rapeseed	36
Groundnut	35
Cucumber & gherkin	31
Millet	29
Oats	27

**Table 2** The world's major food crops for human consumption ranked by production (from FAO Statistical Database, http://apps.fao.org, 2002). Banana and plantain have been combined, and the pooled category 'Vegetables Fresh not elswhere specified' has been omitted (between positions 6 and 7).

major role in the negotiations, contrasting with the involvement of environmental and other non-governmental organisations (NGOs). There were discussions about the outcrossing of GM oilseed rape, the potential development of resistance in target insects and the potential effects on non-target organisms from the cultivation of Bt maize, the 'Pusztai' case, and a few other high-profile science-related developments.

The key elements of the Protocol are wide-ranging. Article 4 states that the Protocol applies to the transboundary movement, transit, handling, and use of all LMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health although there are other international routes for this aspect. Article 5 exempts pharmaceuticals for humans. Article 6 notes that the Advance Informed Agreement (AIA) procedure does not apply to the movement of LMOs for contained use or to LMOs in transit. The AIA procedures were thought to be at the centre of the biosafety talks that led up to the formal Protocol, with a 'need to know' and 'prior informed consent' deemed to be necessary by LDCs, and essential to build up confidence in biotechnology. Risk assessments (Article 15 and Annex III; level of detail, costs, scope, transparency, etc) and management, explicit versus implicit consent, the issue of precaution, and minimal national standards were considered in detail. LMOs intended for direct use as food, feed, or for processing (LMO-FFP) - agricultural commodities were a key issue in the Protocol negotiations, as the area devoted to GM crops increase year by year. Unique identifiers will be required for a central database in the Biosafety Clearing House so that the transgenic crops agreed for release in the marketplace (many are already in international trade) can be employed for monitoring supply chains and usage. Socio-economic considerations for importing countries are dealt with in Article 26, allowing them to protect their biological diversity. Issues of liability and redress (Article 27) were contentious, similar to the situation when the wording of Articles 19(3) and 19(4) of the Convention on Biological Diversity (CBD) were discussed and eventually adopted at the United Nations Conference on Environment and Development in Rio de Janeiro, Brazil (known as the Earth Summit) in June 1992. In the absence of any specific known cases of damage caused by the transboundary movement of LMOs, the Protocol is preemptive. Time scales for liability and insurability, impact assessments, definition of 'damage', identity of the person or organisation to be held liable, the relationship between the Protocol and Article (14)2 of the CBD remain to be resolved.

Principle 15 of the Rio Declaration states "In order to protect the environment, the precautionary principle shall be widely applied by States according to their capabilities. Where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimise such a threat". This influenced the role of the 'precautionary principle' (or properly precautionary approach) in the Cartagena Protocol (see Articles 10 and 11) but there is in Annex III, general principles, point 4 a counterbalancing statement: "Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable level of risk". Importantly, the Protocol refers to 'potential adverse effects' as a precondition for possibly triggering precautionary measures. The question then arises as to the extent which the precautionary principle represents a principle or possible a customary rule of international law. Thereafter, the WTO Agreements on Sanitary and Phytosanitary Measures (SPS), and on Technical Barriers to Trade (TBT) need to be analysed in the context of the Cartagena Protocol and trade in biotechnology products. At the present time, the WTO's case law is uncertain, and the criteria of unilateral risk assessment and risk management, taking into account also socio-economic factors, would allow for extensive trade restrictions in GMOs and LMOs. It likely that the WTO dispute resolution mechanisms will be under pressure.

GM Crop Debate In previous Reviews, I have dwelt at length on the debate about GM crops. The surreal situation in Europe and the UK has occupied the broadcast and printed media in sensationalist outpourings. In the absence of "bad" actual accounts of GM crops, scare tactics have been used based exclusively on scenarios that are concocted to be damaging to biodiversity and nature, human health, agriculture and horticulture, and international trade. D. L. Kershen, Earl Sneed centennial Professor of Law in the University of Oklahoma, released in 2002 a landmark article Innovations in Biotechnology - Public Perceptions and Cultural Attitudes : An American's Viewpoint (dkershen@ou.edu). He argues cogently that the debate about agricultural biotechnology is of equal importance as Galileo versus Ptolemy, or Darwin versus Lysenko, drawing on the examples of China, Europe, India, and the USA. It should be obligatory reading for European policy makers. Not unrelatedly, the reading should also extend to In Defence of Global Capitalism by J. Norberg (ISBN 91-7566-503-4) published in 2002. As pointed out by J. D. Gwartney, R. Lawson and D. Samida, in Economic Freedom of the World Annual Report 2000 (088975 202 8, Vancouver, B.C. Fraser Institute, see also reports for 2001 and 2002, J. D. Gwartney, R. Lawson, W. Park and C. Skipton, at http://www.fraserinstitute.ca/publications) , the mostfree countries with open economies had the highest per capita incomes, the highest percentage growth through the 1990s, rapid technological uptake, and the lowest number of poor people as a percentage of the population based on the UN Human Poverty Index. They are also the least corrupt.

Around 14 million people in southern Africa are threatened with starvation, a figure likely to rise throughout 2002. On the eve of the UN World Summit on Sustainable Development in Johannesburg in August 2002, a major dispute erupted when the EU rejected a plea by the USA to offer reassurance to the affected southern African countries about the safety of GM emergency food relief. Mozambique, Zambia, and Zimbabwe turned back food shipments of GM grain - the USA supplies over 50% (500,000 tonnes) of the region's humanitarian aid - evidently preferring death by starvation than a theoretical risk to human health, and crop and livestock exports to the EU. Scientifically and morally, the action of the EU was reprehensible. Zambia has around 1.75 million of its population facing starvation, and suffers 20% HIV/AIDS infection rates. Its President, Levy Mwanawasa, stated that "we will rather starve then give something toxic". Zimbabwe's chaotic political situation, HIV/AIDS pandemic, and drought, means that it too faces a prodigious humanitarian crisis. Three countries accepted GM maize: Lesotho, Malawi, and Swaziland. The UN World Food Programme has urged needy countries to accept the imports.

Sensitivity over GM crops extends to their use as animal feeds. The major UK retailers attempt to source non-GM products but recognise that for such products as fats and oils, carbohydrates, micronutrients, and additives it is difficult to have full traceability. Current and future GM crop market dynamics : the case of soybeans, published by Brookes West (graham.brookes@btinternet.com), noted the rise in the demand for non-GM soybeans and warns of the possibility of price rises whilst the prices of GM soybeans and meal fall in line with its global increase in production and yield efficiency. The extra costs will either have to be transferred down the supply chain, or further pressures will be placed on the feed and livestock-production sectors. A thriving market is developing for authenticity testing, essentially driven by pressure groups.

A useful, albeit incomplete, forward look on biotechnology was produced by the Agriculture and Environmental Biotechnology Commission (AEBC) in April 2002, entitled *Looking Ahead. An AEBC Horizon Scan* (aebc.contact@dti.gsi.gov.uk). It was intended to be an internal awareness document, strongly focused on the UK, and drew on a series of consultations. Although virtually all the points raised in the consultations were relatively well-rehearsed in numerous debates and articles, including this series of Annual Reports, over the past six years, there was a polarisation of views, interesting because no commercial production of GM crops is permitted in the UK. Mention was made of mergers and acquisitions in the crop seed, agrochemical, pharmaceutical, and smallcompany biotechnology sector, such that there are now very few multinational agrochemical and seed companies (e.g. Syngenta, Bayer/Aventis, Monsanto, DuPont/Pioneer, Dow, BASF, Advanta) - but there was no detailed appraisal of the sharp contraction of the UK R&D effort in these areas in both the public and private sectors, a trend likely to continue for the short to medium term. Likewise, there was no audit of the UK intellectual property and competitive position. Transboundary issues, such as product segregation, smuggling, gene flow etc. were considered in brief. Herbicide tolerance, pest resistance, fungal resistance, viral resistance, bacterial resistance, abioticstress resistance, increased yield, food-product quality, animal-feed quality, plants as factories, other nonfood crops, smart plants, trees, fish, insects, other animals, bioremediation and phytoremediation were assessed fairly naïvely in terms of the aim of the work, possible benefits, possible risks, and "further issues". Noteworthy of comment is the way in which the AEBC volunteered itself to cover all aspects of biotechnology, including social, economic, legal, and political trends, and offer what it regards as authoritative reports. Meanwhile, as science budgets have been squeezed, an increasing proportion of R&D spend has been directed to policy issues relating to potential adverse effects of GM technology, reducing the research effort in cutting-edge innovation. Presently, the diversity of approach to GM crops throughout the world and the low level of harmonisation of regulatory processes, means that the debate will continue, unresolved, for a few years yet. Many of the arguments for and against GM food are at cross-purposes; absolutes (as in virtually all areas of human activity) are rare; emotions run high; political and economic viewpoints can rarely be reconciled; rational analyses of risks, benefits, and existing technologies tend to be ignored; intolerance is rife; trust undermined; and some confuse research with commercialisation. The reader is recommended to consult Genetically Modified Food and the Consumer, edited by A. Eaglesham, S. G. Pueppke, and R. W. F. Hardy, National Agricultural Biotechnology Council Report 13, 2001. In this, an

account is given of a meeting held to discuss the safety, ethical, marketing, and environmental issues that influence the acceptance of agricultural and food biotechnology by consumers. The risks of not researching and developing biotechnology are greater than failing to develop or impeding its development without just reason. In the UK, the Prime Minister's Strategy Unit produced a Scoping Note, The Costs and Benefits of Genetically Modified (GM) Crops, following an announcement by the Secretary of State for the Environment, Food and Rural Affairs in May (http://www.strategy.gov.uk 2002 and GMCrops@cabinet-office.x.gsi.gov.uk). This study will run alongside a review of the scientific issues raised by GM crops and feed into a protracted debate on the potential impacts of growing GM crops in the UK. In 1998, the EU stopped approving new applications for imports of GM food, a moratorium that was only lifted in October 2002, and which was de facto illegal under EU law and WTO rules. In the absence of commercially grown crops in the UK, the results of the Farm-Scale Evaluation trials in which SCRI is involved, are eagerly awaited. Homologous allelic recombination or replacement technologies (HARTs) are under active consideration by plant molecular geneticists as a way to amend specifically by site-directed processes the sequence of a particular gene or controlling DNA so that the plant remains in all other ways in its original genetic condition, with the exception of the targeted introduced subtle change, and there would only be a minor allelic variation. When HARTs emerge, ideally apomixis by HARTS, they will spell the single greatest technical breakthrough since DNA transformation of plants was announced (see R. A. Jefferson, Transcending Transgenics – Are There "Babies in the Bathwater," or Is That a Dorsal Fin? In The Future of Food. Biotechnology Markets and Policies in an International Setting, edited by P. G. Pardey, International Food Policy Research Institute, 2001).

The Marketing Battle over Genetically Modified Foods by B. Wansink and J. Kim (American Behavioral Scientist, 44, 1405-1417, 2001), describe current models of consumer behaviour to point out the ineffectiveness of both proponents and opponents of biotechnology in educating consumers. Their analysis of fallacious and accurate assumptions is relevant to the UK debate. In this vein, food labelling is being actively pursued in some MDCs as a socio-political tool to help 'ethical' or belief-related perceptions of 'desirable' agricultural production (*i.e.* food produced

'organically' and locally, with free-range-based animal welfare, as opposed to food produced with the involvement of GM technologies ionising radiation, or multinational companies) subsume fundamental descriptions (e.g. composition, additives, nutritional values, safety, phytosanitary controls, audit controls, value-for-money etc.). Some pressure groups hoped that legally enforced labelling on their terms will help prejudice food-production systems they abhor, rather than simply inform dispassionately and offer freedom of choice. Agricultural development and production in exporting LDCs will be modified by complex labelling requirements, potentially to the detriment of those countries. By and large, producers of conventional agricultural products and GM produce were relaxed about more detailed labelling so long as it was concise, fair, accurate, and balanced, and extended to other products (e.g. medicines). There was evidence, however, that the impact of some of the proposed labelling recommendations, emblems of a perceived lack of trust, would not be as effective as assumed.

### **The Environment**

Environment in 2001 According to the National Aeronautics and Space Administration (NASA), based on terrestrial and oceanic data from January to October 2001, the year was on schedule to be the second warmest on record. Lower tropospheric temperatures, however, were close to the 1979-1998 mean, indicating that the warming trend was located at or close to the surface. By April 2001, equatorial temperatures returned to normal, indicating that La Niña (the below-normal ocean-surface temperatures in the central and eastern equatorial Pacific) which had begun in 1998 had petered out. La Niña was thought to have accounted for long-term drought in the southeastern USA. Drought continued in the Middle East and south-central Asia, northern China, and North and South Korea. Storms affected the Northeast, Great Plains, the South and mid-Atlantic coast of the USA, Beliza, Honduras, Nicaragua, Cuba, Algeria, Taiwan, Philippines, China, and Japan. Monsoon floodwaters badly affected India.

**Climate Change** At the Sixth Conference of the Parties to the 1992 UN Convention on Climate Change held in The Hague in November 2000, there was sharp disagreement over carbon "sinks" and nuclear power. Whole-plant physiologists and soil scientists who are able to provide base-line data and determine carbon fluxes are surprisingly rare these days. Australia, Austria, Canada, Japan, New Zealand, Norway, Russia, Switzerland, Ukraine, and the USA wanted forests and agricultural land to be counted as carbon-dioxide-absorbing sinks, a stance opposed by the EU on the basis that this would obviate positive measures to meet Kyoto targets. In March 2001, President G. W. Bush of the USA stated on the grounds of cost that he would not accept mandatory controls on carbon-dioxide emissions. Later, in July 2001, the conference of parties met in Bonn leading to the acceptance by 178 countries to rules for meeting Kyoto targets. The USA did not accept the agreement even though there were concessions on the inclusion of carbon sinks. In November, details of the treaty were finalised: 40 MDCs would be required to reduce greenhouse-gas emissions by an average of 5% below 1990 levels by 2012. Even so, the Kyoto Protocol needed to be ratified by at least 55 countries before it could come into force. Words are usually preferred to actions in international politics.

In July, a massive report from the Intergovernmental Panel on Climate Change predicted that global temperatures might rise by 1.4 to 5.8°C by 2100 on the assumption that levels of atmospheric carbon dioxide would reach 540-970 parts *per* million. Significantly, the combustion of fossil fuels and the emission of synthetic chemicals were deemed to have "contributed substantially to the observed warming over the last 50 years."

Pollution Marine and freshwater pollution events in 2001 came from (a) spills of lubricant, diesel, and bunker fuel oil; (b) pesticide and fertiliser run-offs from agricultural land and emissions from livestock farms and silage; (c) human sewage (contains diverse bioactive components); (d) contamination from mining and logging operations; and (e) domestic and industrial wastes. Toxins produced by naturally occurring "blooms" created difficulties for fisheries and shellfish industries. In June, a meeting in Spain of the parties to the Ospar Convention agreed on reductions on the discharge of oils into the northeast Atlantic, and raised to 29 the number of listed substances to be phased out from discharges onto the ocean by adding ethenyl ether, neodecanoic acid, and triphenyl phosphine to the list.

Around 500,000 tonnes of obsolete pesticides have been dumped in many LDCs and countries in economic transition. In responding to prompting from the FAO, the Global Crop Protection Federation (GCPF) awaited a scheme for safe dispersal of pesticides from the FAO that would be confined only to the products derived from the companies that contributed financially to the scheme. Of greater hazard than pesticides was the unsafe storage of radioactive wastes from nuclear weapons, nuclear power stations, hospitals and laboratories, and industrial sources. The greatest such problems remained in the former Soviet Union. Since the 1950s, a remarkable improvement has taken place in agrochemical efficacy, specificity, and targeting. In several countries, there are official disposal schemes. Changing legislation in the EU aimed at reducing the number and type of pesticides permitted for agricultural and horticultural use, is leading to concerns about the building up of resistance in pests and diseases. Consumer, pressuregroup, and retailer demands for lowered pesticide usage, coupled to environmental obligations to reduce pollution will inexorably lead to a greater focus on plant breeding to deliver pest and disease-resistant cultivars in conjunction with integrated crop production (ICP) strategies, crop mixtures, companion plants, and quality-audited production systems. Aspects of this approach were favoured in July by the agriculture ministry in The Netherlands, strengthened by an ICP certification plan, a tax on pesticides to begin in 2003, and a proposal to ban pesticide usage on non-ICP-certified farms. (See also Agriculture and Food Section)

In May 2001, an international treaty to reduce or preferably eliminate the production and application of aldrin, chlordane, dieldrin, endrin, heptachlor, mirex, toxaphene, and hexachlorobenzene, was opened for signature in Stockholm. It will enter into force once 50 countries had ratified it; apparently this will not be a problem given that representatives of 122 governments had finalised the treaty in Johannesburg in December 2000. Tropical countries would be allowed to continue to use DDT for malaria control, the most cost-effective method, until suitable substitutes become available. Technological advances mean that there is no longer any reason to use DDT in agriculture. Likewise, the slow phasing out of polychlorinated biphenyls would permit the use of equipment containing them until 2025.

**MEA** A \$21-million multinational, multiorganisational quadrennial study of the condition of the global environment, the Millennium Ecosystem Assessment, was announced on World Environment Day, June 5, 2001, by Kofi Annan, the UN Secretary-General.

**Fossil Fuels** The combustion of fossil fuels continued to cause concern, most notably the use of coal. The environmental effects of mining coal, transporting it and its pollutant emissions are well known and docu-

mented, but perversely it is an industry which received subsidies (e.g. Germany, Spain), or is exempted from compliance with emissions regulations (e.g. USA). According to the International Energy Agency, there are 1.6 billion people worldwide who are unable to access sources of modern energy, and along with the rest of the world's population will create a need for new power plants; many of these plants are likely to burn coal despite the quality and effects of their carbon emissions unless firm international accords are brought into play. For LDCs, coal can be a relatively cheap source of energy, and many (e.g. China, India, South Africa) have substantial reserves. Wood and charcoal are still the only sources of heat for many in the LDCs, leading to the depletion of forests and scrubland, soil erosion and desertification. Poor people can often be condemned to travel vast distances to gather fuel and collect water. Figures from UNDP World Energy Assessment 2000, and Howard Herzog, Massachusetts Institute of Technology (see The Economist July 6 2002, pp 93-94) point to a global resource base of over 5000 GtC (billion tonnes of carbon equivalent) for coal, compared with 509 GtC for unconventional natural gas (e.g. coal-bed methane), 253 GtC for conventional natural gas, 407 GtC for unconventional oil sources (e.g. tar sands), and 241 GtC for conventional oil resources. Various technological solutions need to be developed and implemented in order to avoid or minimise the major but not the sole consequence of combusting (oxidising) the carbonaceous fossil fuels - the release of carbon dioxide which thought to be an important anthropogenic greenhouse gas. These range from "sinks" or "buffers" e.g. photosynthesising forests, captive processes during or immediately after the combustion pressure, or sequestration. Integrated gasifier combined cycle systems which separate carbon dioxide formation from the heat-generation process, and the "scrubbing" of carbon dioxide from exhaust gases could deliver the greenhouse gas in a way that could enable it to be disposed of in the seas and oceans, in depleted oil and gas reservoirs in porous rocks capped by impermeable rock, in coal seams, and in deep saline aquifers. The geological route may play a role in emissions-reduction credits trading. Of the nonphotosynthesis-based sequestration options, oceans are estimated to have a global capacity in orders of magnitude of 1,000s GtC, deep saline formations 100s-1,000s GtC, depleted oil and gas reservoirs 100s GtC, and coal seams 10s-100s GtC. Photosynthesis is a remarkable sequestration route, especially into longlived perennials and wood products.

**Information** The Aarhus Convention – the UN Economic Commission for Europe (UNECE) Convention on Access to Information, Public Participation in Decision Making and Access to Justice in Environmental Matters - was adopted in June 1998 (see http://www.unece.org/env/pp/documents/cep43e.pdf) by 37 UNECE members, including the 15 EU member states and the European Commission (EC) itself. In order to ensure that EC environmental legislation is consistent with the legislation, the EC proposed in July 2000 a revised Directive on Access to Environmental Information. During 2001-2002, member states reviewed the original 1990 EC Directive 90/313/EEC in the freedom of access to information on the environment and the national legislation that gives effect to the Directive.

**Groups** According to the American Association of Fundraising Counsel Trust for Philanthropy, donations to environmental groups in the USA alone were more than \$6.4 billion in 2001; globally, the income of environmental organisations was at an all-time

high. The focus of attention from these advocacy groups has been business, economic growth, financial markets, profits, governments, regulators, mainstream scientists and technologists, modern agriculture, the aeroplane, and the internal combustion engine. American multinationals, fossil-fuel producers, mining companies, forestry companies, and chemical companies are particular targets of ire. Campaigning has become an industry in its own right, pressuring democratic governments and companies alike. Undoubtedly, the emphasis on the environment has led to improvements in the quality of water and air in many MDCs, and the areas of national parks and reserves, and conservation activities generally have all increased. In a climate of intolerance and inflexibility, though, it may be difficult for companies to embrace innovation to deal with the environmental costs of their actions when the pressure groups require the 'mandate, regulate, and litigate' approach rather than foster market-based environmental reforms as well.

## **UK Perspectives**

Environment Environmental protection in the UK is shaped by a combination of over 50 international conventions and protocols, over 300 European Directives, legislation and strategies of the UK Government, and the actions of charities and voluntary bodies. Various individuals, organisations (and companies also) are actively engaged in environmental protection. Regulation is the responsibility of the Environment Agency, the Scottish Environment Protection Agency, and the Environment and Heritage Service for Northern Ireland. At the EU level is the Sixth Environmental Action Programme, Environment 2010: Our Future, Our Choice which has the intent of improving the implementation of current legislation; integrating environmental concerns into other policies; working with the market; empowering private citizens and helping them modify behaviour; and taking account of the environment in land-use planning and management decisions.

Sustainable development is the main focus of the environmental agenda in the UK, considering social and economic development as well as environmental issues, in the worthy understanding that sustainable development meets the needs of the present generation without compromising the ability of future generations to meet their own needs. Starting with the first UK sustainable development strategy in 1994, and the first set of sustainable development indicators in 1996, the latest strategy A Better Quality of Life was published in May 1999. It has four objectives (social progress, environmental protection, prudent use of natural resources, maintenance of high and stable levels of economic growth and employment), 14 headline indicators, and 150 other indicators against which progress is measured. Separate indicators are being developed for Scotland. Related to governmental strategies is the commitment of local authorities to formulate sustainable development strategies for their own areas, driven by Local Agenda 21 which came out of the 1992 UN Conference on Environment and Development in Rio de Janeiro.

The Framework Convention in Climate Change, signed and ratified by 184 countries including the UK, governs the UK's response to climate change. It came into force in March 1994 and is intended to reduce the risks of global warming by limiting the emission of 'greenhouse' gases. Progress at meeting targets are assessed regularly at conferences, the most famous being the 1997 meeting in Kyoto, Japan, where a protocol (The Kyoto Protocol) to the Convention was adopted. Six greenhouse gases are covered: carbon dioxide, methane, nitrous oxide, hydrofluorocarbons, perfluorocarbons, and sulfur hexafluoride. MDCs agreed to cut emissions of greenhouse gases by 5.2% below 1990 levels by 2008-2012, but EU members agreed to an 8% reduction, and the UK specifically to a 12.5% reduction. Three ways (the Kyoto mechanisms) were devised to increase the flexibility and reduce the costs of meeting the targets, viz. the clean development mechanism; emissions trading; and joint implementation. Unfortunately but not surprisingly, the Sixth Conference of the Parties, held in The Hague, in November 2000, where it was hoped to clarify the methodology of using "sinks" such as vegetation and a compliance regime to monitor performance of countries, was suspended.

A programme setting out how the UK intends to meet its Kyoto target, and progress towards meeting a domestic aim of a 20% reduction in carbon-dioxide emissions by 2010, was published in November 2000. Measures already or about to be implemented are (a) a climate-change levy on sales of electricity, coal, natural gas, and liquefied petroleum gas to the business and public sectors; (b) agreements with energy-intensive users to meet reduction targets; (c) integrated pollution prevention and control; (d) reducing transport congestion and pollution; (e) energy-efficiency initiatives and standards of performance; better countryside management; cuts in fertiliser use; new targets for improving energy management of public buildings; and emissions trading. The Scottish climate-change programme was published in November 2001.

Air pollution is being addressed under other conventions, policies, strategies, and duties. The Convention on Long Range Transboundary Air Pollution, adopted in 1979, came into force in 1993 and involved protocols covering various pollutants including nitrogen and sulfur oxides. Within the UK, the Environmental Protection Act 1990 specified two regimes: integrated pollution control to regulate emissions to any environmental medium from certain types of industrial process; and local air-pollution control to regulate emissions from smaller processes to air. In fact, the recent European Integrated Pollution Prevention and Control Directive was largely based on the UK's Environmental Protection Action, but will cover more types of installation and site reinstatement.

The first National Air Quality Strategy published in 1997 was revised in January 2000 with air-quality

objectives to be met by 2003-2008 for reducing the levels of eight main pollutants (benzene, 1-3 butadiene, carbon monoxide, lead, ozone, nitrogen dioxide, particulates, and sulfur dioxide). All district and unitary authorities have a duty to monitor air quality in their areas and to assess whether the air-quality objectives are being met. Specific air-quality targets to be met by 2010 in the UK included reductions in emissions based on 1990 levels of sulfur dioxide by 63%, nitrogen oxides by 41%, volatile organic compounds by 40%, and ammonia by 17%.

Under the auspices of the EU Water Framework Directive, the EC Bathing Water Directive, (which applies to 391 coastal and nine inland bathing waters in the UK), river-quality objectives set by the Environment Agency, and abstraction and discharge licences relating to the European Urban Waste Water Treatment Directive, mean that water-quality targets are set for drinking water sources, wastewater discharges, rivers, coastal waters, and bathing water. These include ensuring that 97% of bathing waters meet European directive standards consistently by 2005, provision of secondary treatment for discharges above 15,000 population equivalent by March 2002, and provision of secondary treatment by 2005 for all significant coastal discharges from settlements of above 2,000 population.

Policies and strategies to deal with commercial, industrial, and domestic wastes have evolved from the old "the solution to pollution is dilution" approach to the current principles of (i) a waste hierarchy of reduce, reuse, recycle, and dispose; (ii) the proximity principle of disposing of waste close to its site of generation; and (iii) national self-sufficiency in dealing with wastes. The EU Landfill Directive has been brought into the public eye through its stringent targets for reducing the amount of wastes sent to landfill sites, and particularly through its planned integrated products policy which aims to internalise the environmental costs of products throughout their life-cycle through market forces by emphasising "eco-design" and incentives to promote "greener" products. Producer responsibility directives for packaging waste and the end-of-life vehicle directive adds greater responsibilities and therefore costs on to producers, and consumers ultimately. Waste strategies operate for England and Wales, Scotland, and Northern Ireland. The main UK targets include (a) reduction of industrial and commercial wastes going to landfill by 85% of 1998 levels by 2005; (b) recovery of 40% of municipal waste by 2005, 45% by 2010, and 67%

by 2015; (c) recycle or compost 25% of household waste by 2005, 30% by 2010, and 33% by 2015; (d) reduce biodegradable waste sent to landfill by 75% of 1995 levels by 2010, 50% by 2013, and 35% by 2020; (e) increase the proportion of waste paper in UK newspaper feedstock to 65% by 2003 and 70% by 2006; (f) reduce the amount of waste going to final disposal by 20% in 2010 and 50% by 2050; and (g) proposed re-use and recovery of 85% of the mass of discarded vehicles and a minimum to 80% recycling by 2006, rising to 95% re-use and recovery and 85% recycling by 2050. Increasing costs of disposal worry farmers whose land is vulnerable to fly-tipping, but most of the public and business seem blissfully unaware of the implications of legislation on wastes.

Bioremediation of the atmosphere, land, and waters is patently a key technology in addressing the various tough and ambitious targets set by the EU and the UK government. Several of the targets require entirely new approaches unless there are substantial changes in lifestyle or even disenchantment of the electorate. Biotechnological advances in generating and propagating novel organisms to handle and exploit wastes, improving and maintaining planting schemes, the development of new manufacturing processes and recyclable materials, new biofuels and other renewable energy schemes will need to be introduced soon, and will require underpinning R&D. Although sporadic small-scale public-sector investments have been made in this area, fresh and concerted effort must be made in the near future. Private-sector investments have been minimal.

In addressing many of the political and social issues on the environment, biodiversity, and recreation and leisure, there has been growing appreciation of the roles of the National Parks; Areas of Outstanding Natural Beauty in England and Wales, and Northern Ireland; National Scenic Areas in Scotland; the National Forest; Sites of Special Scientific Interest; National Nature Reserves; Local Nature Reserves; Forest Nature Reserves; and Marine Nature Reserves. The ten National Parks of England and Wakes set up after the National Parks and Access to the Countryside Act 1949 received the Royal Assent, were created to conserve and protect scenic landscapes (visual amenity) from inappropriate development and to provide access to the land for public enjoyment. Designation of National Park status does not directly affect ownership of the land or remove the rights of the local community - indeed, only 2.3% of the land is owned by the National Park Authorities. A special

statutory authority, the Broad Authority, was established in 1989 to develop, conserve, and manage the Norfolk and Suffolk Broads, and there is an intention to give the New Forest a status equivalent to a National Park. In August 2000, the National Parks (Scotland) Bill received Royal Assent, leading to the recent formation of the first Scottish National Park of Loch Lomond and the Trossachs, soon to be followed by the Cairngorms.

In England and Wales, the National Parks and Access to the Countryside Act 1949 made provision for deregulating Areas of Outstanding Natural Beauty (AONB) by the Countryside Commission – now the Countryside Agency in England, and the Countryside Council for Wales. The 41 AONBs emphasise the protection of flora, fauna, geological, and other landscape features but have less emphasis on the provision of open-air enjoyment for the public than the National Parks. There are nine AONBs in Northern Ireland. In Scotland, there are 40 National Scenic Areas which have a broadly equivalent status to AONBs.

As of March 2001, there were 4,115 Sites of Special Scientific Interest (SSSIs) in England, 1,449 in Scotland, and 1,008 in Wales, covering more than 2 million hectares. In Northern Ireland, there are 161 Areas of Special Scientific Interest (ASSIs) covering over 83,000 hectares. Both SSSIs and ASSIs have been identified by English Nature, Scottish Natural Heritage, the Countryside Council for Wales, or the Department of the Environment for Northern Ireland, as being of special interest because of the flora, fauna, geological, or physiographical features; some are managed as nature reserves. Owners or occupiers of the sites must gain written consent before they can undertake certain listed activities on the site, but may receive funds to protect the site, and may benefit from increased valuations of their sought-after properties. Related to the SSSIs and ASSIs, under the same forms of control, are the National Nature Reserves designated for the study and preservation of the flora, fauna, geology and physiographic features. As of March 2001, there were 208 National Nature Reserves in England, 72 in Scotland, 65 in Wales, and 46 in Northern Ireland, totalling 221,000 hectares. Local authorities have designated 665 Local Nature Reserves in England, 34 in Scotland, and 51 in Wales, totalling around 45,000 hectares, as well as 38 km of 'linear' trails. Forestry Enterprise, an executive agency of the Forestry Commission, manages 46 Forest Native Reserves in England, Scotland, and Wales, and

18 Caledonian Forest Reserves in Scotland to protect and expand 16,000 hectares of native oak and pine woods in the Highlands. There are 35 Forest Nature Reserved in Northern Ireland administered by the Forest Service.

Three statutory Marine Nature Reserves for the protection of marine flora and fauna, and geological and physiographical features on land covered by tidal waters or parts of the sea in or adjacent to the UK, have been set up in Lundy, Skomer, and Strangford Lough, with two other areas proposed – the Menai Strait, and Bardsey Island (visible from Aberystwyth on a good day) with part of the Llyn peninsula. In addition, there are non-statutory marine reserves established by conservation groups.

UK Flora Changes in the UK flora have been monitored and reported in the 910-page New Atlas of the British and Irish Flora, Oxford University Press, 2002. A decrease was noted in species associated with northern (Arctic and Boreal) vegetation zones, and an increase in Mediterranean-zone plants. This shift in vegetation composition has been blamed on habitat destruction and global warming, as the 1,396 native species that arrived by natural colonisation following the last Ice Age, and the small group of 149 archeophytes (ancient introductions before AD1500 - probably by early farmers), are being displaced by the more recent introductions (neophytes) after AD1500 mainly horticultural and forestry species. Changes to the 'natural' flora of the UK in recent years reflected (a) a substantial increase in the areas down to plantation forestry; (b) fungal diseases of trees leading to premature death; (c) encroachment on lowland heathland; (d) restoration of fenlands; (e) reduction in peat extraction which ceased at Thorne Moors near Doncaster and Wedholme Flow in Cumbria following compensation to the owner by the UK government; and (f) suburban expansion.

**Red Tape** Bureaucratic impositions – red tape – afflict all UK organisations adversely affecting their international competitiveness and profitability, although the phenomenon is not confined to the UK. The British Chambers of Commerce estimated that since 1997 the total cost to business of compliance with regulatory measures had risen to £15.6 billion by May 2002, the most expensive being the Working Time Directive (£7.65 billion), the Data Protection Directive 95/46/EC (£3.1 billion), the EU Pollution Directive 98/69/EC (£1.6 billion), the National Minimum Wage (£674.5 million), IR 35 (£523.5

million), Disability Discrimination (£364 million), Stakeholder Pensions and Welfare Reform Bill (£314 million), Part-Time Workers Directive (£306 million), Working Families Tax Credit (£262.8 million), Student Loan Repayment (£255.8 million), Young People-time off for studying/training (£273 million). In 2001, 4,642 sets of regulations were introduced, yet to be tested in case law. Proposed legislation on maternity and paternity benefits, dismissal procedures, the rights of temporary workers, and health and environmental controls will add to the considerable burden on employers not only to balance their accounts but to act as unpaid tax collectors, providers of statistics for government departments, providers of social and welfare services, and - particularly in food and agriculture - key players in expensive quality-assurance systems. Concerns were expressed specifically about meeting the administration costs of addressing information requests from staff and customers expressing their rights under the Data Protection Act. Employers and company profitability are now especially vulnerable to legal challenge. The OECD, however, observed that in recent times the UK has had a regulatory environment that is among the most supportive of market openness and global competition in the world, with entrepreneurs operating in a better business environment than in most of the other 30 member countries. Particular praise was given to competition policy, trading arrangements in the electricity sector, and reform of the telecommunications sector. Recent positive developments such as reductions of tax on profits, taper relief on the sale of businesses, and R&D tax credits are helpful to small- and medium-sized companies, especially in biotechnology. Entrepreneurial behaviour, though, is closely linked to the generation and exploitation of intellectual property, and it is readily suppressed by an oppressive and turbulent regulatory and taxation environment.

**R&D Scoreboard** In the 2002 R& D Scoreboard, the twelfth consecutive annual report in the series produced by the Department of Trade and Industry (DTI), it was clear that the UK still lags behind its international competitors in several areas of R&D support. The report is based on an analysis of the R&D investment data in the audited annual reports and accounts of the top 600 UK and top 600 international R&D-investing companies, with classification based by FTSE sectors. R&D-active UK companies in pharmaceuticals & biotechnology account for 37% of total R&D, and aerospace and defence for 10%, both investing above international levels. The UK is

also strong in food processing and oil & gas (9% in total compared with 2% internationally) but only has 4.5% of R&D in electronic & electrical compared with 10.4% internationally. IT hardware (25%), automotive (16.5%), and pharmaceuticals & biotechnology (16.3%) dominate international R&D. An important measure is R&D intensity as defined by R&D spend as a percentage of sales. Within the international group of companies, the Americas (principally the USA) have an R&D intensity of 5.1%, the rest of the world (mainly Japan) 4.1%, and Europe 3.6%. Although within Europe, the UK has the lowest R&D intensity (2.2%), it has increased from the particularly low level of 1.8% in 1998. Further analysis shows that UK company R&D intensity is above international levels in pharmaceuticals & biotechnology (14.6% versus 13.0%), aerospace and defence (6.5% versus 4.3%), and health (6.5% versus 4.9%), but in the electronic & electrical, software & IT services, chemicals, and engineering, the UK figures are lower than its international competitors. Moreover, as many universities and research institutes ruefully realise, UK subsidiaries of overseas companies have a higher average R&D intensity than UK-listed companies. At an international level, the five sectors comprising electronic & electrical, health, IT hardware, pharmaceuticals & biotechnology, software & IT services have the highest R&D intensity values and account for over 60% of all R&D. The US dominated in four sectors, and Japan in one (electronic & electrical).

For many years, it has been clear that R&D is a key to successful growth; companies with above-average R&D spend tend to show above-average sales-growth, productivity, and market value or shareholder return. Companies mainly grow by acquisition or by organic means through R&D or other investments. The acquisition route is frequently much inferior to the organic route in terms of total shareholder return, although it has been the preferred route in recent times, and could account in part for the recent poor performance of many companies that have used the merger and acquisition route. Even so, competitive advantage fundamentally requires a suite of attributes: management and worker skills, capital investments, market development, originality, adaptability, and commitment. The Scoreboard report recognises that its analysis is not and cannot be comprehensive. It does not include those companies that undertake R&D but do not declare the amount invested in their accounts, nor does it include UK companies investing less than £260,000 per annum in R&D. Importantly, it excludes publicly funded R&D but it would be useful to try to relate this component of R&D expenditure to the various FTSE sectors, and to assess over a suitably long period, e.g. 30 years or more, the societal impacts of that R&D. It is the aspiration of government and scientists that the various incentives for R&D investment in addition to improvements in the science base and business environment should accelerate the process of success through innovation. The UK needs more small- and middle-sized companies in the high-R&D sectors, and more of them with high R&D intensities: it is from this category that the new major international companies will emerge. From the work of D. B. Audretsch and R. Thurik in 2001 ((i) Linking Entrepreneurship to Growth, OECD STI Working Papers 2001/2.DSTI/DOC (2001)2. OECD Paris. (ii) What's New about the New Economy? Sources of Growth in the Managed and Entrepreneurial Economies, Institute for Development Strategies, Indiana University, Centre for Advanced Small Business Economics and Tinbergen Institute at Erasmus University Rotterdam, EIM Business and Policy Research, Zoetermeer), it is clear that smallfirm economies tend to have higher growth than large-firm economies, as the industrial economy gives way to the knowledge economy, one characterised by (a) investment in innovation, (b) gaining from creativity rather than investment, (c) being consumer- rather than product-oriented, (d) having short product lifecycles, (e) constantly adapting to change *i.e.* turbulent, and (f) enjoyment of both jobs and high wages. N. Crafts and M. O'Mahoney, in A Perspective on UK Productivity Performance (Fiscal Studies, 22, 271-306, 2001), made the point that compared with Europe, the UK and Scotland invest less in physical capital (plant, buildings, and machinery), and in human capital (skill levels). Compared with the USA, the UK and Scotland invest less in innovation and communication technology, and are less entrepreneurial. They also point out that business R&D expenditure as a percentage of output over the period 1990-1998 was 1.98% in the USA, 1.77% in Germany, 1.45% in France, 1.35% in the UK, and only 0.62% in Scotland. Another measure to consider is exploitation of R&D. Over the period 1996 to February 2002, the numbers of US utility patents per million population were 976 for Taiwan, 400 for France, 376 for the Republic of Korea, 287 for the UK, 208 for the Irish Republic, and 74 for Scotland. This figure for Scotland is out of kilter with the research output and citation impact of its scientists, and the fact that R&D

expenditure by higher education as a percentage of GDP over the last three years was 0.6% in Scotland, 0.5% in Finland, 0.4% in Denmark and the UK, 0.3% in the Irish Republic, New Zealand, and Wales. Spin-out companies have been viewed as a major route for exploitation of IP arising from the public sector. According to the Bank of England Finance for Small Firms - A Ninth Report (Domestic Finance Division, London, 2002), venture capitalists and UK universities alike regarded the top issue as accessing suitably qualified management. Fast-growth companies tend to have better qualified managers than slowgrowth businesses. As market valuations decline, however, there is increasing danger of mergers and acquisitions by companies from overseas competitors, or a shifting of decision-making staff to other countries, or even a firesale of potentially valuable intellectual property.

### **UK Agriculture**

Overview An authoritative and clearly presented overview of the UK agricultural industry is provided by the keynote publication Agriculture in the United Kingdom 2001 produced by the Department for Environment, Food and Rural Affairs; Scottish Executive Environment and Rural Affairs Department; Department of Agriculture and Rural Development (Northern Ireland); and National Assembly for Wales Agriculture Department. This publication was the fourteenth in an annual series, and relates also to other publications, including Agricultural Census Statistics in the UK, the Agricultural Atlas, the June Census Analyses, Historical Agricultural Data, and Farm Incomes in the United Kingdom 2000/01. Particularly useful websites include www.defra.gov.uk, www.wales.gov.uk, www.scotland.gov.uk, www.dardni.gov.uk, www.food.gov.uk (Food Standards Agency), www.potato.org.uk (British Potato Council), www.hgca.com (Home Grown Cereals Authority), www.statistics.gov.uk (Office for National Statistics) and www.europa.eu.int (European Union – Eurostat).

**FMD** The most severe outbreak of foot and mouth disease (FMD) since 1967-1968 took place during the period February 20, 2001 to January 14, 2002, directly affecting Dumfries and Galloway, Cumbria, Northumberland, North Yorkshire, Durham, Lancashire, Powys, Gloucestershire, and Devon, but dramatically affecting the entirety of the UK rural economy. Official figures note that 2,030 FMD cases were confirmed, 6.1 million animals were slaughtered, 4.1 million for disease-control purposes, and 2 million

for various welfare reasons; most, 4.9 million, were sheep, and 526,000 were slaughtered under the Light Lamb Scheme. Some sources claim that the official figures excluded many young animals and the real figure was around 9-10 million. Between June 2000 and June 2001, the total cattle population declined by 4.8%, the dairy herd by 3.4%, the beef breeding herd by 7.3%, sheep and lambs by 13%, the sheep breeding flock by 12%, the number of pigs by 9.8%, and the pig breeding herd by 2.0%, figure influenced by the incidence of FMD, movement restrictions, the outbreak of Classical Swine Fever in pigs in 2000, and the ongoing downward trend in the market. Debate persisted as to the legality and cost of the official preemptive slaughter activity. A total of £1.2 billion was paid in compensation to farmers for value of stock lost in the outbreak, with considerable other disposal and disruption costs, estimated by some commentators to be in the order of £5-6 billion. Exports of livestock and livestock products were banned, and thereby the reputation of the UK livestock industry was severely dented.

Economic Contribution Provisional data in the calendar year 2001 edition indicated that the contribution of agriculture to the total UK economy gross value added (GVA) as a percentage of total GVA at current prices, declined yet again from 0.8% in 2000 to just 0.7%, contrasting with a figure of 1.5% in 1996. At current prices, the GVA in 2001 equated to £6,418 million, compared with a downwardly revised figure of £6,535 million in 2000. Using a new basis for calculating the workforce in agriculture, that now for the first time includes spouses of farmers, partners, and directors, about 2.2% of the total workforce was employed in agriculture (550,000); the relentless decline in rural employment continued apace. As in previous years, there was no media attention on this issue contrasting with less diffuse redundancies announced in manufacturing, mining, and service industries. As before, the data do not take into account a large tranche of the UK workforce involved directly with agriculture, ranging from parts of the public sector (staff in government departments, agencies and institutes; Research Councils and their institutes; higher-education and further-education bodies; and groups related to the EU); to several parts of the private sector; food-processing, storage, distribution, and retail sectors; the industrial feedstock industry; restaurants, hotels, and the tourist trade. Contrasting with agriculture, the food and drink manufacturing, wholesale, retail, and food-service sector accounts for

circa £57 billion GVA, or 6.9% of GDP, and is responsible for around 3 million jobs. Although the food and drink manufacturing industry is the second largest of any manufacturing sector in the UK in terms of output, it does not receive the same level of publicly-funded R&D support as agriculture. The Total Income From Farming (TIFF) measure in 2001 was estimated to be £1.7 billion, some 11% in real terms higher than in 2000, and comprised business profits plus income to farmers, partners, and directors, and those with an entrepreneurial interest in the business. At £7,861, TIFF per annual work unit of entrepreneurial labour was 13% higher in real terms, compared with 2000, but TIFF itself was forecast to be 72% below its peak in 1995. Average subsidies per annual work unit amounted to £11,293 in real terms, and were unequally distributed according to the sector of agriculture. Total-factor productivity decreased by 6.1% in 2001, reflecting the impacts of FMD and the effects on the arable sector in 2000 of the wettest Autumn for at least 230 years. The percentage of total household final consumption expenditure (formerly 'consumer's expenditure' until the European System of Accounts was adopted in 1998) on household food was provisionally 9.7% compared with 5.9% for alcoholic drinks.

The component countries of the UK varied in their contribution to agriculture according to the local economic, political, and social roles of the industry. Thus, the Gross Value Added at basic prices in 2001 was £4,757 million in England, £824 million in Scotland, £312 million in Wales, and £526 million in Northern Ireland. The TIFF estimates were £1,206 million in England, £273 million in Scotland, just £47 million in Wales, and £190million in Northern Ireland; only the figure in England was lower than in 2000. Estimates of the share of agriculture of total regional gross value added at basic prices, continued recent trends in that it was lowest in England (0.6%), followed by Wales (0.9%), Scotland (1.1%), and then Northern Ireland (2.9%) where agriculture had grown in relative importance since the previous year (2.6%). As before, a slightly different pattern persisted for the share of total regional employment by agriculture; the official data include employees in direct agricultural and horticultural employment, the relevant selfemployed workforce, and those engaged in work-related government training schemes, but because the industry includes a relatively high proportion of temporary and part-time workers, any comparison on the basis of full-time equivalents with other sectors of the economy would yield significantly lower percentages. For 2001, 1.9% of total employment was officially attributable to agriculture, with the lowest level in England (1.5%), followed by Scotland (2.9%), Wales (4.7%), and Northern Ireland (7.6%).

Land Areas In June 2001, the total area of UK agricultural land plus common rough grazing was estimated at 18,549,000 hectares, of which 4,454,000 hectares were down to crops, and 43,000 hectares were bare fallow. These figures compare with an average of 18,862,000 hectares devoted to agriculture, and 4,984,000 hectares down to crops in the period 1990-1992. More detailed analysis of the cropping data reveals that the area devoted to cereals declined by 10% from 3,348,000 hectares in 2000 to 3,014,000 hectares in 2001. This was mainly due to the decline in the wheat area from 2,086,000 hectares in 2000 to 1,635,000 hectares in 2001, although there was an increase in the barley area from 1,128,000 hectares to 1,245,000 hectares. Only small changes were noted in the areas devoted to oats (109,000 hectares in 2000; 112,000 hectares in 2001), rye and mixed corn (10,000 hectares in 2000; 7,000 hectares in 2001), and triticale (16,000 hectares in 2000, 14,000 in 2001). The potato area decreased slightly from 166,000 hectares in 2000 to 165,000 hectares in 2001. Other arable crops, excluding potatoes, increased in area to the levels of the early 1990s, from 979,000 hectares in 2000 to 1,103,000 hectares in 2001. This was mainly as a result of an increase in the area down to oilseed rape (332,000 hectares to 404,000 hectares), and to peas for harvesting dry and field beans (208,000 hectares to 276,000 hectares). There was a sharp decline in the area of linseed (71,000 hectares down to 31,000 hectares), and hops fell from 2,000 hectares in 2000 to just 1,000 hectares in 2001. Sugarbeet not for stockfeeding rose slightly from 173,000 hectares to 177,000 hectares. Although there was a slight increase in the area of horticultural land, from 172,000 hectares in 2000, to 173,000 hectares in 2001, this was still down markedly from the average of 203,000 hectares cultivated in the period 1990-1992.

The areas of vegetables grown in the open (120,000 hectares), orchard fruit including non-commercial orchards (28,000 hectares), soft fruit including wine grapes (9,000 hectares), ornamentals including hardy nursery stock, bulbs, and flowers (14,000 hectares), and glasshouse crops (2,000 hectares), were closely similar to those in 2000.

Production In terms of production, the decreased area of land given over to cereals accounted for the decline in the volume of harvested production (23,990,000 tonnes in 2000 to a provisional 18,990,000 tonnes in 2001). The value of production fell from £2,338 million to £2,019 million. Exports to the EU and the rest of the world amounted to 2,470,000 tonnes, and imports were 2,719,000 tonnes; domestic usage was 20,816,000 tonnes. Wheat production fell from 16,700,000 tonnes in 2000 to 11,570,000 tonnes in 2001, and yield declined from 8.01 tonnes per hectare to 7.08 tonnes per hectare, similar to the average yield in the period 1990-1992. The value of UK wheat production was at a new low since the 1980s of £1,222 million. Of the 13,256,000 tonnes of wheat used domestically, 5,627,000 tonnes were used for flour milling, 6,480,000 tonnes for animal feed, 330,000 tonnes for seed, and 819,000 tonnes for other uses and waste. Barley, one of SCRI's mandate crops, recorded an increase in production from 6,490,000 tonnes in 2000, to 6,7000,000 tonnes in 2001, although yield declined from 5.75 tonnes per hectare to 5.49 tonnes per hectare. The value of production was £726 million. Domestic consumption was 5,583,000 tonnes, of which 1,939,000 tonnes were used in the brewing and distilling industries, 3,429,000 tonnes were used as animal feed, 169,000 tonnes for seed, and 46,000 tonnes for other uses and waste. The major beneficiary from the UK malting barley is the Treasury which receives on current excise duty and value-added taxation rates more than 200 times the profit realised by the producers, ignoring indirect taxation on producers. Oat production declined to 615,000 tonnes in 2001 from 640,000 tonnes in 2000, still greater than the average of 518,000 tonnes in the period 1990-1992. Yields were 5.60 tonnes per hectare, and the value of production was £64 million. UK domestic consumption was 527,000 tonnes, with 282,000 tonnes used for milling, 227,000 tonnes for animal feed, 16,000 tonnes for seed, and 3,000 tonnes for other uses and waste.

The production of potatoes, one of SCRI's major mandate crops, was down by 1.9%, from 6,652,000 tonnes in 2000, to 6,528,000 tonnes in 2001. Reflecting the declining market for earlies, and delayed planting due to wet conditions, the area devoted to earlies declined from 12,200 hectares in 2000 to just 7,5000 hectares in 2001, whereas the maincrop area increased from 153,800 hectares to 158,400 hectares. With yields of 23.2 tonnes *per*  hectare for earlies and 40.1 tonnes *per* hectare for maincrop potatoes, the volume of harvested potato production was 6,528,000 tonnes, with 175,000 tonnes for earlies, and 6,354,000 tonnes for maincrop cultivars. The total value of production on farm was estimated at £600 million in 2001, compared with £454 million in 2000. A total of 7,643,000 tonnes were used in UK markets in 2001, down from 8,155,000 tonnes in 2000, with 6,429,000 tonnes for seed tubers, 833,000 tonnes as chats, waste, and retained stockfeed.

Of the other arable crops, oilseed rape production at 1,159,000 tonnes was closely similar to 2000, and had a value of £167 million. Yield was 2.57 tonnes per hectare. Interestingly, that portion of the UK crop containing unauthorised genetically modified (GM) plants was destroyed and compensation payments were made. Linseed production declined to 39,000 tonnes, contrasting sharply with the 302,000 tonnes recorded in 1999. Although the area of land in linseed had declined from 74,000 hectares in 2000 to 31,000 hectares in 2001, average yield had increased from only 0.58 tonnes per hectare to 1.24 tonnes per hectare; in the period 1990-1992, yield had average 1.73 tonnes per hectare. Sugar beet production based on "adjusted tonnes" at standard 16% sugar content stood at 8,180,000 tonnes, with a value of production of £255 million. Sugar content averaged 17.15%. Sugar production in the UK on a '000 tonne refined basis fell to 1,200,000 tonnes, and imports amounted to 1,310,000 tonnes.

The combined value of peas for harvesting dry and assumed to be used to stockfeed (approximately 80% of pea production) and field beans used mainly for stockfeed rose from £113 million in 2000 to £141 million in 2001; subsidies amounted to £63 million in 2001. With a yield of 3.54 *per* hectare, pea production reached 280,000 tonnes; field bean production was 590,000 tonnes with a yield of 3.50 tonnes *per* hectare. Vegetable protein production will become an important issue as around 70% of the protein used in the EU is imported.

Horticulture remained an industry dominated by financially stretched, small-scale producers without ready access to subsidies and lacking both capital and influence in a market place dominated by a few supermarkets. Vegetables were cultivated on 145,100 hectares, only 1,100 hectares of which were protected. Nevertheless, the value of production was £970 million, of which £659 million were for vegetables grown in the open, and £311 million for vegetables grown under protection. The principal vegetable crops were cabbages, carrots, cauliflowers, lettuces, mushrooms, peas, and tomatoes. Fruit production in the form of commercial orchards only, and soft fruit excluding wine grapes, took place on 33,400 hectares, with a total value of production of £243 million, inclusive of glasshouse-produced fruit, orchard fruit accounting for £97 million (mainly desert and culinary apples, and pears) and soft fruit £133 million (mainly strawberries and raspberries). Ornamental production on just 20,000 hectares was valued at £708 million, accounted for by £395 million for hardy ornamental nursery stock, £282 million for protected crops, and £31 million for flower bulbs, including forced flower bulbs, in the open.

**Livestock Feed** Purchased livestock feedingstuffs, valued at £2.297 billion, rose from 19,698,000 tonnes in 2000, to 19,855,000 tonnes in 2001. Compound feedingstuffs for cattle, calves, pigs, poultry, and other livestock rose in value by 9.3% in 2001 to £1,404 million.

Seeds Total purchased seeds totalling 1,030,000 tonnes and valued at £292 million represented a decline from the average of 1,162,000 tonnes valued at £318 million in the period 1990-1992. Agriculture, horticulture and plantation forestry are dependent economically on a flow of improved cultivars, as end-user demands change, and pests and diseases overcome genetic resistance systems and pesticide efficacy. Throughout the EU, the plant-breeding industry was under severe financial pressures, not aided by regulations and the threat of even more regulations to monitor GM crop seeds. In the UK, the virtual elimination of public-sector plant-breeding R&D, and consequently the training and education of plant breeders, and development of commercially relevant germplasm resources, bode ill for the future of the UK's competitive position and the ability to capitalise on the range of new technologies to improve the efficiency of plant breeding, selection, and propagation.

**TIFF** Total Income From Farming (TIFF) is an oftcited figure but should be used with caution. It refers to business profits plus income to those with a direct entrepreneurial interest in the agricultural industry (*e.g.* farmers, growers, partners, directors, spouses, and most other family workers), but it is sensitive to relatively small changes in the values of outputs and inputs, compounded by the provisional nature of the 2001 data, and the fact that as a result of the perfectly rational decision by the Office for National Statistics that livestock destroyed for FMD and associated welfare purposes should be treated as "exceptional losses" under capital transfers and not as part of income. As in many areas of central statistical analyses, TIFF is also sensitive to changes in statistical methodology and services of the data. It is derived by deducting interest, rent, and paid labour costs from Net Value Added (NVA) at factor cost, an appropriate measure of value added by the agriculture industry because it accounts for NVA at basic prices plus other subsidies (less taxes) on production. According to Agriculture in the United Kingdom 2001, TIFF was estimated to have risen by 13% (11% in real terms) to £1,710 million compared with its level in 2000. In real terms, TIFF was stated to be 72% below its peak in 1995, after more than doubling in the period between 1990 to 1995. The revised figure for 2000 was £1,513 million, contrasting with £3,016 million in 1997. The NVA at factor cost in 2001 was £4,433 million compared with £4,275 million in 2000, and £5,823 million in 1997. According to the aggregate balance sheets in terms of assets and liabilities for UK agriculture, at current prices the net worth in 2001 was £103,357 million, compared with £99,972 million in 2000, and £49,444 million in 1997, reflecting in large measure the leap in the valuations of land and buildings, but yet fully to show the impact of FMD. Perhaps the private satisfaction of owning land and farming it as a garden or other type of recreation should be regarded as an output element of agriculture.

In the short to medium term, the downward pressure on the prices of agricultural products is likely to continue. Technological advances in agriculture and food processing and storage have facilitated new purchasing and marketing strategies, especially those capitalised on by the large, market-dominant supermarket/discount retailers, which have successfully adjusted to consumer demands. In Germany, the major retailers have driven down prices of food and non-food products, a trend likely to spread throughout Europe, aided by trade liberalisation, adoption of systems that utilise the efficiencies from increasing the scale of operation, and learning from the USA. Innovation in the form of R&D could be regarded as a potential victim of this trend towards price deflation, as much as the small-scale producer acting alone, or inefficient and unimaginative retailer. A switch in agriculturally related R&D priorities in both the public and private sectors is likely, as new market developments are introduced.

## Director's Report

**Productivity** Productivity of the UK agricultural industry can be assessed in various ways: volume of output, labour productivity, profitability, import substitution etc. Total factor productivity in terms of the volume of output leaving the industry per unit of all inputs, including capital and paid labour (a significant portion of agricultural labour is unpaid), has increased by 35% since 1973. This has arisen by increased output without corresponding increases in capital and labour inputs. During the 1990s, however, gross output less transactions within the agricultural industry remained relatively static, but inputs decreased slightly. In 2001, output decreased and inputs were more or less static. The trend in productivity growth since 1973 relates in large measure to a doubling in labour productivity as measured by the volume of NVA per unit of paid and entrepreneurial labour. Even so, in 2001, the NVA per Annual Work Unit (equivalent of an average full-time person engaged in agriculture) of all labour, using volume indices 1995=100, was 102.5, compared with 124.2 in 2000. Around 40% of the agricultural workforce now comprises low-paid part-time workers. Total factor productivity as given by the final output per unit of all inputs, including labour and fixed capital, using 1995=100, fell from a revised figure of 109.2 in 2000 to a provisional 102.6 in 2001. Fortunately for the taxpayer, the minimum wage legislation does not apply to the self-employed that make up the bulk of agriculture and horticulture.

Across the Member States of the EU, there was again great variation in the percentage changes in income derived from agricultural activity as measured by Eurostat's Indicator A (see Eurostat-Statistics: Statistics in focus. December 2001), which is based on NVA per whole-time person equivalent. Provisionally, rises were noted in Denmark (12.5%), Portugal (9.5%), Austria (8.5%), Republic of Ireland (7.3%), Belgium (6.2%), Germany (5.7%), The Netherlands (4.3%), UK (4.3%), Finland (3.0%), Sweden (2.8%), Spain (2.7%), Greece (1.4%), and France (0.8%). Declines were recorded for Italy (-0.8%) and Luxembourg (-2.4%). Across the 15 Member States incomes rose by an average of 2.7% concomitant with a continuation in a reducing volume of labour. Parenthetically, in 2000, the UK suffered an alarming decline of -10.8%.

**Subsidies** Support from the public purse for agriculture takes many forms. When the UK joined the European Economic Community in January 1973, the Common Agricultural Policy (CAP) came into force, with the laudable aims of guaranteeing food security, increasing agricultural productivity, safeguarding the livelihoods of farmers, and stabilising markets that historically had been prone to wide fluctuation. In 1973, the UK had the most dynamic agricultural industry of the Member States, as it entered a system of greater market regulation in peacetime than it had experienced before. To counteract commodity surpluses and escalating costs of the CAP, both of which attracted adverse publicity, a series of reforms to the CAP were introduced, leading to direct-aid payments, set-aside, environmental and social measures, encouragement of organic farming and diversification grants, with lessened emphasis on price and market price support mechanisms. At present, there are three main types of public-sector agricultural support: (a) intervention purchases and import tariffs; (b) direct payments for production; and (c) direct payments for rural development. The direct payments are made under three main headings: (i) direct subsidies and levies, which in 2001 were provisionally £2.457 billion less levies, some 1.9% more than in 2000, and increasingly the subsidies were less related to the amount produced; (ii) arable-area payments, which in 2001 amounted provisionally to around £1billion, including agrimonetary compensation; (iii) direct support to livestock producers, which in 2001 amounted provisionally to around £1billion, including agrimonetary compensation. Agrimonetary compensation evolved from the original "green rates" arrangements originally introduced to protect farmers from the vagaries of currency exchange-rate turmoils, through to special arrangements to take account generally of the introduction of the euro in January 1999, and specifically to allow for the relative strength of sterling against the euro. For many farmers, though, one of the most contentious issues is the topic of modulation, essentially a recycling or virement of direct CAP payments under the various commodity regimes. Thus, in 2001, modulation was introduced at a flat rate of 2.5% to help fund the Rural Development Programme, which on an accruals basis effectively reduced arable and livestock subsidies in the year by £44 million. Proposals to limit payments to farmers were another contentious matter -Scotland has the largest average farm size (131 hectares) in the EU, compared with an average of 72 hectares in the UK as a whole, and an average of 27 hectares in the EU. Other forms of support for agriculture include export subsidies, including food aid; payments made under structural objectives, especially relevant to Objective 1 areas; and various kinds of tax relief.

Subsidies to help UK organic growers were announced in July 2002, with a €140 million package of maintenance payments under the EU's Rural Development Programme spread over five years, supplementing compensation payments for losses sustained while converting from conventional farming. Around 70% of organic food sold in UK supermarkets is imported; sales of organic food in 2000-2001 reached £802 million, and circa £950 million in 2001-2002. Top fruit (apples, cherries, pears, and plums) farmers will benefit most, with subsidies of up to £600 per hectare, compared with £30 per hectare for arable farmers. There are no subsidies for conventionally grown fruit, vegetables, or flowers in the UK. Organic farming - Guide to Community Rules published by the EU Directorate-General for Agriculture outlines briefly the history of organic farming and explains the development of the community legislation that supports it (see also http://europa.eu.int/ comm/agriculture/qual/organic/index\_en.htm).

The CAP-related subsidy categories supporting UK agriculture (Table 2) are not the only sources of support. Additional costs came from operating market regulation, animal health, education, research, advice, food standards, and relevant public-sector staffing and associated facilities; some of such costs, of course, do not directly benefit producers but are designed to benefit consumers and trade interests. As a result of the FMD episode and establishing the Livestock Welfare Disposal Scheme, the total UK expenditure in 2001-2002 was forecast to increase substantially from the previous year by £2.2 billion to £5.3 billion. Spending under the CAP regime was forecast to increase from £2.7 billion in 2000-2001 to £2.9 billion in 2001-2002, divided into seven main headings: Arable Area Payment Scheme (36%); Beef and Veal (non-BSE measures) (27%); Beef and Veal (BSE Measures) (13%); Sheepmeat (8%); Milk (6%); sugar (4%); and other activities including processed goods (6%). A Rural Payments Agency, an Executive Agency of the Department for Environment, Food & Rural Affairs, was established in October 2001 as a single accredited EU paying agency with responsibility for CAP schemes in England and for certain UKbased schemes, although the Scottish Executive, the National Assembly for Wales, and the Department of Agriculture and Development for Northern Ireland retain administrative responsibilities for schemes within their respective bailiwicks. Expenditure on conservation grants, Exchequer funding of accompanying measures, assistance for agriculture in special areas, and in particular FMD compensation and related disposal costs, amounted to around £2.4 billion in 2001-2002, compared with £324 million in 2000-2001.

**Environmental Impact** Further developments took place in 2001 in considering the environmental impact of agriculture and how it relates to the environmental accounts compiled by the Office for National Statistics. Many international bodies are beginning to offer guidance on environmental accounting frameworks, and it is becoming an area of active academic study, not least as the multipartite concept of sustainability continues to gain credibility. In Agriculture in the United Kingdom 2001, positive and negative impacts of farming were considered briefly, and recognition was given to the subjective nature of valuing the environment. According to the report by O. Hartridge and D. Pearce, Is UK Agriculture Sustainable? Environmentally Adjusted Economic Accounts for UK Agriculture, July 2001), the provision of environmental services by agriculture as practiced currently, is outweighed by damage caused by the release of greenhouse gases and water pollution. It was based on some contentious and incomplete assumptions, scrutiny of agriculture should be seen in the context of mass urban-related habitat destruction, squalid urban public and private places, and urbanderived wastes and activities blighting the countryside. Using the DEFRA's Towards Sustainable Agriculture: a pilot set of indicators (see www.defra.gov.uk), the environmental accounts for UK agriculture are in three related sections as follows. (i) The state of the agricultural environment as adjudged by bird populations on the basis of their wide habitat distribution and proximity to the top of the food chain *i.e.* the bird population index is used as a biodiversity indicator for sustainable agriculture. Although 105 native species have remained fairly constant over the period 1970-2000, there was a marked decline in farmland bird populations between 1977 and 1993, stabilising thereafter. DEFRA has a Public Service Agreement target to reverse the long-term decline in farmland birds by 2020, but the reasons for the decline are manifold, and are not simply due to land-management practices. (ii) The levels of damaging emissions, e.g. fertiliser and pesticide contamination of water, gaseous pollution from animals, manure and slurry, and emissions from energy expenditure. Most of the emissions are diffuse, difficult to quantify and their impacts unclear. Integrated Farm Management (IFM) whole-farm policies offer the most immediately attractive route to minimise and ameliorate adverse environmental

#### Arable payments: wheat

barley other cereals (mixed corn, oats, rye, triticale) oilseed rape linseed peas and beans other crops other crop subsidies (hops and herbage seed support, hemp and flax aid, oilseed rape and linseed support, British Potato Council compensation payments) **Livestock subsidies:** beef special premium suckler cow premium scheme sheep annual premium scheme

extensification payment scheme dairy agrimonetary compensation

### Set-aside

### Other animal disease compensation Less-favoured areas support schemes

### Agri-environment schemes:

Environmentally Sensitive Areas Countrywide Stewardship Nitrate-Sensitive Organic Conversion Areas Habitat Moorland Woodland Schemes English Heritage Sites of Special Scientific Interest (SSSI) Tir Cymen (Wales) Tir Gofal (Wales) Countryside Council for Wales (SSSI) Scottish Natural Heritage

### Table 3 CAP-related Subsidy Categories for UK Agriculture

impacts by focusing on soil management, crop nutrition, crop protection, pollution control and waste management, energy efficiency, animal husbandry, and landscape and wildlife features. It attempts to integrate economic viability with environmental sustainability. The best non-ideological exponent of the system is Linking Environment and Farming (LEAF) that has over 13,000 members and operates with a self-assessment audit. DEFRA has issued its Codes of Good Practice for the Protection of Soil, Air and Water, and the Scottish Executive has issued the Prevention of Environmental Pollution for Agricultural Activities. (iii) The use of finite (nonrenewable) and sustainable (renewable) resources in agriculture can be assessed at several levels. Consumption of finite resources (petroleum, coal and gas in energy generation; metals and plastics in equipment manufacture; certain chemicals; excessive use of natural resources such as water and soil. It was estimated that in 2000, the direct and indirect energy consumption of UK agriculture was 191.6 PetaJoules (less than 1% of total UK energy consumption), compared with 240.3 PetaJoules in 1985. Agricultural biomass was estimated to account for 15% of renewable energy in the UK. By having a two-pronged strategy of small-scale on-farm combustion for heat production and electricity generation for sale under the forthcoming Non-fossil Fuel and Renewables Obligations programme, it is expected that agricultural biofuels will contribute to the generation of 10% of UK electricity from renewable resources by 2010.

With the three objectives of improving and extending wildlife habitats; conserving historic, geological and landscape features; and restoring traditional aspects of the countryside, support through agri-environment schemes is set to increase substantially. In June 2001, 623,202 hectares of land in the UK were registered as being farmed organically, aided by Organic Conversion schemes.

# Historical Perspectives: Agriculture in Britain 1700-1900

By 1700, many foods and crops were exchanged between Europe, Asia, and the Americas. In Britain, the potato, tea, coffee, chocolate, tobacco had become commonplace, and sugar was a cheap commodity. From 1700 to 1800, there were fundamental societal changes and turmoil, including the Union of England and Scotland on May 1 1707 and adoption of the Union Jack; the incorporation of the South Sea Company (1711); the Treaty of Utrecht (1713); the "Fifteen" Jacobite rising in Scotland (1715-1716); the war with Spain (War of Jenkin's Ear; 1739-1748); War of the Austrian Succession (1740-1748); the Second Jacobite Rebellion (1745-1746); adoption of the Gregorian calendar (1752; "Give us back our eleven days" - the days between September 2 and September 14 were omitted); land and naval war between Britain and France (1756-1763); the Treaty of Paris (1763); the resignation of Lord North for failing to subdue the American colonies (1782); and the War of American Independence (1775-1783). At the beginning of 1801, the legislative union of Great Britain and Ireland was enacted, under the name of the United Kingdom.

The Eighteenth Century is thought by many historians to represent artistically the shift from the Classical to the Romantic ages, but it was the initiator of the Industrial Revolution and the Agricultural Revolution. Robert Burns died in 1796, by which

time there had been a war with Revolutionary France for three years. Science and technology had already made great strides, e.g. John Tuberville Needham (1713-1781) in Observations upon the Generation, Composition, and Decomposition of Animal and Vegetable Substances, published in 1748, reported that boiled, sealed flasks of broth teemed with "little animals", but Lazzaro Spallanzani (1729-1799) devised properly controlled experiment to test such factors as the amount of heat needed to kill contaminating microorganisms. In 1749, George Leclerc, the Compte de Buffon (1707-1788) published the 54-volume Histoire naturelle, focusing on the main elements of evolutionary biology up to the beginning of the 20th century - geographical distribution, development, isolation, transmutation, correlation, and variation of organisms. In the tenth edition of Systema naturae, published in 1758, Carl Linnaeus (1707-1778) catalogued all the then-known flora and fauna, including humans, consistently using a binomial nomenclature, thereby laying the basis of modern taxonomy. Agriculture had been advanced by the publication in 1732 of Jethro Tull's (1674-1741) New Horse Hoeing Husbandry describing his inventions and introductions, such as the seed drill (1701), the horsehoe, and soil pulverisation. Robert Bakewell (1725-1795) introduced selective breeding of livestock. Charles Townshend (1674-1738) introduced crops such as turnips and clover for winter fodder. These early pioneers, whose efforts were publicised by Arthur Young (1741-1820), produced the Agricultural Revolution that led to fundamental changes globally in the production of foodstuffs and animal materials such as wool and hides. By 1801, Franz Achard (1753-1821) built the first sugar-beet factory in Silesia, enabling the sugar beet industry to develop in France and Germany. In 1834, Cyrus H. McCormick (1809-1884) patented his reaper. Plough technology was developed by Jethro Wood (1774-1834) with the cast-iron plough in 1819; John Lane in 1833 with the steel-bladed ploughshare; John Deere (1804-1886) introduced the steel plough in 1837; the chilled plough of 1855 by James Oliver (1823-1908) was improved in 1857 by the Marsh brothers; cable ploughing was introduced in 1850 to be followed by the steam plough by John Fowler in 1858. The revolving disc harrow was introduced in 1847, the binder in 1850, two-horse straddle-row cultivator in 1856, combine harvester in 1860, combine seed drill in 1867, and the sheaf-binding harvester in 1878. Pasteurisation as a preservation technique for beer, wine, and milk was introduced in 1861 after the

pioneering work of Louis Pasteur (1822-1895). Chemical fertilisers came into widespread use in the 1880s following the pioneering research at Rothamsted by J. B. Lawes. Mechanical freezing, long-distance rail travel, internal-combustion-engined tractors were introduced in the latter half of the 19<sup>th</sup> century. Between 1850 and 1900, about 200 million hectares of grazing land in the USA were converted into grain fields; similar conversions to arable land took place in Australia, Russia, and several countries in South America.

In the first part of the 19th century in the UK, the ending of the Napoleonic wars was associated with economic depression, and as a form of remedial action, the Corn Law of 1815 was introduced to protect agricultural landlords from imported grain until home-grown corn reached the "famine price" of 80 shillings a quarter. Bread prices rose and there was widespread malcontentment. In the 1820s, William Huskisson, the then President of the Board of Trade, breached the protectionist mercantile system by reducing duties in several imports (e.g. coffee, sugar, cottons, woollens, silk, iron etc.). In 1828, the Corn Law was modified to permit grain to be imported at any time and fixing duties on a sliding scale. Parliamentary reform characterised the period 1830-1846, introducing important social developments (abolition of slavery in the colonies, the Factory Act, growth of trade-unionism, new Poor Law etc.). In 1845-1846, there was considerable agitation against the Corn Law, fostered by Richard Cobden and John Bright, political leaders of the Manchester School, building on the Manchester Anti-Corn Law Association (1838) and Anti-Corn Law League (1839). The ruin of the Irish potato crop and the threat of famine were instrumental in gaining the support of Robert Peel, leading in turn to the Repeal of the Corn Laws on June 6 1846. By June 29 1846, the government led by Peel was overthrown by a parliamentary revolt led by Benjamin Disraeli. In 1879, there was a severe agricultural depression arising from the worst harvest of the century, a period associated with a general economic downturn, several strikes, the unpopular Afghan and Zulu wars, and problems with Ireland. The remainder of the 19th century, like the rest of the century, was marked by growing internationalisation of trade, and scientific and technological achievement.

UK agriculture was transformed with enclosure (inclosure) of 'waste' and common land. During 1702 to 1802, 577 Acts were passed for enclosing over 8 million acres of such land. The First Report from the Select Committee of The Honourable The House of Commons Appointed to take into Consideration the Means of promoting the Cultivation and Improvement of the Waste, Uninclosed, and Unproductive Lands of the Kingdom, 1 January 1796 (price one shilling and replete with interesting spellings and the long s(f)!) resolved that "the cultivation and improvement of the waste lands and commons of the Kingdom is one of the most important objects to which the attention of Parliament can possible be directed, and that the granting of a bounty to encourage the cultivation of potatoes, in lands at present lying waste, uncultivated or unproductive, would not only be the means of augmenting, in a considerable degree, that valuable article of food, but might also be the means of promoting the improvement of extensive tracts of land, at present of little value". The Select Committee also opined that "certain legal liabilities that stand in the way of division and enclosure of waste lands should be removed". Reference was made to the idea of lands in common was "derived from that barbarous state of society, when men were strangers to any higher occupation than those of hunters or shepherds, or had only just tasted the advantages to be reaped from the cultivation of the earth". "Those who live in the neighbourhood of great wastes are still an idle and lawless set of people", "that such commons are the frequent resort of thieves and other depredators on the public", "and are on that account but particularly near the capital, a public nuisance". The Bishop of Landaff stated "That whilst there is an acre of such waste improveable land in Great Britain, it may be hoped that when the Legislature shall turn its attention to the subject, no inhabitant of this island will be driven, by distress, to seek a subsistence in Africa or America". The report estimated that there were 7,888,777 acres of uncultivated land in England and 14,218,224 acres in Scotland giving a total of 22,107,001 acres of waste land, compared with 39,027,156 cultivated acres in England, and 12,151,471 acres in Scotland. The uncultivated area was regarded as a great source of future national wealth.

"The waste lands above enumerated are not only uncultivated themselves, but they have a tendency to make the farmers in the neighbourhood neglect the improvement of the lands they enjoy in severalty".

"If any person entertains an idea, that a General Inclosing Bill is an impractical measure, his doubts will probably be removed, when he is informed that such an act was passed about a century ago in Scotland, and has been found to answer the purpose thereby intended". "Act concerning the dividing of commonties, passed in the Parliament of Scotland, 17<sup>th</sup> July 1695".

"In regards to the climate of such wastes, it is evidently worse in consequence of the want of cultivation. -At the same time, from the insular situation of Great Britain, the climate is infinitely milder and better than in any part of the continent of the same latitude. It is stated in one of the Reports, on the most respectable authority (George Dempster Esq), that very fine barley and oats ripen in due season, on the summit of a hill in Forfarshire, elevated 700 feet above the level of the sea; and that in Invernesshire, at an elevation of 900 feet above the same level, wheat of a good quality has been grown. - Hence it may be inferred, that grain, and other articles of a similar nature, may be raised to such a height upon the sides and summits of all the hills in the island; and, in regard to grass, it is well known, that luxuriant crops of hay are obtained at the lead hills of Lanarkshire, elevated 1,500 feet above the sea. The climate of this country, therefore, can hardly be urged as an objection to the improvement of the greater part of our wastes, either for grain or grass; as to trees, it is not to be questioned, that the larch grows in Italy on higher mountains than any we have in this island."

"Lastly, at least a million acres of the Waste Lands in the kingdom may certainly be brought to an astonishing height of produce by watering or irrigation. – This great means of improvement, though long established in some parts of the kingdom, yet in others has been unaccountably neglected. But when once that art is extended as it deserves, the advantages thence to be derived cannot easily be calculated, - for by it land is not only rendered perpetually fertile without manure, but the luxuriant crops which it raises, produces manure for enriching other fields; and the manure obtained from the produce, it another source of national wealth, that otherwise could not be looked for."

"Nor ought the wealth to be derived from the improvement of our Wastes to be alone taken into consideration. The increase of population, and above all, of that description of persons who are justly acknowledged to be the most valuable subjects that any government can boast of, merits to be particularly mentioned. His mind must indeed be callous, who feels himself uninterested in measures, by which not only the barren Waste is made to smile, but of which

the object is, to fill the desart with a hardy laborious, and respectable race of inhabitants, the real strength of a country; being the fruitful nursery, not only of our husbandmen, but also of the fleets, the armies, and the artists of the nation. The additional number of inhabitants, who might thus receive occupation and subsistence, cannot easily be ascertained; but if the present population of Great Britain amounts to about ten millions, these Wastes and Commons, properly improved, might be the means of adding, at least, from two to three millions; - a number, it may be proper to observe, equal to that possessed by the United States of America, when they first erected the standard of independence against the Mother Country. The evils were then felt from the creation of such an extent of population at a distance: but from such an increase of people at home, instead of similar consequences being to be apprehended, additional strength and prosperity of every description may be looked for with certainty".

"There is another point of view in which this subject ought also to be considered. - The improvement of Wastes not only adds to the wealth and population of a state, but also renders it more defensible. An inclosed country is, perhaps, the strongest of any. Every hedge and ditch becomes a rampart, through which an enemy cannot easily penetrate, and which there is little difficulty in defending. Were this kingdom completely inclosed, and no opportunity afforded of fighting any pitched battle (the only thing to be dreaded in the event of an invasion) we should have little reason to apprehend the landing of any body of men, however numerous, or however well disciplined. They might do some mischief on the coast, but could never penetrate into the interior of an inclosed country. The best defence the capital can have, is not to suffer a spot of unenclosed ground to remain between it and the coasts in its neighbourhood."

"Before concluding this Address, it is necessary to take notice of one important circumstance. For some years past, this kingdom has been under the necessity of importing grain from other countries; and the importation seeming to increase, rather than otherwise, it was seriously apprehended that the agriculture of this island could not furnish grain sufficient for the use of its inhabitants. – Many reasons may be assigned for the scarcity of grain; the seasons, since 1754, have certainly been in general unfavourable. We have seldom had two successive good seasons, but often two successive bad ones. To this natural cause may be added the increased population, consumption, and the luxury of the people, and the greater attention that of late has been paid to the improvement of stock, in consequence of which considerable tracts of arable land have been converted into pasture. This, instead of being a public loss, was undoubtedly a national benefit. Land that for ages had been kept in tillage, and produced but scanty crops of grain, required rest, and was usefully appropriated for pasture."

### "Appendix D.

### On the culture of POTATOES in Waste and Boggy Lands; from the Publications of the Board of Agriculture, and other Authorities."

"Old pastures have always been considered extremely favourable to the culture of potatoes, and even upon bogs partially or wholly drained, and upon such rough soils as are difficult to plough, this method has been successfully adopted; - pare and burn the surface: add lime to the ashes: strike the lands into straight beds, six feet wide, with intervals of two feet, and two and a half. Lay the sets twelve inches square on the beds, and cover them two or three inches deep, with spades from the intervals: when the plants appear, cover them again in the same manner, one and half or two inches more. Keep them clean by one hand-hoeing, and successive weeding. They may be taken up with the plough; by splitting the beds, and filling the former intervals; converting the open furrows, left in the center of the former beds, into drains, deep enough to leave the land dry in winter."

"In Dumbartonshire, the potatoe culture for improving waste and mossy soils, universally acknowledged to be of great advantage. Cutting down brush-wood, and removing great stones, is the whole preparation, previous to planting."

"In West Lothian, it is observed, that the upland parts of the country produce larger crops, than the more cultivated and low situations."

"In East Lothian, in the high district, the crops more productive than in the low part of the country. Sixty bolls, barley measure, is not reckoned a great crop; but in the low district, rarely more than forty or fifty."

"Sir W. Stirling, in Perthshire, has often raised forty bolls of potatoes on an acre of light moor, not worth 1s. – Rent at 5s. only: here are two hundred rents."

"In Roxburghshire, Baron Rutherford has found them the best means of bringing Waste Lands into culture."

"In Dumbartonshire, Sir James Colquhoun improved a peat moss, by planting potatoes in the lazy bed ways; sowed after them, meadow soft grass, with the oats, which is excellently adapted to mossy soils, as it spreads quickly. This was mown every year; the bog, being ten or twelve feet deep, could not be pastured." "In the highlands of Scotland, the fairest and largest potatoes are produced upon the spongy mosses, planted in lazy beds."

"They find in Perthshire, that mosses, when drained, are a favourable soil for potatoes."

POTATOES on WOODLANDS

"Mr. Abdy, of Essex, an honorary member of the Board, grubbed a wood, dunged it with twenty wagon-loads an acre, and planted potatoes: produce 563 bushels *per* acre; expence £.16. 13s. 6d.

*Observation.* – This single experiment may be of use to those who grub up woods, as it may probably be found that no other crop is better adapted to be first had resource to on such occasions. – It merits a trial to discover whether dung is necessary in such cases."

Improvement of Waste, by TURNIPS and POTATOES "The following practice seems to be well entitled to particular attention, as an excellent mode of improving Waste Lands. First burn the surface of the coarse grounds, such as the outskirts of bogs, and lands overrun with furze, heath, fern, &c. in the months either of March of April, or even May, and spread the ashes about the beginning of July. Then plough and harrow the lands, and sow it with turnips, the crop from which will probably pay the rent and expences. In the Spring following plough and harrow the land, and without any farther manure plant the potatoes with a small plough. The crop from new ground treated in this way is very great: Sometimes a second crop of potatoes is taken, but in general the sooner new land can be laid down in pasture, the better, until it has acquired strength sufficient to yield crops of grain in regular rotations. According to the nature of the soil, it may be laid down with grass-seeds, either with barley, if the land is light, or with oats, if it is strong or heavy."

At the end of the 18<sup>th</sup> century, food security and agricultural technology were patently high-priority issues in government. In the next report in this series, I shall comment on the period 1900 to the present.

### **Concluding Comments**

Research in the life and environmental sciences is progressing at a phenomenal rate, producing unprecedented numbers of discoveries, inventions, concepts, products, and new processes that are having substantial societal and scientific impacts. The life science industry in the USA alone raised \$44 billion between mid-1999 and mid-2001. A widening gulf is opening up between those elite organisations and companies that have attained a critical mass of talented scientists and state-of-the-art physical resources, and lesser wellresourced or inappropriately managed organisations and companies striving unsuccessfully to be world class. Innovation, as some of the major pharmaceutical companies now realise, is a precious asset that requires careful custodianship and sensitive management that allows intellects to blossom. Micromanagement, excessive short-term target setting, constant reviews, and persistent financial instability provide the wrong environment.

Over the next five years, agriculture is likely to remain under massive price pressures in order to meet the demands of urban populations and politicians for cheap and wholesome food, all-year-round, regardless of the weather. Food prices globally have progressively declined in real terms. Irrespective of the generic nature of the science, engineering, and technology used in the life and environmental sciences, agricultural innovation is likely to be one of the casualties of these pressures, as profitability and investment decline. Agricultural production is essentially a private-sector activity - it has been badly affected in the past by unnecessary regulation, intervention by heavy layers of bureaucracy, taxation, subsidy, and an underlying willingness of certain states to become almost a monopoly supplier - collectivisation, after all, has led to starvation. A stagnation or suppression of agricultural R&D – R&D well supported in the past by both the public and private sectors - is a potential disaster as governments have become complacent on the back of technology-dependent agricultural successes, forgetting the vagaries of the weather and the adaptability of pests and diseases, and population pressures in various parts of the world. In Agriculture and rural extension worldwide. Options for institutional reform in the developing countries, FAO, Rome, 2001, agricultural extension systems in various countries are now described as failing, moribund, in disarray, or barely functioning. Access to water, water-use efficiency, and nutrient-use efficiency by crops, and cultivar performance are huge issues. Numerous studies have shown that agricultural improvement reduces poverty and inequality. In Africa, agriculture employs around 66% of the labour force, and accounts for 37% of GNP and around 50% of exports (World Development Report 2000, World Bank, Washington DC). N. Nagarajan in The Millennium Round: An economic appraisal (European Commission Economic Papers, European Commission, Brussels) estimated that developing country gains from a 50% cut in mainly agricultural tariffs, by both MDCs and LDCs, would be in the

order of \$150 billion, about three times the aid given to LDCs. These gains would help improve LDC agriculture. The CGIAR Centers are now under financial pressure. Even international genebanks and germplasm collections are suffering from declining funding - according to FAO, there are about 6 million samples of plants held in around 1,300 repositories, many of which in the LDCs may be lost, despite the worldwide commitment to safeguard biodiversity. I urge policyholders in the UK and elsewhere to consult Global Food Projections to 2020. Emerging Trends and Alternative Futures by M. W. Rosegrant, M. S. Paisner, S. Meijer, and J. Witcover, International Food Policy Research Institute, August 2001, and related documents from that CGIAR Center. As P. Pinstrup-Andersen points out, there is one inescapable conclusion: "even rather small changes in agricultural and development policies and investments, made in both developed and developing countries, can have wide-ranging effects on the number of poor and undernourished people around the world. The policy choices we make now will determine to a considerable degree what kind of lives the next generation will lead".

Another particular pressure in the UK is the policy environment surrounding agriculture and the countryside, with the involvement of a wide range of stakeholders, some holding irreconcilable opinions. Agriculture in the UK remains in the doldrums and faces further harsh times. It tends to be judged on farm-gate prices rather than its underpinning role in supporting other sectors of the economy; few realise that there are more people employed in rural manufacturing than in urban manufacturing (A. Michael MP; November 9 2002), or that the profitability of the food processing industries, the catering and resturant, and retailing is dependent on low-cost primary produce. Industries directly related to agriculture, such as plant breeding and agricultural chemicals are under stress. Many companies are disinvesting from the UK, and their associated R&D activities terminated. There is talk of a 'post-agricultural' countryside such that farming is but one of several participants and often regarded merely as a recipient of rural social therapy (i.e. public funding) to maintain 'environmental goods'. New thinking is required. Farming is eminently capable of producing new types of crop and livestock; new ways of helping to provide the lungs, kidneys, visual amenity, and recreational base for the urban masses; as well as producing the usual food and non-food items. Most important, agriculture can be, should be, and must be, a successful business. A UK agricultural roadmap should be synthesised. Allied to the present decline in UK agriculture is a decline in plant science (botany). From a position of international pre-eminence in numerous universities, institutes, and companies, botany has diminished rapidly in national importance, such that there is a loss of scholarship in certain key areas of the subject. It is in the scientific, diplomatic, and economic interest of the UK that the situation is rectified. Fortunately, the generic nature of the research conducted in certain institutions such as SCRI, and its applicability to a range of environmental, industrial feedstock, regulatory, and health-related industries and activities, mean that there remains a thriving core of basic, strategic, and, to a lesser extent, applied research in the UK.

# **Science Overview**

### Wayne Powell

In today's era of new paradigms represented by tech-Inological advances, perceived market and social opportunities, shifting public concerns and government expectations we must ensure that we do not lose sight of our primary Institute obligation: the delivery of scientifically relevant, high-quality research. The scientific articles outlined in this report articulate a selection of our achievements and progress. However, we must not be complacent. To be successful in continuing to raise our national and international standards we aspire to have research programmes that are considered, by both our scientific colleagues and our funders, to be amongst the best in the world. Sustaining high levels of excellence requires an emphasis on quality and high levels of investment in staff and facilities to attain a critical mass of resources. Indeed, the gap between world-class organisations and the remainder is widening and those organisations that fail to respond to this challenge run the risk of 'missing the boat' and may never catch up. Our new management and organisational structure is designed to meet this challenge, raise the bar for performance, assume greater risk, be more responsive to emerging opportunities, and provide a better interface with the public and private sectors. Our aspirations have been significantly facilitated by increased support from SEERAD via the "Outer Core" initiative, providing an additional £1.9m funding over the next 3 years, enabling us to invest in new areas of scientific capability to ensure international leadership that provides the maximum benefit to our sponsors and stakeholders. Based on this new investment in science we are in the process of making 15 new appointments. In addition, during the current year SEERAD also provided a capital grant of £2,483,000 for major items of building, plant and equipment.

Our official mandate crops (potatoes, barley, blackcurrants and raspberries) are largely overlooked by the ag-biotech industry, giving us the opportunity and responsibility to reinforce our efforts to establish strategic product orientated research. Private sector led progress in genomics and biotechnology capability for these species is therefore not an option. Our plan is to build genomics and plant breeding capability around relevant germplasm and to ensure that we are uniquely positioned to capture the range of social and market opportunities arising from integrating genome science with plant breeding. Indeed, competency in

plant breeding and the ability to convert traits to products is a major global technology platform that will provide a delivery vehicle for health, diet, food, renewable resources and the bio-industries. In order to realise these opportunities we must ensure that our mandate crops do not lag behind in the application of biotechnology. In addition, we must reconsider the way in which plant breeding is planned, conducted and communicated to ensure that the benefits arising from it are fully recognised, more visible and relevant to the needs of society. Part of our strategic plan will therefore to be to coordinate and expand our plant breeding efforts through a Crop Improvement Centre that will link activities across our three scientific themes and Mylnefield Research Services (MRS). Underpinning research on transgenic crop technologies and production will also be consolidated within the Centre. This new management approach will aid cohesion and ensure that we optimise our research and commercialisation infrastructure to add value to our crop improvement efforts.

Facilitating synergistic interactions between fundamental, strategic and policy-relevant research means that our scientists must be proactive in the management of projects at various stages of their development and evolution. Individual creativity and originality is paramount but we cannot afford to base our future success exclusively on serendipity. Integration of research activities across disciplines is optimised by our organisation into Themes, which also reflects our determination to attain an appropriate balance between discovery science, hypothesis driven research and strategic product orientated research.

During the past 12 months, we have established a formal agreement with the University of Dundee to relocate four senior members of University staff (Dr Andy Flavell, Dr Claire Halpin, Professor Hamlyn Jones and Professor John Raven FRS) and their research teams to the SCRI campus. This exciting development is part of our strategic intent to establish effective partnerships and forms part of a shared vision with the University for the future development of research, education and training in the plant bio-sciences. Already, we are seeing the benefits of this venture through closer interactions, and this enhanced synergy is providing the basis for further productive and mutually beneficial initiatives with the University sector. Preparations for our 2003 Visiting Group exercise, or more formally the Research Organisation Assessment Exercise (ROAE), in May 2003 are underway. This event will coincide with the fiftieth anniversary of James Watson and Francis Crick's identification of the structure of DNA. The 20<sup>th</sup> century witnessed the phenomenal success of the reductionist approach to biology. A major opportunity for the 21<sup>st</sup> century will therefore be to integrate the reductionist view of biology with genome-wide approaches and whole organism biology. To realise these opportunities will require focus, and the development of computational support for data acquisition, analysis and model building and validation. Alignment of research effort and appropriate resource allocation is therefore critical. The establishment of a scientific advisory board, and particularly the input of Dr J Antoni Rafalski from DuPont USA has helped us focus and resolve key issues of relevance to our current and planned research programme. We are excited and optimistic about our future and look forward to engaging in constructive dialogue with visiting group members and presenting our aspirations for the future development of the Institute.

## **Biodiversity research in relation to crop improvement and conservation genetics**

J. Russell, A. Booth, M. Woodhead, K. Caldwell, H.V. Davies, D.F. Marshall, J.R. Hillman & W. Powell

**B**iological diversity – biodiversity – has many meanings, ranging from the number of species in an ecosystem, a country or the world, through to the levels of genetic diversity within a species, family, or Phylum. It tends to refer to the phenotype - the biological form we see, in essence, the manifestation of gene expression in the organism as influenced by various components of the environment.

The conservation of wildlife and habitats is integral to the public debate and government policies on biodiversity. In 1994, the UK ratified the international Convention on Biological Diversity (www.conbio.org. uk), building on the 1971 Ramsar Convention on Wetlands of International Importance especially as Waterfowl Habitat, which entered into force in the UK in 1976. A national Biodiversity Action Plan was published in 1994 (www.ukbap.org.uk) followed in 1995 by a report of the UK Biodiversity Steering Group proposing the monitoring of 1,252 species to gauge biodiversity trends. In March 2001, Sustaining the Variety of Life: 5 years of the UK Biodiversity Action Plan was published. At present, there are nearly 170 local biodiversity action plans in preparation or in progress in the UK. Other relevant international conventions include the 1973 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) which came into force in the UK in 1975; the 1979 Convention on Conservation of Migratory Species of Wild Animals, which came into force in the UK in the same year; and the 1979 Bern Convention on the Conservation of European Wildlife and Natural Habitats which came into force in the UK in 1982. The application of CITES is reinforced by the European Council Regulation on the Protection of Species of Wild Fauna and Flora by Regulating Trade Therein, which came into force in the UK in 1997. In terms of UK legislation, the Wildlife and Countryside Act 1981 offers legal protection to a number of specified wild animals and plants; any variations to the listing requested by the Secretary of State for the Environment, Food and Rural Affairs are dependent on parliamentary approval.

Maintenance of biodiversity is generally regarded as an important aspect of the quality of life, fundamental to

the natural heritage, and the basis of important economic and social activities. Biodiversity is therefore regarded as natural capital, but vulnerable to the impact of humans on the natural environment. Even so, it is difficult to measure biodiversity, or identify the optimum level of biodiversity in a habitat, let alone assess the optimum level of genetic diversity within a species. Microbial biodiversity, especially in soils, is poorly understood, and there has been a tendency to underestimate the role and relevance of biodiversity studies in economically important species and in agriculture, horticulture, and forestry, despite the current focus on the concept of sustainability, as well as initiatives assessing the potential impacts of climate change and the introduction of invasive nonnative ('alien') species.

Concerns about biodiversity have become increasingly relevant to the way we manage biological systems. This is both because the diversity within species or ecosystems plays a major role in determining both their resilience to short-term environmental pressures and their survival over evolutionary timescales. In addition, biodiversity has increasing relevance as a resource that can provide valuable and often novel products at scales ranging from the pharmaceutical to the commodity. Biodiversity, in term of intra-specific variability, has a key role in underpinning the ability of global agricultural systems to keep pace not only with the biological arms race imposed by pest and pathogen burdens but also with the increasing demands both for volume and processing quality associated with the food industry as well as the increasingly diverse range of industries that utilise plant products. Plant biologists must therefore not only face up to the biodiversity challenge but also consider creative ways to exploit biodiversity using the contemporary tools of genome science and informatics.

Genetic diversity within species has long been the mainstay of population genetics and in the last century an extensive body of mathematical theory, built initially on the elegant work of Fisher, Wright and Haldane described the key factors that determine the patterns of genetic variation within and between populations. It is however only with the recent development of 'omics' technologies that the potential to

	RFLPs	RAPDs	Microsatellites (SSRs)	AFLPs
Pros	Effectively infinite in number Single protocol for all markers Codominant Detects homeologous loci Robust Transferable	Quick Easy Primers available cheaply Many markers per run Simple Unlimited number of markers	Simple PCR based Codominant Highly polymorphic Quick Robust	Detects large numbers of loci PCR based Highly polymorphic Robust
Cons	Requires large quantities of DNA Slow-hybridisation based Variable level of polymorphism Difficult in large genomes Generally uses isotope	Dominant markers Experimental conditions are critical Poor transferability Poor reliability	Long development time Need specific primers Expensive to establish May use isotope	Dominant May use isotope

Table 1 Associated advantages and disadvantages with 4 popular DNA marker technologies

both measure and understand these processes at a genome-wide level has become possible. This together with the second generation 'functional genomics' approaches built on a range of technologies such as microarrays, knockout, proteomics, metabolomics etc., is providing us with unique resources with which to build our understanding of 'functional biodiversity'. The purpose of this article is to review some of the key developments of relevance to biodiversity research in relation to crop improvement and conservation genetics.

**Intra-specific diversity** The discovery of DNA-based genetic markers by Botstein *et al*<sup>1</sup> in 1980 fundamentally changed our ability to detect, analyse, describe and manipulate sources of genetic variation. Since that time, various molecular assays have been developed (Table 1) each possessing a specific set of

attributes ranging from simplicity e.g. RAPDs, informativeness e.g. SSRs, through to high multiplex ratios e.g. AFLPs. All the methods outlined in Table 1 have one common feature in that they represent indirect approaches to detecting DNA sequence differences. Developments in genome science, particularly highcapacity DNA sequencing technology coupled with powerful informatics tools have allowed the genomes of many organisms to be either fully or partially sequenced. A re-focusing of effort in the intra-specific diversity of crop plants is now emerging in both private and public research organisations (Rafalski<sup>2</sup>). The extent of polymorphism differs substantially between species and sampled loci. For example, each copy of the human genome differs from any other copy in the population by roughly 1 in 1,250 nucleotides. Although far less advanced, data on the extent and distribution of sequence polymorphism in

Genome	Common Name	Haploid size (Mb)	Intra-specific sequence diversity
2ea Mays	Maize/Corn	2,292	1 SNP per 83bp
Glycine max	Soybean	1,115	1.64 SNPs in coding and 4.85 SNPs in non- coding regions per kb.
Arabidopsis thaliana	Thale Cress	125	1 SNP per 3.3 kb

Table 2 Estimates of sequence diversity in some crop plants

Haplotype	The sequence configuration of two or more alleles on a single chromosome of a given individual.
Linkage Disequilibrium	Non-random association of a particular haplotype for two or more loci.
Nucleotide Diversity	A measure of DNA sequence variation for a given region influenced by the number of variable sites and their population allele frequency.

Table 3 Definition of some terms used in this article.

#### Cultivated

#### Landraces

#### H.spontaneum

Figure 1 Sequence polymorphism at Best608, with homology to a jasmonate-induced protein

crop plants is progressing and estimates of sequence diversity for maize, *Arabidopsis* and soybean are given in Table 2. Recent studies cited by Rafalski<sup>2</sup> have identified for elite US maize germplasm 1 SNP per 48 bp in non-coding regions of the maize genome.

Similar studies are being conducted for barley and an example of SNPs identified in a 246 bp amplified region of the barley genome is shown in Figure 1 for a

sample of 24 accessions. There are a number of key features of this figure, foremost of which is the organisation of SNPs into haplotypes (Table 3). Overall there is a limited number of haplotypes (Figure 2) in the cultivated gene-pool and it would appear that barley breeders are shuffling a restricted range of haplotypes in current barley germplasm. The structure of haplotype blocks in crop germplasm is important since it represents a fossil record of the history and structure of ancestral populations together with a

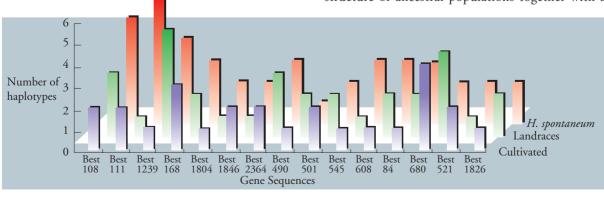
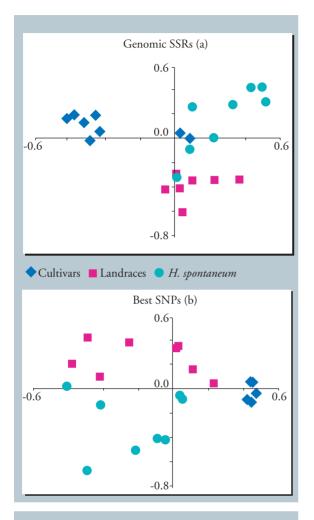


Figure 2 Comparison of haplotypes among the three gene pools of barley.



**Figure 3.** Principal Coordinate Analysis (PCO) analysis using distance matrices generated by genomic SSRs (a) and SNP haplotypes (b).

means of describing and quantifying functional polymorphism. Haplotype analysis may therefore be used to analyse the genetic structure and relationships of various gene pools. In Figure 3 we present data comparing the genetic relationship between H.vulgare cultivars, landraces and H.spontaneum accessions based on SNiP-derived haplotype data and genomic SSRs. Although there are similarities between both plots, the hapolotype-based analysis clearly discriminates between the three gene pools with the H.vulare cultivars being represented by a very similar and distinctive cluster. In particular, Figure 3a graphically illustrates the narrowing of the genetic base or "bottle neck" experienced during the domestication of cultivated barley. Well-characterised and sampled products of the domestication process may provide an experimental framework to identify and eventually validate the association of genes with phenotypes that have been exposed to selection during crop domestication. The

products of the domestication process together with their ancestors therefore provide a rich resource to isolate genes involved in domestication and adaptation. Exploiting the products of domestication in conjunction with population-based genetic analysis of relevant genes can give rise to new insights into the evolutionary processes that sculptured the formation of crop (and animal) species.

The frequency of SNPs in some plant genomes together with their occurrence in the vicinity of plant genes make this class of polymorphic marker an ideal choice for meiotic map construction. We have initiated a programme to map both SNP and other functional classes of polymorphism in double-haploid (DH) populations of barley. The highly successful BBSRC/SEERAD-funded cereal transcriptomics programme has generated a wealth of EST sequences providing a substrate for SNP discovery. Amplification by PCR followed by direct sequencing of DNA regions from the parents of the mapping population has been used to identify SNPs and insertion/deletion events. Preliminary data on the map location of ESTs are shown in Figure 4 for the Steptoe x Morex mapping populations. Microsatellites also occur in reasonable high frequency in ESTs (Cardle et al 2000<sup>3</sup>, Morgante et al 2002<sup>4</sup>) providing an opportunity to map ESTs based on length polymorphism. The advantages of this marker system are that the polymorphism is physically associated with a coding region and the amplification products are robust with a high degree of reproducibility.

Conservation Genetics The tools of genetic science can also be applied to species of high conservation priority, by constructing transcriptome libraries from rare and scarce species, to offer novel insights into biodiversity, including sequence diversity and its relationship to biological function. As part of a collaborative project with the Royal Botanic Gardens, Edinburgh, we have generated transcriptome libraries from three rare species, Anastrophyllum joergensenii, a dioecious leafy liverwort restricted to cool montane high-rainfall areas; Athyrium distentifolium, a diploid out-crossing fern of montane areas and Koenigia islandica, a diminutive annual, which in the UK is restricted to Skye and Mull. Over 1000 ESTs have been sequenced from two of the three species and functional SSR markers have been developed (Table 4), to allow a detailed genetic assessment of populations from different environments and across a major continental disjunct (Figure 5). The patterns of variability detected with this type of functional gene analysis will provide the baseline scien-

A	. jorgenser	nii A.	distentifoli	um K. islandica
No. of Clones Sequenced	1152		1152	384
No. of Quality Sequences (>100bp)	1050		1065	133
Total No. of SSRs	41		165	24
Percentage of SSRs	3.8%		15.5%	18%

**Table 4** Details of the three cDNA libraries and the overall percentage of quality sequences containing  $SSRs \ge 11$  base pairs.

tific background to develop informed conservation management and species recovery programmes.

This approach is also being applied to important genetic resource collections held at SCRI e.g. Commonwealth Potato Collection (CPC) and soft fruit (*Rubus* and *Ribes*) collections.

**Metabolomics.** This relatively new field of scientific discovery deals with chemical processes in living organisms which result in a metabolite production. The term "metabolome" usually refers to the entire complement of all low molecular mass, non-peptide metabolite molecules in cells and tissues at a particular physiological and developmental state. To place metabolomics in context, four types of metabolic investigation have been defined (Fiehn, 2001<sup>5</sup>):

*Target compound analysis* (analysis of specific compounds most directly affected by a modification or experiment).

*Metabolic profiling* (analysis of selected compounds from the same chemical group or compounds linked by known metabolic relationships).

*Fingerprinting* (rapid screening for sample classification, e.g. by global analysis of spectroscopic data, not identification and quantification of individual compounds).

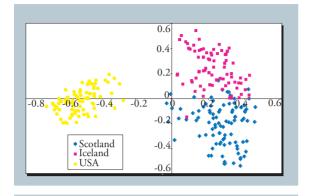
*Metabolomics* (identification and quantification of as many individual compounds as possible across all compound classes).

Several hundred metabolites can now be screened using appropriate analytical tools such as GC-MS, LC-MS, NMR and FTIR. The metabolites cover many metabolic pathways, increasing the chances of

7 <b>H(1</b> 0.0 ∖	/*ABG704
3.3 \	*RISIC10A
3.8 —	— *MWG036B
9.1	/ *BCD129
10.1	~*ABG320
14.1	<pre>/*iEst5</pre>
16.4 ~ 20.2 ~	*His3A *WG789A
20.2 < 22.1 -	~*MWG089
24.7	$\sim$ *dRcs1*ABC167A
26.8	ABG380
29.7	*Mad1 <b>SNP286</b>
33.5 -	*ABC158
38.2 -	×*ksuA1A
40.3	*ABC154A
42.0	└ *MWG836
49.5 —	_*Brz
56.1 —	— SNP123*ABC156D
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75.5 -\\	/_ *ABC308
76.4 🖯	- SNP957
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81.7 -	\_*KFP194
85.0 -/	\_*Amy2
98.2 \	∕ *Ubi1
99.0 —	- *TLM1
103.2 —	— *KFP190*ABC310B
107.9 -	- *RISP103
109.5 —	- *bBE54E
110.7 /	` *ABC305
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1 12.0	IONIOOD

**Figure 4** Map location of EST-SNPs on chromosome 7H (Steptoe x Morex)

detecting significant perturbations in metabolic networks that give rise to phytochemical and functional diversity. Because the approach is broad-based, additional functionality properties may be detected even in cultivated plant species which have undergone intensive selection for specific traits of commercial value.

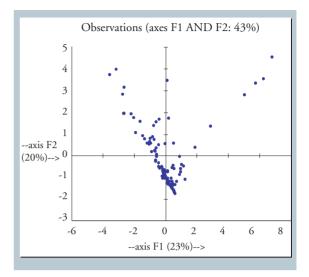


**Figure 5** Principal Coordinate Analysis (PCO) of Diversity detected with EST-SSR in *Athyrium distentifolium* populations from Scotland, Iceland and North America.

Work to date has demonstrated that the application of data-mining tools to metabolic profiling analysis allows insights into the relatedness of certain genetic situations. Correlation analysis allows confirmation of established hypotheses concerning metabolic interactions within these systems. As an example, Principal Component Analysis (PCA) of metabolomic data derived from GC-MS has been used to differentiate, through hierarchical data clustering approaches, *Arabidopsis* ecotypes and mutants (Fiehn *et al.*, 2000<sup>6</sup>). However, complete elimination of environmental effects on metabolite profiles is never possible, thus data clusters for a genotype will have a spread of points determined by the 'environmental' influence.

The SCRI is using similar approaches to assess the capacity of metabolic profiling to differentiate between potato accessions maintained in the Commonwealth Potato Collection. Preliminary work using LC-MS of leaf extracts coupled with PCA analysis is already indicating the utility of this approach (Figure 6); In this particular example (an analysis) of 90 accessions of the CPC, two accessions, TBR MPG 501 and CPH 5844, cluster away from the main body of germplasm analysed. This differentiation can be achieved with a dataset based on as few as ten variables (metabolites). When analysis is repeated with all 160 visible metabolites from LC-MS, near total differentiation is achieved for all 90 accessions. The ability to capture such information by observing the broadest possible class of metabolites and relating phytochemical diversity to DNA sequence and transcriptional /translational profiles remains a key challenge.

**Connecting sequence Diversity to heritable pheno-typic differences** An avalanche of DNA information derived from both model and crop plant genomes is



**Figure 6** Principal Component Analysis (PCA) of data acquired (LC-MS) from potato leaves sampled from the Commonwealth Potato Collection (CPC)

currently being released into the public domain (http://www.ncbi.nlm.nih.gov/dbEST\_summary.htm) As more plant and crop genomes are either being fully or partially sequenced more emphasis is being placed on the discovery and exploitation of intra-specific sequence diversity. Associating and validating the occurrence of sequence polymorphism with phenotypes of strategic relevance to UK agriculture therefore represents a major scientific opportunity of immense relevance to our future.

Access to more molecular markers or ideally fully or partially sequenced genomes provides an opportunity to examine the global patterns of polymorphism. This allows the correlation of allele frequency or nonrandom distribution of alleles at adjacent loci to be determined (linkage disequilibrium (LD) see Table 3). The extent and magnitude of LD is a prime determinant of the feasibility of association mapping. Association studies based on LD can provide a novel route to the identification and isolation of genes controlling quantitative sources of phenotypic variation. Candidate gene and whole genome scans have both been advocated. For the former approach, a limited number of candidate genes whose biochemical functions hint at a role in controlling a given phenotype is used to identify and test gene-phenotype association. In contrast, genome scans attempt to survey the entire genome for regions implicated in the control of a phenotypic trait. The success of either approach will depend on the extent and distribution of LD together with haplotype structure. Thus, regions with high lev-

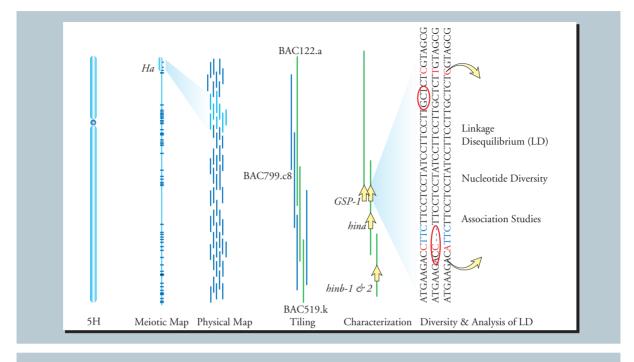


Figure 7 Schematic representation of candidate gene approach for grain texture in barley

els of LD will require few markers but the resulting resolution is likely to be low favouring a whole genome scan to association mapping (Rafalski<sup>2</sup>). In contrast regions, with low LD are likely to require large numbers of markers with the potential for high resolution. A major goal for our research must therefore be to better understand LD distribution and recombination in crop plants.

An example of a candidate gene approach for grain texture in barley is shown in Figure 7. In this case candidate genes hordoindoline-a (hin-a), hordoindoline-b (hin-b) and grain softness protein (GSP-1) have been proposed based on homology to puroindolines genes identified in wheat. A comparative genomic approach has therefore been taken to identify barley BACs clones that harbour the three candidate genes and these have been mapped meiotically to the distal end of chromosome 5H. In the first instance, this approach allows us to anchor BACs to meiotic maps and initiate a forward genetics approach. Surveys of nucleotide diversity across the three genes have also been undertaken to determine haplotype content as a prelude to determining the feasibility of association mapping for quality traits in barley.

In conclusion, we have outlined examples of where biodiversity research is benefiting from opportunities arising from new areas of genome science. However, to fully capture these opportunities the fundamental role of genetics as a tool to analyse biological processes must be reinforced. Genetics together with expertise in whole-organism biology, analytical chemistry, genetic resources and plant breeding are key competencies for the future.

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# Sustainability in Agriculture

### D.K.L. MacKerron, J.R. Hillman & J.M. Duncan

**Definition** For our purposes, it is hard to better the definitions provided by Webster's 3rd New International Dictionary (p2304) viz., *Sustainable*: capable of being sustained. And *Sustain*: (from *tenere* to hold) - (2) to supply with sustenance - nourish; (3) to keep up especially without interruption, diminution or flagging; (6) to endure.

### General context:

The trouble with 'sustainability' is that it has become a fashionable term. Worse! It is politically correct! As a result the term is used to qualify and justify courses of action or policies that are thought subjectively to be desirable for other reasons. It has become a term that is forced to mean any one of many things and, when used by some people, can change meaning within the course of a single speech or article.

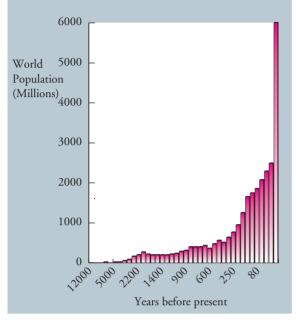
The quality of being sustainable is so attractive to us in our world where we have a nagging realisation that our current practices cannot be continued indefinitely, that the concept has generated a huge academic and pseudo-academic literature, full of large words and abstruse arguments but with little effect on the real world. Sustainability is a term that is used in the hope of conferring general acceptability on policies or actions that are seen by their proponents as being desirable for other reasons, many of which have little or nothing to do with sustainability. These include sustainable growth, development, and consumption. Examples include the UN, the World Bank, the UK Department of Trade and Industry<sup>1</sup>, and even the Royal Society<sup>2</sup>. There are even periodicals on the issue of 'sustainability' e.g. Sustainable Development UK<sup>3</sup>. In the course of a conference on 'Sustainable Consumption'<sup>2</sup>, organised by a group of European Academies of Science in March 2000, questions were raised on the unsustainability of certain agricultural systems. The issue spread readily into wider concerns for biodiversity, quality of surface and ground waters, and the consequences for woodlands whether timber is seen as a source of fuel, fibre, or construction material.

These opinions of ours may seem to be cynical but the purposes of this short review are simple. They are to step back and consider what is sustainability. In other words, to get off the bandwagon and walk round it, kick the tyres, really look at it and then suggest a coherent and simple (if possible) definition / description of what is sustainability. Is it necessary, is it even desirable? Can it be recognised in advance by asking what are the qualities and characteristics of agriculture that will allow it to be 'sustainable', and can one suggest those characteristics that make for unsustainability. Are there really such things as 'indicators of sustainability'; can one establish some simple guiding principles? And it is our purpose to do these things in a simple and direct language.

In a sense, and in the long run, nothing is sustainable. Not even life. To deny that is to deny the laws of thermodynamics. So! When we say or write 'sustainable' we should have at least an outline idea of the boundary conditions for our statements<sup>5</sup>.

The world's population is estimated at 1-10 million people 10,000 years ago, between 170 and 400 million in the time of Christ, 250-350 million 1,000 years ago, ~1 billion in 1800 and 1.5-2 billion in 1900. Today, it is in excess of 6 billion and climbing – rapidly! The 20th century was not one of unparalleled fecundity, merely an era when many more children survived to adulthood and to reproduce. That trend will continue in the 21st Century with populations exceeding 10 billion by 2050. What can be held to be sustainable against such a background? Possibly the never failing ability of human beings to reproduce but certainly little else in the biosphere; exceptions will be animals and plants that live in harmony with human activity such as rats, mice, cockroaches, and *E. coli*.

There is evidence that mankind in low numbers, 10,000 years ago and before, had considerable and usually undesirable impacts (from a modern viewpoint) on his environment<sup>4</sup>. For instance, the extinction of many large animals around the world coincides exactly with the first appearance of man. Thus, the disappearance of hippos, elephants and deer, often in pygmy form, and other even more unusual animals from the islands of Crete, Cyprus, Corsica and Sardinia in the Mediterranean coincides almost exactly with the first signs of colonisation by hunter-gatherers. There is some debate about whether all such extinction was due directly to predation or indirectly to man-made alterations of the local environment but it easy to see the attraction that barbecued pigmy hippopotami might have had for our forefathers! If primitive man in his small numbers could have such effects, what of us in our billions - mining, logging, hunting, farming and just generally getting by.



Is the ability to last 10 years enough for a system or a practice to be called 'sustainable'? Or must something be able to last 100 years? Or 1000 years?

Some people have the idea that for a system to be sustainable it must be in some kind of balance or equilibrium. That is a superficially plausible idea but is it one that can stand close scrutiny? We doubt it and will examine the concept later. We can find agricultural systems that are more fertile now than they were formerly. How has that come about? These systems have developed because inputs were greater than outputs. That is, they were not in balance during that development.

Even a (living) system in an almost static equilibrium - if we can think of one, a remote non-eroding island perhaps - requires at least the input of energy from the sun.

We will explore the boundaries that need to be set before a discussion on sustainability can be meaningful. One such, that we will use from here on, is that we will consider sustainability of agriculture. Sustainability in fishing, motor manufacture, or candlestick making are outside the scope of this paper. On the other hand, such enterprises may attract a mention where they can be seen clearly to make agriculture unsustainable.

### Agricultural context:

The government of the UK, the EU and indeed the whole of the developed world are exercised to achieve 'sustainability in agriculture'. See, for example, the web site http://europa.eu.int/comm/agriculture/ foodqual/index\_en.htm on the future of agriculture and food in Europe, which asks:

"What does our society expect from the agricultural sector and from the food we eat? How can EU policy enhance economic, environmental and social sustainability in agriculture? What does food quality mean and how does quality relate to price?

"According to a recent Commission poll, for EU citizens the priority of the CAP should be to ensure that agricultural products are healthy and safe, promote the respect of the environment, protect medium or small sized farms and help farmers to adapt their production to consumer expectations.

"The aim is transparency, quality and safety and a farm sector in tune with the environment and animal welfare. We need to develop an even more sustainable farm and food sector for the future."

In this short example alone we see confusion in meanings and a blurring of issues.

In December 2002, the UK government launched its strategy for farming and food in England <sup>6, 7</sup> in which it set out how industry, government and consumers could work together to secure a profitable and internationally competitive future for our industries, whilst contributing to a better environment, improving nutrition and public health and prosperous communities. *Is that what is meant by sustainability or is there more to it?* 

The government plans to develop a scheme to pay farmers to farm in a more sustainable way, a core recommendation of the Curry Commission<sup>8</sup><sup>†</sup>. *That presumes that we know what is sustainable.* 

The strategy combines a complex of measures: Continued expansion of rural and environmental schemes like Countryside Stewardship; a new 'whole farm' approach to management and regulation, helping farmers plan their business as a whole to meet commercial and regulatory needs; an audit- based approach to identify a farm's strengths and weaknesses as a basis for cutting red tape and the number of inspections required (Is not that more red tape?); a new Agricultural Development Scheme intended to improve competitiveness and marketing, and to spread 'best practice'; assistance to small regional food producers; extra support for skills and training to make a profit while 'respecting the environment'; a network of demonstration farms to share best practice and experiences; a new animal health and welfare strategy; a Food and Health Action Plan; and other

<sup>†</sup> Farming & Food – a sustainable future. "The Curry report" - The Policy Commission on the Future of Farming and Food was set up by the Prime Minister in August 2001. The Commission was chaired by Sir Donald Curry. The Curry Commission report was presented to the Government on 29 January 2002. features. Most, or even all, are generally thought desirable but do they comprise sustainability? Are they both necessary and sufficient for sustainability?

### What is Sustainability in Agriculture?

'Attempts to define sustainability miss the point that, like beauty, sustainability is in the eye of the beholder ...' A. Campbell  $^9$ 

We tend to test 'sustainable agriculture' against a notion, perhaps ill defined, of long-term behaviour that neither depletes the resources of the land nor accumulates products to a toxic or pollutant level. Thus, adding an organic manure such as seaweed to land would be sustainable unless you were seriously worried that you would either wipe out the seaweed population and that which it sustains, or that you would deplete the sea of its mineral reserves(!). Additionally, we would consider it sustainable if the principal effect of that practice were to increase the organic matter content of the soil without increasing the levels of minerals that might be leached. We offer these ideas simply as a starter and to indicate our own leanings but we recognise that the issue is wider than that.

The population of the world is greater than ever and growing faster than ever and it appears to be outstripping the Earth's ability to feed it. Paradoxically, many no longer consider that intensive, western-style agriculture with high levels of inputs to support high yields is an appropriate model on which to base the agriculture of developing countries with burgeoning populations. Some even question that it is appropriate for Europe and North America. Yet the highest yields of most staple crops are achieved in intensive systems whether industrialised as in Europe or based on high inputs of labour and tight re-cycling of nutrients as in the traditional cultivation of rice in south east Asia. Sustainable agriculture is the Holy Grail of our times. However, sustainability is a complex and contested concept and it is important to clarify what is being sustained (Pretty, 1995) <sup>5</sup>. The popularly accepted meanings implied by the term 'sustainable agriculture' are that levels of yield should be maintained and also that the ability of the land to continue to grow food should not be impaired. These are sensible interpretations to make but some individuals and governments may tack a range of other ideas onto that basic pair.

Debates on issues such as the use of external inputs such as fertilizers, <sup>10</sup> tend not to resolve the issues but

to illustrate how the judgmental values of the debaters can seriously affect their conclusions and recommendations, e.g. on the significance of soil erosion. However, to be sustainable, agriculture must provide the farmer with a living. Not just in the future, but now, and in between as well, or the farmer will not reach the future as a farmer. The idea of sustainability must distinguish between, yet be required to accommodate, both 'ecological' or 'biological' or 'environmental' sustainability and 'economic' sustainability, or it is not a useful concept.

The pursuit of efficiency in farming can, potentially, sidetrack one's thinking on sustainability. The requirements for ecological / biological / environmental sustainability have little to do with efficiency. These aspects of sustainability hinge, principally, on effectiveness - how well the job is done. Consider the use of water. Water Use Efficiency, the production of dry matter per unit of water evaporated, is generally recognised to be greatest where water is in short supply<sup>11</sup>. - Indeed this is true of any commodity, including money. But, in the right conditions, an irrigated crop will yield far more than an unirrigated one. This is an example where lower efficiency in the use of one resource - water - leads to greater production because the irrigation enabled the crop to increase its use of other resources e.g. sunlight. Efficiency - the output expressed as a fraction of the input - is really only relevant to the economic sustainability of the system. And the economics of agriculture are influenced by many non-agricultural factors, including politics.

As stated earlier, whatever the definition of 'longterm' sustainability, a sustainable agriculture must allow or enable the farmer to survive now, and in each succeeding 'now' between the present and the future target date for sustainability. Farming must be profitable if it is to be sustained.

Yet the products of our agriculture are sold in commodity markets that are increasingly open to global competition. That competition is usually based on price alone and, with the principal buyers being relatively few and large, these buyers are able to maintain a continuous downward pressure on prices.

Under a global free-market, production will move to (or survive in) areas where the climate is benign, soil suitable, and land and labour are cheap. What enables this global economy is the relative cheapness of fuel. – Transport over long distances appears to be hardly a consideration. As long as these conditions obtain, UK agriculture is scarcely sustainable; not because it uses fossil fuels but because others do. But these conditions, themselves, may not last. When they change, when fuel becomes more expensive, the measure of sustainability in UK agriculture will change also – and possibly change for the better.

Since 1973, agricultural productivity in the UK has increased by 36% and the key factor has been labour productivity, which has doubled since 1973 <sup>12</sup> (at Indicator 13). (That is almost entirely accounted for by the fact that staffing has reduced by a third - see box) <sup>12</sup>. Yet DEFRA says that it is committed to making food production 'more sustainable' and that part of restoring economic sustainability will be making farm diversification easier. – In other words, enable farmers to spend less time on food production and do other things instead. – This is an example of the juxtaposition of two ideas, each of which seems laudable but which simply cannot co-exist.

Time 1:	Produce 120 units using 3 men.	
	Productivity = 40 units / man.	
Time 2:	Produce 120 units using 2 men.	
	Productivity = 60 units / man.	
Labour productivity has risen by 50%		

The real cost of food has declined since 1973<sup>12</sup>. One reason stated is greater efficiency in the food chain [sic]. - They really mean the supply chain but that point will be considered later. The principal reason, not mentioned, is that farm-gate prices have been kept low and pushed even lower, e.g. for potatoes, a crop grown without any subsidies, the prices over-winter in seven of the ten years 1979 -88 were within the range of the average prices over-winter in the years 2001/02 and 2002/03. That is, they are as low now as they were twenty years ago. The challenge is seen to be keeping food cheap while keeping production sustainable. This is a perversion of priorities. Household expenditure on food is decreasing (see box) and while people are spending less and less on food they are being manoeuvred into spending more and more on mortgages, consumer durables, not-so-durables, and entertainment.

The proportion of household expenditure that is spent on food (excluding catering) has fallen from 12.4% in 1989-91 to 9.7% in 2001. The proportion spent on alcohol has increased to 5.9%. <sup>13, 14</sup>

**Industrialised Agriculture** European agriculture is industrialised in that it is mechanised and is dependent on fossil fuels for machinery, fertilizers, and agrochemicals. These practices cannot be sustainable in their present form in the sense that they cannot go on forever. Supplies of petroleum are finite. However, it is neither useful nor realistic to suggest that agriculture should abandon the use of these products while they are available. A more practical question to ask would be whether it is sustainable to raise livestock on grain feeds, particularly on grain produced in other regions, even other continents. (This is discussed under 'Farming animals').

However, it is salutary to consider how arable agriculture would perform without the use of fossil fuels for machinery or some other sources of energy that might be devised. Consider how much land a horse can cultivate then consider how much more land it takes to feed and maintain that horse – probably between one and two hectares when allowance is made for grazing, hay, grain and bedding.

From time to time, one reads statements that several countries, notably those of central and Eastern Europe, could increase grain production and expand their exports if they would adopt economic policies to realize their full potential. Such calls imply the belief that the practices of modern Western agriculture are indeed sustainable, and ignore the eventual limitations imposed by the supply of fossil fuels.

### What inputs are allowed to sustain the system?

NUTRIENTS. In the SCRI Annual Report for 1998-1999, the article on 'Organic' Farming <sup>15</sup> touched on sustainability in a few places and had a short section specifically headed 'Sustainability'. Here we précis part of that article:

It is easy to describe goals for a more sustainable agriculture, it is more problematic to define it. It can imply persistence and the capacity to continue for a long time. Applied to the environment, it involves actions that do not damage or degrade natural resources. In any discussion of sustainability, it is important to clarify what is being sustained, for how long, for whose benefit and at whose cost, over what area, and measured by what criteria. Answering these questions is difficult as it means assessing and trading off values and beliefs (Pretty, 1995)<sup>5</sup>.

What do <u>we</u> mean by sustainable? All the food and other products taken from the land represent an abstraction of resources. Unless these are replaced that land will become depleted and infertile. So, agriculture must

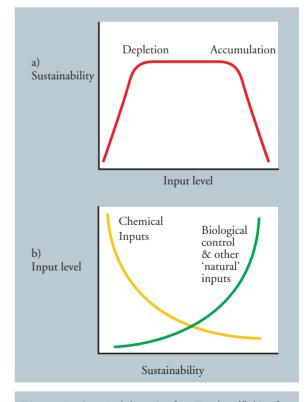
look to what resources are drawn from the land and how these are to be replaced. (It must also look to what is lost inadvertently from the land through e.g. leaching and soil erosion, and it must consider changes brought about by its very operation e.g. soil compaction and changes in the abundance of associated species). The principal commodities taken from the land are water, carbon, energy, and minerals. The first three of these are renewable although the energy balance has wider implications through the current dependence on fossil fuels. (And the carbon balance is modified by agricultural practices). The ability of plants to fix carbon and energy is dependent on the fertility of the soil and its physical properties. The organic matter in the soil influences these and so it is a legitimate concern that an adequate proportion of the assimilated carbon should be left in the soil. The supply of water as rainfall may be inadequate for maximum crop production, and then irrigation may be considered. Unless the irrigation water is drawn from on-farm reservoirs, filled by winter rain, the practice of irrigation is arguably not strictly sustainable; and this must be a consideration where water for irrigation or other purposes is drawn from ground water.

The main concerns over sustainability must lie in the ability to replace the nitrogen and other mineral elements that are taken from the land in a crop. (Also consider any excesses of inputs over abstraction in the forms of crops or livestock). Nitrogen and some other minerals are replaced from the atmosphere but not at rates that even approximate the rate of abstraction in a crop. So, the fixation of atmospheric nitrogen is in the order of 10 - 80 kg N / ha / y over most crops and 80 - 280 kg N / ha / y over a clover-rich sward. But a well-grown crop of potatoes (60 t / ha) takes 160 kg N / ha off the field in the tubers and a 5 t / ha crop of spring barley would remove about 85 kg N / ha in the grain and perhaps 40 kg N / ha in the straw. The minerals removed in a harvested crop are not readily replaced in rainfall (Allen)<sup>16</sup> and the growth of green manures only serves to cycle them.

AGRO-CHEMICALS There is a common presumption that 'sustainable' farming will require a reduction of inputs, particularly those that are 'chemical'. Yet this is not a useful idea until such time as we can foresee synthetic chemicals becoming unavailable. The fundamental points at this part of the debate are that farmers use agrochemicals because they do the job e.g. controlling disease, and it is economic to use them. That is, the financial benefits outweigh the financial costs. Late blight of potatoes, caused by the fungus *Phytophthora infestans*, is one of the most serious diseases of food crops and despite years of attempts to breed resistance into the crop, it is still controlled by the use of fungicides. The yield of potatoes in 'organic' systems where copper-based fungicides are used as protectants, are only 60% of those achieved by conventional agriculture. Leaving aside the point that copper itself is highly toxic and that approval for its use is now being withdrawn, the yield of organic potatoes without its use falls to 40% of conventional.<sup>17</sup>

That is only one example but it serves to pose the question, "Can the use of fungicides be sustained?" We do not know. But what we can say is that the world will be a far hungrier place without them.

Zandstra <sup>18</sup> described sustainability as a non-linear function of input levels of chemicals (Figure 1). He described systems in which excessive inputs degraded the system through accumulation while inadequate levels degraded the system through depletion. Between those extremes, the system is 'sustainable'. This contrasts sharply with the concept held dearly by the proponents of 'organic' systems in which sustainability is increased with reduced dependence on chemical inputs (Stinner & House) <sup>19</sup>.



ENERGY Can the inputs of synthetic materials be sustained? Providing only that regulations permit, it would seem that as long as the modern energy systems survive then chemical products can be synthesised. If the sources of energy collapse then a whole lot more than agriculture will be in difficulty, and agriculture is not a principal user of fuel.

Between 1985 and 2000, the consumption of energy, both direct and indirect (Indirect includes fertilisers, pesticides, manufacture of equipment and animal feeds), in UK agriculture dropped from 240 PJ to 192 PJ (PJ = PetaJoule =  $10^{15}$  Joules) while the consumption per capita remained almost constant at around 345 GJ <sup>14</sup>. While such figures do not assure us of sustainability of agriculture, they do reveal both the dependence of agriculture on energy supplies and the small part it has (1%) in the national appetite for energy. We have sought comparable figures for other industries but have not been able to find them.

Sustainable use of the land Land has been farmed in Europe for several thousands of years. Over the centuries there has been a huge investment of effort and resources in the land to improve drainage, correct acidity using lime or chalk, which are plentiful, minimize flooding, and enable irrigation. That work has ensured, not only that European agriculture has been sustained but the level of productivity has been raised, and some soils can recover from a degree of misuse. <sup>20,</sup> <sup>21</sup> In the earliest times, agriculture was very small scale and in some places shifting agriculture was practised - an indication of unsustainable, short-term exploitation of the nutrient reserves of a site and then moving to another place while the first site recovered. The developments of higher populations, more structured societies, and ideas of property were enabled by the combined practices of fertilizing land and of fallow (which, in essence, is really only a fractional equivalent of shifting agriculture). But where did the fertilizers come from? And was agricultural production sustained? In most cases if not all, arable land was fertilized with nutrients gathered from surrounding lands. The practice of grazing beasts on common land or 'outer toons' and bringing them in at nights worked to an extent, because it concentrated nutrients that had been deposited on a wider area than the cultivated land. But even these devices could not be guaranteed to sustain production. The practice of spreading 'night soil' from towns on the neighbouring farmlands not only solved a waste problem but also resulted in fertile soils in market gardens around most towns and cities in the country. The towns would have imported nutrients, not only from those gardens but also from further afield. Those nutrients were then applied to neighbouring land. In some rural coastal areas, seaweed was used to improve the soil with significant effect in many areas; again drawing nutrients from a wider source. Yet it is a matter of record  $^{22}$  that agricultural production in Europe was nutrient-limited before the introduction of the first imported mineral fertilizers and later, manufactured fertilizers.

As reported in the abstract from 'Organic Farming etc. <sup>15</sup> harvesting a crop removes significant quantities of nutrients. These have to be replaced if the soil is not to degrade. Over the latest 50 years or so, applications of fertilizer in Europe have probably exceeded the 'offtake' in the crops. This is almost certainly true of nitrogen and phosphate, so that applications that are a bit lower can only have a beneficial effect on the environment and no adverse effects on the crops. That does not obviate the need to apply some fertilizer if yields are to be maintained and this focuses attention on what is meant by sustainability. If appropriate levels of fertilizers are not applied, current levels of yield are not sustainable. If fertilizers are used then there is every reason to believe that current levels of agricultural performance can be sustained. The manufacture of fertilizers, particularly nitrate, is dependent on supplies of energy, principally from fossil fuels. We can recognise that there must be a finite end to producing fertilizers as at present since fossil fuels are a finite resource. But should that mean that we should stop using these fertilizers? Certainly not. Which extra persons would be unfed because there is less grain in the world and it is more expensive? - The farmers among others. Should we avoid using fossil fuels to conserve these for future generations? No. These fuels would simply then be used for other purposes and any associated reduction in demand would provide only a marginal delay in the depletion of fossil fuels. And this leads us to the homily from the 'Brundtland' Commission <sup>23</sup>, "meeting the needs of the present without compromising the ability of future generations to meet their own needs". While we should not squander resources and impoverish others whether in the future or the present, we must feed ourselves and others now, and we must do it next year and the one after. When the oil runs out it runs out. The needs of future generations will be their problems. (To be tackled by those same characteristics of acquiring and developing new technologies that has brought humanity and the world to their present condition).

There are plenty of examples where our society is indeed squandering irreplaceable resources for its present ease and gratification (see box) but agriculture is not among them. On the contrary, the most glaring example of ignoring Brundtland's dictum is in the destruction of good, and not-so-good agricultural land for urban and suburban building developments. Developers favour flat sites and sites near the edges of cities. These are frequently the very best agricultural land. If we wish to ensure that agriculture and its ability to feed and clothe us is to be sustained, then it is the planners and politicians who need to be re-educated, not the agriculturalists.

In 1988-1993, Dongguan, in China, converted 10.4% of its total land area into urban land uses. The city tried to maintain the best agricultural land by directing the large proportion of the expansion toward the area of less fertile land, but it is recognised that too much agricultural land was lost.  $^{24}$ 

Results of a remote-sensing-based survey of the tropical forests in Africa using high resolution satellite data of two dates, one close to 1980 and the other close to 1990, showed a compound annual rate of deforestation of 0.8% over the ten years. In Latin America and the Caribbean, and in Asia and the Pacific, the equivalent figures were 0.8% and 1.2%.  $^{25}$ 

In the UK the total area of a rable land has declined 574,000 ha over the twenty years since 1981  $^{26}$ 

Year	Area (1000 ha)
1981	5071
1991	5020
2001	4497

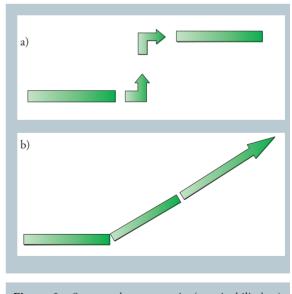
It is the politicians and planners who state that they want rural communities to thrive and be inclusive but then confuse such communities with urban communities in a rural setting. So it is that small rural villages are expanded, at the cost of arable land, into small towns from which the residents travel to neighbouring large towns or cities each day to work. It is this disconnected thinking that declares that we should reduce our use of the motor car for environmental reasons and then encourages families to commit themselves to the use of two or more cars.

We need to recognise that the loss of arable land is not sustainable – no more is being made – and so we should be very sparing indeed with approval for the change of land use from agriculture to domestic or industrial use. Houses are a once-only crop, only once-<u>ever</u>, even if they do pay the developer.

Beyond such an obviously unsustainable use of agricultural land, the real questions that we should address in assessing whether an enterprise fits with sustainable agriculture are: (i) Whether current levels of production are being achieved without 'consuming' the land and reducing its ability to serve the needs of present and future populations <sup>20</sup>. (ii) What are the levels of inputs required to sustain production at current, or other, levels and can those levels of inputs be sustained? (iii) What ecological side effects of modern agriculture will have adverse effects on current or future agriculture? (Notice that we did not write, 'what ecological effects will agriculture have?' We deliberately asked what ecological effects would rebound on agriculture).

Can cereal yields be increased further without damaging the environment? Remember that, within the genetic potential of a crop, the limits to yield are set by the limitations of temperatures on crop development and interception of radiation on dry matter fixation. It follows that simply increasing those inputs that have enabled the current levels of production will not increase production unless current levels are still limiting. If they are no longer the limiting factors, then increasing their inputs will prove uneconomic and so normal commercial constraints will operate.

There are ancillary questions concerning which inputs and outputs can be out of balance and for how long can they be out of balance. In the simplest case, it might be sufficient to attain balance within the period



**Figure 2** Sense and nonsense in 'sustainability', a) sustainability at two levels, b) 'sustained' development.

of a crop rotation and this leads to a consideration of equilibria.

Sustainability: a stable equilibrium or a dynamic equilibrium? The possibility of 'sustainable development' is a fashionable concept but a shallow one. Like acceleration, development cannot be sustained indefinitely. This does not mean that development is a bad thing or that it cannot take place, for a time. It is perfectly possible to conceive of a situation that is 'sustainable' (i.e. it could endure as it is) and that is then developed to another level that is, again, sustainable (Fig. 2 a) - or not as the case may be. It is the concept of sustained development that is a nonsense (Fig. 2b). An example of (a) might be levels of production before the 'improvements' of the 18<sup>th</sup> century such as drainage and liming. And still, development to new sustainable levels is possible, conceptually at least, in the 21st century.

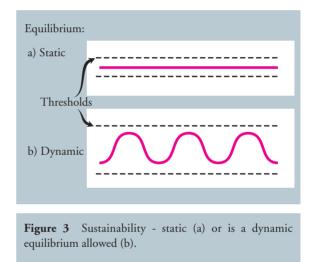
What can be used to indicate sustainability, and can such indicators of have any value to us? There have been a plethora of indicators suggested and tried, <sup>7, 27, 28</sup> just as there have been many interpretations of 'sustainability'. Many of those published reveal a mind set unchanged from the traditional 'growth' pattern, e.g. "producing ... in a <u>more efficient way</u>", "<u>greater</u> efficiency of the food chain [*sic*]...", What is wrong with the present level of efficiency? Others are quite unquantifiable, e.g. "Improved landscape". Most ask for 'more' of good things and 'less' of bad things but few if any set a quantified target.

If 'indicators' of sustainability must be devised then they should be absolute and quantifiable otherwise they can never be attained.

Where a system is understood and certain aspects of the system are recognised to be key ones then it is conceivable that these could be used as 'indicators' of sustainability. Does that mean that, for as long as those parameters had values between certain prescribed limits the system in question could continue to function indefinitely. Surely not! A system might fail from some other cause not measured by the 'indicators'. Similarly, sustainability cannot be guaranteed by ensuring that all the so-called indicators lie within their prescribed limits - and for the same reason. Any set of indicators is simply a kind of model. It is an abstraction of reality and a simplification. The best that can be done is to recognise that if the values of the indicators go beyond the recognised limits, the system will not be sustainable. Whether a system will

or will not be sustained over a period of time then becomes an exercise in probabilities. There have been several reports that explore this idea <sup>29, 30, 31</sup> but, of course, quantifying the probabilities is dependent upon data from the past, not the future.

To identify a few key indicators for a system and to set threshold values for acceptable levels of these indicators is a difficult enough problem and yet the idea implies a fairly low level of understanding of that system. It implies that the system in question is in static equilibrium (Fig. 3a). An alternative, more general, and more realistic model of an agricultural system is one that is in dynamic equilibrium (Fig. 3b).



The concept of a system in dynamic equilibrium makes additional demands on the choice of indicators and on identifying threshold values for them. Here, certain indicators are allowed to reach unsustainable levels just as long as their duration at those levels is limited and provided that they are followed by values that are on the opposite side of those required for sustainability so that the effect on a 'pool' of resources or waste products does not go beyond defined limits. So, not only must the required 'concentration' of an indicator be known, but the pool size must also be known together with acceptable rates of abstraction and return.

Essentially, this is what happens in any system of crop rotation. Each crop in the rotation moves the values of some factor or another, away from the 'average' for the rotation, be it soil organic matter, organic nitrogen, soil microflora or whatever. Succeeding crops shift other factors and allow the first to return to the average value. Equally, the fears over the sustainability of monocultural systems are exactly that they might induce long-term trends that are not reversed or even, in extreme case, may be hardly reversible.

### What is unsustainable?

INDICATORS - OF SUSTAINABILITY OR OF A SYSTEM OR PROCESS THAT IS NOT SUSTAINABLE?

What is to be indicated? (i) That no change is taking place? Is that necessary? And besides, changes may occur in other variables than the ones being monitored. (ii) That there is a wide biological diversity? That may indicate a certain flexibility for the future but at what level is the system not sustainable? (iii) That the imbalance in a variable is within tolerable limits? That will require understanding and suffers from the same drawback as in (i).

Should indicators of sustainability be positive (present = sustainable) or should they be negative (present = unsustainable)? Alternatively, would indicators of unsustainability be a better class of indicators if they can be devised? Would it be better to highlight these?

Is IT BETTER TO THINK OF GUIDING PRINCIPLES RATHER THAN INDICATORS? Yes. Presumably, indicators of sustainability show an impending unsustainability by their disappearance or appearance (depending on whether +ive or -ive). Whereas guiding principles should enable one to avoid unsustainability.

There have been attempts to take a firm, scientific view of the concept of sustainability. Hansen <sup>29</sup> considered the term to have both a philosophical meaning, as an approach to agriculture, and a quantifiable meaning, as a property of a system. He argued that sustainability could only be used as a criterion for assessing farming systems when it was used as a measurable property. Indeed, Hansen proposed that to be effective in guiding future development *"the character-ization of sustainability should be literal, system-oriented, quantitative, predictive, stochastic, and diagnostic"*. That analysis was an excellent one yet did not define sustainability. Rather it revealed weaknesses in current and proposed approaches to achieving sustainability.

The approach taken by Hansen <sup>29</sup> and then by Hansen & Jones <sup>30</sup> was followed by McRoberts et al. <sup>31</sup> who focused on the stochastic aspects of indicators of sustainability. This drew attention to the difficulty of selecting indicators of sustainability. Critical thinking about the approach taken by Hansen and by McRoberts *et al.* suggests another difficulty, however,

with this mathematical approach to sustainability. The difficulty lies with the requirement that sustainability should be considered as a stochastic (i.e. random) property of the system under study. That means that at any time, a system has a finite probability of failure. The simplest such assumption is that the instantaneous probability of failure is constant; value = p. For a cohort of N examples of the system (farms), the value for sustainability in Hansen & Jones' <sup>30</sup> simulation is approximately the expected value of Binomial[N, $(1-p)^t$ ] divided by the initial size of the cohort. Then the probability of a system being sustained declines exponentially over time. This ignores the possibility that a system that survives for one or for ten periods of time (years?) may be better, more sustainable, than one that fails, for reasons not considered in the definition of sustainability - Because of the character of the farmer, for example. McRoberts et al. <sup>31</sup> developed the ideas of Hansen & Jones to include a 'specificity' term that reduced the probability of failure successively at each time interval. That still showed sustainability to be increasingly unlikely over time. How can one provide inputs to a model that will predict a progression that is strongly influenced by factors outside the model?

These mathematical approaches do offer the potential to contribute to the idea of sustainability but that potential has not yet been realised.

One is reminded of the two academics debating how best to invest for the future and one says that he will invest in land. The value of land has increased progressively over the last two thousand years, he says. To which his colleague responds, "Yes. But do you really think that the last two thousand years were typical?"

Pearce  $^{32}$  suggested that the amplitudes of cycles in system variables around their mean values could instead be used as indicators of <u>loss</u> of sustainability, that is of unsustainability. This idea is related to the one proposed earlier in this article that a dynamic equilibrium is acceptable as long as the excursions around the mean are not too large.

What is too large? An excursion from the mean that cannot be recovered is too large. But what the size of that excursion is will depend on the system and on the variable or indicator that is being considered. Then the resilience of a system or, rather, of the variables that define the system may offer indicators of sustainability. A unidirectional change from the mean will suggest unsustainability. Increasing amplitudes in the deviations about the mean would suggest a chaotic system rather than one driven by environment and, again, one likely to be unsustainable.

## WHERE SHOULD ONE LOOK FOR POTENTIAL CHANGES THAT MIGHT WARN OF UNSUSTAINABILITY?

The nature of agriculture depends heavily on the climate and on the soil. The climate defines the type of agricultural enterprises that are conducted; the weather defines the potential for production of crop or pasture; and the soil is the limiting source of nutrients and water that may constrain primary production within that potential. A sustainable agriculture, therefore, must be one that is compatible with the climate and that maintains or, even, improves the quality of the soil. - Or at least, any component enterprises of the agricultural system that reduce that quality must be offset by others that enhance it. That, after all, is the idea that underlies the use of crop rotations. Indicators of possible unsustainability, therefore, include the prolonged reduction of soil organic matter and the accumulation of mineral salts.

We suggest 'possible' unsustainability because some soils that are low in organic matter can be highly productive as long as the necessary inputs of water and minerals can be provided. The accumulation of mineral salts is normally only a problem in areas of low rainfall.

The indicators of sustainability must include economic factors such as simple profitability. Yet the current dependence of many farm enterprises on state subsidies threatens the sustainability of agriculture on political grounds. The issue of subsidies for agricultural commodities and for management of farming systems is too wide to be treated here. However, if profitability could be achieved coupled with the reduction and eventual elimination of subsidies then our agriculture would be well on its way to being both politically and economically sustainable.

### Farming animals

The MAFF publication, *Towards Sustainable Agriculture. - A Pilot Set of Indicators*<sup>27</sup>, included a five-point definition of sustainable agriculture that advocated keeping animals "in a welfare-friendly manner". Attending to animal welfare is good husbandry and is humane but it has little to do with sustainability. ....

Animal-based agriculture is an integral part of our systems for food production, with foods of animal origin

### Food-Chains 35, 36

Higher plants that photosynthesise are the **primary producers** of all human food. Only they can manufacture food from inorganic raw materials. That food feeds herbivores, called **primary consumers**, and then **carnivores** that feed on **herbivores** are called **secondary consumers**. Carnivores that feed on other carnivores are **tertiary** (or higher) **consumers**.

Such a path of food consumption is called a **food chain**. Each level of consumption in a food chain is called a **trophic level**. So, the chains of: grass - grasshopper - toad - snake - hawk, and of: leaves - greenfly - ladybird - blackbird - hawk, each have five trophic levels.

Most food chains are interconnected. Animals typically consume a varied diet and, in turn, serve as food for a variety of other creatures that prey on them. These interconnections create **food webs**.

At each trophic level, net production is only a fraction of gross production because the organisms must expend energy to stay alive and there are substantial losses in net production as energy passes from one trophic level to the next. The ratio of net production at one level to net production at the next higher level is called the **conversion efficiency**. We can take 10% as the average conversion efficiency from producers to primary consumers. From primary consumers to secondary consumers (herbivores to carnivores) conversion efficiencies tend to be much lower, averaging about 1%.

So, when we eat fruit and vegetables we participate in a very short food chain. When we eat meat the chain is longer and we are making less efficient use of energy from the sun. Whether we are efficient or not with the nutrients depends on what we do with the wastes.

representing about  $1/_6$  of human food and  $1/_3$  of human food protein <sup>33</sup>.

The losses that are inevitable in the conversion of plant matter to meat makes meat-eating an inefficient way to use energy from the sun in feeding people (see box). However, this overlooks the fact that many animals, especially ruminants (cattle, sheep, goats) mostly eat food that is not suitable for humans. The traditional distribution of agricultural enterprises e.g. between arable and pastoral had much to do with the environmental resources and constraints of an area. For many reasons, soil-types, slopes, weather, most of the uplands of the UK are unsuited to arable farming but well-suited to raising animals. Similarly, in areas with more extreme environmental challenges – arctic tundra, semi-arid areas, food-production is animal-based.

In areas that are fertile, agriculture may be mixed between arable and animal and the balance between

### Sustainability in Agriculture

the two depends partly on environment (e.g. in the UK the wetter west has more animals) and always on economics.

Populations are high where arable agriculture can be practised (more food per unit area of ground) or, with the economics of modern transport, where the products of agriculture can be delivered.

We should recognise a difference between rearing animals using grazing and fodder from their farm and rearing them on grain or fodder that is bought in. Animal production from grassland has many characteristics of sustainability, relying on perennial plant species and long-term production cycles. <sup>34</sup> This does not immediately say that the other system is unsustainable, although it invites the question, simply that different issues are raised by the two systems.

Much is made of the idea that intensification of agriculture involves loss of micro-habitats and diversity of wildlife and there tends to be a presumption that 'sustainable' farming is less intensive than otherwise. That is a simplistic idea.

The wood / pasture systems of southern Europe involve grazing livestock on permanent pastures and among tree cover. At one time, they were characterised by what is known as transhumance - seasonal migration up and down the hills with livestock - but there is little if any of that these days. The cattle and sheep still graze but there are fewer grazing animals than before - because it doesn't pay. And the consequence is that what were once 'permanent' pastures are invaded by scrub, reducing the grazing (and the diversity of species) and farmers now periodically have to destroy some of the scrub mechanically. In the Transylvanian region of Romania, where traditional systems of farming were maintained until late 20th century, changes following the revolution in 1989 have meant a sharp decline in the level of grazing and a loss of biodiversity. 37

On the other hand, studies by ADAS in Wales, where a trend to a monoculture of sheep has changed grazing pressure and the competitive balance between plant species, leading to a decline in the quality and quantity of heather, have suggested that reduced stocking rates have differing effects in the short- and longterms. In the short-term, reduced stocking rates may lead to increased cover by heathers, increased availability of forage in late season, and an increase in the proportion of productive ewes, an increase in their live weight, and an improvement in their condition. Yet, in the longer-term, reduced stocking rates may lead to an increase in rough, undesirable grasses, decreased quality and quantity of forage, reduced flock performance and reduced weaning weights for lambs. <sup>38</sup>

The point is that there are appropriate stocking rates for each system. Further, there are other options for management besides simply raising or lowering the stocking rate. To argue for higher or lower stocking rates without knowing the circumstances of the place under consideration is to invite not just mistakes, but the collapse of the very things one wants to preserve.

FOOD-CHAINS AND SUPPLY-CHAINS We have explained the proper meaning of the term 'food-chain' (see box, previous page). A supply chain is the set of links from food producer (the farmer) to the consumer and so may include a wholesale merchant, a processor, another wholesale merchant, a retailer, and finally the consumer. Unfortunately, the biologically uneducated among us have pirated the former term and used it for the latter term.

The two kinds of chain have one thing in common. The longer they are the less efficient they are. In supply chains, each middle-man needs to take his 'cut' and so the shorter the chain the more nearly the consumer might expect to pay the cost of production. In a food-chain, each organism has a conversion efficiency of only a few percent.

So it is that while a shorter food-chain may enhance sustainability of food production by being more efficient with the use of primary food production, a shorter supply-chain would also enhance sustainability. In our current, energy dependent, industrial economy, supply chains may be lengthened in several ways, some obvious and some less so. The inherent inefficiency is obvious if one is eating strawberries flown from Chile - a short food-chain, you are eating the primary product - but a supply-chain that is thousands of miles long. The inefficiency is perhaps less obvious if you live in York and are eating ready-prepared Bubble and Squeak from your local supermarket. Again you are eating primary products, and the distance is probably short for both the potatoes and the cabbage, but the number of hands is considerable from farmer to potato processor, to food processor and packer, to retailer and then you, the consumer.

Here there is an obvious conflict between the ideas of sustainability and value-adding employment.

### Is biodiversity important or irrelevant?

Has 'biodiversity' got anything to do with sustainability or is it just a red herring? Nice, but red nonetheless? Does wild life have a role in a sustainable agriculture? Is it necessary and if not, is it tolerable?

Smith et al. <sup>39</sup> reported just 6 animal and 35 plant extinctions across Europe since early in the 15th century. But, even if correct, this gives a false impression of the effect of humanity on biodiversity in Europe. The greatest effect has been on local abundance of species with little effect of the regional species diversity in the strict sense. Whether these changes have had or will have a significant effect on sustainability of our agricultural ecosystems is unknown.

'Biodiversity' and 'wildlife' are terms that the public may interpret to mean higher plants, mammals, birds, and butterflies but which also include other less attractive insects, mosses, and moulds, fungi, and all the microflora and microfauna of the land. Their interactions with each other and with agriculture are complex and not properly understood. Particularly, the potentially overlapping functions in the biology of the soil are only now being appreciated and studied. It follows, therefore, that it must be unwise as well as being uncultured, to dismiss any organism as being insignificant or irrelevant. However, the survival of any organism is dependent, not just on its food supply but upon the survival of its whole environment. The major decline in biodiversity has occurred through loss of habitat. Following our proposition that the key to sustainability is to avoid making changes that are irreversible, we have to allow that biodiversity in its fullest sense should be encouraged, not just tolerated, even where the necessity for parts of that biodiversity are not recognised.

Tolerance is not enough. Extinctions can occur through indifference as well as through deliberate elimination and the biggest threat to the survival of any species is the loss of habitat. It follows, therefore, that in agriculture and in other human enterprises we should ensure that there are adequate areas of unmanaged land and that these should be more or less connected to allow movement of other organisms.

Where species are confined to a shrinking, and disjunct, non-agricultural habitat, it is tempting to think that although their loss is regrettable and from wider ethical points of view, may be unacceptable, they do not affect agriculture. But that would be a presumption based on ignorance rather than understanding. As a hypothetical example, consider a bird that eats insect pests on an agricultural crop during the growing season but that then, in winter, needs to feed on an insect species that lives exclusively on a woodland tree. Loss of that woodland would mean loss of the second insect species, loss of the bird, and so on. Unless we actually know everything we are unwise to dismiss any parts.

As suggested earlier, a sustainable system of agriculture is one whose attributes stay within an acceptable range of states – but a range not at a fixed level. These attributes vary with time and the patterns of variability within the system may change in scale and complexity. The drivers of the system may change and so the factors conferring sustainability are related to the diversity of system components and to the extent and consequences of their interactions. So, a diversity of species and habitats is desirable.

In the same way, a diversity of farm enterprises is desirable, too. If one weakens or fails there is another to tide one over. But each enterprise must potentially be capable of being both profitable (economically sustainable) and environmentally sustainable. If individual farms are able to follow this approach then there is a possibility that the wider agriculture will be sustainable.

### **Concluding Points**

The 'Brundtland' Commission <sup>23</sup> defined sustainable development as, "meeting the needs of the present without compromising the ability of future generations to meet their own needs". This has been a definition of sustainable anything that has been most readily accepted by politicians and policy makers. We judge this quotation to be worthless, pious rubbish because it says nothing but allows those who use it to appear to be politically correct. Instead we propose the much simpler idea that to be sustainable, anything - agriculture, production etc. – should be capable of being continued for a long time and should not make irreversible changes.

There is a fundamental problem to be resolved over the approach to take when considering sustainable agriculture. One option is to think at the macro scale and proceed **from the top down.** - To consider the size of the human population and its growth, then to consider what it needs to feed itself and its increase; how much more food is needed to feed that population better; and whether that could be sustained. The difficulty with that approach is that one quickly gets lost in the unrealistic arithmetic of dividing production in one part of the world by mouths in another part. It is all very well calculating that world production of grain, evenly distributed, could support 2.5 billion people at the American rate of consumption, five billion at a European level, or ten billion at an Indian level <sup>2</sup>. The underlying assumption is preposterous, the world supplies of grain are not going to be spread evenly for reasons of economy, limited resources for transport, etc., and it completely disregards what would be the consequent effect on the world's population in the succeeding years. So, it is better not even to start those sums.

A second option is to consider the problem **from the bottom up.** What can be done to ensure that current agricultural production, here, does not fail? If that is not possible, what must be done to change production practices to ensure that production is sustainable. Alternatively, are there changes that can be foreseen that would enhance production and would still allow production to be sustained at that higher level.

This second option must be the more rewarding one to take. First, the problems that are perceived are real and understandable. Then, they may be capable of solution. Any solution that is to be worked out at a macro scale must be implemented at the micro scale the farm or the field. Given diversity in soils, weather, crops etc. it is evident that **problems must be recognised and resolved at the local scale**. If solutions are implemented then the sum of their effects can build towards the macro scale.

Urban-based cultural perceptions assume that oldfashioned agricultural practices and technology are, somehow, superior and more sustainable than their modern equivalents. In most cases, that is not so although they may offer guidance.

OVER WHAT INTERVAL OF TIME? Sustainable' should mean forever but it is not in our gift to see that far ahead. Rather we should consider that an operation or a system is sustainable if it does not lead to irreversible changes within the period that we can foresee. So, depletion of soil organic matter is not sustainable over a long period but an operation that causes that in the short-term is acceptable if it is to be followed by an operation that reverses that change.

WHAT OF CONTINUITY? Again, continuity is, or should be, a prerequisite of sustainable agriculture. However, this is not to be confused with an unchanged mix of farm enterprises. Indeed the mix of farm enterprises can be conceived as cycling over a longer period just as crops cycle in a rotation. Such changes would be slower because of the capital investment required in any new enterprise but, because true sustainability involves making only reversible changes, they would be manageable. A farm on which the farmer 'came out of' pigs or potatoes in the 1960s might well return to either if the economic conditions warranted it.

IS A BALANCE REQUIRED BETWEEN INPUTS AND OUT-PUTS? We suggest that a system in which nutrients are balanced over a period are part of the sufficient conditions for sustainability – but only part, and that balance is not a necessary condition. A negative nutrient balance is clearly unsustainable over a prolonged interval but a positive nutrient balance might well be sustainable if the nutrients are locked into a non-labile form such as nitrogen in soil organic matter.

The important measures for assessing sustainability will be those that show how far one's system has moved from its mean condition, for how long one can continue, and how long it will take to recover. That is, measures to define permissible cycling in a dynamic equilibrium.

ECONOMIC POINTS Diversification from farming into other enterprises may be currently an economic necessity but it risks the farmer losing focus.

For agriculture to be sustainable the price that the farmer gets for his products must be related to the cost of production plus a margin. To insist that production be achieved within an arbitrary price limit is to condemn farming to failure.

The best philosophy for a sustainable agriculture is to use 'appropriate technology' and to use just enough of it. To eschew the use of modern products or techniques simply because they are not traditional is very short-sighted and makes the farmer dependent upon the goodwill and prosperity of special customers with a penchant for a particular philosophy.

The scarcest resource is our agricultural land. That should only be surrendered under duress.

FINALLY The idea of indicators of sustainability is not practicable. Rather there should be indicators of unsustainability and a set of guiding principles.

We have tried to set out some of these guiding principles in this article. – Maintenance of diversity both agricultural and biological and, principally, no irreversibility.

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# Potato Breeding at SCRI during the last quarter of the 20th century

### George R. Mackay

**Introduction** In 1977, an historical review of potato Lbreeding at Pentlandfield was published (Holden, SPBS Annual Report 1976-77, 66-97). Twenty-five years on, it seemed opportune to review progress since then. Much has changed, both in terms of the organisation and funding of plant breeding in the UK, as well as the technologies and scientific know-how available to modern plant breeders. There is no mention in the 1977 review, for example, of progeny tests, targeted and accelerated breeding, DNA, markerassisted selection nor genomics. Moreover, at that time and during the early and mid-1980s, the institute was commissioned by government to produce finished cultivars. In the 1977 review, discussion on breeding procedures focused almost entirely on aspects of selection and the methods employed to identify superior individual clones, by their phenotypes, amongst large segregating populations generated by sexual hybridisation of parental clones or cultivars, also selected on the basis of their phenotypes.

Although it was recognised that the choice of parents was of equal, perhaps greater significance than selection methods, cultivars or clones with complementary phenotypes were crossed "in the hope" that their progeny might contain superior individuals (recombinants) with the best features of both parents. The need for greater scientific input into parental section was recognised and it is interesting to see if and how this has been achieved, and might be further improved by the application of modern technologies.

It is not possible in a short review of this nature to cover in depth all aspects of research into the genetics and breeding of potatoes at SCRI since 1977, so it focuses primarily on those aspects of organisation and research that have had a direct bearing on the production of cultivars.

**Organisation and funding sources** It would not be possible in a review of this nature not to make some reference to the organisation and funding of the breeding programme, which have obviously had an influence on the objectives and priorities of the breeding team. Up until 1987, all SCRI-bred potato culti-

vars were promoted and marketed by the National Seed Development Organisation (NSDO), which was the sole agency for all state-bred cultivars. The interaction and relationship between SCRI breeders and NSDO was complex and not entirely satisfactory. The breeders' priorities and objectives were, by and large, determined in-house and in accord with the policies of the (then) Department of Agriculture and Fisheries for Scotland (DAFS) [later the Scottish Office Agriculture and Fisheries Department (SOAFD), soon to become the Scottish Office Agriculture, Environment and Fisheries Department (SOAEFD) and now, since devolution, the Scottish Executive Environment and Rural Affairs Department (SEERAD)]. NSDO only became directly involved at the point where SPBS/SCRI decided to submit a clone to National List Trials (NLT), with the exception of their interest in overseas trialling of pre-NLT clones in the mid-1980s. However, NSDO were also responsible for the promotion and marketing of cultivars bred by the Plant Breeding Institute (PBI) in England and the Department of Agriculture for Northern Ireland (DANI). This meant that NSDO had no direct influence on the choice of products of the SPBS/SCRI programme and were often faced with promoting and marketing similar cultivars aimed at the same markets from all three sister institutes. This was sometimes quite frustrating for the breeders. Although there are over one hundred potato cultivars on the UK National List, the acreage is dominated by fewer than ten major cultivars which account for more than 70% of this acreage.

Promoting and marketing a new cultivar in such circumstances is expensive and involves substantial financial risk in building up seed stocks to satisfy a market demand that may not materialise. Thus, NSDO were sometimes obliged to focus their promotional activities on the product of one institution versus that of another, and the lifetime of latter cultivars was often quite short. In an attempt to rationalise this SPBS, PBI and DANI did attempt to co-ordinate the testing and trialling of their advanced pre-NL clones using common origin seed produced at SPBS's high grade seed site at Blythbank (SPBS Annual Report 1979-80, 67). However, each institute retained the right to submit their own choice of clones to NLT, so this was not a complete solution. In 1987, everything changed: NSDO (and PBI) were sold to Unilever to become Plant Breeding International Cambridge (PBIC) Ltd. SCRI was then obliged to seek alternative outlets for the products of their potato, brassicas, beans, barley and soft fruit breeding programmes as public sector funding of the production of finished cultivars was withdrawn. An agreement, excluding soft fruit, was struck between a consortium of Dalgety Agriculture Ltd and Nickersons Seeds Ltd (SCRI Annual Report 1988, 74). Under the terms of this agreement, SCRI continued to breed and select potatoes in accordance with their strategic objectives, the consortium became responsible for testing and trialling of clones reaching the stage at which SCRI would historically have initiated their routine regional trials in England and overseas.

The arrangement with the consortium had little influence on the priorities and objectives of the SCRI breeders, which remained strategically driven. The consortium's objectives were much more market led. Yield and skin finish became priorities and, whilst the companies professed to recognise the need for more disease and pest-resistant cultivars, they held to the view that these objectives were in the public good i.e. to facilitate more sustainable agricultural practice and to reduce reliance on agrochemicals, so that they were more properly the responsibility of the public sector. This was most noticeable in the priorities afforded to the disease testing of potential cultivars. Late blight and cyst nematode (PCN) resistance were assigned some importance, but resistance to the common viruses less so. It has been suggested that the latter facilitates may encourage the use of home-saved seed and, therefore, is of disadvantage to specialist seed producers.

Under the terms of this agreement, SCRI was able to offer third parties any clones not selected by the consortium. Several of these "discards" were taken up by other companies and are becoming commercially available, the most recent being cultivar Thyme (2000). It was also possible for SCRI to negotiate contracts for fully funded breeding programmes with other companies. Whilst there is a degree of commercial confidentiality associated with such contracts, the first product of such an arrangement, cv. Anya, appeared on a major supermarket's shelves in 1996 under an exclusivity arrangement with the company to whom the clone was sold by SCRI's commercial arm, Mylnefield Research Services Ltd (MRS). The clone that became Anya derived from early attempts by SCRI breeders to produce novel types of potato for niche markets using cultivars such as Pink Fir Apple as parents. Although not a strategic objective of the core-funded research programme, this capacity to exploit SCRI's extensive germplasm collections has also now resulted in the listing of two clones from SCRI's long-day-adapted phureja population, selected in association with and funded by private companies who wish to exploit their unique organoleptic qualities in niche markets (Ann. Report 1995, 34-37). Soon, cvs Mayan Gold and Inca Sun, the former as a flavour enhancer for other potato products, the latter for garden and allotment use, will appear in the seed catalogues.

In addition, SCRI breeders were able to capitalise on their research into, and breeding of, clones with superior storage and processing traits to develop and apply a targeted, accelerated breeding programme for crisp and French fry potatoes, initially jointly funded by Golden Wonder plc and McCain (UK) (Ann. Report 1996-97, 40-45).

However, during the period 1990-1999, both original members of the consortium changed ownership. This, allied to the increasingly more fundamental nature of SCRI core research, led to the early, mutually agreed termination of the agreement in 1999. Greenvale A.P., successors to Dalgety Agriculture, continue to have first option on the products of SCRI's core-funded research into breeding until the end of March 2003, and are currently testing and trialling the final products of the state-funded programme. It is gratifying to record that one of the most recent of these, cv. Lady Balfour (2001), is an extremely high-yielding maincrop with good resistance to late blight and PCN (Globodera rostochiensis and G. pallida). Lady Balfour has performed extremely well in independent trials under organic farming conditions. The cultivar is, therefore, currently targeted specifically for organic production, and demonstrable justification for the original, strategic objectives of the historic SCRI "commercial" potato breeding programme.

In accord with current government policy, there is now no core funding of cultivar breeding programmes and SCRI is obliged to produce cultivars solely on a contractual basis with private companies.

**Breeding objectives** For many years, certainly in 1977 and during the 1980s, the main, strategic objectives of the SCRI potato breeding programme were

	onformity Tuber shape, regularity and uniformity
	bsence of growth defects
	Gemmation, hollow heart, growth cracks
2	Juality
	<b>Table and processing</b> - Enzymic browning, after cooking blackening, sloughing, texture, dry matter content, sugars (crisp colour), storage characteristics (dormancy), etc.
R	esistance to mechanical damage
	<b>External</b> - Shatter cracks, scuffing, etc. <b>Internal</b> - Bruising (blackspot).
Ð	ye appeal
	Consumer prefences - skin colour, flesh colour.
V	liscellaneous disorders
	Internal rust spot, wind damage, sensitivity to herbicides, etc.
)	isease and pest resistance
	Late Blight - tuber and foliage, Common viruses (PVX, PVY, PLRV), Cyst nematodes ( <i>rostochiensis</i> and <i>pallida</i> ), Common Scab, Gangrene, Wart, Skinspot, Powdery Scab, Spraing (Tobacco Rattle Virus), Soft Rot, Dry Rot.

Yield

resistance to the common viruses (PVX, PVY and Leafroll), late blight and PCN, whilst also attempting to incorporate resistance to one or other or all of these major biotic constraints, in a background of resistance to other significant potato diseases such as gangrene, common and powdery scab, skin spot, dry rot, spraing and wart (SPBS Ann. Report 1977-78, 42-54). It was a fact then and remains so now that, no matter how resistant a clone may be to late blight, PCN and the common viruses, if extremely susceptible to one of the many other pathogens or abiotic stresses that can affect potatoes, or if of low yield and indifferent quality, it is unlikely to become a successful cultivar. The sheer number of traits that a breeder of cultivars has to take cognisance of has undoubtedly been an overriding factor in dictating the pace of advance in potato breeding methods (Fig. 1).

Moreover, potatoes are put to numerous end uses, each requiring different characteristics of the tuber,

not least in some cases 'cosmetic' features such as flesh and skin colour can determine success or failure. In attempting to select potatoes for all possible end uses, the SCRI 'commercial' breeding programme was of necessity genetically broadly based and designed to screen large numbers of phenotypically different clones for many traits simultaneously, clones deemed unsuitable for one end use would often be retained for further assessment for another and the core breeding scheme was of necessity complex and lengthy (Fig. 2).

Year	Site(s) No. of clones Plot sizes
1	Glasshouses 200-300 progenies
2	Blythbank 40,000 Single plants in Murrays " replicates per progeny
3	Blythbank 4,000 1 x 4
4	Blythbank Murrays } 1,000 1 x 6 2 x 5
5	Blythbank Murrays } 500 1 x 20 2 x 10
6	Blythbank } 200 1 x 100 Murrays } 200 (2 x 10) x 2 lifts
7	Blythbank Murrays UK Regions 60 1 x 300 variable
8	Blythbank Murrays UK Regions Overseas $60^*$ $1 \times 700 (A/S)$ Variable
9 to 12 (variable)	Submission to UK National List Trials (2 years); repeated trialling/testing as in year 8 of the selected clones (1-5); stocks increased to 0.04ha; VTSC initiated by DAFS. Named variety UK National List (Common Catalogue) VTSC 0.4Ha A/S =(AA1) NIAB Rec List Trials COSAC 0.4Ka
Years 4-8 r	ADAS PMB Vew varieties, extension service trials etc. = seed site (Peeblesshire); Murrays = ware (E.Lothian) nost routine disease tests, cooking and quality assessment, etc. nes as year 7 UK.

Figure 2 The historic SCRI potato breeding scheme.

During the last twenty-five years, the UK potato industry has undergone major restructuring and reorganisation, and is now largely dominated by the major supermarkets and their suppliers, and rather few major processors, manufacturers of French fries, crisps and other processed products. The increasing proportion of the crop being processed led to close collaboration between SCRI breeders and the Potato Processors Association (PPA) to whom samples of advanced clones were provided for independent evaluation. By 1982, in recognition of the critical importance of low temperature sweetening, a few advanced clones were stored at low temperature (5°C) as well as at the normal higher temperature (10°C) prior to carrying out fry tests. This soon led to the additional screening of earlier generations, for clones capable of being stored at low temperature without exhibiting low temperature sweetening (Ann. Rep. 1983, 63). Over the next few years, this routine screening consistently identified a small proportion of the segregating population which produced pale fry colours following storage at 4°C. Crosses between clones exhibiting this trait confirmed its heritability and led to the purposive selection of cultivars Brodick and Eden, and eventually the targeted, accelerated breeding of Golden Millennium, Harborough Harvest and Montrose (Ann. Rep. 1996-97, 40-44). Unfortunately, despite very encouraging results from the independent PPA trials and its successful completion of National List Trials in 1980, an earlier potential crisping cultivar, Sheriff, displayed a tendency to growth crack under some conditions and its life as a superior, blight resistant, crisping variety was short lived. Brodick too, despite its excellent qualities, yield potential and general all round disease resistance, also was withdrawn when it exhibited a weakness which resulted in internal problems (spraing and internal rust spot) in commerce; these failures confirming a grim lesson to all potato breeders. No matter how high yielding, disease resistant or superior in quality a clone may be during its 10-15 years of testing and trialling, a single intermittent fault missed by breeders, by the statutory testing authorities and independent trials will quickly result in the demise of a new variety when it occurs in commercial practice.

As the proportion of the crop utilised for processing has increased, so too has the domination of the table sector by the major supermarkets. The pre-pack trade in washed potatoes now demands a standard of 'skin finish' that is extremely hard to achieve. Diseases or opportunistic fungi such as silver scurf and black dot, which have little effect on productivity, and hence were regarded as minor problems, are now of major economic importance. Similarly, there is an increasing demand for speciality potatoes, punnet or salad types for example, which require cultivars that produce numerous small tubers to fit the very tight grading standards of the supermarkets or, at the other end of the scale, larger tubers that can be sold ready to cook, singly or in small numbers as 'bakers'. To an extent, this was anticipated by SCRI breeders, when they retained some flexibility in their choice of objectives, and crosses were made which eventually led to the cv. Anya (1996) selected in collaboration with a major pre-packer. However, these objectives were much lower priority than SCRI's core funded strategic aims, though they are now the target of several market-led, commercially funded contracts.

Late blight and PCN resistance have both remained top priorities for core-funded research into the genetics and breeding of potatoes at SCRI throughout the period of this review, and are recognised as desirable, if not essential, by SCRI's private partners. However, resistance to the common viruses has become a very much reduced priority. Historically, virus resistance was a major objective of the SPBS/SCRI breeding programme (Davidson, SPBS Ann. Rep. 1979-80, 100-108). Indeed, its most successful cultivar, Pentland Crown, was a product of this component of the programme. The resurgence of interest in Pentland Crown following the mild winters and warm summers of the early 1970s, when virus health of the Scottish seed crop was severely affected by early spread of aphid-borne viruses, is indicative of the value of such resistances (to PVY and PLRV) should similar circumstances return (Fig. 3). Industry's current view

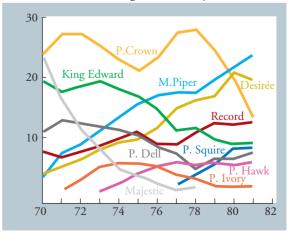


Figure 3 Maincrop potato cultivars percentage area planted (GB).

seems to be that the seed certification scheme and expertise of specialist seed growers are adequate to control the virus health of the crop. One hopes that they are right but questions whether this philosophy is sustainable.

During the mid to late 1980s, SCRI's brief venture into the breeding of cultivars suitable for export also led to a broadening of breeders' objectives to include warm climate diseases such as verticillium and early blight (alternaria) as well as tolerance to abiotic stresses such as heat stress and use of brackish irrigation water.



Figure 4 Great Britain potato statistics.

Breeding for overseas It has long been recognised that seed potato production has an extremely important place in the Scottish potato industry. As ware yields have increased and acreages declined, so too has demand for seed within Great Britain (Fig. 4). However, the Scottish seed industry also exports seed, particularly to the Mediterranean region and North Africa. This provides an opportunity for the industry to exploit its climatological and geographic advantage by developing this trade further still. In recognition of the export potential of British-bred potato cultivars, NSDO, in the period up to 1987, appointed various agents for "their" varieties in potential export markets. However, funding was only provided for the trialling and testing of relatively few cultivars once they had been selected as suitable for submission to the UK NLT. With the support of Matutano S.A., an enthusiastic agent for NSDO in Spain and the Balearic Islands, SPBS were able to supply small samples of pre-NLT clones for trial in Spain and Mallorca (Ann. Rep. 1982, p.74). As was anticipated, it soon became obvious that clones with potential as "export" varieties were being discarded on the basis of their performance in the UK. Eventually, an agreement was reached

with NSDO to finance the trialling of all clones reaching an advanced, but pre-NLT stage of selection, at four locations in Spain and Mallorca and, by 1984, SPBS was finally commissioned to "Breed potatoes for export and select suitable cultivars by overseas trialling" (Ann. Rep. 1984, pp.68). Primary trialling sites were located in Spain, Mallorca, Cyprus and Israel. Clones which performed well in these trials were also sent for trial in Egypt, Tunisia and Algeria by arrangement with Solanex Ltd., a company set up by NSDO to exploit these markets. The routine testing and trialling in Israel led to substantial collaborative links with the Volcani research centre and led to some limited research and development into breeding for resistance to hot climate diseases such as verticillium and alternaria, as well as tolerance to abiotic stresses such as the use of saline irrigation water (Ann. Rep. 1993, pp. 20-23)(Fig. 5).

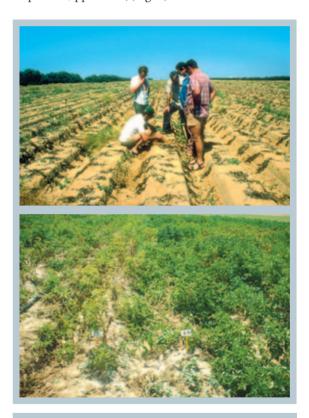


Figure 5 Breeders' trial plots in Negev Desert.

The early results were encouraging, as clones exhibiting resistance or tolerance to these non-indigenous biotic and abiotic constraints were identified. By 1988, specific crosses between such clones were made to produce segregating progenies for research and breeding purposes. However, with the privatisation of NSDO in 1987 and the withdrawal of government support for what was perceived to be "near market" research soon thereafter, this effort, in common with the regional trialling of advanced selections in the UK, was discontinued as a core activity in 1989.

Nevertheless, three cultivars were identified and National Listed during this brief interlude, largely on the basis of their superior performance in the Mediterranean region and thus of export potential: cvs Rhona (1985), Torridon (1989) and Stirling (1991). More recently, cvs Othello (1991), Amour (1998) and Sebastian (2000) have been selected by our commercial partners on the basis of their performances in the Mediterranean region.

**Cultivar breeding procedures** Twenty-five years ago, the then 'Commercial Breeding Programme' was basically similar to other public and private sector potato breeding programmes, worldwide, perhaps differing only from those of private companies in its more strategic emphasis on disease and pest resistances.



Figure 6 Glasshouse seedlings and seedling tubers.



**Figure 7** Visual appraisal and selection of second clonal generation tubers in the field at Blythbank.

Moreover, SCRI breeders were also, of course, obliged to conduct basic research into genetics and breeding methods in order to further the science and technologies associated with breeding *per se*.

Each year, several hundred pair crosses were made between cultivars and parental clones. These crosses were usually made in a series of independent crossing schedules aimed at achieving one or other of the principal objectives being: cultivars with enhanced resistance to late blight or to PCN or to the common viruses PVY, PVY and Leafroll (PLRV). However, in selecting parental clones, due regard was also paid to resistances to other minor, but nonetheless important diseases and, of course, to yield potential and quality (for both table use and processing). Approximately 100,000 seedlings were sown in the glasshouses to produce tubers (clones) upon which to practise selection (Fig. 6). This population would usually comprise first time sowings of samples of speculative crosses made the previous year of family size c. 200, and resowings of crosses from earlier years, selected on the basis of the survival rate of clones from these crosses during the routine testing and trialling procedures. In parallel with this population, approximately 15,000 seedlings bred specifically for resistance to PVX, PVY and PLRV would also be sown. These would be screened for resistance to PVX and PVY by sap inoculation in the glasshouse, then surviving, putatively resistant, clones would be retained and, after screening in the field for resistance to PLRV and PVY, the resistant selections would enter the main, routine clonal trialling system.

Of the 100,000 seedlings sown, approximately 40,000 clones would be selected by visual appraisal of the seedling tubers in their pots, and a single tuber of each

planted in family blocks the following year at the Institute's seed site. At harvest, these 'singles' would be selected, also by visual inspection of their tubers in the field. If selected, originally three but, in later years, four tubers of each selected single would be planted back again at the seed site the next year. These plots were also visually selected at harvest when some attempt to visually compare them with controls (named cultivars) was made (Fig. 7). In subsequent years, selected clones would be grown under ware conditions on the Institute farm, whilst seed stocks were reproduced solely for seed. By this, the fourth year stage, the original population of 100,000 seedlings will have been reduced to fewer than 1,000 clones. Ninety-nine percent of the original genetic variation was thus eliminated on the basis of visual appraisal of tubers produced under the rather atypical growing conditions of a seedling in a pot, a single plant, and then a small unreplicated plot grown under a seed potato regime. Only in the fourth year were selected clones grown under more typical ware conditions and data such as yield, cooking quality and disease resistances collected. Two further clonal years of trialling and selection at the local ware site followed before the remaining clones, c. 40 per annum, were also trialled at regional centres, courtesy of ADAS, in ware growing districts of England. Despite the obvious deficiencies of this system, it was the means by which the very successful Pentland series of cultivars was produced and, in 1977, these occupied approximately 40% of the British potato acreage. In 1977, the latest product of this regime, cv. Croft, received the accolade of a recommendation from NIAB as a high yielding, good quality, blight-resistant maincrop.

By 1981, advances in computing hardware and the inhouse development of suitable software, dramatically facilitated the planning, management, acquisition, analyses and interpretation of data, such that it became possible to introduce randomisation and, where seed stocks permitted, replication of all trials of all clones undergoing selection (Ann. Rep. 1981, 175). This single development was probably the most significant contribution to placing the potato breeding programme at SCRI on more sound scientific grounds. It dramatically increased both the quantity and quality of data and enhanced selection procedures as well as freeing manpower resources from many time-consuming and tedious aspects of label writing and manual record keeping, to focus on research.

In parallel with, and aided by, these developments in logistics, research into the efficiency of early genera-

tion selection continued apace throughout the 1980s. This research and development advanced on two broad but complementary fronts, which not only increased the efficiency with which potential new cultivars can be bred, but also provided the means to estimate breeding value of parental clones and to investigate the genetic architecture of agriculturally important traits. It permitted SCRI breeders to move from phenotypic to genotypic selection.

Early generation selection and parental breeding There are a number of important resistances to potato pathogens governed by major dominant genes. The most well documented example is probably the H1 gene ex. CPC 1673(1), which provides qualitative resistance to pathotype RO1 of PCN (Globodera rostochiensis, the golden cyst nematode), now present in most modern cultivars. There are also genes such as  $\mathrm{Ry}_{\mathrm{sto}}$  and  $\mathrm{Rx}_{\mathrm{adi}}$  which convey extreme resistances to viruses PVY and PVX respectively. However, the complexity of tetraploid tuberosum complicates the use of such valuable sources of resistance. A parental clone simplex for one such gene will only ensure that 50% of its progeny inherit the resistance in crosses with susceptible clones, thus half the populations so produced will lack the desired phenotype. However, a parent duplex for such a gene raises this probability to more than 80% and a triplex or quadruplex parent guarantees resistance in all its progeny. By deliberately intercrossing clones with these genes then test crossing their progeny, it was possible to selectively breed parental clones duplex at their resistance gene loci. The fact that the recently released cv. Spey (1994) is triplex for H1 provides demonstrable evi-

Rrrr x Rrrr ↓ <sup>1</sup> / <sub>4</sub> rrrr <sup>1</sup> / <sub>2</sub> Rrrr	Simplex genotype x simplex genotype Duplex genotype recognized in test cross to rrrr by 5 to 1 (11 to 3 with chromatid segregation) ratio of resistant to susceptible progeny compared
<sup>1</sup> / <sub>4</sub> RRrr	with 1 to 1 (13:15) ratio if simplex genotype
RRrr x RRrr ♥	Duplex genotype x Duplex genotype
<sup>1</sup> / <sub>36</sub> rrrr <sup>8</sup> / <sub>36</sub> Rrrr <sup>18</sup> / <sub>36</sub> RRrr <sup>8</sup> / <sub>36</sub> RRRr <sup>1</sup> / <sub>36</sub> RRRR	Triplex or quadruplex genotype recognized in test cross to rrrr as gives all resistant progeny (or 27 to 1 susceptible with chromatid segregation in triplex)

**Figure 8** Production of parents multiplex for a major dominant resistance gene R with chromosomal segregation.

dence that this approach need not be at the expense of yield or quality (Fig.8). Such useful qualitatively inherited resistances are rare, nevertheless the principle of breeding and selecting parents by the development and application of progeny tests remains the same. During the 1980s, much research effort was devoted to this end (Ann. rep. 1985, p. 63) and the application of such tests became an integral component of the programme (Ann. Rep. 1991, pp. 13-16). By 1985, SCRI potato breeders were sufficiently confident to abandon the philosophy of raising many thousands of individual seedlings upon which to practise selection and to use the power of progeny testing first to identify superior progenies, then to focus attention on selecting the best clones from amongst those best progenies Mackay et al., (Ann. Rep. 19976/97 pp. 40-45). In 1989, transfer to the Mylnefield site provided a unique opportunity which confirmed the robustness of this concept by re-evaluating progeny testing for agronomic potential in an entirely new environment under different growing conditions (J.E.Bradshaw et al. 1998. Early-generation selection between and within pair crosses in a potato (Solanum tuberosum subsp. tuberosum) breeding programme. Theoretical and Applied Genetics 97: 1331-1339.). Finding the best clones from amongst the best progenies does, however, remain a logistical problem in multistage selection for many traits

Initially, progeny tests were applied independently with appropriate sub-populations of the main population being progeny tested for resistance to late blight or PCN or the common viruses etc. In 1990, it was decided to pursue the possibility of combining them in what has become described as the multi-trait selection scheme (Ann. Rep. 1998/99, pp.92-96). An additional major benefit of the progeny-based selection approach is that by using mating designs such as diallel crosses, the data from the progeny tests also provides the means to estimate the breeding value of parents and to partition the genetic variation into its various components, thus, providing insights into the genetic architecture of important traits, and to formulate more scientifically sound breeding strategies.

Moreover, these improvements in the techniques for screening large populations repeatably and efficiently for a range of agronomic, quality and resistance traits are also providing the means to phenotype segregating populations so essential to identifying allelic variation at the underlying Quantitative Trait Loci (QTL) through the associations of traits with mapped molecular markers. Such work is a pre-requisite to markerassisted selection, which would have its greatest impact in selecting between clones of the best progenies, perhaps as early as at the seedling stage in the glasshouse (Ann. Rep. 1997-98, 86-88).

These techniques have also proved invaluable in screening the CPC and identifying putatively novel sources of resistance to PCN and late blight.

Strategic breeding and the Commonwealth Potato **Collection** Strategic breeding during the period of the 1977 review and during the 1980s primarily focused on broadening the genetic base of tuberosum sensu lato by developing long-day-adapted populations of andigena (neo-tuberosum) and of the diploid groups phureja and stenotomum. The Commonwealth Potato Collection (CPC) of wild species and primitive forms was, in 1977, just emerging from the hiatus caused by its screening for Potato Spindle Tuber viroid (PSTV). Discussions were taking place at that time between SPBS, the German-Dutch Potato Collection (at Braunsweig) and the relatively newly-founded International Potato Centre (CIP) in Peru, as to the future of such collections. In the early 1980s, it was mooted that the CPC might be merged with the Dutch-German collection as a precursor to a European gene bank, but difficulties posed by international exchange of potato germplasm due to strict quarantine regulations, eventually negated this possibility. Sources of resistances to late blight, PCN and the common viruses tracing to the CPC were being exploited in the commercial breeding programme, but the CPC itself was by and large held on a care and maintenance basis throughout the 1980s and early 1990s, its immediate exploitation for breeding purposes in abeyance.

However, by 1983, use of neo-tuberosum parents in the commercial crossing programmes re-commenced, after a hiatus also caused by the need to screen all SPBS parental stocks for PSTV (SPBS Ann. Rep. 1983, 69). The diploid populations of phureja/stenotomum also began to be used speculatively as parents in tetraploid breeding programmes (SPBS Ann. Rep. 1985, 68).

The history of the neo-tuberosum programme is a lengthy one, having been initiated at the former John Innes Institute in 1960. By 1981, following a Visiting Group recommendation, a substantial effort was initiated to evaluate this population as parental material for use in breeding cultivars (SPBS Ann. Rep. 1981, 181). In one respect, the admission on to the National List of cv. Shelagh (1986), an F1 hybrid between a neo-tuberosum clone and an unnamed tuberosum parent, provided tangible evidence that it was possible by phenotypic recurrent selection to upgrade the short-day-adapted primitive form andigena to a point at which it could be used directly in breeding cultivars for the UK, without the usual need to carry out several backcrosses to tuberosum to restore the maturity and yield required in a modern cultivar. However, routine screening of the SCRI neo-tuberosum population did not identify any unique or enhanced resistances to the major pests and pathogens that were, by the late 1980s, not already available in the tuberosum parental stocks available to the breeders. This was probably a reflection of the earlier endeavours by SPBS breeders in incorporating such resistances by conventional introgression and selection directly from wild species and primitive forms (Ann. Rep. 1994, 36-39). In 1987, further work on direct improvement of SCRI neotuberosum was terminated and, in 1996, the elite clones of this population were eventually converted to true botanic seed for long-term storage as a potential genetic resource if required in the future. Similarly, the population improvement of the long-day adapted phureja/stenotomum material was halted as resources began to be redirected at more fundamental genetic research. Nevertheless, SCRI has, by obtaining collaboration and funding from the private sector, recently been able to National List two long-dayadapted phureja clones as named cultivars: Mayan Gold and Inca Sun (2001), whose unique organoleptic properties may compensate for their moderate yields in a more discerning market place where flavour, taste and novelty will add value to these cultivars (Ann. Rep. 1995, 34-37).

In 1986, the future of the CPC at SCRI was secured when the decision to retain it was made and arrangements set in place to augment it by incorporating the University of Birmingham's true seed collection of wild species, after passage through quarantine at the S.A.S.A. (Ann. Rep. 1986, 65). A brief hiatus caused by the closure of Pentlandfield and transfer of staff to the Dundee site (1989) followed, but by 1992 the CPC had become reactivated as a valuable genetic resource at SCRI and was being rapidly augmented by pathogen-free stocks of additional species from the Birmingham Potato Collection (BPC) (Ann. Rep. 1992, 13-17). Recent research has identified numerous potentially novel sources of late blight and PCN resistance in the augmented collection and work has been initiated to explore and exploit these valuable

traits using modern molecular technologies to identify the genes controlling them, as well as utilising the elite long-day adapted phurejas to more rapidly introgress these traits into agronomically adapted germplasm more efficiently and rapidly than hitherto. Moreover, the successful acquisition of additional funding in 2001 (FF834) is enabling high-throughput screening of the CPC, in collaboration with Strathclyde University, for biologically active, potentially pharmacologically valuable chemicals, thus raising the possibility of breeding potato cultivars for a range of industrial/pharmaceutical uses as well as consumption as a highly nutritional food source.

**State of the art and the way ahead** The last 25 years has seen major changes in government policy and restructuring of the potato industry into fewer and larger companies and groups of companies representing the different sectors of producers, processors and retailers. Potato breeding at SCRI has also evolved to meet the challenges of the modern world and to maintain its international reputation as a lead centre for research into genetics and breeding of this extremely important commodity.

The Commercial Breeding Department of the Potato Division, as was in 1977, eventually merged with the Brassica breeding department at the time of transfer to the Dundee site, to form the Potato and Brassica Genetics Department in 1988 (Ann. Rep. 1989, 26), shortly after to merge with the other commodity breeding groups, cereals and faba beans, to form the Crop Genetics Department in 1989. In 1999, potato breeding and associated research became part of the Applied Genetics Unit of the Genetics Division and, in 2001, joined the Genome Dynamics Programme in the Genes to Products Theme. In some respects, these organisational changes reflect the move from commodity-led research to more fundamental disciplinary-driven research. However, as a consequence of the research into breeding methods of the 1980s and 1990s, SCRI is now well equipped to explore and exploit the genetic architecture of economically important traits of the potato, armed with the most modern technologies that molecular biology and genomics can bring to bear (Ann. Rep. 1997, 76-80; Ann. Rep. 1997, 86-88; Ann. Rep. 1998, 101-104; Ann. Rep. 2000/01, 75-78). Moreover, it has been possible to capitalise on the wealth of expertise and accumulation of unique enhanced germplasm of the hitherto core-funded breeding programme by attracting investment from private companies to continue to breed and select new improved cultivars.

Listed			
1977	Croft	1993	Buchan
1981	Sheriff	1993	Brodie
1981	Baillie	1996	Othello
1981	Provost	1996	Derek
1982	Kirsty	1996	Claret
1984	Ailsa	1996	Spey
1984	Moira	1996	Kirrie
1985	Morag	1996	Anya
1985	Rhona	1998	Amour
1986	Shula	1998	Blush
1986	Teena	1999	Golden Millennium
1986	Shelagh	1999	Harborough Harvest
1986	Morna	1999	Montrose
1987	Glenna	2000	Sebastian
1989	Torridon	2000	Thyme
1990	Brodick	2001	Scarborough
1991	Stirling	2001	Tay
1991	Eden	2001	LadyBalfour
1991	Glamis	2001	Mayan Gold
1991	Provan	2001	Inca Sun
1992	Cramond	Comme	rcially available in 2002

Figure 9 SCRI-bred potato cultivars National Listed in the UK since 1977.

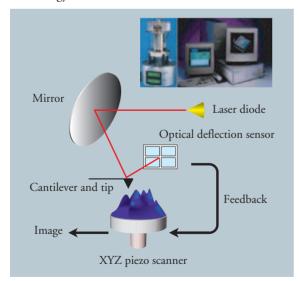
There is no doubt that the objectives of these commercially funded breeding programmes are much more market-led than were the hitherto strategic aims of the core funded programme. Nevertheless, as and when the fundamental researches now directed towards exploitation of the CPC; the use of molecular-marker-aided selection is developed to a point of application; genes are mapped and cloned; the genetics of host-pathogen interactions unravelled; the nutritional value of the potato tuber enhanced; it will become possible to use this technology and know-how to continue to breed high-quality, disease- and pestresistant cultivars required for more sustainable agricultural systems, either in association with and funded by the private sector or to provide industry with the means to achieve these objectives itself. The potato is the fourth most important food crop in the world after rice, wheat and maize. In the absence of disease and abiotic stresses, it is capable of producing a higher yield of highly nutritious food per unit area, more quickly than these grain crops. Moreover, apart from needing to be cooked, potato tubers are immediately ready to consume. Except for a deficiency in the sulphur-bearing amino acids methionine and cysteine, potatoes represent an excellent balance of carbohydrate, minerals, vitamins, protein and fibre – capable, if ever needed, of sustaining human nutritional needs with little need for supplements.

Consumption of potatoes in the developed world is more or less static with an increasing proportion manufactured into processed products. However, the proportion of the world crop grown in the developing cuntries has risen from about 11 percent in the 1960s to 30 percent in the 1990s and continues to rise. Potatoes have a key role in feeding a hungry world but yields are low in the absence of prophylactic chemicals and lack of access to healthy seed. Disease and pest resistances are essential pre-requisites to increasing productivity in less developed countries. Indeed, in developed countries where demands for reduced pesticide use are increasing and the popularity of organic production seems to be gaining ground, intrinsic, genetically-based disease and pest resistances are becoming ever more desirable or perhaps in the case of PCN in the UK, essential. Conventional breeding at SCRI has done much to achieve this objective, but is unlikely to produce cultivars with resistance to all important diseases with the yield, quality (and skin finish) demanded of a modern cultivar. However, in concert with the biotechnologist, the breeder has a much greater opportunity to achieve this aim. SCRI's most important objective in the way ahead is to weld the skills, knowledge and experience of the traditional breeders with those of the molecular biologist. In the 1977 review, it was reported that SPBS had bred and released 24 potato varieties from the UK in the 56 years of its existence. Since 1977, SPBS/SCRI have satisfied the UK National List 'Value for Cultivation and Use' criteria and placed 41 new cultivars on the National List (Fig. 9). A number of these have been withdrawn and no doubt others will be if they do not meet the demanding needs of what is, in terms of varietal choice, a very conservative industry, but only time will tell if the last 25 years of SCRI breeders' efforts can emulate the successes of their predecessors in terms of acreage versus numbers of named cultivars.

# Atomic force microscopy: applications for molecular biology

### M.E. Taliansky & P. Palukaitis

onventional imaging techniques, such as electron microscopy or X-ray crystallography, have provided important information concerning structures of different proteins, nucleic acids, cellular organelles and virus particles. However, the vacuum and radiation environments of electron microscopy are rather restrictive and destructive for analysis of biomolecular complexes that are unstable under extreme non-physiological conditions. In addition, X-ray crystallography requires at least two-dimensional crystals for analysis, and such crystals do not allow sites of interactions with long, disordered RNA or DNA molecules to be identified. A technique that does allow the interactions between biological molecules to be examined, but does not require crystals of such complexes to be produced, is atomic force microscopy (AFM) (Fig. 1A). AFM was developed initially for physical studies of materials and has produced significant achievements in this field. AFM is fast becoming an important tool for biological research, and allows three-dimensional imaging and measuring of individual complexes at a nanometer scale and under ambient and/or physiological conditions<sup>1,2,3</sup>. This technology has been used to examine the structure of



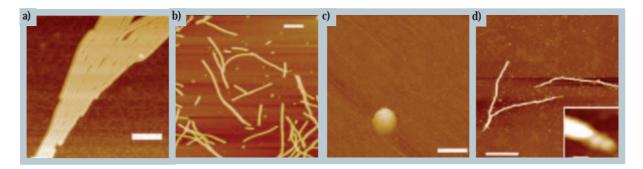
**Figure 1** The atomic force microscope and associated monitoring equipment are used to visualise images produced by the deflections of a cantilever holding a tip and a laser diode.

single hairpin ribozymes in solution<sup>4</sup> as well as virus particles<sup>5,6</sup> and polymerase structures and activities<sup>7</sup>. AFM has also allowed the direct visualisation and analysis of living cells<sup>8</sup>.



The principle of AFM AFM works by measuring tiny contact forces between the surface and a scanning tip (Fig. 1B). The sharp AFM tip mounted at the end of a flexible cantilever can be used to probe a number of properties of the sample, including its topological features and its mechanical characteristics. Precise lateral and vertical displacement of the sample with respect to the probe is achieved by a piezo scanner holding the sample, or by the cantilever holder. Forces acting between the surface and the tip cause deflection of the cantilever that is registered by a laser beam reflected off the back of the cantilever. The cantilever deflections are used to create a topographic image of the sample when the sample is scanned in the x-y (horizontal) direction, or to produce the force curves when the probe is moved in the z (vertical) direction. The AFM can operate in different modes. In the contact (constant force) mode the force between the sample surface and the tip is kept constant. The contact mode can be used to image hard and stable samples that are not affected by frictional forces. The tapping (non-contact) mode uses an oscillating tip. When the tip moves towards the surface, it begins to touch or 'tap' the surface. This leads to an energy loss of the oscillating tip, which reduces the tip

## Atomic force microscopy



**Figure 2** Images generated by atomic force microscopy of different shaped plant virus particles. (a) Aggregated, rigid-rod particles of *Tobacco mosaic virus*; (b) flexuous-rod particles of *Potato virus X*; (c) a single, isometric particle of *Cucumber mosaic virus*; and (d) flexuous-rod particles of *Beet yellows virus*. In (d), note that one end of each virus particle is the same thickness as the rest of the flexuous rod, while the other end is tapered. At higher magnification (see insert), the tapered end is shown to contain morphological features described in the text. The scale bars represent 250 nm (a,b), 40 nm (c), 500 nm (d), or 50 nm.

amplitude. The reduction of the oscillation amplitude is used to identify and measure surface topographic features. The tapping mode operates in air and under liquids.

Applications of AFM Several recent advances in AFM-related technology have enabled the technique to be used under high resolution at the molecular level. Better probes, new imaging techniques, and improved sample preparation methods have all contributed to higher resolution structural imaging. Applications of AFM in molecular and cell biology include the following visualisation and analyses<sup>9</sup>: DNA and RNA, proteins and peptides, proteinnucleic acid complexes, protein-ligand complexes, molecular crystals, and living cells and their organelles. AFM was also employed for studying interactions between different macromolecules, including the interaction between antibody and antigen. Time-lapse, AFM-based techniques for kinetic analysis of protein:nucleic acid and protein:protein interactions in real time also have been developed.

Using AFM, we now have the opportunity to image the surface topography of single cells in aqueous buffer, in real time, as well as at the molecular scale, with spatial and force resolution. In addition, AFM allows us to go beyond topographic imaging by providing information on the physical properties (such as viscoelasticity) of cells and cell organelles, membranes and the cytoskeleton. Specific examples of some of these applications are described below.

**AFM studies at the SCRI** AFM studies on biological macromolecules were initiated in 2001 in collaboration with Prof. A.G. Fitzgerald at the University of Dundee under a research grant obtained from the

Leverhulme Trust. Recently another grant has been awarded to the SCRI from International Association for the Promotion of Co-operation with scientists from New Independent States of the former Soviet Union (INTAS) for the project 'Molecular interactions of a plant virus genome with virus-coded and host cell proteins involved in intercellular virus transport: high-resolution imaging of protein-protein and protein-RNA complexes'. In this project, SCRI will co-ordinate international multidisciplinary collaboration of five teams including the University of Helsinki, Moscow State University, the Advanced Technology Centre (Moscow) and the Centre of Bioengineering (Moscow).

These interdisciplinary studies aim to probe biomolecular 'transport' complexes with AFM. Among the complexes being examined are those formed by viral RNAs and specialised virus-encoded transport proteins involved in processes of cell-to-cell movement through the plasmodesmata (cytoplasmic connections between plant cells), long-distance spread via the phloem, and intracellular nuclear transport. AFM is being used for direct imaging and measuring the biomolecular complexes, as well as mapping proteinbinding sites on target RNA or DNA molecules; and analysing the oligomerisation state of RNA- or DNAbinding proteins (see below). The binding forces between the biomolecules (protein:protein and protein:nucleic acid) can be measured after immobilisation of the interacting molecules on sensor (tip) and surface (substrate for AFM). This enables the stability and elasticity of the biomolecular complexes to be investigated and factors controlling dissociation of the complexes to be identified.

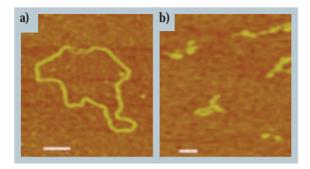


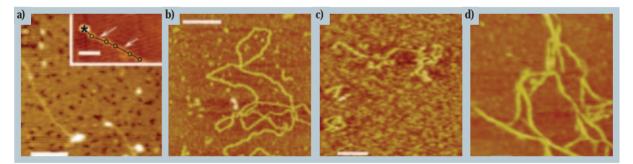
Figure 3 Visualisation of doublestranded DNA (a) and singlestranded RNA (b) by atomic force microscopy. Note that the RNA appears as tangled strands, due to high, internal secondary structure and essential random tertiary structure, while the secondary and tertiary structure of the DNA are more constrained by its doublestranded nature. The scale bars represent 50 nm.

A detailed physical analysis of biomolecular 'transport' complexes and the processes of their formation, trafficking and dissociation is on the cutting edge of plant molecular biology, virology and their interface with physics. Structural and functional characterisation of the 'transport' complexes can provide both novel information on the molecular mechanisms of intracellular and intercellular movement of biological macromolecules, as well as new tools for further studies.

The structure of ordered, biomolecular complexes, such virus particles, can be examined by AFM. For well-characterised virus particles, the structures confirmed those already seen using EM for *Tobacco mosaic virus* (Fig. 2a), *Potato virus X* (Fig. 2b), or

Cucumber mosaic virus (CMV) (Fig. 2c). We have also studied structure of closteroviruses, filamentous plant viruses with more complex structure (in collaboration with Prof. V.V. Dolja of Oregon State University, Corvallis, OR, USA). For these viruses, such as Beet yellows virus, in which additional proteins besides the major capsid protein are present in the particles, morphological differences could be discerned at one end of the virus (Fig. 2d). These gave rise to the 'rattlesnake' structure previously identified by electron microscopy<sup>10,11</sup>, but seen at a higher resolution by AFM, due to the masking of the structure by the staining needed to see the virus particles by electron microscopy. AFM allowed more distinct structures to be seen within the 'rattle' at one end of the particle (insert in Fig. 2d). A detailed analysis of the 'rattlesnake' structure together with the biochemical characterisation of virus proteins present in it are very topical, since it has been postulated that this structure is involved in interaction with components of plasmodesmata allowing the virus to move from cell to cell.

Various macromolecules, which have been examined before elsewhere, such as doublestranded (ds) DNA and singlestranded (ss) RNA, were analysed here as a prelude to examining biomolecular complexes containing these macromolecules. These images are shown in Figure 3a and Figure 3b, respectively. Note that ssRNA can be distinguished from dsDNA by the tendency of the former to form tight coils or balls.



**Figure 4** Visualisation of biomolecular RNA:protein complexes by atomic force microscopy. (a) Complexes formed between singlestranded RNA and the ORF4-encoded movement protein of *Groundnut rosette virus* (GRV) show limited binding (white blobs) between the protein and the RNA, with the RNA molecules exposed between the regions covered with protein, shown by arrows and circles, respectively, in the insert in (a). (b) Complexes formed between singlestranded RNA and the 3a movement protein of *Cucumber mosaic virus* (CMV) show the RNA densely packed with movement protein molecules, which are known to bind cooperatively to the RNA, unlike the GRV ORF4-encoded movement protein. (c) Complexes formed between singlestranded RNA and a modified CMV 3a movement protein in which the C-terminal 33 amino acids have been deleted show RNA much less densely packed with protein, with areas of non-coated RNAs being visible as very as small aggregates of protein. (d) Complexes formed between singlestranded RNA and the ORF3-encoded movement protein of GRV show the RNA densely packed into flexuous, rod-like structures by the ORF3-encoded protein, which is known to bind cooperatively to RNA. The scale bars represent 250 nm (a), 50 nm (insert in a), or 100 nm (b, c, d).

A number of viral transport proteins have been shown to be able to bind ssRNA in vitro. These RNA:protein complexes could also be visualised by AFM. In the case of the 3a movement protein of CMV, the protein was known to bind to the RNA cooperatively<sup>12</sup>. By contrast, the ORF4-encoded movement protein of Groundnut rosette virus (GRV) bound RNA non-co-operatively<sup>13</sup>. Visualisation of these RNA:protein complexes by AFM showed that the GRV ORF4-encoded movement protein bound to RNA incompletely, resulting in various lengths of protein-free segments of RNA (Fig. 4a). By contrast, the CMV 3a movement protein formed highly packed RNA:protein complexes (Fig. 4b). CMV requires its capsid protein in addition to its movement protein for cell-to-cell movement14. However, CMV movement protein truncated in its C-terminal 33 amino acids has the ability to mediate viral movement independently of capsid protein<sup>15</sup>. Biochemical assays showed that although CMV C-terminally truncated, movement protein binds viral RNA cooperatively like the wildtype CMV 3a movement protein, this interaction requires fewer protein molecules per molecule of RNA. AFM visualisation of the complexes formed between ssRNA and the CMV C-terminally truncated movement protein showed structures that were less dense (looser?) than the highly dense structures observed for complexes formed between the wildtype CMV 3a protein and ssRNA (Fig. 4c). The densely packed structures of viral RNA and wildtype CMV 3a movement protein, presumably needed for cell-to-cell movement, may prevent RNA molecules from being for expressed (translated) or replicated, unless some other factor, for example the capsid protein, facilitates the release of the viral RNA. However, in the case of the C-terminally truncated 3a movement protein, the complex is not so densely packed and therefore may allow RNA to be released for expression or replication without the need for additional factors such as capsid protein. In support of this is the observation that the GRV ORF4-encoded movement also does not coat viral RNA completely and that may explain why it does not depend on capsid protein for virus movement (for release of viral RNA). This is also true when the GRV ORF4 replaces the CMV 3a gene in infectious CMV RNAs<sup>16</sup>.

Finally, we have also visualised a complex formed by viral RNA with the GRV ORF3-encoded protein, which previously was identified as a long-distance RNA movement protein<sup>17</sup> that may be involved in transport of viral RNA through the phloem (Fig. 4d).

These latter complexes also exhibit rather dense structures, indicating ordered packaging of the RNA. Further imaging with mutants of the various movement proteins described above will provide a better understanding of the nature of the complexes visualised here, as well as the nature of the forces holding these complexes together and the role of these structures in facilitating virus movement.

While our studies have been limited to those involving viral encoded proteins and their nucleic acids, there is no reason this technique cannot be applied to the analysis of proteins encoded by bacteria, fungi, plants, or animals in various protein or ribonucleoprotein complexes. The applications of AFM to visualising such biomolecular complexes and the effects of alterations in these structures on their functions are just beginning!

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## Meanbh-chuileag - the Highland biting midge

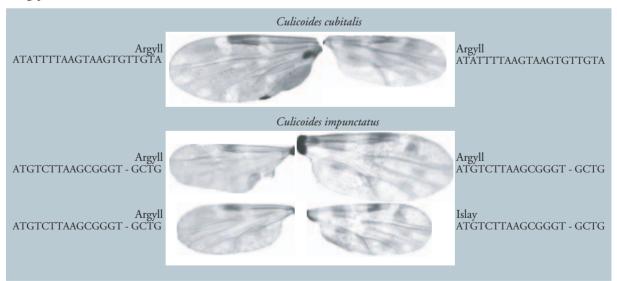
A. Blackwell\*, A. Ritchie, J.R.Hillman & B. Fenton.

**What is a midge?** The Ceratopogonidae are a group of small biting flies ('midges') with a world-wide distribution. Out of 60 genera, Culicoides is the most important in terms of its impact on man and livestock. These insects are a severe biting nuisance in many areas of the world and are also of considerable economic importance. There are 37 species in Scotland but one, Culicoides impunctatus Goethgebuer, outnumbers all others. This species is present throughout the whole of the summer in Scotland, coinciding with much of the country's outdoor industry, including agriculture, forestry, and tourism. The Ceratopogonidae are closely related to the Chironomidae, or non-biting midges, but can be distinguished by the presence of female biting mouthparts, short fore legs and characteristic dark markings on the membranous wings (see Figure 1), which are folded, scissor-like at rest or when feeding (see Figure 2). They are most commonly known as 'biting midges' but can also be called 'sandflies', 'punkies', 'no-see-ums', no-nos', 'moose-flies' or 'biting gnats', depending on geographical location. The Gaelic name for the Highland midge is Meanbh-chuileag (tiny fly) emphasising its diminutive, 1.4 mm wingspan.

The females of most species of biting midge have specialised mouthparts that pierce the hosts skin with finely-toothed mandibles and maxillae. These work in a scissor-like fashion to create a pool of blood, from which the insect feeds. Saliva, containing an anticoagulant to maintain the flow of blood, is pumped into the wound. The host's body responds to the midge's saliva by releasing histamine at the site of the wound,



**Figure 2** *C. impunctatus* feeding on a human. *C. impunctatus* feeds by biting and feeding from a pool of blood and not sucking as a mosquito does. The insect in the Figure is engorged with blood and the abdomen is bloated. The blood-meal provides additional nutrients and blood-fed females can lay more eggs.



**Figure 1** The Figure shows the wing patterns from a number of individuals from two *Culicoides* species: *C. cubitalis* in the top panel and *C. impunctatus* in the bottom. These patterns are one of the most important taxonomic features. Both species show a range of overlapping characters (size, venation, coloration) that makes accurate identification difficult. However, the rDNA sequences of the two species exhibit clear differences (inset panels).

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resulting in the characteristic itching and swelling of the midge bite. Left undisturbed, a female midge will feed for 3-4 min, taking an average of 2  $\mu$ l blood (see Figure 2).

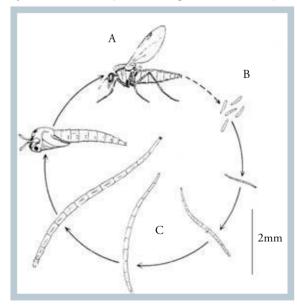
Ceratopogonid midges have a world-wide distribution, with the exception of the Arctic and Antarctic. There are approximately 5000 species in 60 genera. Members of four genera feed on the blood of vertebrates, with the most important genus being *Culicoides*. The remaining genera feed on the tissue fluids of other insects. There are more than 1400 named species of *Culicoides*, 50 of which have been implicated in vectoring various pathogens and parasites to man and other animals. These small flies are quite distinct from the longer and slender mosquitoes that have specialised mouth parts for piercing and sucking from blood vessels.

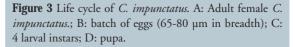
Midges in Scotland There are 37 known Culicoides spp. in Scotland. About 20 of these are mammalophilic with five regularly attacking man, including C. impunctatus (the 'Highland biting midge'), which is responsible for 70-95% of the biting attacks on humans. C. impunctatus probably occurs throughout the UK, breeding in acidic, boggy ground, although it is in Scotland that by far the largest numbers are found. Whether or not different races are present in the UK is unknown. Each species has specific requirements in terms of breeding ground, bloodmeal host etc., resulting in a characteristic distribution. For example, C. obsoletus Meigen (which will also bite man), is perhaps the most abundant Culicoides spp. in the UK, concentrated around domestic gardens, although it is outnumbered by C. impunctatus in western Scotland. Other species concentrate around farmland, breeding in animal manure (e.g. C. nubeculosus (Meigen)), whereas others are found around salt marshes and coastal regions (e.g. C. maritimus Kieffer and C. halophilus Kieffer). In addition to spatial separation, the Culicoides species in Scotland are also separated on a temporal basis. For example, whilst most species are crepuscular, with peaks of activity at dawn and dusk, C. heliophilus Edwards is most active during the daytime. Also, amongst the eight members of the 'pulicaris' group of midges, the largest C. delta Edwards appears in May, C. pulicaris (Linnæus) and C. punctatus (Meigen) occur in late spring-summer, C. lupicaris Downes & Kettle and C. grisescens Edwards appear in late July/August and C. impunctatus can be found for the duration of the summer, appearing first in late May. C. impunctatus remains dominant in upland areas of

Scotland and creates the greatest havoc with regard to agriculture, tourism and other outdoor industry during the summer months.

Midges are not a new phenomenon in Scotland. Midges have been found in 75 million-year old amber and their more recent history in Scotland has been eloquently described by Hendry<sup>1</sup>, including Bonnie Prince Charlie's encounters with the 'mitches' whilst hiding in the hills after Culloden. Scotland's midge fauna was first described at the beginning of the twentieth century but it wasn't until the middle of the century when the Scottish midge problem was investigated more fully, with the University of Edinburgh's 'Midge Control Unit', established in 1952. This era produced some basic knowledge on the biology of midges in Scotland, particularly of C. impunctatus. More recently, after a lapse in research of more than 30 years, modern approaches to the midge problem have produced a wealth of information on their population dynamics and mating activity etc. These studies have highlighted a number of key areas in the midge's life cycle and aspects of its behaviour to which control measures could be addressed, allowing the formulation of a series of potential 'solutions' to the midge problem in Scotland (with possible extrapolation to elsewhere in the world).

*C. impunctatus* Biology *Life cycle and population dynamics* The life cycle of *C. impunctatus* is the key to





its success. Midges lay 30 - 100 eggs in the summer months on the surface of damp soil/vegetation. Egg hatch is rapid (< 24 h) and is followed by 4 larval stages which live as omnivores/detritivores in the water films of the surface layers of the soil. The final, fourth larva acts as the overwintering stage, followed by a short (1-2 d) pupal period in May/June (probably triggered by increasing day-length and temperature) and adult emergence (Fig. 3).

Studies have revealed that *C. impunctatus* lay their first (and largest) egg batch *without* taking a bloodmeal, instead using fat and protein reserves built up during the larval stages). This might be a key factor in the survival and success of *C. impunctatus* in what is often an extremely inhospitable climate, with few readily available bloodmeals. Both protandry (males emerging a few days before females) and bivoltinism (i.e. 2 generations per season) have been recognised for *C. impunctatus*, with a generation period of six weeks.

Genetic identity of C. impunctatus populations Several Culicoides species have been investigated for genetic differentiation. This has resulted in the identification of a number of morphologically similar, sibling species among vector midge populations that varied significantly in their levels of vector competence. These species include the C. imicola complex and C. variipennis. Figure 1 illustrates the difficulty of accurately identifying closely related Culicoides species. Wing patterns are one of the key features, but they can have a wide range of sizes and patterns. Insect pests of plants have many parallels with animal pests. Like mosquitoes aphids pierce plant tissues to reach nutrient supplies. In the process of doing this they can either acquire or pass on viruses. Eriophyid mites are smaller than midges, but they build up in vast numbers on plants causing crop losses. Eriophyid mites have a very limited range of characters with which they can be distinguished. Sensitive techniques have been developed at SCRI for identifying these cryptic species i.e. morphologically indistinguishable species of plant pest insects and mites and these have helped in the study of midges. These use both DNA analysis and ecology, generically referred to as 'molecular ecology'. The main method of analysis is to obtain DNA sequences from informative genes such as ribosomal DNA. Figure 1 includes DNA sequence from a small section of the ribosomal spacer regions of each midge. This type of information is usually easily interpreted when compared with morphology and the DNA sequences clearly grouped individuals from these populations as either C. impunctatus or C. puli*caris.* The results of the complete analysis have yet to be published, but they indicate that there may indeed be races of *C. impunctatus.* 

Economic impact of midges Much of the Highland's outdoor industry cannot be readily carried out in the 'midge season'. These industries include timber felling and tree planting, harvesting, and road construction and repair. Forestry and agriculture are integral parts of Scotland's economy and in addition to the many anecdotal reports of the misery midges can wreak with these activities, there are a small number of official reports. The Forestry Authority have estimated that of the 65 working days each summer, as much as 20% can be lost due to midge attacks preventing men from working. Ironically perhaps, there is far less information available on the effects of midges on the single largest input of income into the Highlands each summer; tourism. Hendry (1996) estimates that 13-14 million tourist trips are made to Scotland each year (mainly in the summer), valued at approximately £2 billion. Many caravan parks, campsites, tourist attractions etc. open only during the 3 months of summer, which thus dominate the local economy. Although again, most reports of midge attacks are anecdotal, it is clear that midges have the ability to restrict and even prevent many tourist activities. Some visitors are undoubtedly driven away, whilst others remain and suffer, being mentally unprepared and provided with no immediate relief other than to apply repellents and avoid the peak midge times. The exact impact of midges in Scotland on 'customer satisfaction' and the loss of income through visitors either leaving, not enjoying their holiday to the full or persuading friends against a visit to the Highlands is undocumented and presently, can only be guessed at.

A press article covering the launch of a 'midge festival' held throughout August 1998 in Argyll wrote of the midge that: "Throughout the 1990s the insect has sustained a highly skilled PR campaign" (Sunday Times, 7/6/98). This period of time has coincided with the introduction of 'midge forecasts' on local radio stations, questions in the House of Lords to the Scottish Secretary concerning Scotland's midge problem, and considerable press coverage (TV, radio and newspaper) of recent research initiatives directed at *C. impunctatus*.

**Ecological impact of midges** An area worth considering is what would happen to food chains if midges were removed. Midge numbers can be drastically reduced by both unusually cold winters and very dry summers but what the effects are on the organisms which rely on them for food is unknown. From the existing data on prey-selection by bats and birds the effects, however, are likely to be minimal, perhaps resulting in a small shift in prey selection in areas of significant midge reduction. This would not be unusual, since the commonest species of bat in Scotland (i.e. pipistrelles) are know to feed unselectively on the available insects, as do many species of warbler and other passerines. Concerning the larval stages, biting midge larvae will only constitute a very small proportion of the total soil fauna and their overall input into the system is likely to be relatively small. It is also inconceivable that any midge control programme would completely remove these insects from the food chain.

**The future** Biting midges have traditionally been difficult to study, both in the field due to the often unpleasant habitats they live in and their biting activity, and in the laboratory, due to difficulties in maintaining them and also, problems with identification. They have also been the poor cousins of mosquitoes and other biting insects regarding research and development funding. In countries where they are primar-

ily a biting nuisance, authorities are often reluctant to admit to their presence for fear of deterring visitors, particularly when there are no successful means of controlling midges. Concerning disease transmission, although vectors of some major livestock pathogens, biting midges are deemed to be relatively unimportant concerning human health. Recent advances in molecular taxonomy and identification (as reported here) and the application of state-of-the-art methods of investigating midge biology (e.g. determining host preferences, identifying novel attractants and repellents and further understanding the influence of climate on midge populations), however, are helping to bring biting midge research into the modern era and midges should be considered alongside other insects of medical and veterinary importance. Areas to concentrate on include the development of effective means of monitoring and control, the identification of taxonomic subpopulations of key species regarding their responses to control measures and their potential to act as disease vectors and also, the influence of climate on midge populations in relation to their changing threat to both human and animal populations.

<sup>1.</sup> Hendry, G. (1996). *Midges in Scotland*. Mercat Press, Edinburgh.

## Mechanisms & Processes

### G.C. Machray, J.W.S. Brown, K. Oparka & L. Torrance

Knowledge of how plants work is essential to safe and rational strategies for their protection and exploitation. This knowledge must extend from the gene, to the cell, to the plant in its environment, and to how the plant reacts in its environment in response to stress and under attack from pests and diseases. At one end of this spectrum lie fundamental biological systems such as plant and pathogen gene expression, cellular responses to pathogens and intercellular communication. To dissect these requires skills in molecular and cell biology, physiology and plant pathology. These disciplines have been assembled across the three Programmes within this Theme. Each has a focus of activity: on gene expression, on the molecular mechanisms underlying pathogen attack and the plant response, and on intercellular communication. The mechanisms driving these processes have already been shown to have key components that operate across them – these are crucial areas from which interdisciplinary projects can derive synergy.

**Gene Expression** Regulation of gene expression underpins plant metabolic processes, growth and development, and responses to and interactions with pathogens, pests and environment. The Gene Expression Programme seeks to delineate the mechanisms and components that govern the expression of plant genes, and how the functions they encode determine, for example, susceptibility to viruses and developmental gene expression in selected biological systems. The regulation of plant gene expression can occur both at transcriptional level and post-transcriptionally. The focus of activity at the post-transcriptional level is on pre-mRNA splicing and alternative splicing, snoRNA gene organisation and expression from multigene families in plants. A unique splicing system has been used to dissect splicing signals and trans-acting factor function. The novel potato invertase mini-exon system has for the first time allowed a quantitative assessment of the contribution of individual nucleotides to the strength of splicing signals. This work is an essential basis for future studies of alternative splicing. Up to 70% of human genes undergo alternative splicing to greatly increase proteomic complexity. Current estimates for plants are between 7% and 20% of genes which undergo alternative splicing such that although less prevalent than in humans, it still represents a very significant regulatory process. The mini-exon system has also yielded the first characterisation of plant intron branchpoint and polypyrimidine tract sequences, and is being exploited to study splicing factors. This work has found practical application in the generation of a novel resistance to nematode infection in a project involving both the RNA processing and nematology groups. By targeting an antisense version of a splicing factor gene in the nematode feeding site in potato roots, from a nematode-induced promoter, 80% resistance to nematode infection was achieved. Future stacking of other genes expressed from other promoters could give full resistance to nematode pests.

The work on RNA processing requires description of RNA-protein and protein-protein complexes, and nuclear and nucleolar architecture and distribution. These structures also feature in our studies of viral gene expression. These have two main thrusts: silencing and suppression of silencing, and virus molecular biology and viral movement proteins. The molecular mechanisms involved in gene silencing and its suppression, and viral movement, and the functions of silencing suppressors and movement proteins are being unravelled using unique systems and tools. For example, we are using cells from the fruit fly, Drosophila melanogaster, to assay silencing suppression. Many plant viruses encode silencing suppressors that act at different stages of the silencing process and it is sometimes difficult to demonstrate silencing suppression activity in planta. The Drosophila system has helped identify potential silencing suppressors and the stage at which they may act. These can be important tools to dissect the silencing mechanism (the study of which is most advanced in Drosophila). In addition, a collaboration with the Roslin Institute has been initiated to explore the utility of these suppressors in animal and human cells.

A second area of virus research centers on the development of amplicon systems that induce silencing. An amplicon [in our case based on *Potato leafroll virus (PLRV)* is a transgene that comprises a full-length biologically active copy of a viral genome. In plants that contain an amplicon, transgene-derived transcripts replicate like RNA viruses and this process could potentially take place in every cell of the plant. However, replication of the transcribed amplicon induces very effective silencing of the transgene (see Annual Report article page 111. This system, based on replicating viral RNA, is likely to more accurately mimic RNA silencing caused by virus infection than are alternative silencing systems that use non-viral transgenes or transient gene expression via *Agrobacterium* infiltration. More recently an amplicon has been developed that comprises a full-length cDNA of PLRV expressing the green fluorescent protein (GFP), and this construct is starting to prove very useful in our studies.



Both plant and viral gene expression overlap in the requirement to study protein components and their interaction with RNA and other proteins in functional complexes. The Programme aims to link the molecular biology of plant and viral gene expression, silencing and suppression of silencing through cell biological and biochemical approaches. This will require analysis of the expression of specific sets of genes identified by conventional or genomics approaches. Such analyses will be promoted by interactions with two new scientific appointments made under a new tranche of outer core funding supplied by SEERAD. Methods for parallel gene expression analyses are being developed by this funding - these will support functional analysis, hypothesis testing and germplasm enhancement, both within the Programme, and through links with other Programmes. Parallel gene expression analysis will provide a broader view of gene expression in a variety of biological systems (e.g. effects of silencing suppressors, germinating barley, alternative splicing, host responses).

We are also developing genetic and cell biological techniques to gain enhanced insight into the function of gene sets. Radiation hybrid mapping utilises fusion of somatic cells from barley, which have been subjected to radiation to induce chromosomal fragmentation, and tobacco. The resulting selected hybrid cells will contain a tobacco genome containing random chromosomal fragments of barley, providing a panel of cell lines for molecular marker analyses of the barley fragments. The establishment and application of this technology allows the physical mapping of the expressed gene (EST) set of barley, linking through genetic maps to phenotype and function. Also under investigation is the potential of male gametic cells, microspores, for the development of gene targeting in plants - the most sophisticated manifestation of transgenic biology, permitting gene knock-outs, down-regulation and over-expression, and allelic substitutions.

**Cell-to-Cell Communication** Research in the Cell-to-Cell Communication Programme is multidisciplinary, ranging from basic molecular studies of plasmodesmal structure and function to whole-plant studies of virus movement. Non-invasive imaging approaches are a hallmark of the Programme, and several innovative strategies have been developed for studying macro-molecular transport in plants based on confocal laser scanning microscopy. This Programme involves extensive interactions with plant pathologists, molecular biologists and biochemists at SCRI to study the basic cell-to-cell signalling processes that underpin plant-defence responses.

The structure/function relationships of plasmodesmata, the small pores that interconnect plant cells, are one focus of activity within the Programme. Plasmodesmata are essential conduits for small solutes and systemic signalling molecules, playing an essential role in plant development, and recent work has shown that they behave as regulatable channels whose permeability is dependent on a number of intracellular cues. A major discovery (published in the prestigious journal Cell) reveals that plasmodesmata can exist in different functional forms, some with the ability to traffic extremely large molecules. Plasmodesmata can also be modified by plant viruses to allow the passage of viral genomes between cells. Cell-cell virus movement has been used as a model system for understanding the regulation of plasmodesmata in response to viral invasion. The study of virus movement in plants using GFP-based technologies has led to the development of new models for virus movement in plants. A highlight is a new functional model for the intercellular movement of tobacco mosaic virus, the most extensively studied of the plant viruses. Unique, noninvasive techniques have been developed for studying virus movement and other basic transport processes in plants and the group is renowned for novel imaging techniques based on confocal laser scanning microscopy.

In addition, considerable contract work is undertaken, most notably with Biosource Genetics Corporation (BGC; California, USA), through Mylnefield Research Services Limited. This joint research programme is aimed at understanding and improving the movement of plant-viral vectors for the production of therapeutics in plants. Stable viral vectors, produced by DNA shuffling of the viral movement protein, represent the first demonstration of this technology using a plant virus. Isolation of novel genes in plants using a viral-based high-throughput functional genomics approach is also under way. In this work, random cDNA-GFP gene sequences are expressed within viral infection sites to identify novel proteins in different plant organelles. To date, several hundred novel proteins have been discovered in plant cells that were of previously unknown function and location. This approach is also identifying novel plasmodesmatal proteins that play a role in regulating intercellular transport of macromolecules and viruses. These studies are currently generating substantial intellectual property that is being exploited via Mylnefield Research Services.

There are further extensive collaborations with other research centres world-wide in joint studies of cell-tocell communication in plants, exemplified by an ongoing collaboration with Professor Chris Hawes (Oxford Brookes, UK) that has been exploring the use of plant-viral expression vectors for studying the endomembrame system in plant cells. A new initiative, funded by SEERAD under outer core funding and undertaken in collaboration with the Gene Expression programme, is examining the utility of viral vectors as tools to determine protein function in post-genomics applications.

**Plant-Pathogen Interactions** The interactions between plants and microbial pathogens are specific, complex and dynamic. They involve recognition and response in the plant leading to signalling cascades and the up- or down-regulation of numerous genes, and in the pathogen to adaptation or evasion. This Programme focuses on plant and pathogen interactions at the cell and molecular level. The aims are to understand and exploit the complex processes involved by using genomics-based approaches to study transcriptional and proteomic changes that occur at key stages of the interaction (recognition, signalling and defence response). The objectives are to discover and functionally characterise plant and pathogen genes involved in pathogenicity and plant defence, to understand the communication mechanisms (elicitation, recognition, response) between plant and pathogen, and to describe the identity and functions of the proteins involved in these processes.

Pathogen characterisation and the molecular basis of pathogenicity involve the identification and functional analysis of pathogen genes associated with pathogenicity, host specificity, avirulence and survival. New approaches to discover novel pathogenicity and host range related genes have been developed. Sample sequencing of selected regions of the Erwinia genome has allowed a detailed analysis of the hrp gene cluster and has uncovered a number of novel gene homologues from other animal and plant pathogens. Molecular analyses of the Phytophthora infestans genome have led to the construction of physical maps across loci containing avirulence genes and the isolation of infection stage specific genes. Genes expressed by potato cyst nematodes following invasion of roots have been isolated and sequence analyses reveals expression from novel nematode genes as well as host genes, many of which are typical of response genes to pathogen attack. Notable among these is the cloning and characterisation of a chorismate mutase from Globodera pallida. The gene encoding chorismate mutase appears to have been obtained by horizontal gene transfer from bacteria. This protein is secreted from the gland cells and may have an important role in the host parasite interaction. Global gene characterisation is also under way with near completion of sequencing of the entire genome of the plant pathogen Erwinia carotovora ssp. atroseptica, with a major bioinformatic annotation exercise to follow.

Description of the plant response to infection requires the discovery, characterisation and functional analysis of plant genes involved in surveillance or activated in response to pathogen invasion. Profiling of differential gene expression has revealed signalling pathways specific to either R gene mediated or field resistance to *Phytophthora infestans* in potato. This information, set in the context of related published research, has been made available by the development of DRASTIC, a web-searchable database of genes up- or down-regulated in plants in response to infection or application of resistance-modifying compounds for the analysis of signal transduction in cells (http://www.drastic.org. uk). Similar catalogues of pathogen-induced plant genes have been assembled for nematode infection where the isolation and functional characterisation of a broad-spectrum potato cyst nematode resistance gene (*Hero*) of tomato features.

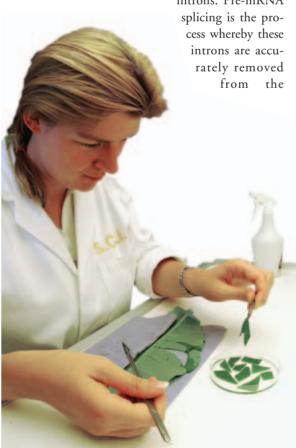
Increasingly, functional characterisation will turn to protein interactions and the role of proteins in disease processes. Physico-chemical analysis of key proteins will be required and protein-protein and proteinnucleic acid interactions will be studied by a variety of methods. Subcellular localization and protein interaction studies have revealed new insights into the coordinate mode of action of Potato mop-top virus triple gene block movement proteins. The lipid binding protein, identified in surface secretions of nematodes and known to counteract the plant defence response, has been purified after recombinant expression for 3D and quaternary structure studies. Further novel regulatory and avr proteins will soon follow. The knowledge gained will help us to understand the molecular bases of disease processes that can be exploited to produce durable resistance to plant diseases. For example, the discovery of common and disease specific responses to pathogens can be used to design novel broad-spectrum control strategies. Already, a number of diagnostic sequences have been made available to aid complementary studies in other Programmes on the co-evolution of plants and parasites at the level of crops and pathogen populations. A new method has been developed using 16 S rDNA decreasing the time taken to identify the soft rot erwinias from 14 days to 1 day. Novel recombinant synthetic antibody assays (ELISA and stem printing) to detect Potato leafroll virus were devised and shown to work as well as tests that utilise reagents obtained by animal immunization. Microsatellite markers have been used with individual nematodes from Globodera pallida populations to distinguish UK populations and a further PCR based diagnostic has been used to distinguish trichodorid nematodes of economic importance in the UK (Paratrichodorus pachydermus, P. macrostylus, Trichodorus similis, T. primitivus). We expect that the genes and mechanisms discovered in this programme will be invaluable to analyses of the durability of resistance and the influence of environmental factors in studies of host-pathogen co-evolution.

## **Detailed analysis of two intron splicing signals**

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

Pre-mRNA splicing is one important area where gene expression is regulated. Alternative splicing, where different mRNA transcripts are made from the same transcription unit through alternative splice site choice, is firmly established in humans and Drosophila for generating protein diversity and for regulating biochemical pathways. Plants show similar alternative splicing events in many tissue and developmental pathways, and alternative splicing is likely to be extremely important in plant growth and development. To understand how alternative splicing is regulated we need, firstly, to understand the basic mechanisms of splicing in terms of splicing signals and factors. This article provides a detailed analysis of two key plant splicing signals, the branchpoint and polypyrimidine tract, which are recognised to have roles in regulating splice site choice.

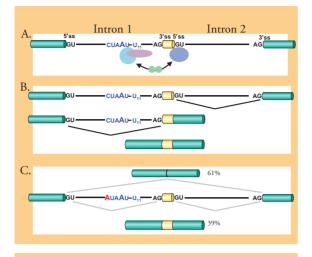
A typical plant gene sequence consists of a number of protein coding exons interrupted by non-coding introns. Pre-mRNA



pre-mRNA to make a mRNA template that is translated into a functional protein. Splicing is an important process, which must be accurate, otherwise non-functional, or dysfunctional proteins can be made. Accuracy of intron removal is achieved by recognition of 5' and 3' splice site signals (Fig. 1) by splicing factors that promote the assembly of the spliceosome. The spliceosome mediates the removal of the introns and the ligation of the exons utilising an intron internal adenosine called the branchpoint found close to the 3' splice site. A consensus sequence surrounding the branchpoint and an associated polypyrimidine tract are firmly established splicing signals in vertebrate introns. They are *cis*-acting signals essential for efficient recognition of the branchpoint nucleotide and for recognition of the downstream splice site. Like the splice site consensus signals, the vertebrate branchpoint consensus is degenerate and poorly conserved (CURAY). The closely associated downstream polypyrimidine tract is a binding site for a protein factor called U2AF<sup>65</sup> that promotes the selection of the branchpoint.

In plant splicing, these signals have never been properly characterised. We showed previously that mutation of a plant branchpoint adenosine, found in a sequence similar to the vertebrate consensus, significantly reduced splicing efficiency (Ann. Rep. 1995, 48-49). Plants do not contain a pronounced polypyrimidine tract as found in vertebrate introns but, have U-rich sequences between the putative branchpoint and the 3' splice site. However, as U- or UA-richness is a feature of efficient plant intron splicing and as U-rich intronic sequences are found throughout plant introns, it has not been possible to distinguish sequences that function as UA-rich elements or as polypyrimidine tracts.

We have been studying the splicing of a 9 nt miniexon from a potato invertase gene and discovered that mini-exon inclusion is dependent on strong constitutive splicing signals. The key elements that determine mini-exon inclusion are a branchpoint sequence, an adjacent downstream U-rich region and the distance between these signals and the splice site downstream of the mini-exon. It is likely that these are sites for factors that help define the mini-exon through an exondefining process that leads to a splicing event that



**Figure 1** A. Schematic representation of the genomic structure of the invertase mini-exon and the exon definition process that promotes splice site selection of the 9 nt mini-exon. Exons are indicated as boxes and introns by lines. The mini-exon box is highlighted in yellow. The 5' splice sites (GU) and 3' splice sites (AG) are shown. The branchpoint sequence and associated U11 in the upstream intron are shown in blue. Exon definition involves the assembly of factors that bridge the exon. These factors are shown as coloured discs and the bridging interaction shown as a double arrow.

B. The splicing pathway for the invertase mini-exon. In the non-mutated construct (inv1, see Table 1) initiation of splicing removes the downstream intron first, followed by removal of the upstream intron and the formation of the fully spliced product.

C. Disruption of the branchpoint and polypyrimidine tract by single substitution mutation changes the efficiency by which the mini-exon is included in a population of pre-mRNA transcripts and leads to varying levels of mini-exon skipping (lacking the mini-exon box) and mini-exon inclusion (includes the mini-exon box). One change to the branchpoint consensus  $C \rightarrow A$ , highlighted in red, leads to 39% of the pre-mRNA transcripts showing inclusion of the mini-exon and the remaining 61% shows skipping (Table 1). Other single nucleotide substitution changes to these signals are shown in Tables 1 and 2.

removes the downstream intron first followed by the upstream intron (Ann Rep. 1997/98, 71-73; Ann. Rep. 1999/00, 111-113) (Fig. 1). The requirement for the branchpoint and U-rich signals for mini-exon inclusion makes this splicing arrangement sensitive to sequencing change leading to skipping of the mini-exon (Fig. 1). The invertase mini-exon system, therefore, provides a system to investigate, in detail, the sequence and spatial requirements of branchpoint and polypyrimidine tract signals for the first time in plants.

A plant branchpoint consensus A systematic mutational analysis of the invertase branchpoint sequence was carried out and the effect on splicing efficiency of the mini-exon in tobacco protoplasts was measured (Table 1). At position -3 from the branchpoint adenosine, a pyrimidine was the optimal nucleotide with a preference for cytidine. A uridine at position -2 was essential as all other nucleotides could not support mini-exon splicing at this position. At position -1 a purine was preferred although pyrimidines could support about 50% splicing of the mini-exon. An adenosine was required as branchpoint nucleotide with a pyrimidine as the nucleotide downstream of the branchpoint. These results clearly support a preference for a consensus sequence which is identical to the vertebrate branchpoint consensus (CURAY) for efficient (>90%) inclusion of the mini-exon. This shows for the first time in plants the importance of the sequence context surrounding the branchpoint nucleotide.

Construct name	-3	m	nchp utati -1		+1	Splicing efficiency %	
inv1	С	U	А	А	U	99	
inv74	A	Ū	A	А	Ū	39	
inv55	G	U	А	А	U	18	
inv54	U	U	А	А	U	80	
inv56	С	A	А	А	U	3	
inv75	С	C	А	А	U	4	
inv57	С	G	А	А	U	2	
inv73	С	U	С	А	U	51	
inv52	С	U	G	А	U	94	
inv53	С	U	U	А	U	47	
inv76	С	U	А	С	U	7	
inv77	С	U	А	G	U	0.4	
inv10	С	U	А	U	U	19	
inv71	С	U	А	А	A	59	
inv51	С	U	А	А	C	99	
inv72	С	U	А	А	G	33	
invertase min	cing efficiency shows the percentage inclusion of the rtase mini-exon (see Fig. 1C). t branchpoint consensus: CURAY						

 Table 1 Splicing efficiency of branchpoint mutations.

A plant polypyrimidine tract To examine whether the U-rich sequence downstream of the branchpoint was functioning as a polypyrimidine tract (U and C nucleotides) or a plant UA-rich element (U and A nucleotides), mutant constructs were tested in tobacco protoplasts (Table 2). The constructs maintained a  $U_4$  element and then increasing numbers (2-5) of C or A nucleotides replaced the remaining Us. Splicing to

Mech	hanisms	$\mathcal{O}$	Processes

Construct name	Polypyrimidine tract mutations	Splicing efficiency %
inv1	U <sub>10</sub>	99
inv38 inv41 inv58 inv60	$\begin{array}{c} U_{4}A_{2}U_{4}\\ U_{4}A_{3}U_{3}\\ U_{4}A_{4}U_{2}\\ U_{4}A_{5}U_{1} \end{array}$	90 69 28 9
inv39 inv42 inv59 inv61	$egin{array}{c} U_4 C_2 U_4 \ U_4 C_3 U_3 \ U_4 C_4 U_2 \ U_4 C_5 U_1 \end{array}$	98 99 83 60
inv42 inv64 inv65 inv8	$egin{array}{c} U_4C_3U_3 \ U_4C_6U_3 \ U_4C_9U_3 \ U_1C_9U_1 \end{array}$	99 72 41 7

Splicing efficiency shows the percentage inclusion of the invertase mini-exon (see Fig. 1C). Plant polypyrimidine tract:  $U_4Y_{2}$ - $_3U_3$ 

**Table 2** Splicing efficiency of polypyrimidine tractmutations.

include the mini-exon reduced steadily with increasing numbers of A nucleotides such that a construct containing a UA-rich element  $U_4A_3U_3$  was splicing the mini-exon below 70% and by  $U_4A_5U_1$  splicing was below 10%. On the other hand, C nucleotides were largely able to compensate for U nucleotides and only by the presence of a run of four Cs was splicing of the mini-exon beginning to fall significantly (83%). Therefore, for splicing to include the mini-exon there is a preference for pyrimidines rather than UA-rich elements.

The U-rich region of the invertase gene contains 11 consecutive Us while other invertase genes contain small groups of Us interspersed with other nucleotides. In our previous analyses (Ann. Rep. 1999/00, 111-113), a run of eight Us spliced at 30%

and reducing the run further to six and four Us virtually abolished splicing. Although a single  $U_4$  group does not support splicing, as shown here a U4 group plus a second U4 or U3 group can support efficient splicing of the mini-exon. This suggests that a single group of Us is not sufficient to support mini-exon splicing, but a combination of two groups of 3-4 uridines is needed. The separation of these groups was investigated by interspersing them with increasing numbers of Cs. This led to a progressive reduction in splicing efficiency (Table 2), despite the ability of Cs to support splicing in a polypyrimidine tract. Splicing reduced to 41% when a  $U_4$  and  $U_3$  pair was separated by nine Cs, but, this splicing was significantly greater than splicing of a construct containing single U nucleotides separated by nine Cs, which spliced at less than 10%. These results support the requirement for two U-rich groups at least 3-4 nucleotides long and that they should be in close proximity to support efficient splicing. The data suggest that for efficient splicing of the potato mini-exon, the polypyrimidine tract should be  $U_4Y_{2-3}U_3$ . This may reflect the binding site of U2AF<sup>65</sup>, which binds to polypyrimidine tracts and has a binding preference for  $U_6C_{2-3}U_8$ .

Using the invertase mini-exon system and an extensive number of mutants we have characterised the sequence surrounding a plant branchpoint and its associated polypyrimidine tract. The identification and characterisation of these signals in plants is significant as these signals are important areas for regulation of splicing and thereby regulation of gene expression. Trans-acting proteins mediate 3' splice site selection and, in vertebrate and Drosophila systems, compete to regulate alternative 3' splice site choice. It is clear that naturally occurring plant introns show great variation in putative polypyrimidine tracts for splicing and these differences may alter the strength or sequence recognition by plant splicing proteins. It remains to be seen to what extent these plant polypyrimidine tracts may regulate alternative splice site choice and consequently gene expression in plants.

## Gene targeting in higher plants

N. Aziz & G.C. Machray

Pene targeting technology (Fig. 1), involving the disruption or substitution of specific alleles, is the most sophisticated tool of reverse genetics. Its use in experimental systems ranging from yeast to mouse allows virtually any cloned gene, even of unknown function, to be specifically mutagenised in vitro and reintroduced precisely into its own chromosomal location. Thus, the effects of null, deleterious or even advantageous mutations to that gene can be directly determined. Gene targeting is routinely used in yeast and has been successfully applied to a range of other lower eukaryotes including filamentous fungi, slime mould and trypanosomes. In these organisms the frequency of homologous recombination (HR) is high (over 10%) compared with the chances of random integration by illegitimate recombination (IR). In most eukaryotic cells, however, DNA integration by IR predominates and the frequency of HR is extremely low, making gene targeting infeasible. In a few ani-

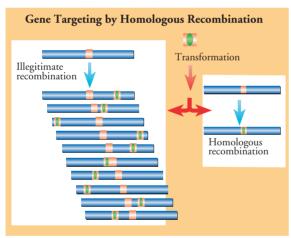


Figure 1 Genetic transformation in higher plants occurs by illegitimate recombination between unlike DNA sequences - in each transformation event the transgene integrates at a different site in the genome. This can result in unwanted effects such as disruption of endogenous gene function or inappropriate transgene expression. Several hundred transgenic lines may have to be generated before a suitable candidate line with the desired phenotype is identified.

In contrast, targeted recombination at a specific site mediated by homologous DNA sequences has no collateral effects and expression level is similar to that of the endogenous gene. Knock-outs, knock-ins and allelic replacements become possible. mal systems, this problem has been overcome by focusing on cell types with the highest HR frequencies and by using powerful negative selection systems. Mouse embryonic stem cells, for example, can now be fairly routinely used to generate targeted gene knockouts, although the ratio of HR to IR in these cells is still 10 to 1000-fold lower than in yeast. Gene targeting has therefore become an invaluable tool for both animal and microbial geneticists for the elucidation of gene function and for the generation of improved strains by genetic manipulation.

By comparison, it has so far proved impossible to establish a feasible gene targeting system for higher plants. This is despite the fact that gene targeting is a key technology both for functional genomics and for the rational, accurate and safe exploitation of plants through genetic manipulation. Current plant genetic manipulation strategies rely on random integration of foreign DNA into the plant genome by illegitimate recombination. Neither the exact site of transgene integration nor the number and organisation of DNA molecules inserted can be experimentally controlled. These factors influence the level, pattern and stability of transgene expression and the likelihood of the transgene eventually becoming silenced. The unpredictability of these processes is one of the major concerns underlying opposition to the use of genetic manipulation for crop improvement. Gene targeting could reduce this concern by offering a more precise, predictable and cleaner method for genetic manipulation of plants. Transgene integration could be directed to particular locations in the genome where unstable expression or other 'position effects' would be minimised. The DNA to be integrated could be very exactly delimited at each end, because integration would be strictly homology-dependent. Moreover gene targeting offers the potential to completely knock-out expression of target genes or to make specific changes to gene function, objectives that cannot be achieved by conventional transgenesis. Given this importance it is clear that commercial biotechnology is active in this area. It is unlikely however that successful gene targeting approaches will be made freely available through this avenue. It is therefore imperative that such research be publicly funded - the importance in the coming years for a clean gene technology

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emanating from public laboratories cannot be overestimated.

Development of a gene targeting system for plants is likely to depend on the identification or development of cell types where the ratio of homologous recombination (HR) to illegitimate recombination is maximal. One of the most hopeful discoveries of recent years has been the finding that HR is highly efficient (over 90%) in haploid tissues of the moss Physcomitrella patens. This may be because, during the haploid growth phase, random integration of foreign DNA would be immediately mutagenic and is therefore protected against, while constraints on HR to prevent recombination between allelic sequences are unnecessary. If these assumptions are correct, then haploid higher plant tissues might also offer high HR frequencies and thus be the best substrate for gene targeting experiments.

Previous work in tobacco (*Nicotiana tabacum* L.) indicated that microspores at late uninucleate/early binucleate stages can be isolated from flower buds and *in vitro* culture methods optimised for their maturation to fully functional viable pollen which, after application to the stigma of emasculated plants *in situ*, led to the generation of large numbers of seed. We have established efficient protocols for the biolistic introduction of a construct containing a reporter gene and selectable marker into these microspores and hence, after *in vitro* maturation and *in situ* fertilisation, for the generation of transgenic plants. Stable transformants of low copy number were generated by this

biolistics	late uninucleate/ early binucleate	to pollen used for fertilisation	
biolistics		via embryogenes to regenerate pla	

#### **Table 1** Transformation of the haploid male microspore.

procedure. The efficiency of transformation achieved allowed the production of large numbers of transgenic plants without selection, dispensing with the requirement for a selectable marker in plant transgenesis (Table 1).

Microspores at various stages of development were further isolated from flower buds of tobacco (*Nicotiana tabacum* L.) and *in vitro* culture methods optimised for their switch to an embryogenic developmental pathway, resulting in the high-throughput production of haploid and double haploid plantlets. Biolistic transformation as above was applied to these microspores and, after *in vitro* embryogenesis, transgenic plants were regenerated. Again, stable transformants of low copy number were generated by this procedure. The efficiency of transformation achieved also allowed the production of homozygous transgenic plants without selection (Table 1).

Having developed highly efficient transformation systems for haploid cells, we next



developed a selection system which would allow the detection of, potentially rare, homologous recombination events. In reciprocal experiments (Fig. 2), two related gene constructs were prepared as targets. In the first, three stop codons were introduced at the start of the aphIV gene, encoding hygromycin resistance, rendering the mutated gene non-functional. In the second, a second non-functional aphIV gene was prepared by the introduction of three stop codons near the middle of

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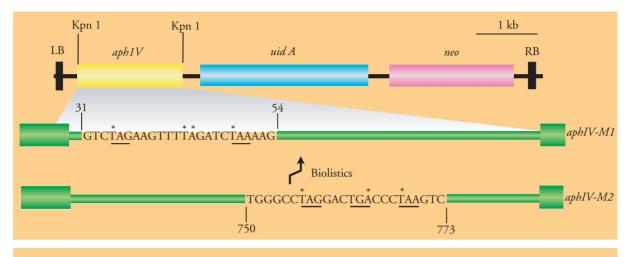


Figure 2 Microspores from transgenic lines carrying either of two mutated hygromycin-resistance genes were bombarded with the reciprocal mutant construct, matured to pollen *in vitro* and used to fertilise emasculated recipient plants. Resulting seeds were germinated and hyromycin-resistant seedlings selected.

the gene. Two sets of tobacco lines were generated (by *Agrobacterium*-mediated transformation) carrying either of these constructs and microspores harvested from these lines. Microspores carrying one mutant gene were then bombarded with the second, and matured to pollen that was subsequently used to fertilise emasculated wild-type tobacco plants. Seed from these fertilisations were germinated in the presence of hygromycin to detect the presence of a rescued hygromycin resistance gene. Initial results from these experiments indicate a ratio of homologous to illegitimate recombination in haploid cells that is two orders of magnitude greater than that observed in diploid tissues. Current experiments are determining whether such a frequency can be achieved using an endogenous gene as a target, to rule out the possibility that the high HR frequency observed resulted from the use of a transgene as a target.

If endogenous genes can be targeted at the frequency observed for transgenes, then gene knock-outs, knockins and allele replacements will be possible, using simple PCR-based screening for allelic constitution. No selectable marker is required. The approach is fast and requires no *in vitro* regeneration of plant tissues hence minimising somaclonal variation. It should be applicable to other species (e.g. cereals) that have developed microspore maturation protocols and will be invaluable for functional genomic studies, as well as more precise genetic engineering (with fewer collateral effects) for germplasm enhancement.

## *Potato leafroll virus* amplicons in the study of RNA silencing in plants

H. Barker, M. Taliansky, M.A. Mayo, K.D. McGeachy, G. Fraser & E. Ryabov

**Introduction** RNA silencing is a type of post-transcriptional regulation of gene expression that is based on the sequence-specific degradation of RNA molecules. Processes related to RNA silencing have been found in a broad variety of eukaryotic organisms including plants, animals and fungi. Thus, cells of 'silenced' eukaryotes are able to degrade RNA species in the cytoplasm that share sequence homology with the triggering RNA molecules. Common to RNA silencing pathways is the generation of dsRNA that corresponds to the target RNA and that acts as a trigger or an intermediate in the degradation process. The dsRNA is cleaved into short interfering (si) RNAs 21 to 25 nucleotides in length that correspond in sequence to both sense and antisense strands of the target RNAs, and these are thought to mediate the specificity for target RNA degradation.

In plants, RNA silencing has been studied most extensively as a post-transcriptional gene silencing (PTGS) mechanism by using transgenic plants. In many studies, plants have been transformed with virus genes in order to determine if resistance is induced against infection with the original virus. However, viruses are both inducers and targets of RNA silencing, and infection of plants with a broad range of viruses results in host defensive RNA silencing responses even in the absence of homologous nuclear sequences. This has led to the idea that RNA silencing in plants may have evolved as a general antiviral defence mechanism. Some viruses encode specific proteins that suppress PTGS, which suggests a co-evolution of defence and counter defence mechanisms between the plant hosts and viruses. [For recent reviews on PTGS see Carrington (2000)<sup>1</sup>, Vance and Vaucheret (2001)<sup>2</sup> and Voinnet (2001)<sup>3</sup>, SCRI Ann. Rep. 2000-2001, 120-123].

We have been investigating the utility of amplicons (virus-based transgenes) as an exciting and innovative tool for the study of RNA silencing. Amplicons were first described by Angell and Baulcombe (1997)<sup>4</sup> for *Potato virus X*, and extended to other viruses such as *Potato leafroll virus* (PLRV) by Franco-Lara *et al.* (1999)<sup>5</sup> and Barker *et al.* (2001)<sup>6</sup>. An amplicon is a transgene that comprises a full-length (biologically

active) copy of a virus genome. In plants that contain an amplicon, transgene-derived mRNA transcripts initiate infection and replication of the amplicon in much the same way as the RNA genome of an invading virus. An important difference is that this process can potentially take place in every cell of the plant. However, such replication also induces very effective silencing of the amplicon. Therefore this system, based on replicating viral RNA, may well prove to be more suitable for obtaining an accurate picture of RNA silencing caused by virus infection than PTGS systems using transgenes. This report describes our recent work to understand the mechanisms involved in amplicon-mediated silencing and our attempts to develop new and more useful amplicons (Fig. 1).

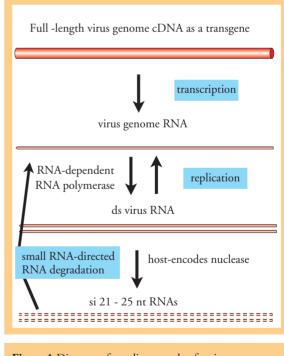
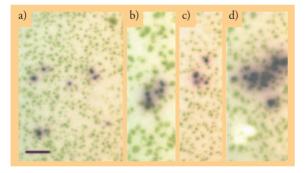


Figure 1 Diagram of amplicon mode of action.

**Transformation of** *Nicotiana* **species with a PLRV amplicon** The first amplicon we developed was based on PLRV, (see *SCRI Ann. Rep.* 1999/2000, 142-145). Like other luteoviruses, PLRV cannot be transmitted to virusfree plants by mechanical inoculation. In nature, PLRV is transmitted by aphid vectors that introduce particles into the vascular tissue of plants where PLRV multiplies and in which it remains largely restricted. PLRV is normally restricted to phloem tissue because the virus lacks movement functions that can operate in epidermal and mesophyll cells and/or because PLRV cannot suppress putative host defence responses in non-vascular tissues. In tobacco plants transformed with the PLRV amplicon, immunoprints of tissue pieces showed that approx. 5% of phloem companion cells were infected, and also that a few mesophyll cells and epidermal cells contained virus. We concluded in 1999 that some mechanism was restricting PLRV multiplication in most cells of these plants whilst permitting replication in a few cells.

In more recent work we have explored this phenomenon further by transforming a better PLRV host, *Nicotiana benthamiana*, with the same amplicon. A small proportion of mesophyll cells (about 2%) in these PLRV amplicon plants contained detectable amounts of PLRV. Surprisingly, we also found that approximately 2% of mesophyll cells in PLRV-infected WT *N. benthamiana* that had been inoculated by aphids, also contained virus. The titres of PLRV, estimated by ELISA, and the extent of mesophyll cell infection were about the same in amplicon plants and in PLRV-infected WT *N. benthamiana*.



**Figure 2** Immunoprints of leaf mesophyll tissue stained for PLRV. Purple spots are PLRV infected cells stained with indoxyl precipitate. (a) PLRV-infected WT *N. benthamiana* showing infected cells, some dispersed and some in pairs; (b) PLRV-infected WT *N. benthamiana* showing a typical cluster of six cells; (c) CW1 N. benthamiana leaf showing a typical cluster of five cells; (d) CW1 *N. benthamiana* leaf showing a typical cluster of approx. 20 cells. Magnification bar in (a) represents 0.5 mm

Because stained mesophyll cells occurred in WT *N. ben-thamiana* plants inoculated with PLRV, we assume that such cells were infected following cell-to-cell movement of PLRV from cells within vascular tissue. In PLRV amplicon *N. benthamiana* plants, mesophyll cells could be infected *via* a similar route, or as a result of RNA transcript from the PLRV amplicon initiating infection

in particular cells. However, because similar numbers of infected mesophyll cells were detected in amplicon transformed and WT plants, it seems likely that the majority of stained mesophyll cells in amplicon plants became infected *via* the same route, as do the cells in WT plants inoculated with PLRV. Immunoprinting (a technique designed to reveal PLRV-infected cells by light microscopy based on a staining method using an anti-PLRV antibody) of leaves from amplicon and PLRV-infected WT *N. benthamiana* showed that a large proportion of stained mesophyll cells were located in clusters (groups of between 2 and 20 cells) (Fig. 2).

Effects of inoculation with PVY on PLRV accumulation in amplicon plants PLRV amplicon *N. benthamiana* plants were inoculated with *Potato virus Y* (PVY) and, 12 days after inoculation, systemically infected leaves were tested by ELISA and immunoprinting. The PLRV titre in these leaves was about 5fold greater and the number of infected mesophyll cells was about 4-fold greater than in uninoculated control amplicon plants (approximately 1 in 24 mesophyll cells were infected in PVY-infected amplicon plants, and in some areas of the tissue up to 1 in 10 mesophyll cells were stained).

After PVY inoculation of tobacco plants transformed with the PLRV amplicon, the amounts of PLRV in leaf tissue samples were compared with those in leaves of non-inoculated plants; the mean titres were 240 ng/g leaf in non-inoculated plants, and 1600 ng/g leaf in PVY-infected plants. From immunoprints of the non-inoculated amplicon tobacco plants, 1 in 3000 mesophyll cells were found to be stained, whereas in PVY-infected amplicon tobacco plants, 1 in 625 mesophyll cells contained PLRV and clusters of up to four infected cells were found.

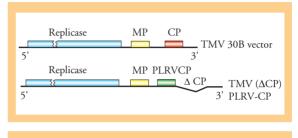
Thus potyvirus infection resulted in a 5- to 6-fold enhancement in the accumulation of PLRV in the amplicon transformed plants of tobacco and *N. benthamiana.* The effect of potyvirus infections on suppressing post transcriptional gene silencing (PTGS) is well known, and so our findings raised the possibility that PLRV accumulation in amplicon plants was limited by PTGS (Fig. 1).

Effects of transgenically expressed *Tobacco etch virus* P1/HC-Pro on amplicon plants The experiment described above used PVY infection to test the effects of silencing suppression on amplicon transformed plants. The HC-Pro protein encoded by potyviruses, such as PVY, is known to suppress silencing. In more

direct tests, we used plants transformed so as to produce high levels of the HC-Pro protein of Tobacco etch virus (TEV), another potyvirus. In progeny plants from crosses between amplicon tobacco plants and tobacco transformed to express TEV HC-Pro, the mean PLRV titres were about 10-fold greater than in progeny plants from control crosses containing only the PLRV amplicon. Immunoprints made from leaves (mesophyll and sections of mid-vein) of plants of these progenies showed that there were many more PLRV-infected cells in the mesophyll and phloem tissue of amplicon plants that also expressed TEV HC-Pro than in control plants. Thus, in the control plants only 1 in 25000 mesophyll cells were infected, whereas 1 in 55 mesophyll cells were infected in amplicon plants that also expressed TEV HC-Pro. Many stained cells were in small clusters of up to six. Thus expression of TEV HC-Pro resulted in an increase in the amounts of PLRV produced in both mesophyll and phloem tissues. This result is consistent with previous experiments in which HC-Pro was produced as a result of potyvirus infection and suggests that expression of HC-Pro diminishes, at least in part, the resistance to PLRV accumulation in the mesophyll.

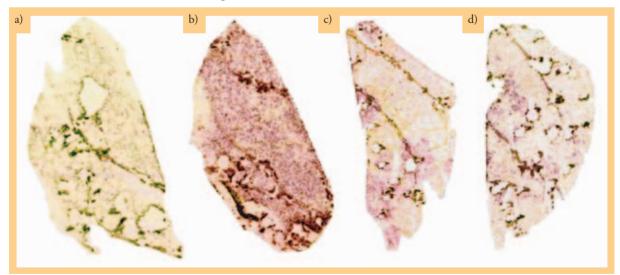
**Inoculation of** *N. benthamiana* **amplicon plants with TMV(\DeltaCP)PLRV-CP** It could be predicted that if PTGS occurs in amplicon plants, it should target all RNAs containing PLRV nucleotide sequences. Therefore, as an alternative system for examining PTGS-like effects, we used the chimeric virus TMV( $\Delta$ CP)PLRV-CP in which the CP gene of TMV

RNA had been replaced by the CP gene of PLRV (Fig. 3). This virus accumulates in inoculated leaves of *N. benthamiana* but does not move systemically, probably because the CP is essential for long-distance movement of TMV and because PLRV CP is unable to provide this function.



**Figure 3** Schematic of TMV( $\Delta$ CP)PLRV-CP genome.

When TMV( $\Delta$ CP)PLRV-CP was inoculated to WT *N. benthamiana* plants, immunoprints made to locate PLRV antigen in leaves showed that large areas (up to 15 mm across) were infected at 4 days post inoculation (p.i.), and at 8 days p.i. approximately 80% of the leaf area was infected (Fig. 4b). By microscopy, it was possible to see that the majority of individual cells in these visibly stained areas were infected. These results suggested that virus had moved from cell to cell in these leaves. The result was different when TMV( $\Delta$ CP)PLRV-CP was inoculated to PLRV-infected WT *N. benthamiana* or amplicon plants. In these plants, by 4 days p.i. antigen-containing cells were in clusters of up to approximately 200 cells. At 8 days p.i. the areas of staining were much larger and



**Figure 4** Immunoprints of leaves stained for PLRV. (a) WT non-inoculated *N. benthamiana;* (b) WT *N. benthamiana* 8 days p.i. with TMV( $\Delta$ CP)PLRV-CP; (c) PLRV-infected WT *N. benthamiana* 8 days p.i. with TMV( $\Delta$ CP)PLRV-CP; (d) CW1 *N. benthamiana* 8 days p.i. with TMV( $\Delta$ CP)PLRV-CP; (d) CW1 *N. benthamiana* 8 days p.i. with TMV( $\Delta$ CP)PLRV-CP.

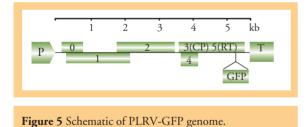
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clusters of up to several hundred cells could be seen in the microscope, and by eye approximately 35% of the leaf area was stained (Fig. 4c & 4d). These results suggest that the delay in spread of  $TMV(\Delta CP)PLRV-CP$  in inoculated leaves of PLRVinfected WT *N. benthamiana* and amplicon plants occurred because of specific resistance against the virus containing PLRV sequence

tance against the virus containing PLRV sequences as a result of PTGS induced by replicating PLRV.

#### Attempts to make a new PLRV amplicon expressing

**GFP** More recently we have made an improved version of the PLRV amplicon consisting of full-length cDNA corresponding to the genome RNA of PLRV, described above, that has been modified to contain a reporter gene encoding green fluorescent protein (GFP) to monitor virus expression (Fig. 5). This plasmid carries a full-length cDNA PLRV clone in which DNA encoding GFP was inserted in the *P5* gene approximately 300 nucleotides from its 3'-end. The insertion did not prevent modified viral RNA from being replicated because virus particles and GFP-P5 fusion protein accumulated in PLRV-GFP-inoculated cells or protoplasts<sup>7</sup>.



Plants of *N. benthamiana* and *N. tabacum* have been transformed with this amplicon. Strong RNA silencing was induced in plants transformed with the PLRV-GFP amplicon, as evidenced by low levels of PLRV-GFP accumulation, lack of symptoms and detection of amplicon-specific si RNAs. Examination by confocal laser scanning microscopy (CLSM) showed that some cells contained GFP but this was restricted to single cells or to small clusters of usually not more than 4 cells that were distributed irregularly

**Discussion** The advantages of the experimental PLRV amplicon-mediated RNA silencing system we have

developed are (i) that PLRV is unable to combat silencing in mesophyll and epidermis (i.e. it is unable to suppress or avoid the effects of silencing), which allows the study of silencing of virus replicating RNAs without any effect from the virus itself, and (ii) that the lack of virus movement

from cell-to-cell allows the study of viral RNA silencing in the absence of possible effects from virus movement. Another advantage of ampliconmediated RNA silencing as an experimental system over conventional silencing systems that use sense or antisense transgenes, is the reproducibility and consistency with which silencing is triggered. Results obtained in this work with the PLRV amplicon, and more recently with the PLRV-GFP amplicon, are enabling us to focus on the various factors, internal and external to the plant, that affect RNA silencing in plants and that could help to improve understanding basic virus defence and counter defence mechanisms. Results of more research on our amplicon plants could prove to be influential in many areas, including the design of improved transgenes for pathogen resistance and understanding the control of natural plant resistance genes.

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# High-throughput localisation of novel plant proteins using virus-based vectors

N. Escobar, S. Haupt, S. Chapman, G. Pogue<sup>\*</sup> and K.J. Oparka

The Arabidopsis genome project has generated a large number of sequences that encode for proteins of unknown function. In the post-genomics era, characterising the location and function of these proteins in plant cells will become a major challenge. In order to ascribe subcellular targeting information to novel proteins, we have developed a high-throughput screening procedure involving viral vectors based on tobacco mosaic virus (TMV). In this system, random cDNA inserts produced from Nicotiana root mRNA were inserted en masse into TMV vectors containing the gene for green fluorescent protein (GFP) to allow subsequent infection and fluorescence screening of leaves infected with the libraries. Each infection site that arose on an infected leaf expressed a unique cDNA-GFP fusion, allowing the subcellular 'address' of a protein to be ascribed at high resolution under the confocal laser scanning microscope (CLSM).

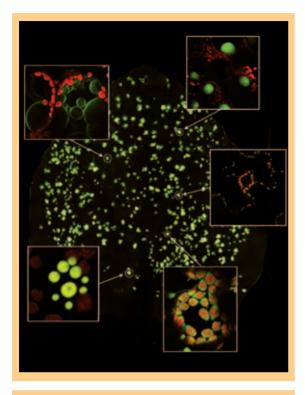
Library construction To prevent masking of the targeting sequences that frequently occur at the ends of proteins, methods were developed to express fusions to both the amino- and carboxy-termini of gfp. For expression of protein sequences fused to the amino-terminus of gfp it was necessary to produce partial cDNAs that contained the initiating methionine codon, found near the 5' end of the mRNAs, but lacked the termination codon, found near the 3' ends. Therefore, to create a library of cDNAs fused to the 5' end of gfp, first-strand cDNA synthesis was performed with a random primer and second-strand synthesis was performed using a method that is dependent upon the cap structure at the 5' end of mRNAs. The cDNAs produced were subjected to size fractionation, so that small inserts containing little targeting information would not be included in the library, and inserted into a TMV vector just 5' of gfp. The natural initiation codon of gfp had been removed so that any cDNAs that were not fused in frame with gfp would not produce fluorescent protein on inoculation of plants. First-strand cDNA synthesis was carried out with an oligo-dT primer, complementary to the 3' ends of the mRNAs, to generate a library of cDNAs fused to the 3' end of gfp. After standard second-strand synthesis, the cDNA was normalized to decrease the representation of cDNAs derived from abundant mRNAs.

Subsequently the cDNA was size selected and inserted 3' of *gfp* in a TMV vector lacking the stop codon of *gfp*.

Screening of infection sites Following inoculation of the cDNA-GFP libraries onto leaves, individual leaves were taped down onto large glass slides and the leaf surface scanned under the CLSM. To avoid the use of coverslips, water-dipping lenses were employed to facilitate high-throughput screening. To image the leaf surface, a water droplet was suspended between the objective and leaf epidermis, and then 'dragged' across the leaf surface between adjacent infection sites. In the case of the 3'cDNA-GFP library, approximately 90% of the infection sites displayed a cytosolic protein localisation; that is, the majority of proteins were not targeted to discrete organelles. However, in the case of the 5'cDNA-GFP library, over 50% of the GFP-tagged proteins were targeted to discrete organelles. This result was expected as many proteins exhibit discrete targeting signals at their amino terminus. An example of the screening procedure is shown in Figure 1. Following the detection of a novel subcellular localisation, the infection sites were excised and the virus passaged onto new leaves to confirm the protein localisation, to remove any contaminating viruses, and to produce material for recloning of the cDNA inserts. Reverse transcription of RNA extracted from the passaged tissue was primed with an oligonucleotide complementary to TMV sequence downstream of the cDNA sequences. The reverse transcribed products were amplified through PCR using gfp- and TMV-specific primers that flanked the cDNA inserts, prior to cloning of the amplified products and determination of the nucleotide sequences of the inserts. Sequencing of the cDNA inserts revealed that many proteins were targeted to the predicted organelle, based on the known functions of the proteins encoded by these sequences.

**Plasmodesmatal proteins** As there are no known genes that encode for specific plasmodesmatal proteins, we were curious to determine whether the screen would reveal novel proteins that are intergral to, or interact with, components of plasmodesmata. In an initial screen of over 15,000 infection sites, we detected 12 unique proteins that accumulated specifically

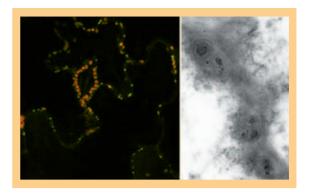
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**Figure 1** Virus-based screening of cDNA-GFP fusion libraries. Each fluorescent infection site that arises on the leaf surface contains a unique cDNA-GFP fusion protein. The infection sites are imaged using a confocal laser scanning microscope fitted with water-dipping lenses. Examples of protein localisations are shown in the inset images. Following detection of a novel protein, the infection sites are excised for subsequent determination of the cDNA sequence.

within plasmodesmata. The sequences encoding these fusion proteins were recloned, sequenced and introduced back into TMV vectors to confirm their subcellular localisation. To establish that the GFP-fusion proteins were unique to plasmodesmata, immunogold labelling was performed, using antibodies against the GFP moiety of the fusion protein. An example of a plasmodesmal-specific protein is shown in figure 2. A major goal of the cell-to-cell communication programme is to study the structure and function of plasmodesmata in relation to plant development and defence. The discovery of unique plasmodesmatal proteins therefore represents a breakthrough in this field, and the functions of such proteins are the subject of further intensive study in our laboratory.

**Nuclear proteins** During the course of screening fluorescent infection sites, we identified over 150 proteins that showed a unique localisation to the nucleus. Many of these proteins were predicted to be nuclear based on



**Figure 2.** Virus-based screening has identified 12 novel plasmodesmatal proteins. These cDNA-GFP fusions show a strong localisation to regions of cell wall containing plasmodesmata. Immunogold labelling of the GFP moiety has confirmed that the cDNAs are located in the plasmodesmal pore.

sequence homology with known nuclear proteins. However, some unidentified proteins revealed unique structures associated with the nuclear envelope or nucleoplasm. For example, one unknown protein decorated the nuclear envelope, and was possibly associated with nuclear pore complexes (Fig. 3A). Another unidentified protein formed discrete sub-nuclear structures that were arranged helically as hollow spheres in non-DNA containing regions of the nucleus (Fig. 3B).

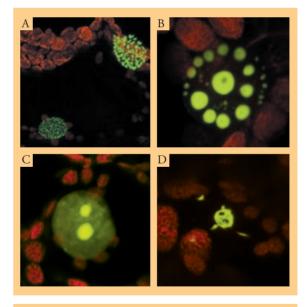


Figure 3 Punctate labelling of the nuclear membrane, possibly associated with nuclear pore complexes. B. An unidentified protein associates with sub-nuclear spherical bodies. C. Localisation of a cDNA-GFP fusion to the nucleolus. D. An unidentified filamentous protein appears to be wrapped around the nucleolus.

Yet other novel proteins were present within the nucleolus (Fig. 3C) or were closely associated with the nucleolus. For example, the protein shown in Fig. 3D appears to be wrapped around the nucleolus in the form of filamentous structures. The further isolation and characterisation of novel plant nuclear proteins forms part of an ongoing collaboration between the Cell-to-Cell Communication and Gene Expression programmes at SCRI.

**Chloroplast proteins** Although many chloroplast proteins were of predicted location and function, some unknown proteins labelled specific structures within chloroplasts. For example, the filamentous structures shown within chloroplasts in Figure 4A contain a protein of unknown function. Some GFP-fusion proteins labelled the thylakoid membranes while others labelled chloroplast protrusions known as 'stromules', which have been shown to interconnect chloroplasts throughout the cell. One of the stromule-labelling sequences was found to encode an aspartate aminotransferase (Fig.4B)

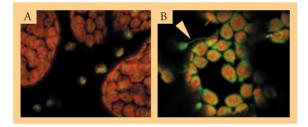


Figure 4. Detection of chloroplast proteins. A. Needlelike structures identified within the chloroplast matrix contain an unidentified protein. B. A cDNA-GFP that labels stromules, hollow extensions of the chloroplast membrane (dart), was shown to have sequence homology with an aspartate aminotransferase.

**Cell wall proteins** Some of the proteins identified through the cDNA-GFP screen were detected in the apoplast. Interestingly, secreted GFP is not normally stable in the apoplast of tobacco leaves, and undergoes rapid degradation by proteases. Thus, some sequences appeared to confer apoplastic stability to GFP. One of the identified apoplastic sequences encoded for a RALF (*Rapid AL*kalisation *Factor*) peptide, a recently discovered peptide hormone involved in defence signalling. Other apoplastic proteins remain unidentified. For example, the GFP-fusion protein depicted in Figure 5 was stable in the apoplast and formed discrete protein 'bridges' between adjacent mesophyll cells.

**Future utility of viral-based cDNA-GFP libraries** The screening of cDNA-GFP libraries is continuing to

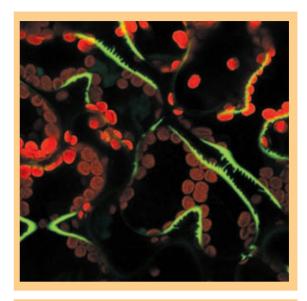


Figure 5. A novel cDNA-GFP fusion labels discrete protein 'bridges' between adjacent palisade mesophyll cells.

identify unique proteins with novel subcellular localizations. While many of the sequences identified by the screen encode for proteins of predicted subcellular 'address', such GFP-fusion proteins are nonetheless likely to find considerable utility as fluorescent tags for different organelles within the cell. In the case of sequences with strong homology to unknown proteins from the Arabidopsis or Nicotiana databases, further characterisation of the function of these proteins is required. In this respect, ascribing a subcellular localisation to such proteins is a useful starting point in identifying their function. The viral-based cDNA-GFP screen is likely to find greatest utility when focussed on a single organelle of interest. For example, the screen has identified putative plasmodesmatal proteins that will form the basis of further functional characterisation of these proteins. During the course of screening the cDNA-GFP libraries, we also noticed that some proteins are expressed specifically in some cell types but not others. For example, the cell-wall wall protein shown in Figure 5 was found extensively between palisade mesophyll cells but not between spongy mesophyll cells. Other proteins were found exlusively in epidermal cells but not mesophyll cells. Thus, the screening procedure outlined here might find utility in identifying proteins that are unique to specific tissues in the plant. In the functional genomics era, ascribing subcellular localisations to proteins will prove to be a useful tool among an armoury of approaches currently being used to assess the functions of unknown genes in plants.

## Phloem development and function probed with a companion-cell marker

K.M.Wright, A.G. Roberts, H.J. Martens<sup>1</sup>, N. Sauer<sup>2</sup> & K.J. Oparka.

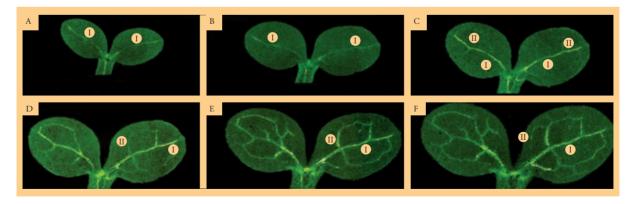
The growth of a leaf from emergence to final size involves periods of cell division, cell and tissue differentiation, and cell expansion. Modern techniques involving the expression of green fluorescent protein (GFP) have enabled us to investigate the structural and functional development of the phloem, the pathway for photoassimilate movement within the leaf.

Transgenic tobacco plants were produced that express GFP specifically in the companion cells of the phloem using the promoter of the Arabidopsis sucrose transport protein, AtSUC21. In Arabidopsis, the AtSUC2 promoter is switched on at the onset of sucrose export, and is only expressed in the functional phloem of source leaves. A similar pattern is seen when the AtSUC2-GFP gene is expressed in tobacco plants, suggesting that the expression of GFP occurs only in those veins that are exporting sucrose<sup>2,3</sup>. The AtSUC2-GFP plants were examined using the confocal laser scanning microscope to locate the expression of GFP, thus enabling us to identify the pattern of functional phloem development in leaves of different developmental stages. GFP expression was taken as

evidence of the vein being functionally mature. Structural maturity preceeds functional maturity. Therefore, if GFP was not being expressed, the electron microscope was used to look at the structural maturity of the veins.

> Basipetal maturation of phloem loading in veins of cotyledons Following germination, the first leaves to appear on a plant are the cotyledons, which provide a source of assimilate for the growing plant. Initially only one vein, the midrib (class I), is present and shows GFP expression (Figs. 1A and B). However, as the leaf grows additional vein classes are formed and these, once mature, also express GFP (Fig. 1C). The pattern of expression shows that phloem maturation occurs basipetally, with the class II veins near the tip of the leaf showing fluorescence before

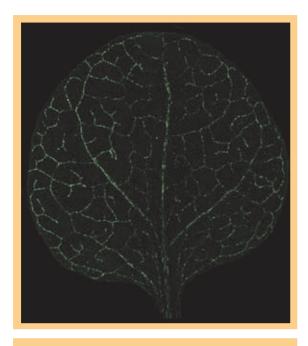
those at the base (Figs. 1C-E). Similarly, class III veins mature basipetally (Fig. 1F). Tobacco has five vein classes; classes I, II and III are called major veins and, in mature leaves, these are normally used for phloem unloading. Classes IV and V, the minor veins, mature once a leaf undergoes the sink-source transition and assumes source status, and these vein classes



**Figure 1** Basipetal development of major veins in cotyledons. AtSUC2-GFP coyledons imaged daily to show the progression of vein maturation. GFP is expressed first in the midrib (I) (A,B), then in class II veins (II) near the tip (C) and then towards the base (D,E,F) of the leaf.

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**Figure 2** A mature AtSUC2-GFP cotyledon expressing GFP in all the major vein classes.

are involved in phloem loading and assimilate export. In cotyledons, which must immediately function as photosynthetic sources, prior to the structural maturation of the minor veins, phloem loading occurs not from the minor veins (which are absent during early development) but from the major veins (Fig. 2). In fact, in young cotyledons, phloem loading occurs from whichever vein classes are functionally mature at the time.

Acropetal maturation of phloem unloading in immature sink leaves In AtSUC2-GFP plants, the GFP synthesised in the companion cells is able to move into the sieve elements and be transported, along with photoassimilate, to developing leaves and other sink areas of the plant. When sink leaves first emerge they have only the midrib to facilitate the transport and unloading of assimilate (Figs. 3A and B). However, as the leaf grows other vein classes are formed and mature in an acropetal direction; the class II veins at the base of the leaf (Fig. 3D) maturing in advance of those at the tip. By the time the leaf is a complete sink, a network of class I, II and III veins has formed. Within this major vein network, phloem unloading of GFP takes place predominantly from the class III veins  $(Fig 4)^4$ .

**AtSUC2-GFP-ER plants** A second line of transgenic plants has been produced in which GFP, again expressed from the AtSUC2 promoter, is targeted to the endoplasmic reticulum. In these plants, GFP is unable to traffick into the sieve element, producing companion-cell autonomous GFP expression which is restricted only to source tissue undergoing phloem loading, In these AtSUC2-GFP-ER plants the cotyledons showed an identical pattern of phloem development to those plants expressing free GFP in the companion cells (Fig. 5A), but in this case the developing sink leaves showed no GFP expression until they commenced the transition from sink to source.

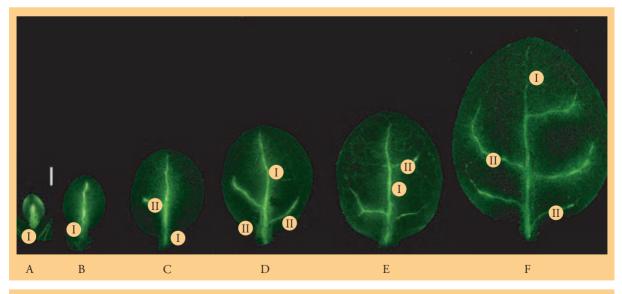
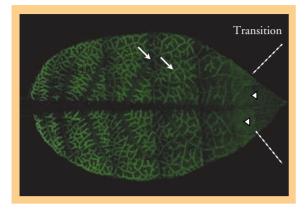


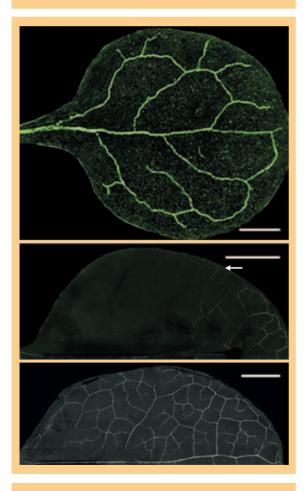
Figure 3 Acropetal development of major veins in sink leaves.

Unloading of GFP into developing AtSUC2-GFP sink leaves takes place initially from the class I vein (I) (A,B), the basal class II veins (II) (C,D) and finally the apical class II veins (E,F).

## Mechanisms & Processes



**Figure 4** Unloading of GFP into an AtSUC2-GFP sink leaf from the major veins (arrows). The transition from sink to source has commenced at the tip of the leaf, as shown by the punctate GFP expression in the minor veins (darts).



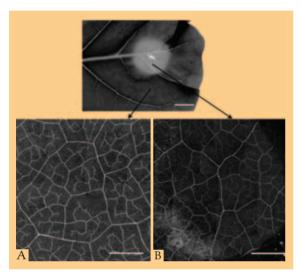
**Figure 5** GFP expressed in the companion cells of AtSUC2-GFP-ER plants. (A) Cotyledon expressing GFP in the major veins. (B) Leaf undergoing the transition to source (arrow) expressing GFP in the major veins at the tip and (C) throughout the leaf. The minor veins in these small leaves have not yet formed.

In plants, the sink-source transition commences with the cessation of photoassimilate unloading from the major veins, accompanied by maturation of the minor veins<sup>4</sup>. Following the functional maturation of the minor veins, phloem loading, as shown by GFP expression (Fig. 5B) begins at the tip of the leaf and progresses basipetally, towards the petiole. It is interesting to contrast the acropetal development of the major veins and subsequent phloem unloading in an emerging leaf, with the basipetal development of minor veins and phloem loading that occurs later during the sink-source transition. However, in the case of cotyledons, there is no other source tissue to support them and so these leaves do not have an initial phase of phloem unloading. Rather, phloem loading must be initiated at the same time as structural and functional maturation of the major vein network proceeds. This makes it necessary for cotyledons to utilise the major veins for phloem loading; a task normally reserved for the minor vein network.



Figure 6 Shading treatment on plants at day 1 (top) and day 12 (bottom). The black disc is 19mm in diameter.

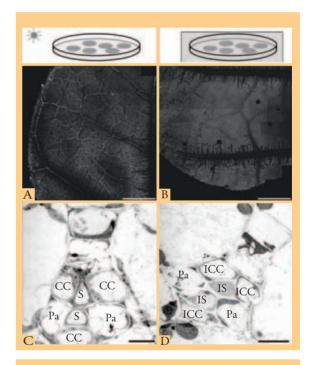
The effect of shading on the sink- source transition In order to study the effect of light on the progression of the sink-source transition, areas of tissue near the tip of AtSUC2-GFP-ER sink leaves were sandwiched between discs of opaque plastic to produce localised areas of shading (Fig. 6). The discs were left in place for 12 days, during which time the leaves passed through the sink-source transition. Following the period of shading, areas of leaf under the discs were examined for GFP expression and compared to neighbouring unshaded tissue. In the unshaded area, GFP was expressed in all the veins (Fig. 7A), both major and minor, as expected. However, in the shaded area, the major veins expressed GFP (Fig. 7B) but the minor veins, although they had matured structurally (data not shown), did not become fluorescent.



**Figure** 7 The effect of partial shading on an AtSUC2-GFP-ER leaf. GFP is expressed in all vein classes in the non-shaded area (A) but only in the major veins within the shaded region (B).

Does the signal for phloem maturation come from the unshaded area? In a parallel experiment, discs were excised from near the tip of sink leaves, floated on water and kept either in the light or dark for 12 days to mimic the unshaded and shaded treatments on intact leaves. However, in this case the leaf discs were isolated from neighbouring leaf tissues. In discs maintained in the light, GFP was expressed in the major veins but not the minor veins (Fig. 8A), although the latter had once again matured (Fig. 8C). In contrast, the minor veins in the shaded discs remained structurally immature (Fig. 8D), and in these discs GFP was not expressed by either the major or the minor veins (Fig. 8B). These data suggest that the signal to initiate expression of the AtSUC2 promoter within the phloem of major veins does not require communication with the rest of the leaf, provided the tissue remains in the light. However, light is not the signal required to initiate AtSUC2 expression in the minor veins. The signal to initiate minor vein development (structural maturation) is potentially light and this signal is different from the one that initiates AtSUC2-GFP expression (functional maturation) in companion cells.

By expressing GFP in tobacco plants, under the control of a companion-cell specific promoter, we have been able to investigate the patterns of vein development in maturing cotyledons and sink leaves. We have also been able to identify a variety of structural and functional control points that regulate the maturation of the minor veins and the expression of sucrose transport proteins within the major and minor veins.



**Figure 8** Leaf discs isolated from AtSUC2-GFP-ER plants. GFP is expressed in the major veins in the light (A) but not in the dark (B). The minor veins have matured in the light (C) but not in the dark (D). (Pa = phloem parenchyma, CC = companion cell, S = sieve element, ICC = immature companion cell, IS = immature sieve element)

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## Investigating gene expression in nematodeinfected roots:

V.C. Blok, M.R. Armstrong, B. Banks, P.R.J. Birch, G. Bryan, L. Castelli, J.T. Jones, A. Kumar, M.S. Phillips, A. Purvis, G. Ramsey, R. Waugh & J. Wishart

Potato cyst nematodes (PCN) cause significant eco-nomic losses in potato production in the UK (>£50million/annum, Parker, 1999). A recent survey has shown that the distribution of the two species of PCN (Globodera rostochiensis and G. pallida) found in the UK is increasing with the latter becoming more prevalent (Minnis et al. 2000). To control PCN, crop rotations, resistant cultivars and nematicides are used. The H1 resistance gene has been incorporated into various commercial varieties and is used effectively to control G. rostochiensis. However, as a consequence G. pallida, for which this resistance gene is not effective, has become more prevalent. No single major resistance gene against G. pallida has been incorporated into UK varieties. Several varieties with polygenic resistance to G. pallida are available but these are not widely grown in the UK. Finding new strategies to control G. pallida is of increasing importance. Research at SCRI, which is funded through SEERAD and EU collaborative projects, is addressing this using different genomic approaches.

Incorporating novel resistance into UK varieties, including the recently isolated *Hero* gene, which gives resistance to *G. rostochiensis* and *G. pallida* in tomato, is underway at SCRI. In addition, understanding the molecular events underpinning resistant responses to pathogens is expected to offer opportunities for enhancing the protection of crops and provide alternative resistance to economically important pests and diseases.

Pathogens vary in their capacity to induce disease depending on the genetic constitution of the host. When the pathogen expresses a specific avirulence (avr) gene and the plant contains the cognate resistance (R) gene, a form of localised programmed cell death called the hypersensitive response (HR) can occur at the site of infection, inhibiting the pathogen. Typical responses associated with the HR include protein phosphorylation, production of reactive oxygen species, ion fluxes and G-protein signalling, all of which contribute to the activation of defence-related genes. Defence-related genes have been shown to encode low molecular mass antimicrobial compounds

called phytoalexins, hydrolytic enzymes such as chitinases and endoglucanases, new cell wall compounds, systemically accumulated proteinase inhibitors and other defence proteins of known or unknown function. In nematode-plant interactions, the classic *R*/*avr* rapid HR is seen with avirulent root knot nematode juveniles (*Meloidogyne*) within 6 hours of invasion of tomato roots of plants with the single dominant *Mi* resistance gene. Strong induction of gene expression is observed using the cDNA-AFLP technique (Fig. 1).

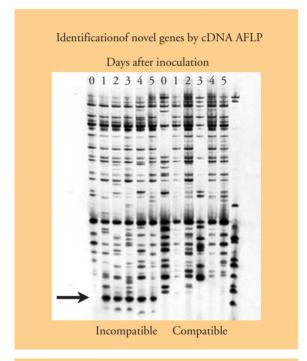
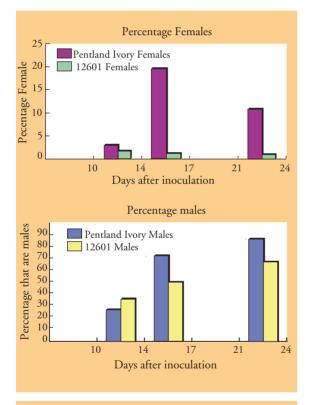


Figure 1 Electrophoresis pattern from cDNA AFLPs showing induction of gene expression in incompatible interaction with Meloidogyne javanica infected tomato with Mi resistance gene but not in uninfected or infected compatible interaction

However, for plant parasitic cyst nematodes the resistant reaction is more typically a delayed necrotic response. Invasion and intracellular movement through the root cells causes wounding and damage; the nematodes eventually settle and begin to modify the host root cells to produce enlarged and metabolically highly active feeding sites. Histological examina-

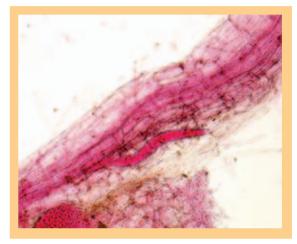
## Mechanisms & Processes

tion of roots shows that, initially, the incompatible/resistant interaction appears like a compatible interaction. However, after several days the feeding site development is restricted and necrosis surrounding the developing feeding site is often observed. Development of invasive stages of the nematodes is delayed and, typically, development of females is poor with males predominating in the adult stage thus limiting the reproductive potential of the pest (Fig 2). The histological responses of the host to cyst nematodes with a single major resistant gene or polygenic resistance have thus far been found to be similar. Currently, additional sources of resistance to G. pallida in the Commonwealth Potato Collection are being examined histologically to determine if different mechanisms of resistance can be found.



**Figure 2** Percentage of females A) of males B) at different time points in a susceptible (Pentland Ivory) or a resistant (12601) host.

We are examining changes in gene expression in infected potato roots resistant to PCN with the aim of identifying components of the plant response pathways. This is being done in parallel with similar studies comparing responses in potato to other nematodes (i.e. *Meloidogyne chitwoodi*) and to the foliar pathogens *Phytophthora infestans* and *Erwinia carotovora*. By contrasting these different pathosytems of potato during incompatible and compatible interactions, candidate genes for potential exploitation in developing broad-spectrum resistance are being identified. Host responses specific to incompatible interactions have not been well characterised at the molecular level. Induction of genes typically associated with plant defence responses, such as peroxidases, chitinases, lipoxygenases and proteinase inhibitors have been observed in incompatible interactions but these have also been observed to accumulate in compatible responses. We are attempting to identify specific responses in resistant interactions.



**Figure 3** Feeding PCN juvenile nematode in potato root tissue stained with acid fuchsin.

One approach we have used is a PCR-based method, suppressive subtractive hybridisation (SSH), which enriches for rare, differentially expressed transcripts. The enriched libraries were sequenced and database searches were conducted using FASTA, BLASTN and BLASTX on c. 2000 sequences. Many of the sequences obtained, when compared to those in databases, were similar to either plant or nematode expressed sequence tags (ESTs). However, many showed no reliable sequence similarities. In some experiments, a significant number of sequences (25-72%) were similar to potato cyst nematode ESTs, indicating that considerable enrichment for rare sequences had occurred, as nematodes comprise a tiny proportion of the root biomass (Fig 3). These include genes coding for proteins expressed in the oesophageal and dorsal glands (Fig 4), such as  $\beta$ -1,4-endoglucanase, which is thought to play a role in root invasion and feeding site development and is believed to have been acquired by horizontal gene transfer from bacteria. Other genes code for structural proteins such as 40S and 60S ribosomal proteins, calponin, and other functionally interesting genes such as surface-expressed fatty acid binding proteins.



**Figure 4** In situ hybridisation of preinvasive PCN juvenile nematode with cellulase gene.

During the development of the feeding site of PCN, cell walls degrade giving rise to a multinucleate, highly metabolically active syncytium. Consistent with these changes in cell wall structure, xyloglucan endo-transg-lycosylase, an important component in cell wall metabolism has been identified in SSH libraries from incompatible interactions between different potato genotypes and PCN and RKN. Interestingly, from the compatible interactions, plant-derived  $\beta$ -1,4-endoglucanases, similar to those also produced by nematodes, were identified. From an incompatible interaction, germin-like proteins were identified. These proteins are believed to be involved in defence responses to fungal pathogens (Lane 2002).

In an alternative approach to identify gene expression differences in resistant and susceptible roots, the 10 most susceptible and resistant clones from a F1 cross of PCN resistant and susceptible parents were bulked. AFLPs performed on these bulks have revealed numerous AFLP bands that segregate with PCN resistance. Expressed sequences that also segregate with PCN resistance may be involved in a resistance pathway. Using the same bulks, cDNA-AFLPs were performed and bands present in the resistant and absent in the sensitive bulks were excised, cloned and sequenced. PCR primers were developed to each of these sequences and used to find single nucleotide polymorphisms (SNPs) in the parents of mapping populations. Having verified that the SNP markers segregate with the resistance phenotype, the map locations of the SNPs are being determined.

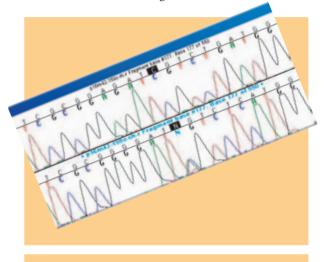


Figure 5 Sequence of a cDNA from a susceptible and resistant host showing a SNP.

Through these approaches candidate genes that may have utility both as markers to assist selection in breeding programs, and which can be manipulated through genetic engineering to provide novel sources of resistance, are being found. In the future providing novel resistance to important crop pathogens which leads to reduced reliance on pesticides is in line with environmentally sound and sustainable agriculture objectives.

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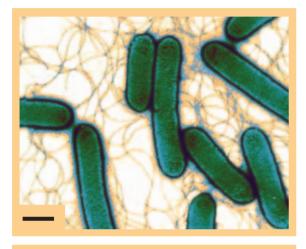
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## *Erwinia* Genomics: A new era in the battle against potato disease

Toth I.K., K.S. Bell, M.C. Holeva, G.J. Bryan, A.O. Avrova, L.J. Hyman and P.R.J. Birch

**Evinia research at SCRI** For over 30 years SCRI has been a world-leader in research on the potato pathogen *Erwinia carotovora* subsp. *atroseptica (Eca)* (Fig. 1), the causal agent of blackleg disease of field



**Figure 1** Electron micrograph of *Erwinia carotovora* subsp. *atroseptica* following colour enhancement. Bar =  $1.0 \mu m$ .

crops and soft rot disease of stored tubers (Fig. 2). This research has focussed on the development of diagnostics, both as predictive tools for growers and for use in epidemiological studies, allowing pathogen movement to be followed through the growing season and between crop generations. The research has had a major impact on the way growers manage their crops and, as a consequence, on decreasing disease inci-



Figure 2 Potato plant with symptoms of blackleg disease.

dence. However, it remains a major problem in many potato-growing regions and more research is thus needed to continue the fight against this disease.

The growth of genomics and microbial genome sequencing Since the structure of DNA was first described in the 1950s, molecular biology has revolutionised plant pathology. Less than 50 years on, in 1995, the genome of *Haemophilus influenza* was the first free-living organism to be completely sequenced (Fleischmann *et al.* 1995). Seven years later there are over 70 complete genome sequences, and almost 200 in progress, providing information relevant to many aspects of microbial life. These sequences are being used successfully to find genes involved in disease, new targets for drugs, and a plethora of other basic physiology, cell biology and evolutionary functions (Doolittle 2002).

Microbial genome sequencing has led to a number of exciting new discoveries, e.g. how microbes have evolved and adapted to different environments and ways of life, and how genomes lose and acquire genetic information. Currently, around a quarter of the genome sequences are from medically-related pathogens. These genomes have allowed comparisons between virulent and non-virulent strains of the same organism to pin-point the genetic bases for these differences in virulence (Perna et al. 2001). Sequence comparisons have also shown clearly how organisms from a common ancestor can adapt to different niches during divergent evolution (Cole et al. 2001). Common mechanisms between pathogenic bacteria have been discovered, such as the Type III secretion system used to inject proteins into plant and animal cells or pathogenicity islands, which are large regions of DNA carrying clusters of genes correlated with virulence. Microbial genome sequences are the basis on which new drugs, vaccines and diagnostic tools are now being developed (Doolittle 2002), and some genomes may prove useful in bioremediation and biotechnology.

The first plant pathogen genome to be fully sequenced was *Xylella fastidiosa* (Simpson *et al.* 2000), and there are now a handful of others, *i.e.*, *Agrobacterium tumefaciens*, *Ralstonia solanacearum*, Xanthomonas campestris pv. campestris and X. axonopodis pv. citri, with more on the way. New insights into the life of these pathogens are emerging. For example, the genome sequence of X. fastidiosa appears to lack avirulence genes and the Type III secretion system needed for their injection into host cells. R. solanacearum, however, does have a Type III system and over 40 candidate proteins for secretion via this mechanism, many of which had not been identified prior to genome sequencing (Salanoubat et al. 2002).

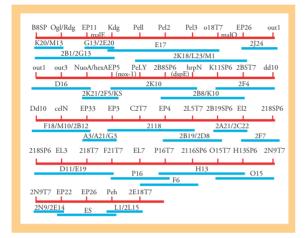


Figure 3 Physical map of part of the *Erwinia carotovora* subsp. *atroseptica* genome. Blue bars represent individual BAC clones. Red line represents map with a number of known genes and other sequences marked.

**Molecular research on** *Erwinia* at SCRI In 1998, through collaboration with the plant genomics group at SCRI, physical maps of selected regions of the genomes of *Eca* and *Phytophthora infestans* were made using bacterial artificial chromosome (BAC) libraries (Bell *et al.* 2002; see article by Whisson *et al.*). The *Eca* library, consisting of DNA fragments of approximately 100 kb (from a total of around 5 Mb), was ordered using AFLP fingerprinting, and many published *Erwinia* genes and genes from our research were placed on the physical map through hybridisation (Fig. 3). From this mapping exercise, a Type III secretion system (*hrp* cluster) was identified within the genome of *Eca* and is now being assessed for its role in pathogenicity and host range.

The genomic region spanning the hrp cluster was examined in detail by sample sequencing of two overlapping BAC clones (ca 200 kb) ( Bell et al. 2002). These sequences revealed the presence of 28 hrp genes previously found in the fruit pathogen Erwinia amylovora (Ea) but also a number of unknown potential pathogenicity genes. Targeted sequencing was used to close gaps between these gene sequences and produce the entire hrp cluster sequence, which was analysed using bioinformatic software, including ARTEMIS and BioEdit to predict the positions, and structural and functional properties of individual genes within the cluster (Fig.4). A number of structural genes involved in the formation (*hrcC* and *hrcV*) and genes involved in the regulation (hrpL) of the Type III system were mutated, along with others thought to be exported via this system (dspE, hrpN and hrp W). These mutants are currently being tested on both tobacco (non-host) and potato (host) to determine the role of the hrp cluster in host range and pathogenicity. Potential pathogenicity genes that were previously unknown in *Eca* included *dsp*EF (essential for pathogenicity in Ea), adhesin-, haemagglutininand haemolysin-like genes, (involved in attachment and pathogenicity in animal pathogens and also present in the genome sequence of Xylella fastidiosa), and sequences similar to opine catabolism genes (synthesised in planta during infection by Agrobacterium tumefaciens to provide a specialized nutrient source).

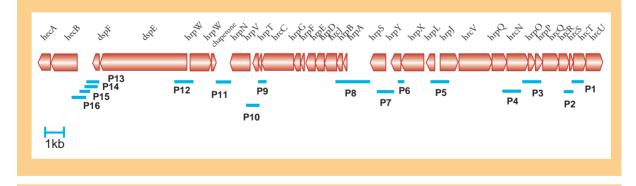


Figure 4 Size and position of genes within the hrp cluster in *Erwinia carotovora* subsp. *atroseptica* together with the position of amplification products P1-P16 used to close gaps in the sequence.

## Mechanisms & Processes

**Erwinia** genome sequencing Collaboration with the Wellcome Trust Sanger Institute in Cambridge (http://www.sanger.ac.uk/Projects/E\_carotovora/) was initiated in 2002, to sequence the complete genome of *Eca.* This project also involves high throughput gene functional analyses based on the complete sequence. Shotgun sequencing of the genome to 8-fold coverage is now complete and almost all sequence gaps in the genome have been closed. Once complete, the sequence will be annotated using the software ARTEMIS to predict structural and functional properties of genes within the sequence. Computer-based

predictions relating to evolution, regulation, secretion, plant interaction, protein folding and many other factors will be examined in collaboration with both BioSS and the University of Dundee.

The *E. chrysanthemi* (*Ech*) genome is also being sequenced in the USA. This project, led by Dr. Nicole Perna from the University of Wisconsin, is being undertaken by an

international consortium, of which

SCRI is a member (http://www.ahabs.wisc.edu:16080 /~pernalab/index.html). Once completed, the *Eca* and *Ech* genomes will be compared with each other to identify variations in genomic content that may account for differences in host range or disease symptoms. They will also be compared with genome sequences from the closely related animal pathogens *E. coli, Salmonella* sp. and *Yersinia pestis*, and a variety of plant pathogens to identify genetic similarities and differences that may be responsible for common or unique modes of attack.

**Future research** From studying the *Eca* genome sequence, we already have a number of new lines of research. These include proteomic analyses, currently being undertaken in collaboration with the Moredun Research Institute (MRI) to identify, for example, proteins secreted *via* the Type III system that may interact directly with the plant to cause disease (effectors) or trigger a resistance response (elicitors). We

have also made a mutation grid of 5,000 transposon mutants, with plans to expand to 40,000, to allow rapid selection and screening of gene mutations in plant tests (assessing changes in disease symptoms and non-host reactions). Gene expression during the early *Eca*-potato interaction, currently being studied within the group using cDNA-AFLP (Dellagi *et al.* 2000), will continue to be an important part of our research, and will include techniques such as suppression subtractive hybridisation (SSH) and microarray analyses.

#### The potential of genomic approaches for blackleg con-

trol Understanding the interaction between Eca and potato is paramount to controlling blackleg disease and we have been doing this on a fieldscale for many years. The new research aims to complement this by looking at the potato - Eca interaction at a molecular level. Genes from Eca proteins found interact to directly with the plant will be used to investigate resistance responses in potato. For

example, expression of secreted

pathogen proteins in potato species within the Commonwealth Potato Collection (CPC) may identify novel sources of resistance, and rapidly facilitate introgression of such resistance into Solanum tuberosum. Type III secretion systems are found in many pathogens, both plant and animal, and have potentially important biotechnology applications. For example, homologous genes in Ea have been patented, as their proteins (under the name "Messenger"), when used as crop sprays, both elicit resistance responses in the crop and enhance plant growth(http://www.edenbio.com /tk/tkmain\_whitepaper.html).Potential elicitors in Eca similar to "Messenger", and other as yet unknown targets, will be investigated for commercial use as well as a role in disease reduction. Ways are also being sought to prevent Eca attacking the plant, e.g. by identifying an "achilles heel" within the pathogen that may respond to small increases in levels of natural plant anti-microbials or oxidative stress (Lopez-Solanilla et *al.*, 1998; Reverchon *et al.*, 2002) or disrupting the disease process by altering natural levels of chemicals/proteins, such as homoserine lactone (HSL), within the pathogen (our work and Mae *et al.* 2001).

In summary, genomic research offers a new and very powerful approach to investigating both the pathogen and its host, offering the potential to provide biotechnological solutions to blackleg control. The research is also expected to lead to the discovery of targets for commercialisation, as well as providing high quality science, forming the core of future *Erwinia* research at SCRI.

For further information visit the *Eca* sequencing web site at http://www.scri.sari.ac.uk/TiPP/Erwinia.htm

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# Late blight in the 21st century: new tools for an old problem

S.C. Whisson, A.O. Avrova, M. Armstrong, E. Campbell & P.R.J. Birch

ver 150 years have elapsed since the first epidemics of a plant disease called late blight devastated the potato crops of North Western Europe. The severe impact of these epidemics on communities that relied heavily on potato as a staple food provided a major impetus to the study of plant pathology. Late blight disease was shown to be caused by a 'fungus' called *Phytophthora* (plant destroyer) *infestans* and since these early epidemics of late blight, intense efforts have been directed to identify new ways to control the disease. Potato cultivars containing major gene resistance (single resistance genes conditioning complete resistance) were used initially to control late blight, but P. infestans rapidly overcame the deployed resistance genes. Use of fungicides has also had some success (metalaxyl for example), but P. infestans can adapt to become resistant to them. In addition, agrochemicals require many repeat sprayings throughout the growing season, sometimes as often as every 5 to 7 days, to be effective. The cost of chemical treatments can greatly diminish the monetary returns from a potato crop, and spraying of chemicals is not viewed by many to be environmentally friendly.

Currently, late-blight is considered the most serious constraint to the production of potato, the world's fourth most valuable crop plant. Worldwide losses due to late blight and control measures are estimated to exceed £3 billion annually. *P. infestans* is thus regarded as a threat to global food security. Despite its economic importance, relatively little is known about the fundamental molecular biology underlying *P. infestans* development, its capacity to cause late blight (pathogenicity), and the factors that restrict late blight to potato and tomato (host-specificity and avirulence). Modern molecular and biochemical studies have placed *P. infestans* in the oomycetes, a class of organisms distinct from the true fungi, and more closely allied to heterokont algae. The oomycetes contain many other important plant pathogens such as *P. sojae* (soybean root rot), *Peronospora parasitica* (Arabidopsis downy mildew), *Bremia lactucae* (lettuce downy mildew), and *P. ramorum* (sudden oak death).

> In recent years, research efforts directed at P. infestans have begun to utilise the latest genomics technologies to further the knowledge of this plant pathogen. At SCRI there is an integrated program of genomics research targeted at understanding the molecular mechanisms involved in the P. infestans/potato interaction. As a result, P. infestans is fast becoming the model oomycete plant pathogen for conducting such studies, as resources such as expressed sequence tags (ESTs), bacterial artificial chromosome (BAC) libraries, and genetic linkage maps have been developed. In addition, techniques for targeted gene discovery, transformation for analysing gene function, and gene silencing have also been greatly improved (reviewed in

Birch and Whisson, 2001). We aim to use all the resources and techniques available for studying *P. infestans* to better understand the development of late blight, and exploit this knowledge for more durable control measures in future.

**Life cycle stage specific gene expression** The life cycle of *P. infestans* is complex, involving differentiation into as many as eleven different cell types. These cell types are highly specialised for life cycle stages involved in sexual and asexual reproduction, propag-

ule dispersal, spore germination, host penetration, and biotrophic and necrotrophic phases of infection. The differentiation of so many cell types requires the upor down-regulation of many genes. Of particular interest are those cells formed shortly before and early in the interaction with the host plant, such as germinated zoospore cysts, germ tube, appressorium, infection peg, and infection vesicle. *P. infestans* is hemi-biotrophic and can form most of these cell types in the absence of a host plant. We anticipate that many genes required for successful infection of the host plant will be up-regulated in these stages. Indeed, preliminary results indicate that some of the genes isolated to date are up-regulated in these cell types and also *in planta*.

Crucial to investigating and exploiting natural resistance mechanisms to *P. infestans* is an understanding of the molecular events occurring during early interactions between *P. infestans* and plants. This stage of the interaction of the pathogen elicits plant defences (Birch *et al.*, 1999). The target elicitors are critical signalling molecules whose identity is central to understanding the outcome of the interaction.

Suppression subtractive hybridisation (SSH) and amplified fragment length polymorphism (AFLP)based mRNA fingerprinting (cDNA-AFLP) have been used to identify genes up- and down-regulated during specific stages of the life cycle and infection. These powerful, PCR-based techniques are routinely used at SCRI for discovering genes in a range of pathogen/plant interactions (see articles by Blok et al. and Toth et al.). SSH cDNA has also been labelled as a complex probe and used to screen a BAC library generated at SCRI that contains the P. infestans genome arrayed on filters (Whisson et al., 2001). In this approach, BAC clones containing clusters of coregulated genes were identified. Full-length genes, derived from both cDNA-AFLP and SSH, can be determined from corresponding BAC clones, or from EST databases.

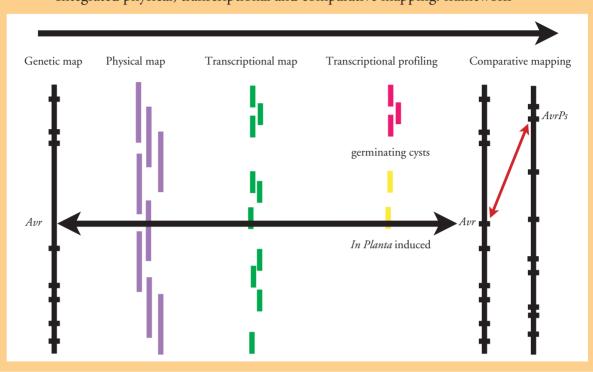
From both cDNA-AFLP and SSH of pre-infective stages, we have identified many *P. infestans* genes that are up-regulated during the infection process. Many of these show significant sequence identity to pathogenicity factors such as cell wall degrading enzymes, but many are more closely associated with stress and defence responses. This suggests a hostpathogen interaction where both pathogen and host are simultaneously attacking and defending. A more

targeted gene discovery approach focussing on the two major phases of infection, biotrophic and necrotrophic, has resulted in the discovery of two novel gene families in *P. infestans* that are very tightly regulated during infection. One of these has been shown to be highly up-regulated at the infection time point corresponding to the transition phase from biotrophy (parasitism) to necrotrophy (cell killing). Technical advances in the detection of gene expression using real-time RT-PCR technology has allowed us to identify and quantify levels of gene expression in P. infestans/potato interactions. This is particularly important at the very early stages of infection, where the fate of the interaction (resistance or susceptibility) is decided. This is the first report quantifying pathogen gene expression at this early infection stage in any plant/pathogen interaction.

Avirulence genes *P. infestans* has a narrow host-range and a hemi-biotrophic mode of infection. Host species and race specificities are determined soon after penetration of the host plant (12 - 24 hours) and are reliant on complex signalling between host and pathogen, and within the pathogen itself. Resistance (whether mediated by the products of resistance [*R*] genes in the host, or as yet uncharacterised receptors in the non-host) is based, primarily, on recognition of a particular elicitor component (or avirulence factor) from the pathogen. Recognition of pathogen avirulence factors triggers the hypersensitive response (HR), a form of localised programmed cell death that restricts further spread of the pathogen, in both resistant host and non-host plants

At least 11 R genes active against races of P. infestans have been introgressed from Solanum demissum into the cultivated potato, S. tuberosum. As yet, only one potato R gene (R1) responsible for race specific recognition of P. infestans has been isolated. However, the positions of R1, R3, R2, R6, and R7 have been genetically mapped. Only a few avirulence genes have been cloned from fungi (e.g. from Cladosporium and Magnaporthe) and one from the oomycetes. In the biotrophic fungi, which often form structures called haustoria that exist within the living plant cell, nothing is known about the mechanisms of delivery of potential pathogenicity or avirulence gene products.

We have adopted two approaches to identify avirulence genes in *P. infestans*. Firstly, we are using a map based (or positional) approach to target avirulence gene Avr2 (matching the resistance gene R2). Here we are characterising the genetics of avirulence to pin-



#### Integrated physical, transcriptional and comparative mapping: framework

**Figure 1** Overview of the SCRI *Phytophthora infestans* integrated genomics programme. Genetic and physical maps will yield insights into overall genome architecture. A transcriptional (gene) map, coupled with transcriptional profiling (gene discovery) will yield information on genes expressed at specific life cycle stages, or during infection of potato. Comparisons with other oomycete pathosystems are possible through a comparative genomics approach. The example given is for a hypothetical avirulence locus (*Avr*), located in a cluster of genes that are up-regulated during infection of potato; a similar locus would also exist in a comparative system (called *AvrPs*).

point the genome region containing *Avr2*. We will then use this genetic knowledge as a basis for identifying the gene from our BAC library.

A second approach is using association genetics. From a large set of P. infestans ESTs we, in collaboration with S. Kamoun (Ohio State University, USA), have identified a small selection of P. infestans genes that will be assayed for single nucleotide polymorphisms (SNPs) associated with avirulence towards specific potato R genes. These genes were selected on the basis of similar features to avirulence genes cloned from other plant pathogens, and the presence of a predicted signal peptide for secretion. Since the avirulence molecules themselves must cross the plasma membrane of the pathogen to be perceived by the host plant, avirulence gene sequences are predicted to encode a signal peptide for secretion. This has been a highly rewarding exercise, with our first screen of sequences identifying a SNP completely associated with Avr3 (matching R3). Further experimental work is now required to characterise the function of this candidate Avr gene.

**Structural genomics** Similar to the project aimed at reconstructing the genome of the potato pathogen *Erwinia carotovora* (see article by I. Toth *et al.*), efforts are underway at SCRI to understand the organisation of the *P. infestans* genome. We aim to link genetic, physical and transcriptional maps for *P. infestans* to allow rapid positional cloning of genes involved in avirulence, and to aid identification of genes involved in sexual compatibility and resistance to agrochemicals.

A genetic map, consisting of AFLP markers and avirulence loci, has been constructed. The SCRI *P. infestans* BAC library was based on an  $F_1$  individual from the same cross used to construct the genetic linkage map (Whisson *et al.* 2001). Mapped AFLP markers are being used to anchor the physical map by AFLP fingerprinting pools of the BAC clones, and non-polymorphic amplified fragments will be used to extend the physical map. A physical map of the *P. infestans* genome will serve as a central resource for the international *P. infestans* research community to locate biologically derived genomic data such as ESTs, and anchor any future genome sequencing for *P. infestans* (Figure 1). By locating ESTs on BAC clones, generich regions of the genome will be revealed. It will also be possible to assess if co-location of co-regulated or related genes occurs in *P. infestans*, as well as any gene duplication. When the transcriptional map is combined with a genomic physical map, a broader view of genome organisation will also emerge.

Integration of the published genetic linkage map with a physical map of contiguous BAC clones is underway and is expected to cover a large proportion of the *P. infestans* genome. Many ESTs from Phytophthora sequence databases, and from the SCRI gene discovery program, have also been placed on BAC clones. Initial results indicate that genes specifically regulated during pathogenesis on potato are clustered in the *P. infestans* genome.

**Comparative genomics** *P. infestans* is a hemibiotrophic oomycete pathogen that predominantly infects the foliage of potato plants. Related oomycete pathogens such as P. sojae and Peronospora parasitica have very different life styles to P. infestans. P. sojae is a hemi-biotrophic pathogen of soybean that infects predominantly through the roots, and *Pe. parasitica* is a strictly biotrophic pathogen that infects Arabidopsis thaliana foliage. Our gene discovery programme also includes comparative analyses with the pathogens mentioned above, especially Pe. parasitica. Comparisons of genomic organisation for similar genes in the P. infestans genome with other oomycete genomes will reveal common or distinct regions that may include genes involved, respectively, in pathogenicity and host-specificity. Conservation of genome organisation in these regions may suggest a central role in host infection.

A comparison between genomes involving a series of genes linked to the *Atr*1Nd avirulence locus in *Pe. parasitica* (Rehmany *et al.*, 2002) has shown that the order of genes in *P. infestans* is conserved. However, we do not know if this locus is also involved in avirulence in *P. infestans*. Other genome comparisons between these pathogens are ongoing.

**Summary** Diseases of crop plants have a significant impact on the economics of agriculture in the UK and many other countries. Current late blight control measures relying solely on major resistance genes in potato are not effective, and spraying with chemicals is expensive and increasingly less socially acceptable. A greater understanding of plant resistance and its underlying biochemical mechanisms will be of use in the development of durable resistance in potato (see article by V. Blok *et al.*). However, conducting an integrated programme of genomics research in parallel on the pathogen will yield those components of the interaction that must also be considered in any future resistance deployment.

Implementation of this integrated research programme at SCRI, studying both sides of the interaction between host and pathogen will lead to an intimate knowledge of the mechanisms and processes involved in pathogenicity, avirulence and resistance, and pathogen life cycle. Identification of biochemical or signalling pathways involved in infection and recognition will be important in designing strategies or targeting chemicals that allow broad-range control of these important pathogens. Exploitation of this knowledge will enable novel control strategies for late blight to protect potato production in Scotland, and many other potato producing countries worldwide.

#### Acknowledgments

Some of the work described was done in collaboration with Sophien Kamoun and Trudy Torto at Ohio State University, USA, and Anne Rehmany, Peter Bittner-Eddy and Jim Beynon at Horticulture Research International, Wellesbourne, UK.

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## Genes to Products

#### H.V. Davies & R. Waugh

The reorganisation of SCRI's science strategy and management structure has brought together, under the 'Genes to Products' Theme, the key disciplines and approaches required to deliver added value products into the food chain. The primary research thrust of the Theme is the development and application of tools and resources required to address, in a novel way, biological questions which limit the development of next generation crops and plant-derived products. The Genes to Products Theme incorporates two research programmes, 'Quality, Health and Nutrition' and 'Genome Dynamics'.

A primary goal of the Theme is to develop added value products by combining the power of contemporary gene discovery and germplasm enhancement tools with targets that can be delivered through a comprehensive understanding of plant biochemistry and phytochemistry. Genetic, molecular, phytochemical and biochemical diversity will be explored to identify the important regulatory mechanisms which govern the traits targeted. These include enhancement of the nutritional value of foods, the introduction of novel texture and flavour and improved quality for specific market outlets e.g. the malting industry. This will be achieved through a combination of basic and strategic research with contemporary plant breeding as the primary vehicle to deliver products to the market place. Links and collaborations with the other scientific themes will be imperative to deliver scientific novelty and products which fulfil the important requirements of sustainable production practices Clear goals of the Genes to Products Theme include:

The exploitation of genomics to provide critical information on the importance of genome organisation and architecture, gene function, allelic variation and gene dosage in trait establishment using species of distinct relevance, but not unique to, the Scottish economy.

The identification of plant products, processes and underlying regulatory elements relevant to the development of produce that will enhance consumer health and well-being.

The exploration of genetic, biochemical and phenotypic diversity to nurture and develop new niches for plants and plant products (new habitats, new functionalities, novel compounds). Germplasm enhancement using all available and relevant technologies which provide a competitive advantage to SCRI in terms of both research and development remains a very high priority.

Consumers will be important end users and beneficiaries of the products developed (enhanced nutritional content/health promoting properties of foods, improved/novel organoleptic properties, advanced food safety testing procedures). However, added value should be gained throughout the production and processing chains (e.g. seed producers [new culti-

### Genes to Products

vars], niche markets for growers, reduced processing costs, new products for food manufacturers, processors and retailers e.g. branded products and improved choice). The basic science components will make a significant contribution to our current knowledge of processes and mechanisms.

Quality, Health & Nutrition Programme In an international retail world that is becoming even more competitive and consumers much more demanding of products, both in its design and value for money, the marketability and success of a product is becoming more complex. Two areas being given much more attention are: (1) nutritional profile and value and

(2) quality and functional properties (for food, drink and industrial market sectors). These parameters need to be explored via collaborative research between scientific bodies and private industry. Traditionally crops have been bred for high yield, and disease resistance and certain components of quality e.g. waxy maize and low sugar potatoes for processing. Far less attention has focused on the enhancement of crops for nutritional value in its broad-

est sense, although consumers are now becoming very discerning with respect to the food they buy and eat from both plant and animal origins.

Opportunities exist to improve the quality, uniqueness and nutritional value of raw materials entering the food chain. This includes commodities important to the Scottish economy and particularly where the commodity e.g. potato, contributes significantly to the daily diet. Further opportunities exist to develop germplasm which is differentiated through improved flavour and texture or which produces clear efficiency gains in downstream processing e.g. malting. Genetic diversity in the attributes under investigation will play a crucial role in formulating our understanding of the fundamental processes and the genes or allelic variants involved. This will be achieved through key interactions between scientists with expertise in phytochemistry, biochemistry, genomics and applied genetics. Interactions will be driven by common goals and targets and by integrated delivery platforms, which translate knowledge gained at the level of gene and cell into germplasm enhancement and performance evaluation in a commercially relevant environment. The programme supports four primary research topics: antioxidants and bioactive pigments, mechanisms and processes regulating organoleptic properties, cereal quality and metabolomics (as an underpinning platform technology).

**Genome Dynamics Programme** The programme aims to exploit genomics and informatics technologies and resources to explore the inherent dynamics of the plant genome through an analysis of diversity and evolution under natural selection, and through domestication, plant breeding and historically imposed selection. Investigating the dynamics of the genome is central to understanding developmental and evolutionary mechanisms and to provide a rationale for crop improvement or diversification via tradi-

> tional or biotechnological means. A key challenge is to relate variation at the level of the gene and of the genome to variation in biochemical and other important phenotypes such as adaptation to environmental stress. Detailed knowledge of, and accessibilappropriate ity to, germplasm is essential, and bio-diverse germplasm collec-(such tions as the Commonwealth Potato Collection)

coupled with genomics and profiling technologies provide the basic platform for our research.

The Programme supports three primary research areas; Contemporary Genetics, Environmental Genomics & Biodiversity and Crop Evolution which are underpinned by a significant commitment to Genomics Resource and Enabling Technology development. The entire Pogramme focuses on the exploitation of modern genetic enabling technologies such as Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs) to address a range of end-user relevant biological/genetical questions. In addition, resources such as comprehensive EST collections, large insert Bacterial Artificial Chromosome (BAC) libraries and experimentally developed populations including Doubled Haploid (DHs), Recombinant Chromosome Substitution Line (RCSLs) and mutant populations for barley and multi-trait selection pedigrees for potato are key components of our strategy to link genes to phenotypes. Technology exploitation through a focused germplasm improvement programme is a key mechanism for delivering the products of our research to the commercial sector and ultimately the marketplace.

## Ascorbic acid biosynthesis in higher plants and micro-organisms

#### R.D. Hancock & R. Viola

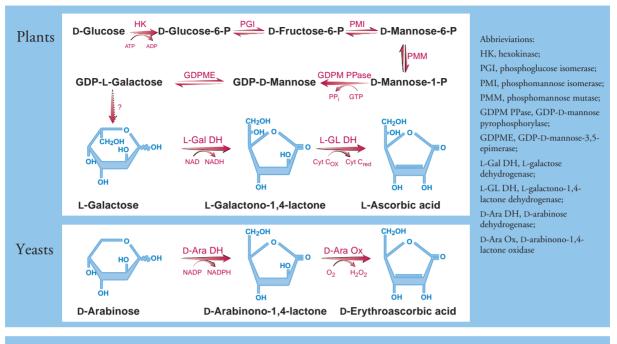
Inlike most animals, humans and a small number of other species are unable to synthesise L-ascorbic acid (L-AA; vitamin C) and must obtain the vitamin by dietary intake. As L-AA is the major soluble antioxidant found in higher plant tissues, plant foods represent the primary source of this essential compound in the human diet. Together with flavonoids, polyphenolics and water insoluble compounds such as  $\alpha$ -tocopherol (vitamin E), L-AA contributes to the overall intake of 'free radical scavengers' or 'antioxidative metabolites' in the human diet. There is now convincing evidence that such metabolites singly and in combination, benefit health and well-being, acting as anti-cancer forming agents and protecting against coronary heart disease. There is increasing pressure for increasing the recommended daily intake (RDA) of L-AA currently at 60 mg/day in the UK but recently raised to 90 mg in the US. Although, vitamin C intake through synthetic supplements is widespread, it is accepted that intake of vitamin C from foods has the great advantage of simultaneously providing many other health promoting nutrients, such as bioflavonoids, carotenes and iron whose absorption is facilitated by vitamin C.

The goal of developing 'functional foods' or products with enhanced 'nutraceutical' qualities has become a major goal world-wide - not necessarily to prolong life but to ensure a high quality of life. Therefore one objective of our research at SCRI is to optimise the level of L-AA in the edible parts of crop plants based on a sound fundamental knowledge of the genetics, biochemistry and physiology of L-AA biosynthesis and distribution in plants. This strategy may therefore be beneficial not only for the increased quality value of the crop as a commodity but also through increased performance of crop plants in the field. In fact L-AA is also essential for plant life through detoxification of peroxide, ozone, and free radicals and providing protection from damage caused by the accumulation of ROS generated under adverse environmental conditions. Moreover L-AA plays a key role in photosynthesis by detoxifying superoxide anions and hydrogen peroxide in chloroplasts and by regenerating the membrane-soluble antioxidants ( $\alpha$ -tocopherol) and zeaxanthin.

In addition to naturally synthesised L-AA, over 80,000 tonnes of synthetic L-AA are produced each year and used to manufacture vitamin supplements, in the food processing and beverage manufacturing industries as well as a supplement in animal feeds. The majority of L-AA is currently produced by primarily chemical means via the seven-step Reichstein process. Alternative methods use prokaryotic fermentations to synthesise intermediates of the Reichstein process, as these industrially useful micro-organisms lack the ability to synthesise L-AA. However, progress in our understanding of L-AA biosynthesis in plants have resulted in the development of alternative strategies for the synthesis of this important commodity with a global market in excess of U.S. \$ 600 million.

L-AA Biosynthesis and Distribution in Plants The L-AA biosynthetic pathway in animals was elucidated during the 1950's following experiments with unlabelled and <sup>14</sup>C-labelled precursors in rats. Progress in the area of L-AA biosynthesis in plants has been much slower. Early pathways proposed for the biosynthesis of L-AA in higher plants<sup>1</sup> based on the animal model conflicted with biochemical observations from radiolabelling experiments. However, in recent years a novel pathway has been proposed by Smirnoff and coworkers, which fits all the currently available data<sup>2</sup> (Fig. 1). The proposed pathway differs substantially from the animal pathway and involves the synthesis of L-galactose a rare sugar never prior described in plants in its free form. Since its proposition, support for the pathway has been obtained from the study of Arabidopsis mutants in which several of the proposed pathway enzymes are down-regulated and that show a substantial decrease in total L-AA content<sup>3</sup>. The biosynthetic pathway identified in Arabidopsis has since been confirmed in a wide range of other plants including crop plants such as potato, celery, barley and blackcurrant (Table 1). Evidence of its operation has been obtained from both green tissues and nonphotosynthetic storage organs such as fruit, tubers and developing seeds. The current consensus is that this pathway represents the major route for L-AA biosynthesis in higher plants, although the presence of other minor pathways has not been dismissed.

### Genes to Products



**Figure 1** The L-AA Biosynthetic Pathway in Plants and D-EAA Biosynthetic Pathway in Yeasts. Plants synthesise L-AA from D-glucose via a ten-step pathway involving the sugar nucleotides GDP-D-mannose and GDP-L-galactose. The pathway also utilises the rare sugar L-galactose. Yeasts synthesise the 5-carbon L-AA analogue D-erythroascorbic acid (D-EAA) from the structurally related 5-carbon sugar D-arabinose.

The resolution of the pathway of L-AA biosynthesis has paved the way for the identification of genetic and biochemical factors controlling L-AA accumulation in plants. As a first step to achieving this objective, we undertook biochemical analysis of the pathway using the unicellular chlorophyte Chlorella pyrenoidosa as a model system. The advantages of this system included the relative simplicity of the organism compared with multi-cellular and multi-organ higher plants and the availability of L-AA hyperaccumulating lines produced by chemical mutagenesis<sup>4</sup>. For our studies we investigated the two mutant lines H1 and H2, which contained ca. 5.1 and 9.8 fold more L-AA than the wild-type respectively. Firstly, we established that both higher plants and C. pyrenoidosa cells share a similar pathway as deduced by the pattern of label

(Table 2). In all cases, the observed incorporation of					
radioactivity into L-AA from each substrate was con-					
sistent with its position within the proposed L-AA					
biosynthetic pathway (see Fig. 1). In the C. pyrenoi-					
dosa mutants the higher incorporation of D-[U-					
<sup>14</sup> C]glucose or D-[U- <sup>14</sup> C]mannose into L-[ <sup>14</sup> C]AA					
compared with the wild-type strain confirmed that L-					
AA biosynthetic capacity is up-regulated. However,					
both mutant strains showed an equivalent or lower					
incorporation of L-[1- <sup>14</sup> C]galactose into L-[ <sup>14</sup> C]AA					
than the wild-type strain, suggesting that the rate of L-					
galactose conversion into L-AA is unaffected in the					
mutants. The only reasonable explanation for these					
results is that up-regulation of L-AA biosynthesis in C.					
pyrenoidosa mutants occurs as a result of increased L-					

incorporation into L-AA from radiolabelled substrates

·	Substrate				
Tissue	D-mannose	L-galactose L	-galactonolactone		
Potato tuber	$1.29\pm0.04$	$3.39 \pm 0.45$	$6.07\pm0.40$		
Celery parenchyma	ND	$6.04 \pm 0.70$	$1.74\pm0.16$		
Celery vascular	ND	$11.06\pm0.79$	$7.59 \pm 1.21$		
Barley leaves	ND	ND	$2.18\pm0.09$		
Blackcurrant leaves	$1.27\pm0.04$	$9.99 \pm 0.87$	$4.48\pm0.26$		
Blackcurrant berries	$0.70\pm0.24$	$1.97\pm0.34$	$2.74\pm0.25$		

Tissues were incubated for 18 h with agitation in 25 mM D-glucose or the appropriate substrate as described. Samples were extracted in 5% metaphosphoric acid containing 5 mM dithiothreitol and total L-AA content determined by HPLC. Relative values are expressed compared to the L-AA content of tissues incubated with D-glucose  $\pm$  standard deviation. ND – Not determined

Table 1 Effect of potential precursors on L-AA content of higher plant tissues.

Substrate	% Metab Blackcurrant Leaves	rporated into L-A . pyrenoidosa H1	AA H2	Dark grown <i>C. pyrenoidosa</i> cells were incubated with 3 $\mu$ Ci of the appropriate precursor and the same quantity of radioactivity was introduced into blackcurrant leaves
D-[U- <sup>14</sup> C]glucose D-[U- <sup>14</sup> C]mannose L-[1- <sup>14</sup> C]galactose			$6.23 \pm 1.64$	5% HClO3 containing 10 mM L-AA. Cell debris was
				quantified by HPLC with flow scintillation analysis.

Table 2 Incorporation of <sup>14</sup>C-labelled precursors into L-[<sup>14</sup>C]AA by algae and higher plant tissues.

galactose formation. This hypothesis received confirmation following incubation of C. pyrenoidosa with Dglucose, L-galactose or L-galactono-1,4-lactone (Table 3). The mutants accumulated substantially more L-AA compared with the wild-type when incubated with D-glucose but all three C. pyrenoidosa strains synthesised similar quantities of L-AA from L-galactose or Lgalactono-1,4-lactone. These data support the hypothesis that the mutation is prior to L-galactose on the biosynthetic pathway and results in the biosynthesis of more L-galactose in the hyperaccumulating strains. This hypothesis was confirmed by the finding that both C. pyrenoidosa mutant strains contained significantly greater amounts of free L-galactose than the wild-type strain (4.04, 15.36 and 13.91 nmol gDW<sup>-1</sup> for wild-type, H1 and H2 respectively). Further biochemical investigations in the mutants revealed that the increased L-AA phenotype is correlated with upregulation of the activity of a novel enzyme involved in L-galactose biosynthesis and work is in progress aimed at the isolation and characterisation of the corresponding gene. Further work is also in progress to characterise the biochemical and molecular regulation of the L-AA biosynthetic pathway using a metabolic engineering approach. Genes coding for enzymes involved in the biosynthetic pathway are being cloned from available sources and expressed transiently in Nicotiana xanthii protoplasts to test the effect of the up-regulation of individual enzymes on the L-AA biosynthetic flux.

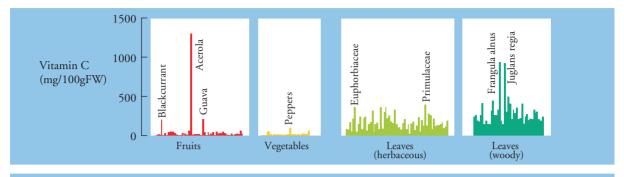
Although we are close to achieving a clear understanding of the biochemistry and genetics of the biosynthetic pathway at the cellular level, our knowledge of the factors affecting L-AA distribution at the whole plant level and particularly to the storage organs remains poor. Whilst leaf L-AA content is generally high with relatively little variability between herbaceous and woody plants, a huge and unexplained variability is observed in the L-AA content of non-green edible plant tissues (Fig. 2). In fruits, for example, the levels vary from 27 mg gFW<sup>-1</sup> L-AA (in the camu camu, produced by the low-growing tropical shrub Mirciaria dubia) to less than 3 µg gFW<sup>-1</sup> L-AA (in the medlar produced by Mespilus germanica). The variability has no apparent taxonomic reason. For example the medlar is a member of the Rosaceae family, to which rose hips also belong and which contain over 4000-fold more L-AA. In addition the L-AA content of food crops is also affected by environmental conditions such as location, weather conditions, mineral fertilisation, growth habit and post-harvest storage, which can result in a reduction of up to 80-90% of the initial L-AA content. One explanation for the variability of L-AA content in plant storage organs is that synthesis of this metabolite in these tissues is controlled much less strictly than in leaves where it fulfils essential and critical functions in photosynthetic metabolism. This suggests that it may be possible to design specific strategies aimed at enhancing the L-AA content of the edible parts of food crops without dras-

	L-AA Concentration (mmol gDW-1)					
Precursor	Wild type	H1	H2			
D-glucose	$4.65 \pm 0.49$	$12.07 \pm 1.57$	$19.40 \pm 4.95$			
D-mannose	$0.28\pm0.09$	$0.40\pm0.05$	$3.38 \pm 1.64$			
D-galactose	$97.70 \pm 7.88$	$99.20 \pm 12.37$	$95.64 \pm 6.82$			
L-galactono-1,4-lactone	$82.27 \pm 12.39$	$75.65 \pm 9.90$	$79.24 \pm 4.08$			

*C. pyrenoidosa* was grown to mid-logarithmic phase in the dark at 35°C. Carbon substrate was 5 g L<sup>-1</sup> D-glucose. Cells were harvested, washed in C-free medium and resuspended with the appropriate carbon substrate (5 g L<sup>-1</sup>). Incubation was continued for 24 h and the culture divided into two aliquots. One aliquot was used for determination of cell dry weight and the other for L-AA determination by HPLC.

Table 3 Effect of potential precursors on L-AA content of Chlorella pyrenoidosa strains.

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**Figure 2** Variability of L-AA Content in Plant Storage Organs and Photosynthetic Tissues Leaves have a higher average L-AA content than fruits and vegetables, however there is a lower degree of variation in photosynthetic tissues than that in fruits and vegetables. This data suggests some degree of flexibility in the L-AA content of storage organs. One aim of the current research is to understand the factors controlling L-AA concentration in sink tissues and to exploit these mechanisms to enhance the quantity of L-AA in food crops.

tically affecting plant growth or crop performance. Another option is that L-AA is actively transported between plant tissues. We have recently demonstrated the presence of high L-AA concentration in the phloem of many plants including rose, willow, barley and potato. The high concentration of L-AA in the phloem of storage organs (Fig. 3) may be indicative of L-AA transport from L-AA producing sources (leaves?) to non-photosynthetic tissues. We are currently characterising the uptake and transport of L-AA in plant phloem and evaluating the contribution of phloem L-AA on the overall L-AA content of storage organs.

L-AA biosynthesis by micro-organisms At present, the majority of commercially manufactured L-AA is synthesised via the seven step Reichstein process utilising D-glucose as a starting point (Fig. 4). The process involves six chemical steps and one fermentation step for the oxidation of D-sorbitol to L-sorbose. Overall, the yield of L-AA from D-glucose obtained by the Reichstein process stands at ~50%. Although the Reichstein process has all the advantages to be expected after more than 60 years development, it is still highly energy consuming and requires high temperatures and/or pressures for many steps. In addition, most of the chemical transformations involve considerable quantities of organic and inorganic solvents and reagents such as acetone, sulphuric acid and sodium hydroxide. Although some of the compounds can be recycled, stringent environmental control is required, resulting in significant waste disposal costs. These and other economic factors have generated a substantial interest in the industry for the exploitation of microbial biotransformations in the manufacture of L-AA. Yeast, in particular is being considered for this process and our increased understanding of L-AA biosynthesis in plants has provided tools for the development of a novel single-step process for L-AA manufacture

Yeast does not synthesise L-AA but its 5-carbon analogue D-erythroascorbic acid (D-EAA) which has similar antioxidant characteristics to L-AA but it lacks antiscorbutic activity and thus has limited industrial applications. However, the pathway of D-EAA biosynthesis in yeast shares many similarities with the 'committed' steps of the L-AA biosynthetic pathway in plants with the 5-carbon sugar D-arabinose replacing L-galactose (Fig. 1). We tested the specificity of the D-EAA pathway in *S. cerevisiae* by supplying cell cultures

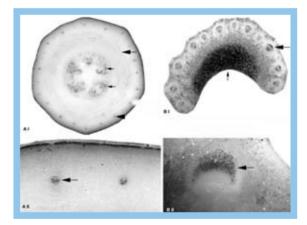


Figure 3 Silver Staining of Plant Storage Organs to Demonstrate Tissue Specific Variability in L-AA Content. Slices of plant tissue were cut and washed briefly. Samples were then stained in an alcoholic silver nitrate solution (pH 2.5) in the dark at 3°C. Excess silver was removed with alcoholic ammonia prior to recording images. In both courgette (A) and celery (B), high concentrations of L-AA can be seen associated with the vascular regions (large arrows) and specifically the phloem (B II). In addition, L-AA staining can be observed around the developing seeds in courgette and in the storage parenchyma in celery (small arrows).

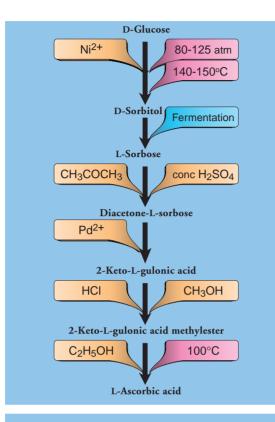


Figure 4 The Reichstein process for Bulk Manufacture of L-AA

In the widely used Reichstein process D-glucose is converted to L-AA via a series of chemical steps and a single bacterial fermentation for the conversion of Dsorbitol to L-sorbose. Catalysts for the development of alternative processes for L-AA synthesis include the use of environmentally hazardous chemicals (orange boxes) or steps requiring high energy consumption (purple boxes).

with a number of potential L-AA and D-EAA precursors (Table 4). D-EAA was present in the cells under all conditions tested and was strongly accumulated when cells were supplied with the natural precursor Darabinose. Conversely, L-AA was only observed in cells incubated with either L-galactose, L-galactono-1,4-lactone or L-gulono-1,4-lactone (the immediate L-

> Pre D-g

D-1

D-g

L-g

D-a

D-g L-g L-g AA precursor in the animal biosynthetic pathway). Data obtained regarding the specificity of the enzymes involved in D-EAA biosynthesis in *S. cerevisiae* supported the hypothesis that the pathway could be 'hijacked' to produce L-AA if supplied with the appropriate precursors. This was further supported by *in vivo* labelling experiments which showed a six-fold decrease in the proportion of radioactivity incorporated into L-[<sup>14</sup>C]AA from L-[1-<sup>14</sup>C]galactose if the cells were preincubated with 5 g L<sup>-1</sup> D-arabinose<sup>5</sup>.

Our results demonstrate that yeast cells are capable of direct fermentation of L-galactose to L-AA. However, given that L-galactose is an extremely rare and expensive sugar a process using L-galactose as a starting material could never be economical. In order to overcome this problem, we are currently developing new yeast strains with extended metabolic competence for the synthesis of L-galactose directly from inexpensive substrates. This project is supported by the Scottish Enterprise Proof of Concept Fund and aims at developing an industrially acceptable yeast-based singlestep fermentation process for the manufacture of L-AA from cheap and readily available starting materials. If successful, this development will form the basis of a new biotechnology for the manufacture of vitamin C, eventually offering a viable alternative to current production methods<sup>6</sup>.

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5 g L<sup>-1</sup> sucrose as the

opriate C-source (5 g

ed and washed then

Samples were mination of cell dry

by HPLC

ecursor	L-AA (mg gDW-	<sup>-1</sup> ) E	P-EAA (mg gD₩	(-1)
glucose	Nd		$1.67\pm0.26$	
mannose	Nd		$3.21 \pm 0.38$	Cultures were grown in the dark with
galactose	Nd		$0.20\pm0.06$	carbon source. Cultures were harvester resuspended in medium with the appro
galactose	$658.0 \pm 94.5$		$0.18\pm0.08$	L <sup>-1</sup> ) and incubated for a further 24 h.
arabinose	Nd		$200.8\pm21.2$	divided into two aliquots for the deter
galactono-1,4-lactone	Nd		$0.60\pm0.14$	weight or L-AA/D-EAA concentration Nd = Not detected
galactono-1,4-lactone	$865.5 \pm 113.6$		$0.19\pm0.04$	
gulonono-1,4-lactone	$138.2\pm10.3$		$0.22\pm0.05$	
	·		·	

 Table 4 Effect of Potential Precursors on Soluble Antioxidant Content of S. cerevisiae.

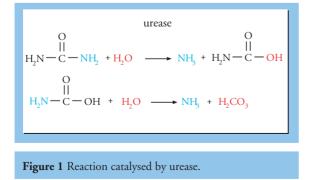
## Molecular manipulation of urea metabolism in potato

#### H.V. Davies, M.A. Taylor, S. Tiller & C-P. Witte

Trea is the most frequently used N fertilizer globally. For example, in China and India, urea accounted for 53% and 83% respectively, of total N fertilizer consumption in 1998. Together, both countries consumed 41% of all N fertilizer used worldwide in that year<sup>1</sup>. Urea is a cheap source of fertiliser with a high N content, it can be applied as a foliar application thus eliminating the groundwater pollution associated with nitrates, and potentially allowing targeted fertiliser applications to meet only the demands of a growing crop. Potential disadvantages include leaf damage at high concentrations and losses due to volatilisation. This article outlines a transgenic approach to more fully assess the potential to improve the efficacy of foliar applied urea as a plant and environment friendly nitrogen source.

In the plant, urease, which is a nickel requiring enzyme, catalyses the hydrolysis of urea to carbamate and ammonia (NH<sub>3</sub>). Carbamate is unstable and yields a second molecule of ammonia and carbonic acid (Fig. 1). The release of ammonia during the urease reaction leads to a pH rise, since at neutral pH most NH<sub>3</sub> becomes protonated (NH<sub>3</sub> (aq.) + H<sup>+</sup>  $\leftrightarrow$ NH<sub>4</sub><sup>+</sup>). Ammonia may escape from the system (volatilise). The action of urease requires several accessory proteins for activation

Potato urease and urease accessory protein genes Prior to transformation, potato urease and urease accessory protein (UreG) genes were isolated and extensive genomic sequence data generated. One allele of the gene has been sequenced completely and a second allele partially. Potato urease was shown to be a single copy gene present on chromosome 5 with a truncated Ty1-*copia* retrotransposon located in an



intron of one of the alleles<sup>1</sup>. In addition, five different cDNAs encoding isoforms of urease accessory protein G (*ureG*) were cloned. The 5'-coding region of these cDNAs is highly polymorphic within *Solanum tubero-sum* ssp. *tuberosum*, containing mainly a simple sequence repeat encoding histidine and aspartate. All ureG isoforms contained a P-loop motive and were similar to other plant and bacterial ureG sequences. Mapping on an ultra high density map of the potato genome and Southern blot analysis showed that the isoforms arise from allelic differences of a single copy gene located on chromosome  $2^2$ .

**Distribution of urease activity in the plant** Urease activity was detected in all potato tissues. A detailed analysis of urease activity in the potato canopy during plant development showed that urease is a classic housekeeping enzyme, maintained at constant levels throughout the plant's life. Messenger RNA coding for urease accessory protein G was also found in all tissues tested and at fairly constant levels. In Western blot analysis, ureG could be detected in most tissues. These findings are consistent with the ubiquitous expression of potato urease. An attempt to correlate

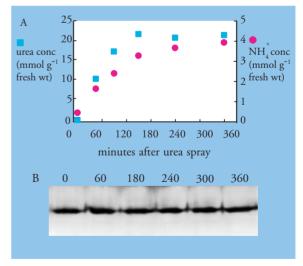
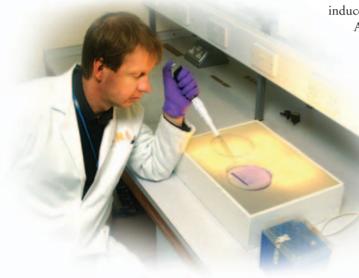


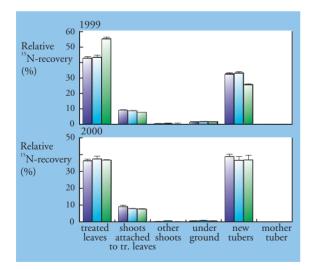
Figure 2 Urea, ammonium and urease levels in leaves at different times after a 2% foliar urea treatment. A, urea ( ) and ammonium () concentrations in the leaves. B, native PAGE gel stained for urease extracted at different times after a 2% urea application. Samples were loaded on a constant fresh weight basis.

### Genes to Products

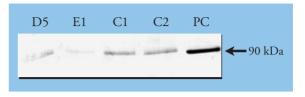
the amounts of urease and ureG from different tissues showed that there are likely to be other factors important for the activation of urease and/or the maintenance of urease activity.

**Metabolism of foliar applied urea** The levels of urea and ammonium increase drastically after foliar urea application, showing that urea readily penetrates the leaf surface and enters the leaf cells where it is hydrolysed by urease (Fig. 2). However, urea neither





**Figure 3** Urea <sup>15</sup>N recovery from field-grown plants. Plants in 1999 (upper panel) were harvested 36-48 hours after urea application. In 2000 (lower panel) plants were harvested 8 days after. Results are shown for three replicate plants indicated by different column shading. Error bars indicate the confidence interval (P = 95%) for the mass-spectrometric determination of <sup>15</sup>N.



**Figure 4** Western blot of protein extracts from leaves of urease antisense transgenics and controls. D5 and E1 are antisense lines. C1 and C2 are control lines, transformed with pBIN19 only. PC is a positive control containing purified jackbean urease (Boehringer).

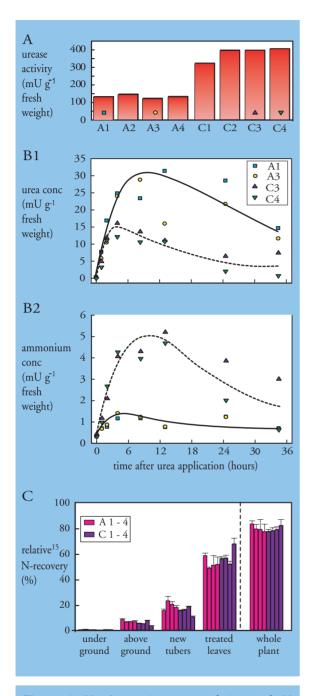
induced nor reduced the amounts of leaf urease. Accumulation of ammonium after urea spray demonstrated that the urease reaction was, at least initially, not rate limiting for the assimilation of urea.

> Nitrogen losses by volatilisation from foliar applied urea did not exceed 18% in recovery experiments on field-grown potato plants. These relatively high recoveries are in accordance with findings from other studies in which the amounts actually intercepted by the leaves of the plant were known. Based on this data, it seems likely that nitrogen losses over

20-30% are generally caused by factors other than volatilisation. Urea nitrogen is initially quickly distributed throughout the plant, possibly because urea itself has access to the vascular system. Once the urea is hydrolysed and the nitrogen assimilated, the redistribution slows down. A significant proportion of the nitrogen remains in the treated leaf and is slowly mobilised for transport into the sink tissues (tubers) during leaf senescence (Fig. 3).

**Urease and Ure G transgenics** Transgenic potatoes have been generated with urease activity down-regulated (antisense urease or Ure G) and up-regulated (over-expression of potato urease). Plants with a range of leaf urease activities from c. 20 mU per gram fresh weight up to approximately 650 mU per gram fresh weight were produced. A reduction of urease antigen in the best antisense lines was demonstrated for both the urease (Fig. 4) and *ureG* antisense plants. RT-PCR results for the *ureG* antisense lines showed a reduction in *ureG* specific mRNA in lines with low urease activity. The results for *ureG* antisense lines confirm that this protein is involved in urease activation in plants.

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**Figure 5** Urea/ammonium accumulation and N distribution/losses following urea application in control plants and transgenic plants with urease activity down-regulated. A, urease activities in leaves of transgenic plants (A1-A4), vector-only control plants (C1-C3) and wild type (C4). B1, urea concentration in leaves after urea application. B2, ammonium concentration in leaves after urea application. C, 15N distribution and recovery 8 days after application of non-labelled urea followed by 15N urea. Percentages refer to total amount of 15N applied. The error bars indicate the confidence interval (P = 95%) for the mass-spectrometric determination of 15N.

Using the transgenic plants generated the influence of urease activity on the losses of foliar applied urea fertiliser was investigated (Fig. 5). Results from <sup>15</sup>N pulse-chase experiments using a simplified leaf disc model system indicated that urease activity was not correlated with nitrogen losses. However, clear differences in leaf urea and ammonium content were demonstrated which were associated with urease activity. Experiments with intact plants confirmed these results. Despite the higher levels of endogenous ammonium after urea application in plants with low urease activity, there were no increased nitrogen losses from these plants. Ammonia volatilisation was not correlated with leaf urease activity. Apoplastic ammonium concentration, which is a major determinant of the plant's ammonia compensation point, might be unaffected by modifications to symplastic ammonia concentration due to the compensating action of NH<sub>4</sub><sup>+</sup> transport systems in the cell membrane.

Conclusions Urea has long been known as an alternative nitrogen source to nitrate for crop production. It is advantageous in avoiding nitrate losses when used in foliar applications as it is taken up by the plant directly and should rarely come into contact with the soil. Urea is readily assimilated via the leaves and incorporated into the plant's nitrogen pool. Although urea hydrolysis leads to greatly increased ammonium concentrations in the canopy there are no obvious harmful effects for the plant if the urea concentrations in applications are not too high. Importantly, there does not seem to be a connection between the level of urease activity in the leaf cells and volatilisation losses from the canopy after urea spray. Therefore, it appears that urea metabolism by the potato leaves does not lead to any additional nitrogen loss to the environment.

#### Acknowledgements

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## Application of a potato UHD genetic linkage map for BAC landing and contig initiation in a region of linkage group V

G. Bryan, D. Milbourne, E. Isidore, J. McNicoll, I. Tierney, A. Purvis, S. Williamson, L. Ramsay, K. McLean & R. Waugh

onstruction of local or global physical maps is essential for many plant genome research applications, such as map-based gene isolation and genome sequencing. Physical maps are assemblages of overlapping genomic DNA clones, or 'contigs' which can be constructed by a host of different approaches. Existing physical maps have been based (at least in part) on sets of genetically mapped STS (sequencetagged site) markers, which serve both to assemble contiguous BAC clones, and to anchor these contigs onto the genetic map<sup>1</sup>. Unfortunately, the numbers of characterised STS markers required to make it an effective approach (generally tens of thousands for complex eukaryotes) currently makes it prohibitively expensive for all but model genomes. More typically, physical maps are constructed by random clone fingerprinting, which requires considerable levels of redundancy and sophisticated analytical software. Whole-genome physical mapping studies are costly, and even more challenging in the case of complex heterozygous genomes like potato.

At SCRI, we have set ourselves a less daunting, and more targeted goal – to generate physical maps for key regions of the genome containing genes of prime agronomic and biological importance. For example, the majority of potato disease resistance genes (and several QTL) have been found in 3 or 4 'clusters', on linkage groups IV, V, XI, and XII. Therefore, targeted physical mapping of relatively few genetically defined intervals could yield the resources necessary rapidly to clone the majority of disease resistance genes in potato. We have opted to build an integrated physical and genetic map of a region of potato LG V, defined by RFLP markers GP21 and GP179, that contains a plethora of genes and QTL of agronomic importance. For example, the late blight race specific resistance gene R1, recently isolated, resides in this region<sup>2</sup>. This work is being performed through two EU-funded projects (FAIR-5-PL97-3565 and APOPHYS), the first

of which led to the construction of an ultra-high density (UHD) genetic map of the potato genome comprising ~10,000 AFLP markers (<u>http://www.dpw.</u> <u>wau.nl/uhd/</u>). As part of the UHD project, we developed strategies, combining the use of large insert bacterial artificial chromosome (BAC) libraries of the mapping parents, to increase the utility of the UHD map as a versatile resource for potato genetics and genome analysis. The LG V physical mapping work relies heavily on the UHD map, and is aimed at demonstrating its utility in the production of 'seeded' physical maps of defined chromosomal regions of the potato genome.

**BAC library construction** As a first step towards this goal, we constructed a BAC library of the male parent (RH) of the diploid mapping population used to construct the UHD map. This library consists of 35,712 clones arrayed into ninety-three 384 well microtitre plates. An analysis of approximately 200 random BAC clones showed the RH BAC library has an average insert size of 102kb, with insert sizes range from 30 to 180-kb. This suggests that the library is equivalent to approximately 4-fold coverage of the haploid potato genome, assuming a genome size of 850Mb.

**BAC pooling** In order to be able to screen the library using PCR, we developed a simple six-dimensional pooling strategy allowing the entire BAC library to be screened in j u s t

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Six-dimensional pooling strategy used for screening potato BAC library comprising ~37,000 clones. Library divided into 8 stacks of 12 384-well plates prior to pooling.

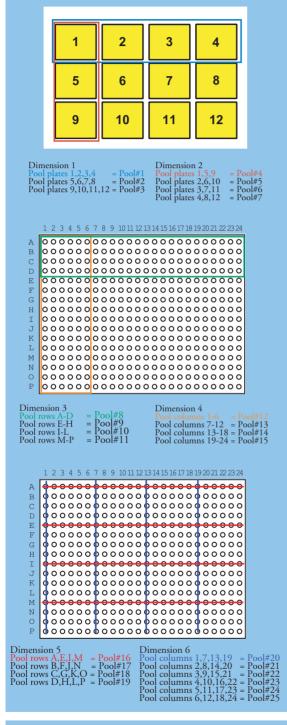


Figure 1 Pooling strategy used to generate 6 dimensional pools of RH potato BAC library, comprising ~35,000 clones.

200 PCR assays (see Figure 1). The strategy relies on sub-dividing the library into sections representing approximately 0.5x genome equivalents, so that the likelihood of 'hitting' a section more than once with any single copy probe is quite low. Pooling in sixdimensions was then applied to each half-genome stack, allowing the complete address of any single copy sequence in the section to be obtained in 25 PCRs. A single hit in a section yields six co-ordinates, one corresponding to each dimension, which allow the complete address of the relevant clone to be determined. Multiple hits in a section will result in more than six co-ordinates, which requires some degree of 'deconvolution' to identify the individual BACs. The pooling strategy used here attempts to minimise this problem by reducing the probability that multiple hits will be detected in a section. For a section of the RH BAC library containing 12 plates, or 4096 individual clones, this probability is approximately 0.56. Thus, the probability of obtaining two hits in a single section can be approximated to 0.31 ( $0.56^2$ ), assuming that the two hits are independent events. Furthermore, when considering using segregating AFLPs to screen a library, it is important to take into account that the necessarily heterozygous state of these markers (they must be heterozygous in one or both parents to map in an F1 population) effectively halves the genome equivalency of the library. Thus a 4x genome equivalent library effectively becomes a 2x genome equivalent library when screening for sequences representing only 1 of the 2 possible alleles at a locus, which gives an even lower probability of obtaining two hits in a section.

Screening the BAC pools The BAC pools were used in conjunction with information from the UHD map for the targeted isolation of BAC clones from the GP21-GP179 interval. This was initiated by mapping GP21 and GP179 as CAPs markers on the UHD map. This allowed the identification of 14 AFLP markers in the interval, of which all but two originated from different primer combinations. A total of 12 AFLP primer combinations, corresponding to 13 AFLPs were deployed in the 6D BAC pools and 8 individual BAC clones were successfully identified. Four of the markers screened on the library yielded no hits, thus 70% (9/13) of the markers screened in the BAC library successfully identified at least one BAC. The RH library was also screened using RFLP markers known to map to this interval (e.g. GP21, GP179, SPUD237, SUT2), a process which identified an additional 10 hits in the library.

Chromosome walking using BAC end sequences The ends of all BACs identified from the library have been sequenced using the T7 and SP6 sites flanking the cloning site in the BAC vector. Resulting sequences were used to search BLAST databases, and for PCR primer design. Primers were tested on genomic DNA, and if a single PCR product of the expected size was amplified, they were tested on the PCR pools. For example, the primers designed to the T7 derived end sequence of RH BAC 56A14 (identified using the primers for SPUD237) were used to screen the pools. Screening the library with this primer identified a total of 4 BACs. One of these was 56A14, the BAC the end sequence was derived from, and another was BAC 13I15, also previously identified by the SPUD237 primers. However, the other 2 BACs identified (48O18 and 64N22) were previously unidentified. This demonstrates that contig extension using the pooled library is feasible, and can be used, in

principle to complete the physical map of the region. Problems will occur when BAC end sequences correspond to repetitive elements within the potato genome but these can usually be identified. Interestingly, the end sequences obtained show several homologies to various plant genes, confirming as expected, that this interval is relatively 'gene-dense'.

Towards a physical map of the GP21-GP179 interval of linkage group V Our goal is to illustrate the 'seeding' of an interval on the UHD map of potato LG V with BACs. The results of the first phase of these experiments are summarised graphically in Figure 2, which displays the 'BAC-seeded' genetic map of the GP21-GP179 region. A total of 20 BACs were identified using markers located in the GP21-GP179 interval, and where possible, the relative positions of these BACs are illustrated on the map, by reference to the marker used to identify them. Interestingly, of the three markers that identified BAC 8E5, two co-segregate, but are separated from the third marker by a single recombination event. Examination of the graphical genotype file for the interval supports the validity of this recombination event. This suggests that, in this portion of the interval at least, the genetic to physical distance ratio is relatively high, a favourable situation for physical mapping. Partial sequencing of 8E5 suggests that it contains alleles or homologues of the R1 gene.

Apart from the genetic order of the markers that were used to identify them, very little is currently known about the relationship of the 'seeded' BACs to each other. Groups of BACs identified by the same Use of the UHD map of potato to initiate contigbuilding across the GP21-GP179 interval of chromosome V. The genetic map of RH chromosome V region in the GP21/GP179 interval is shown, with the two bars representing the two RH alleles. Numbers in the middle are recombination events. Genetic markers on each side (in black) are the markers mapped on the UHD map in the same phase. Individual BAC clones, identified by screening the BAC pools, are displayed in red next to the marker used to identify them.

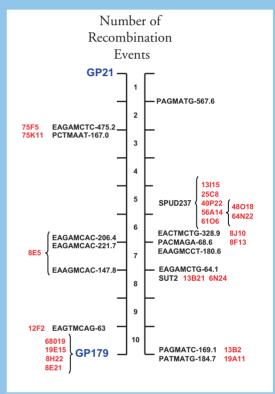


Figure 2 Seeded physical and genetic map of GP21 – GP179 interval of potato LG V, following initial round of screening with AFLPs and STS markers (in E:\POTATO\_LGV\map.ppt but needs redrawing for Ann Report)

primers have a very high probability of overlap. Restriction digest fingerprint analysis has confirmed extensive overlap for BACs isolated using the same target sequence (e.g. SPUD237, GP179). An important aspect of the map is that, due to the heterozygous nature of the AFLP markers on the map, BACs identified by these markers are also 'phase' specific. A consequence of this is that primers designed to end-sequences for these BACs will potentially identify not only overlapping clones, but also BACs from both chromatids. Thus, contigs based on these BACs have the potential for rapid growth in terms of both expan-

sion and depth. Moreover, this will facilitate the joining of BACs or contigs based on clones identified by AFLPs alone.

Conclusions This article outlines the construction of a 3-4 fold genome equivalent BAC library of the diploid clone RH, and its successful use to devise a six-dimensional BAC pooling strategy. We have successfully combined the use of AFLP and STS analysis on BAC pools, to identify specific markers present in the interval of interest on the RH genetic map. End sequencing of some of the BACs from the interval has allowed the demonstration of chromosome walking in the region to identify overlapping BAC clones. The identification of 20 BAC clones spanning 11 recombination events from the GP21/GP179 interval is a first step in the construction of a physical map of the region. Its completion relies on use of the end and internal sequences from BACs already identified as 'initiation' points for further BAC isolation from the library. Once a sufficiently large number of BACs has been identified, they can be fingerprinted to identify clone overlaps, which will group the BACs into larger contigs. Yet more chromosome 'walking' can then be performed to fill in the potential remaining gaps in the physical map. This map will be used to identify a

minimum 'tiling path' across the region (i.e. the least redundant set of BACs spanning it), which will be used for determining the gene content of this important genetic interval. A further objective of this work is to isolate a Potato Cyst Nematode resistance gene (*Gpa5*) conferring resistance to *G. pallida*, present in the GP21-GP179 interval in the diploid clone JP, for which we also have a BAC library. This will demonstrate further the utility of the markers in the UHD genetic map and will rely on further development of locus-specific markers from the RH BAC clones present in the region.

### Acknowledgements

We gratefully acknowledge the financial support of the Scottish Executive Environment & Rural Affairs Department (SEERAD) and the European Union.

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# Assessing potato germplasm for resistance to late blight

### H.E. Stewart, J.E. Bradshaw & G.R. Mackay

**Introduction** Late blight, caused by the oomycete **L***Phytophthora infestans*, is the most important disease of potatoes, and occurs world-wide wherever they are grown. Black or brown lesions develop on the leaves, and in warm, humid weather, these can expand and increase in number to destroy the foliage of the whole crop very rapidly (Fig.1). Spores are washed from the leaves into the soil by rain, and the tubers become infected whilst in the ground or during lifting. This can result in severe or total loss of the crop, as happened in Ireland in the 1840's, causing the famine and subsequent emigration. Today, control of the disease depends on the use of costly fungicides and the world-wide economic cost of protection and crop loss is estimated at \$3 billion per annum. However, in the developing world, where potato production is increasing, the cost of protection is unaffordable for many farmers. In Europe, on the other hand, there is an increasing demand for reduced use of agro-chemicals because of concern for the environment. The way to overcome these problems is to develop varieties with a higher level of durable resistance for use as part of an integrated control system. There are two kinds of resistance; one is race specific, governed by one or more major (R) genes, and is inherited in a simple Mendelian manner. It confers immunity to the disease or a very high level of resistance. The other is effective against all races and reduces lesion size, frequency and the amount of sporulation. It is more complex in inheritance and is termed field resistance. Major gene resistance has proved short lived, for



Figure 1 Potato crop infected with late blight.

example cv. Pentland Dell, bearing three R-genes, was immune to blight when it was released as a commercial cultivar in 1961, but succumbed to extensive infection by new races 6 years later. Consequently, for some time potato breeders have opted for field resistance. SCRI has a long history of breeding for resistance to blight, beginning at the Scottish Plant Breeding Station in the 1920s. Several cultivars with good levels of resistance have been released, and three more recent ones, cvs Stirling, Torridon and Teena, have performed well in blight trials in Europe, South America, and the United States<sup>1</sup>. In order to become commercially successful, resistant cultivars also need to possess other desirable agronomic traits and recent research has aimed to combine resistance to blight with resistance to potato cyst nematodes and high yield, and the quality demanded by processors and supermarkets. Moreover, in order to widen the genetic base of the resistance as insurance for the future, novel sources have been sought amongst accessions of the wild species of the Commonwealth Potato Collection (CPC), which is maintained at SCRI. Success in achieving such aims depends on reliable methods of selecting the best parents and the most resistant genotypes from crosses between them. The tests need to enable assessment of large numbers of genotypes, to ensure as far as possible that durable field resistance is selected and not short-lived R-gene resistance, and laboratory or glasshouse tests need to reflect actual performance in the field. Laboratory tests were initiated by William Black in the 1950s, when selection for field resistance began. Since then they have been developed and improved by SCRI pathologists, in particular Jean Malcolmson, Roger Wastie and Helen Stewart, and a field site established which has given reliable epidemics every year for over 20 years. This brief review records the most relevant factors for achieving success in assessing potato germplasm for resistance of both foliage and tubers to this devastating disease.

Foliage resistance *Progeny test* True seedlings, 5 to 7 weeks from sowing and about 10 cm tall, are sprayed with a suspension of *P. infestans* containing  $5 \times 10^4$  zoospores per ml. A complex race capable of overcoming any R-genes present is used, and the amount of



Figure 2 Progeny test for foliage resistance.

blight recorded 6 days later<sup>2</sup> (Fig. 2). The test has been shown to consistently rank the seedling progenies with their subsequent performance in the field, although it is not a very effective means of identifying resistant individual genotypes within progenies. It has been successfully used in breeding programmes at SCRI to select the best progenies within a year of making crosses, to estimate breeding value of parental genotypes, and to identify resistant accessions of wild species of the CPC for genetic studies as well as introgression into the cultivated potato (*S. tuberosum*).

Glasshouse Whole plant test Plants are raised in the glasshouse until just prior to flowering, the stage of growth at which resistance is best expressed. They are sprayed to the point of run-off with a zoospore suspension (5 x  $10^4$  spores/ml) of a complex race of P. infestans, incubated at 15°C, and kept at high humidity for the first 24 hrs to keep the leaves wet to enable infection. The amount of blighted foliage is recorded 7 days after inoculation on a 1-9 scale of increasing resistance<sup>3</sup> (Fig. 3). The test broadly agrees with field assessment, but the difference in score between the most resistant and most susceptible genotypes is smaller in the glasshouse than in the field trial, leading to underestimation of both resistance and susceptibility. It is therefore important that the glasshouse plants are reduced to a single stem and raised well spaced out in a cool glasshouse in order to produce sturdy plants that most closely reflect field behaviour. The test enables assessment of several hundred individual



Figure 3 Glasshouse whole plant test.

genotypes, and is effective in identifying resistant ones and eliminating the most susceptible in the intermediate stages of a breeding programme. It has been used to study the genetics of resistance in the wild species Solanum verrucosum and S. papita, and in progenies of cv. Stirling. It has also provided phenotypic data to enable molecular work to locate a QTL (quantitative trait locus) responsible for a significant proportion of the high level of field resistance of cv. Stirling and the mapping of its R-gene. Furthermore, it provides a reliable method of identifying R-genes and the virulence characteristics of P. infestans isolates. Detached leaflet tests traditionally used for this purpose can give variable results depending on factors such as the age of the plant and inoculum concentration. These factors have less influence on the whole plant test, which is therefore preferable as clear, repeatable results are achieved. Genotypes can be assessed using different races, including non-indigenous ones which can be safely used in the enclosed incubation facility.

The field trial This is carried out on a farm near the west coast of Scotland, where the climate favours  $blight^3$  (Fig. 4). It is inoculated with a single race of



Figure 4 Field trial.



Figure 5 Test for resistance of field grown tubers.

P.infestans, virulent against any R-genes present in the germplasm being assessed. Infected glasshouse-grown plants are spaced at metre intervals along drills of a susceptible cultivar situated on either side of pairs of drills of the genotypes being assessed. One complex race is used because mixed inoculum of complementary races can lead to overestimation of the resistance of genotypes bearing R-genes that only one component of the inoculum can overcome. The R-gene differentials, 11 genotypes each bearing one of the S. demissum derived resistance genes, are grown in the trial to monitor the virulence of the pathogen and hence confirm its ability to defeat R-genes borne by the genotypes being tested. The epidemic progresses more rapidly in early maturing genotypes (because they tend to be more susceptible) than in maincrops and so the two maturity groups are planted in separate blocks, each with control cultivars of appropriate maturity and covering a range of resistance. This enables the genotypes of each group to be assigned a 1-9 score, based on the amount of blighted foliage on the date which shows best discrimination between the control cultivars. The resistance score of a genotype is affected by the resistance of its neighbour, but variation between plots of the same genotype within the trial is low, probably because the test plants are all adjacent to the highly susceptible plants of the spreader drills. However, some differences in resistance between years occur which cannot be attributed to the presence of R-genes, particularly in some cultivars, and repeated testing is needed for accurate assessment of small differences. Severe epidemics have developed every year since the site was first used in the mid 1970's and the trial provides the best ultimate assessment of field resistance. Advanced selections from breeding programmes are thus assessed, and data collected for genetic studies and to develop marker-assisted selection (detecting resistance through testing for the presence of molecular markers associated with it).

**Tuber resistance** Although resistance of foliage and tubers is correlated, genotypes with resistant foliage do not always bear resistant tubers and selection for resistance of both has been SCRI policy and is advocated. Both are important because whereas susceptible genotypes are soon dead, partially resistant ones provide a source of inoculum for tuber infection over a prolonged length of time and hence with a greater chance of coinciding with weather conditions favourable for tuber infection.

Glasshouse progeny test for resistance of tubers True seedlings are sown, raised in individual pots in the glasshouse until flowering, and two samples of single tubers of 25 seedlings of each progeny dip-inoculated in a suspension of a complex race of P. infestans containing 2.5 x  $10^4$  spores/ml. The tubers are kept at high humidity for 24 hrs and the percentage of blighted tubers recorded 10 to 14 days later, ignoring infections through the artificial wound of the stolon scar<sup>4</sup>. The test has been shown to predict successfully the susceptibility of the same progenies assessed using field grown tubers, and hence is an easy and reliable way of assessing large numbers of progenies. It has been used to select progenies with tuber as well as foliage resistance, and to combine these with other agronomic traits.

Test of field grown tubers Immature tubers are hand dug in early August before the skins set, as this is when natural infection is most likely to occur and because they become more resistant as they mature. First-early maturing genotypes are generally lifted a week before second-earlies and two weeks before the maincrop genotypes. The rose-end of replicate samples of 20 to 40 damage free tubers of each genotype is sprayed with a zoospore suspension  $(5 \times 10^4)$ spores/ml) of a complex race of *P.infestans* on the day of lifting and incubated at high humidity for 24 hrs. The percentage of blighted tubers is recorded 10-14 days after inoculation<sup>5</sup> (Fig. 5). Inoculation on the day of lifting mimics natural infection and ensures meaningful discrimination between genotypes. Nevertheless, differences in resistance of the same genotypes within and between years of test do occur, and hence more than one year's results are required for a reliable estimate of resistance. Glasshouse grown tubers were found to be more susceptible when harvested from dry compost than from wet or moist compost, and a further study attributed this to the



Figure 6 Glasshouse test for tuber resistance.

presence of a higher number of bacteria antagonistic to *P.infestans* on the surface of tubers grown in wet compost. Environmental factors such as this may account for some of the variation found in the field test results.

Glasshouse test of genotypes for resistance of tubers The test of field grown tubers is highly labour intensive and assessing large numbers of genotypes in this way is impracticable. So a test of glasshouse-grown tubers was developed, in which replicate samples of 20 tubers harvested from flowering plants were inoculated the same day with a zoospore suspension  $(2.5 \times 10^4)$ spores/ml) of a complex race of P. infestans. They are inoculated, incubated and scored as described for the progeny test<sup>6</sup> (Fig. 6). This glasshouse test shows close agreement with the test of field grown tubers; indeed comparison of the two suggested that results of the glasshouse test would be more consistent over years than those of the field test. The glasshouse test is particularly valuable for assessing the hundreds of genotypes under evaluation during the intermediate stages of a typical breeding programme and for providing phenotypic data for genetical studies on tuber resistance.

**Remaining challenges and prospects for the future** Ensuring that the race of *P.infestans* used is capable of overcoming all the R-genes present is not always possible. Although blight populations are becoming more virulent (able to overcome more R-genes) in some parts of the world, races able to overcome all of the recognised eleven R-genes from the wild species *S. demissum* are still often unavailable, especially for use in field trials for which indigenous races are essential. Other R-genes also exist, and since R-gene resistance does not always confer immunity, it can be phenotypically indistinguishable from a high level of field resistance. Therefore ensuring that the resistance selected is field resistance and not race specific R-gene resistance is difficult. At present, the only way of distinguishing them apart with confidence is to examine the way that the resistance is inherited. This can take at least three years. However, both R-gene and field resistance interactions with *P. infestans* are now being studied at the molecular level at SCRI by Paul Birch, and the potato genes up-regulated in the two processes identified.

The expression of resistance is influenced by differences in the environment as well as the physiology of the plant. Therefore the susceptibility of a genotype to blight can differ from test to test and year to year, to a greater extent in some tests and in some genotypes than in others. This means that genotypes have to be tested in several successive years in order to obtain an accurate estimate of resistance. Development of molecular marker assisted selection should eliminate the need for repeated testing since it will be unaffected by environmental differences. The tests described here are being used to develop the molecular techniques and will be used to verify them. Should this prove successful, assessing potato germplasm in future will be much more rapid, and less labour intensive.

### Acknowledgement

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# **Barley Transcriptome Resources**

L. Ramsay, P. Hedley, D. Caldwell, H. Liu, N. McCallum, S. Mudie, L. Cardle, B. Harrower, G. Machray, D. Marshall & R. Waugh

As part of the SEERAD/BBSRC-funded 'Investigating Gene Function' (IGF) programme, SCRI is creating and extending genomics resources, expertise and infrastructure available to the UK cereal community and beyond. SCRI's contribution to the project is analysis on barley, with our collaborators (JIC and IACR-LARS) concentrating on wheat. These are the two cereals of most importance to the UK economy. However, due to extensive genetic similarity, information from other species such as maize and rice are also of great significance. A major part of the cereal IGF programme is the development of first class transcriptome resources.

The transcriptome is a recently coined 'omics'-derived term that is used to denote the population of mRNA transcripts in the cell, weighted by their expression levels. It essentially gives a snapshot of the genome in action within a particular cell type or tissue under particular conditions. The transcriptome represents regions of the genome that code for proteins and thus the portion of the genome of primary importance.

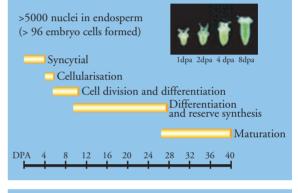
Comparisons of the transcriptome from different tissues, or from the same tissue at different times or under different conditions, gives an indication of the relative importance of certain genes in the expression of traits of interest. Development of transcriptome resources at SCRI is, therefore, a primary goal of the Genome Dynamics Programme.

In particular, within the IGF programme the development of barley transcriptome resources entails:

A. Creation of a wide range of cDNA libraries from various grain developmental stages and abiotic-stressed root material.

C. Utilisation of a high-density array containing unique selected ESTs. This work on the barley transcriptome closely mirrors similar work on wheat at IACR-I ARS within the cere-

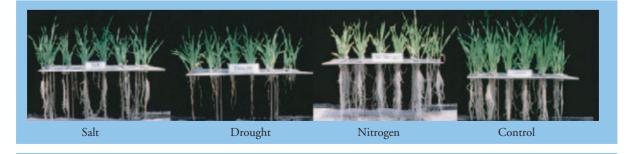
similar work on wheat at IACR-LARS within the cereals IGF project, allowing us to take advantage of the extensive genetic similarity between the two species.





**Construction of wide range of cDNA libraries** The main focus of the transcriptome programme is the molecular dissection of important events of grain development (Fig. 1) and germination, which are processes of primary biological and commercial interest (e.g. for the Scottish malting and whisky industries). For these studies we have used the cultivar Optic that is currently the most widely grown malting quality spring barley grown in the UK. A secondary focus of our work is on various abiotic-stressed and normal barley root tissue grown in hydroponic environments (Fig. 2).

Directional cDNA libraries have been generated in plasmid vectors utilising customised protocols for rapid and reliable library production. Aliquots of each



B. Generation of EST sequences from ~40,000 clones.



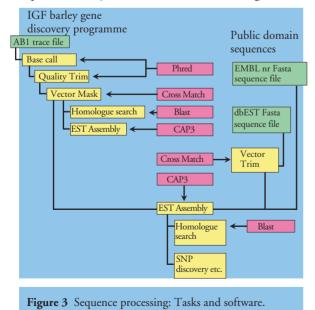
library have been plated, picked and immortalised using the high-throughput robotic facilities now available on-site at SCRI.

A total of 34 libraries have been produced from a range of tissues at different stages of grain development and from root and shoot tissues subject to a number of abiotic stresses.

**Generation of EST sequences** Clones from the stored cDNA libraries have been used for plasmid preparation and sequencing. This has taken advantage of the robotics for picking/replicating, liquid handling and capillary sequencing facilities on-site. Over 40,000 cDNA clones have now been sequenced in a single pass from the 5'-end, generating a large number of Expressed Sequence Tags (ESTs), which have been submitted to GENBANK dbEST.

The high throughput sequence generation has necessitated the development of software tools to deal with the information generated. The downstream processing, searching and databasing of the EST sequences has been automated by establishing a high throughput pipeline for the analysis of EST sequence data. This pipeline has been developed using Perl scripts to automate and 'glue together' a range of well established Sun Solaris software tools for the processing and assembly of sequence data (Fig. 3).

The 40,000 sequenced cDNA clones have been analysed and submitted to dbEST, the public access database for expressed sequence tags at NCBI. This represents a major contribution to the cereal genomics



community. Placing sequence information in the public domain has already resulted in requests for specific clones and information from both national and international colleagues.

**Utilisation of a high-density array** The redundancy inherent in the generation of EST data allows the barley sequences generated at SCRI and elsewhere to be assembled into a 'unigene set' that gives a more complete picture of the genes when compared to the single pass ESTs themselves. The targeted tissue approach taken within the IGF programme and the relatively low redundancy within the libraries has resulted in SCRI's sequences playing an essential role in the generation of a 'global' unigene set from all publicly available barley ESTs. Over 5,000 unique clones developed within the IGF programme has been sent to the University of Arizona (Rod Wing) for 3'-end sequencing.

This additional information will be utilised in the development of a barley Affymetrix 'gene chip' constructed with oligonucleotides designed from the assembled EST data. This microarray will allow the barley community to monitor the expression profiles of over 22,000 barley genes in any single experiment.

The use of microarray technology will augment studies already initiated using the EST information generated within the programme. In addition, insight into gene function is being gained through the use of parallel transcriptional analyses (such as cDNA-AFLPs to measure temporal and spatial regulation of expression) and RNA-based *in situ* tissue hybridisations to identify sites of transcriptional activity.

With the initial generation of transcriptome resources nearing completion, the focus of our work is now progressing to their utilisation to answer questions of relevance to the biology and cultivation of the crop.

### Acknowledgements

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# Genomics of the root-soil interphase

B.P. Forster, G. Bengough, R.P. Ellis, W.T.B. Thomas, S. Clark, D. Gordon, H. El-Menaie, R. Keith, R. Waugh & P. Hedley.

Roots provide a dynamic interface between plants and the soil. Root systems anchor plants, enable them to acquire water and nutrients, generate and modify soil structure, and determine the distribution and quality of carbon fluxes into the soil microbial community. Root growth and distribution is determined both by plant genotype and the soil environment. For example, we have found previously that root growth is slowed by drought and salt stresses, but promoted by nitrogen deficiency - the extent of the response varying among barley lines tested. Mutants have provided insight into the genetic control of root traits such as the occurrence of root hairs. However, the study of metrical traits has lagged behind, because of the difficulty in screening large populations of root systems. Our aim is to study the genetic controls of root traits in barley, and to understand their functional significance. This work is integrated between the Plant-Soil Interface, Genome Dynamics and Gene Expression Programmes at SCRI.

We designed a rapid screening technique to select for root development during seedling establishment. Root length and depth, root number, and root angular spread were chosen as key parameters that determine the efficiency and rate at which root systems explore and exploit the soil volume. The two-dimensional seedling test consists of growing barley in the dark between two plates coated with thin layers of gel and recording root traits manually or using a desktop scanner with image analysis software (Fig. 1). A genetic mapping population of barley (Derkado x B83-12/21/5) has been subjected to this test and the loci affecting the 2-D root traits identified (Fig. 2) with, for example, loci affecting root length on four of the seven barley chromosomes: 2H, 4H, 5H and 7H. The only association with a known gene is that with decreased 2-D root length due to the ari-e.GP dwarfing gene on the short arm of chromosome 5H. In addition to 2-D root assays, data from several studies have also been mapped in this population e.g. salt tolerance in hydroponics, seedling response to gibberellic acid, field measurements of yield and laboratory assessment of grain nitrogen content<sup>1</sup>. From these studies we can say that the two dwarfing genes sdw1 and ari-e.GP affect many traits but are distinctive in their action as the sdw1 locus has fewer physiological

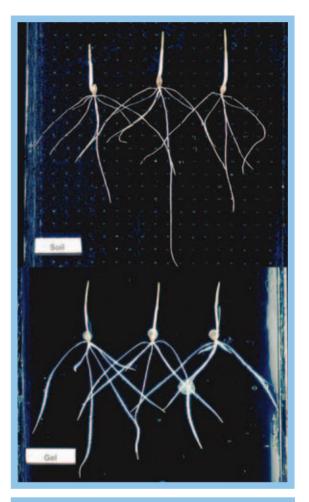
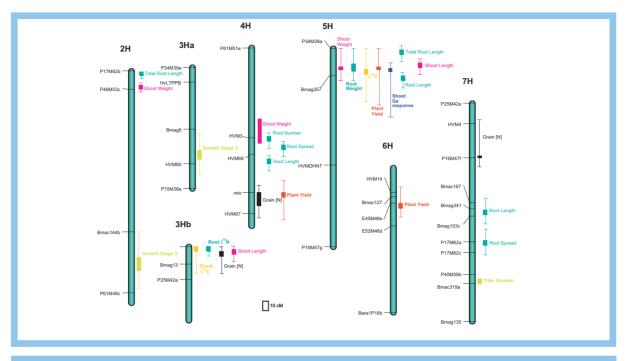


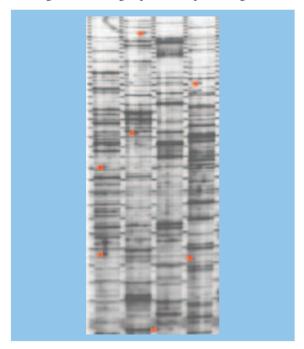
Figure 1 Barley seedlings growing in a 2-dimensional system to investigate root structure, the top panel shows the root system of the cultivar Chime after soil has been washed away, the bottom shows a similar developmental pattern when grown between gel coated plates.

effects. Field performance is only affected by the *ari-e*.GP locus which is association with a QTL x E effect for grain yield. Otherwise QTLs for seedling root traits, whether from hydroponic studies or from the 2-D test, show no association with yield QTLs. This is also the case for other seedling traits such as GS2 (the rate of leaf emergence) and TN2 (the rate of tiller emergence) and illustrates the lack of any direct relationship between early seedling development and yield in this population. There is, therefore, potential to alter root structure to improve root traits such as nutrient uptake without incurring a yield penalty.



**Figure 2** Genetic map of Derkado x B83-12/21/5 DH population. The position of QTLs is indicated by box and whisker plots. The box indicates 1 LOD distance and the whisker the map distance for which the QTL exceeds the threshold for SIM. No QTLs were located on chromosome 1H so it has been omitted.

In order to directly assay which genes may be of importance to abiotic stresses, gene expression in roots is being studied using expressed sequence tags (ESTs),



**Figure 3** cDNA-AFLPs derived from barley root RNAs used to identify differentially expressed genes (examples highlighted) between different abiotic stresses.

cDNA-AFLPs and cDNA microarrays, as part of the Investigating Gene Function (IGF) programme (see article on Barley Transcriptome Resources). ESTs have been derived from root mRNAs of barley plants subjected to various hydroponic treatments (control, salt stress, nitrogen deprived, drought, waterlogging and etiolated) to identify common and specific responses. Over 1,000 ESTs from each treatment have been generated, allowing us to compare root gene expression among treatments. cDNA-AFLPs have been utilised to generate an independent comparison of expression (Fig. 3), and microarrays derived from unique root ESTs (~ 1,300) will be used to produce detailed expression profiles during root development and under stress. This will allow us to identify potential candidate genes for such stress responses in barley.

### Acknowledgements

The authors acknowledge the financial support of the Scottish Executive Environment & Rural Affairs Department.

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# An assessment of gene flow in red raspberry measured by SSRs

### J. Graham

Regrown commercially though little is known about the interactions of commercial cultivars with wild species. Domestication has resulted in a reduction of both morphological and genetic diversity<sup>1,2,3</sup> with modern cultivars being genetically very similar. Future advances will require the incorporation of novel germplasm, as this lack of genetic diversity can result in vulnerability to biotic and abiotic stresses. It is important therefore to safeguard any existing diversity, and for this an understanding of what is available in the wild populations is required, and of how this is being influenced by cultivation.

Until recently, no data was available on the genetic makeup of wild species, in terms of how similar wild populations were to each other and to cultivated raspberries. Recent studies<sup>4,5,6</sup> now suggest that local populations are genetically distinct from cultivated raspberries and that the populations themselves have differentiated genetically and physiologically. The extent

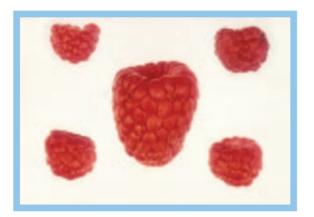


Figure 1 Wild and commercial raspberry fruit.

of gene flow has been inferred from genetic diversity and from morphological studies to be small, but has not been directly measured. However, this is not what would have been predicted for wild raspberry populations. As an out-breeder, with the potential for wind and insect-mediated movement of genes by pollen possibly over large distances, high levels of gene flow and little population structuring would have been expected.

In order to examine gene flow directly, this study set out to identify any new alleles entering one wild raspberry population via pollen flow from other wild populations or from cultivation. A system was therefore required that allowed characterisation of both alleles at each locus examined in the parental population. Once the allele status of the parental population had been determined, new alleles arising in the progeny could then be identified.

To achieve this, one wild population ('Site 12')<sup>6</sup> with 48 individuals was selected for study being of moderate size, readily accessible to SCRI and situated within an area of commercial raspberry cultivation. Leaf material was collected from all 48 individuals and all red fruit from the whole population was removed for seed extraction and plant germination. DNA was then extracted from each of the parental plants and from all progeny.

To identify both alleles at a particular locus, single locus polymorphic simple sequence repeat (SSR) markers were developed<sup>7</sup>. Repetitive DNA sequences such as short tandemly-repeated motifs account for a considerable proportion of the plant genome and

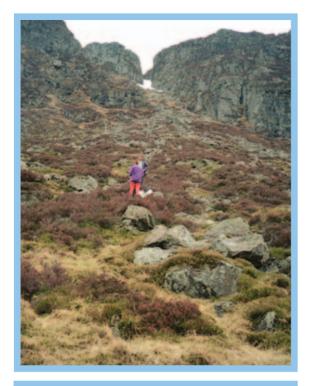


Figure 2 Glen Doll.

therefore provide an ideal marker system for this study. Changes in the number of the repeat units in the SSR, arising from mutation, results in different sized alleles at that site. These length–polymorphisms can be identified by designing primer pairs to the sequences flanking the SSR. By fluorescently labelling one of the primer pairs, PCR product identification and thus visualisation of the alleles can be achieved on an automated DNA sequencer.

From the 48 parental plants in the population, 20 produced red fruit from which seed germination occurred. A further 12 plants produced red fruit from which no seed germination occurred. The remainder either did not set fruit or the fruit did not ripen beyond the green fruit stage. Two hundred and seven-ty-nine plants germinated for analysis. Progeny from the same parent were analysed together.

A total of twelve loci were examined, the number of alleles identified at each of these ranged from one to five. For 19 of the 20 parental plants no new alleles were found in the progeny. For one parent, evidence of pollen flow resulting in pollination from out-with the site was detected with one new allele identified.

It is possible that other pollination events from outwith the site had occurred, but could not be identified due to all alleles being the same as those found within the site. However, given the high levels of genetic diversity found between populations in previous studies, it is possible to conclude that any underestimation of gene flow would be small.

Allele sizes were determined for the same 12 loci in the most widely grown commercial raspberry cultivars, 'Glen Moy' and 'Glen Ample'. From these, we can conclude that no pollination was detected from these raspberries and, therefore, erosion of the genetic diversity from this wild raspberry population by frequent gene flow from cultivation is unlikely.

### Acknowledgements

The authors acknowledge the financial support of the Scottish Executive Environment & Rural Affairs Department.

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# A physical / chemical mutation grid for barley functional genomics

D. Caldwell, N. McCallum, S. Mudie, P. Hedley, L. Ramsay, H. Liu, D. Marshall, R. Waugh

Progress in DNA sequencing has propelled a shift in genome analysis from structural to functional genomics (ie. the genome-driven systematic study of gene function). As a result, rapid methods for ascertaining the function of large numbers of genes are highly desired. With the development of sensitive methods for the detection of 'aberrant' DNA fragments in pooled samples, the use of chemical or physical mutagens to facilitate targeted gene inactivation has recently been shown to have significant potential in a reverse genetics program. Strategies deploying mutagens which induce random point or small deletion mutations coupled with PCR-based DNA mismatch screens therefore theoretically facilitate the detection of mutations in any specified target region. Some inherent attractions of this approach, over alternative biological ones such as insertional mutagenesis, include the ability to manipulate the mutagen and its dose (influencing mutation type and frequency) and to scale up or down easily. We are investigating the potential of chemical / physical mutagenesis to facilitate reverse genetics in barley. In a necessary parallel set of experiments, mutation detection protocols are being evaluated with the specific objective of being able to routinely identify mutant alleles in pooled DNA samples at various pooling depths. If we are successful in our objectives then the mutant populations that we develop will be available to all in the global research community to screen for mutations in their favourite gene. The process is outlined graphically in Figure 1.

Production and management of a large collection of mutagenised plants In pilot studies we have generated M1 test populations for each of three mutagens (gamma-irradiation, DEB, EMS). Grain from the M1 plants from four different dose rates of each mutagen (12 populations, 50 families from each = 600 families) has been carried forward to the M2 generation (comprising c. 36,000 plants). Mutation frequency in the different M2 populations has been calculated based on lethality, visible phenotypes and genome-wide mutagen-induced DNA sequence polymorphism (using AFLP). As expected, M<sub>1</sub> lethality increased with a corresponding increase in mutagen dose and ranged from <10% to over 90% kill. We observed little correlation between lethality in the M1 and M2 generations. Of the mutagens tested, EMS generated the highest frequency of visible mutations with up to 15% of the families tested showing obvious phenotypes (mainly chlorotic or albinos which tend to be seedling lethal). The AFLP analysis - while not quantitative - provided an overall 'guestimate' of the relative mutation frequency in the various populations at the level of mutations per base pair. Based on the appearance of new bands or disappearance of existing AFLP bands, frequencies of 1 mutation per 50,000 bp to 1 per 450,000 bp were estimated (i.e. 5,500 -50,000 mutations per mutant genome) in the different populations. Correlation with the 'kill' and visible phenotype frequency was high. Taken together, the results indicated that in barley the overall approach outlined in Figure 1 will be an appropriate reverse



 $M_0$  grain was mutagenised with EMS. The mutagenised  $M_0$  grain gave rise to  $M_1$  plants. The  $M_1$  plants yielded  $M_2$  grain. No more that two  $M_2$  grain per one  $M_1$  parent was taken forward, self polinated,

and grown to maturity. Young leaf tissue was harvested for genomic DNA isolation and the  $M_3$  grain was harvested. Individual  $M_2$  genomic DNA was 1-Dimensionally pooled and stored in 96 well titer plates.

Regions of interest will be amplified with gene specific primers and scanned for mutations. Individual M2 lines will be identified and mutations confirmed.



genetics strategy. These results prompted the development of a large scale "EMS Mutant Population".

In this case, approximately 80,000  $M_1$  plants were grown in the glasshouses at SCRI in 2001 and tillers harvested. From these, 50,000 grains (no more than 2 grains from each  $M_1$  plant) were selected and grown in the field. Leaf material was collected from each of the remaining 25,000 viable  $M_2$  plants and DNA is being isolated from optimally sized pools using high throughput (HTP) protocols. As indicated above, because of the necessary population size, mutations in target sequences will require to be

identified by the analysis of pooled samples. Individual mutants will then be identified from the de-convoluted pools.

Rapid and systematic identification of mutations in target sequences In parallel with the development of the populations, a critical aspect in implementing our reverse genetics

approach is the choice of a mutation detection method which will allow us to screen PCR-amplified target gene sequences with diagnostic sensitivity, high specificity and low cost. It should preferably provide some information on the location of the mutation. Through a collaboration with Dr. Tony Yeung (Fox Chase Cancer Center), we have investigated the efficacy of enzymatic cleavage of heteroduplex DNAs using CEL I, a mismatch specific endonuclease. The CEL I system is a simple assay that requires PCR amplification of the target sequence, denaturation and annealing to allow formation of heteroduplexes between the wild type and the mutant allele, enzymatic mismatch cleavage, and analysis of the cleaved products by gel electrophoresis. In addition, we have investigated the use of denaturing HPLC which is advantageous over other mismatch detection systems as it requires no post amplification template modification, is not gelbased, and as a result is both inexpensive and HTP. Both systems work well. We are currently comparing and improving the detection methods in the framework of high-throughput procedures for plant functional genomics to identify the most robust (in terms of specificity and sensitivity) and cost-effective protocols for the targeting of candidate genes. This decision will largely be based on the number of samples which can be pooled while still allowing single base pair mutations to be detected at an optimal rate. At the moment, a pool depth of 1:24 alleles is routinely allowing us to identify single bp mutations by dHPLC.

> Database development and the correlation between mutation data and phenotype The choice of target sequences and the type of mutations sought for further analysis will be dictated by the biological questions being asked by individual research groups who wish to screen the mutation grid. Those in turn will depend upon the availability of EST, cDNA and genomic sequences and is essentially case-specific. Embodied in our strategy is the recognised need that

multiple steps in the process require informatics and Laboratory Information Management tools (LIMS) which monitor the tracking of  $M_2$  plants, their DNA and their grains; the design of primers for PCR amplification of targeted regions, the interpretation and databasing of mutant alleles; and the integrated analysis of mutant alleles and phenotypic information. We are, therefore, in the process of developing a relational database to assist in the management of the populations and their progenies and to record, store and exploit all data generated within the project. Our objective is to have the whole system 'open for business' by the end of 2002.

### Acknowledgements

This work was funded as part of the SEERAD/BBSRC jointly funded project "Cereal Community Resources for investigating gene function" as part of the IGF (Investigating Gene Function) Initiative.

# QTL Mapping in Autotetraploid Species: Theory and Application to Map QTL Affecting Blight Resistance in Potato

C. A. Hackett, J.E. Bradshaw, Z. W. Luo<sup>1</sup>, H. E. Stewart, B. Pande, G. Bryan, R. Waugh & J. W. McNicol

**ntroduction** Linkage maps based on molecular Lmarkers are an important tool in plant genetics, especially for locating the quantitative trait loci (QTL) controlling important traits such as yield or disease resistance. Methods for linkage analysis and QTL mapping are well established for diploid species, but the extension to polyploid species is complicated by the large number of possible genotypes in polysomic inheritance. One method for locating QTL, in diploids or polyploids, is to compare the mean trait values for individuals with and without a marker allele. This is a useful preliminary analysis, but less informative than an analysis using all markers on a chromosome simultaneously. Here, we outline the statistical methodology underlying this latter analysis, and apply it to examine the genetic control of maturity and the resistance of the foliage to late blight (Phytophthora infestans) in a tetraploid potato population comprising two parents (Stirling and 12601ab1) and their full-sib offspring.

**Statistical methodology** There are three main stages to the statistical analysis. The first stage is to construct a linkage map of the markers for each parent and to deduce which of the four homologous chromosomes has each marker allele i.e. the marker phase. The second stage is to construct a 'graphical genotype' for each offspring, showing which chromosome segments it has inherited from which parent and where recombinations between chromosomes have occurred. The final stage is to relate the trait data to the graphical genotypes to locate QTLs. We discuss each stage in turn.

LINKAGE ANALYSIS Molecular markers are first separated into linkage groups by identifying groups of markers that do not segregate independently in the offspring. For each linkage group, the probability of a recombination event (the recombination frequency) and the likelihood of linkage (the *lod* score) are calculated for every pair of markers in each possible phase.

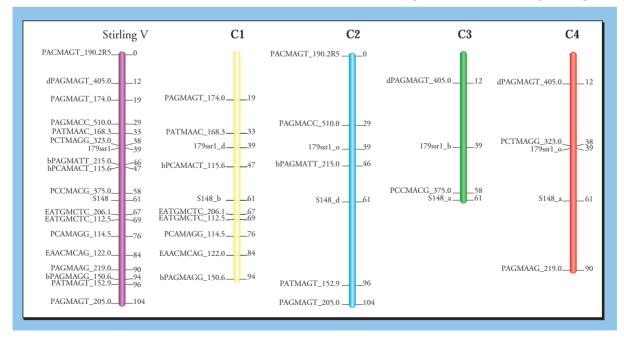


Figure 1 A map of linkage group V for Stirling, showing the overall marker order and the positions of the alleles on the individual chromosomes C1-C4. Marker positions are in centiMorgans (cM).

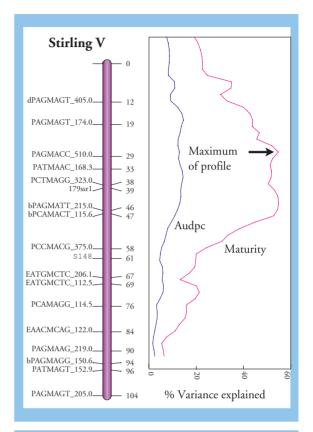
<sup>&</sup>lt;sup>1.</sup> Current address: School of Biosciences, The University of Birmingham, Birmingham B15 2TT

locus	All and a start of a s				
			_	_	
PACMAGT_190.2R5	А	0	0	Α	
dPAGMAGT_405.0	О	12.8	0	Ο	
PAGMAGT_174.0	А	19.0	A	Ο	
PAGMACC-510.0	А	28.8	0	А	
PATMAAC_168.3	А	33.0	А	Ο	
PCTMAGG_323.0	0	37.5	0	Ο	
179ssrl	BD	38.7	D	Ο	
bPAGMATT_215.0	А	45.8	0	Α	
bPCAMACT_115.6	А	46.9	A	Ο	
PCCMACG_375.0	0	57.9	0	Ο	
S148	ABD	60.5	A	D	
EATGMCTC_206.1	0	66.9	Ο	Ο	
EATGMCTC_112.5	0	69.2	Ο	Ο	
PCAMAGG_114.5	0	75.8	Ο	Ο	
EAACMCAG_122.0	0	83.6	Ο	Ο	
PAGMAAG_219.0	А	90.4	A	Ο	
bPAGMAGG_150.6	0	93.5	Ο	Ο	
PATMAGT_152.9	А	96.0	Ο	А	
PAGMAGT_205.0	А	103.9	Ο	А	

Figure 2 A graphical genotype for the inheritance of chromosomes from linkage group V of Stirling. The B allele of 179ssr1 and the B allele of S148 are from the 12601ab1 parent.

If both markers are single dose markers, with an allele present on one chromosome of one of the parents, there are two possible phases: the alleles are both on the same chromosome (coupling phase) or they are on different chromosomes (repulsion phase). For markers such as simple sequence repeat markers (SSRs), where several alleles can be identified, there can be up to 24 possible phases for each parent. The EM algorithm enables recombination frequencies to be calculated efficiently for every phase. The recombination frequency for the phase with the highest likelihood is used to order the markers. There are a large number of possible marker orders: 10 markers can be ordered in 1,814,400 ways! However a computer search algorithm, simulated annealing, compares orders to find the optimal one. Figure 1 shows a linkage map for chromosome V of Stirling, based on these methods.

GRAPHICAL GENOTYPING For QTL interval mapping it is necessary to infer the genotype for each offspring at possible QTL locations between the mapped markers. To do this, we create a 'graphical genotype' for each offspring, to show how chromosome segments have been inherited from each parent. Consider, for example, the S148 SSR locus on the map of Stirling, which has four different alleles in this population. The parental genotypes are BDAA for Stirling and CBBB for 12601ab1, so an offspring with



**Figure 3** Interval mapping of maturity and resistance of the foliage to late blight (AUDPC) for Stirling linkage group V.

the A, B and D alleles at this locus must have received the A and D alleles from Stirling, and two copies of the B allele from 12601ab1 i.e. at this locus it has received from Stirling segments from chromosome 2, and either chromosome 3 or chromosome 4. We identify possible segments for each locus in turn, and then use a branch and bound algorithm to identify the chromosome configurations that give the observed phenotypes with as few recombinations as possible. One such graphical genotype for chromosomes from linkage group V inherited from the Stirling parent is shown in Figure 2. This individual's phenotypic data is consistent with it having inherited chromosome 2 without recombination, and a recombined chromosome with part from Stirling chromosome 1 and part from Stirling chromosome 4. The recombination is located between the 179ssr1 and the S148 loci.

QTL MAPPING The graphical genotype enables us to infer the genotype at a possible QTL at any position along the chromosome, assuming that there are no double recombinations between markers. For the individual shown in Figure 2, we deduce that it

receives alleles from chromosomes 1 and 2, i.e. a QTL genotype Q<sub>12</sub>, with probability 1 at positions from the start up to locus 179ssr1, a genotype of Q24 with probability 1 at positions from S148 to the end, and a mixture of these two genotypes at positions between 179ssr1 and S148, with probabilities depending on the position. For interval mapping, we consider the possibility of a QTL at a grid of positions along the chromosome, using steps of 1-2 cM. At each position, a mixture of normal distributions is used to relate the trait value of each individual to its inferred QTL genotype(s) at that position, using the QTL genotype probabilities. The mixture model can be fitted by weighted regression in an iterative manner, modifying the initial weights from the graphical genotype to include information on the trait. We obtain a profile of the percentage of the trait variance accounted for by a QTL for the trait at each location.

**Maturity and resistance of foliage to late blight** Figure 3 shows a QTL profile for resistance of the foliage to late blight (measured as the area under the disease progress curve, AUDPC) and maturity for Stirling linkage group V. There are significant QTLs for both traits, with the QTL for maturity accounting for 55% of the variance, and that for blight resistance accounting for 14%. The most likely position is between markers PATMAAC\_168.3 and PCT-MAGG\_323.0. The QTL analysis also shows that the allele on chromosome 1 has a significantly different effect on the trait from those on chromosomes 2, 3 and 4. There is a strong linear relationship between blight resistance and maturity. When this effect is removed by a linear regression analysis, no further variance in blight resistance is explained by a QTL on linkage group V. These results indicate that we have a QTL for maturity on chromosome V, with offspring carrying the allele from chromosome 1 maturing earlier than those without this allele and that this QTL has an indirect effect on blight resistance, through the change in maturity, with early maturity associated with increased susceptibility.

Another QTL for blight resistance is found on Stirling linkage group IV, and this accounts for 24% of the trait variance. However there is no evidence for a QTL affecting maturity on linkage group IV: the resistance mechanism is different from that on chromosome V. For this QTL, offspring receiving alleles from both chromosomes 1 and 4 have the highest AUDPC scores, while offspring receiving alleles from both chromosomes 2 and 3 have the lowest scores. This indicates a double-dose locus for blight resistance, with offspring with either the allele from chromosome 2 or from chromosome 3 having some resistance, and those with alleles from both chromosomes 2 and 3 being most resistant. These results are directly relevant to potato breeding and cultivar production at the tetraploid level. For example, it should be relatively easy to identify progeny clones that combine the blight resistance alleles from linkage group IV with early maturity from chromosome V, an important goal in potato breeding.

### Acknowledgements

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# Molecular markers for agriculturally important traits in barley

W.T.B. Thomas, R. Waugh, L. Ramsay, J.R. Russell, W. Powell, T. Konishi, R.C. Meyer, G.R. Young, P.E. Lawrence, A. Booth, J.S. Swanston & A.C. Newton

The advent of comprehensive molecular marker maps means that breeders can use DNA sequences directly to select for the presence of particular genetic factors rather than attempting to measure the effect these factors have on the appearance (phenotype) of the character under selection. This technique is called Marker Assisted Selection (MAS). When selection for donor alleles at target loci, where changes are desired, is combined with recipient alleles in the remainder of the genome, problems associated with linkage drag, i.e. the inadvertent selection of unwanted traits, in the introgression of new genes are greatly reduced. This potentially makes great savings in time and cost in a breeding programme, and offers more accurate selection when

reliable measurement of phenotype is difficult. The large library of molecular markers available to SCRI is, therefore, a valuable resource in potential MAS applications. Over the past 4-5 years, we have identified markers that can be used to select for a number of key traits in barley.

**Barley Yellow Mosaic Virus** The Barley Yellow Mosaic Virus complex (BaYMV) is spread by a soilborne fungus and can cause severe loss in yield and quality in winter barley crops grown in certain parts of the world. Reliable assessment of resistance to the disease can only be done with specialised testing facilities and, even then, is not totally effective. A number of resistant cultivars have been developed in Europe, utilising resistance genes located on the distal portion of the long arm of chromosome 3H. The two main sources are the *rym4* gene derived from Ragusa, which is effective against BaMMV and BaYMV-1 but not BaYMV-2, and the *rym5* gene derived from Mokusekko 3, which is effective against all three races. BaYMV-2 is gradually becoming more prevalent and it is therefore important for breeders to be able to distinguish between the two resistance genes in their selection programmes. A Simple Sequence Repeat

(SSR) marker developed by SCRI can not only distinguish between resistant and susceptible genotypes at the locus on 3H but can also distinguish between the two different sources of resistance. This marker is now being used by a number of commercial breeders in their selection programmes and has proved to be very reliable. We have recently identified Single

Nucleotide Polymorphisms (SNPs) that can also be



Susceptible rym5 rym4

Figure 1 Identification of BaYMV resistance using a molecular marker.

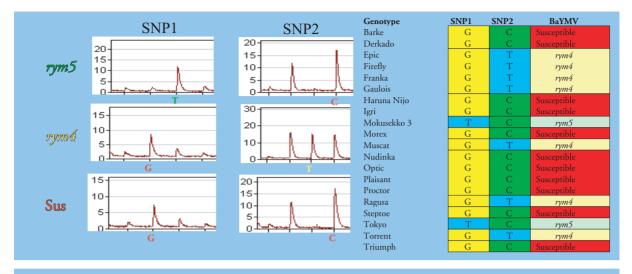


Figure 2 Pyrosequencing detection of Single Nucleotide Polymorphisms Identifying Cultivars Resistant or Susceptible to BaYMV.

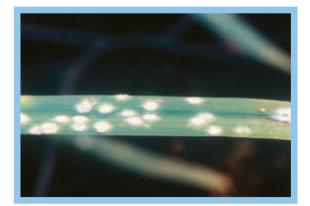
used to identify the two sources of resistance. Rapid, non-gel based methods, such as Pyrosequencing<sup>TM</sup> (www.pyrosequencing.com), can easily be applied to detect SNPs. SNPs therefore offer the possibility to screen germplasm on a far greater scale than previous molecular marker systems and have great potential utility in MAS programmes.

**Rhynchosporium** Rhynchosporium (Leaf Blotch or Scald) is becoming more prevalent in UK spring barley but the resistance of currently recommended cultivars is, with the exception of Pewter, poor. Some sources of resistance are available but a specialised disease nursery is required to select resistant types from amongst the progeny of crosses. Results from the UK Cereal Pathogen Virulence Survey show that the cultivar Digger, which was on the UK Recommended List from 1986 to 1991, has consistently good levels of resistance. The SCRI cultivar, Livet, also had a good level of resistance to scald and inherited a resistance gene from Digger. From studies of a mapping population, we have identified an SSR marker that is closely



linked to this resistance gene and have been able to trace the allele back to Osiris and forward to Pewter. We have used this marker to correctly predict the resistance of a number of other cultivars and it therefore can be used in MAS schemes to detect lines carrying a particular gene for resistance to scald.

**Powdery Mildew** Powdery mildew is the most prevalent disease of spring barley in Europe. Breeders have incorporated the *mlo* resistance gene into a number of cultivars and it has provided effective resistance for over 20years. Selection for resistance in the field is generally effective in most European countries but incidence of the disease can occasionally be low, causing problems in identifying resistant lines. A number of breeders now utilise Doubled Haploidy to develop populations of inbred lines, in which selection is more effective than in products of a pedigree programme. Doubled Haploid plants are initially multiplied in the glasshouse before field assessments but, in crosses between resistant and susceptible lines, half the plants will be susceptible to powdery mildew. Early identifi-



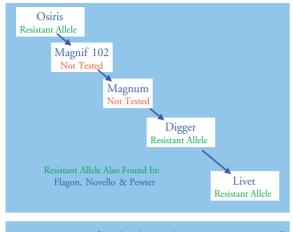


Figure 3 Use of molecular markers to trace source of scald resistance in Livet.

cation of such plants would save time in culturing and glasshouse facilities and is possible through MAS being applied to leaf tissue being sampled at the stage of green plant isolation. A barley SSR marker can identify the *mlo11* allele possessed by cultivars carrying this resistance and is therefore ideal. There are two main sources of *mlo11* resistance and evidence from the UK Cereal Pathogen Virulence Survey suggests that the L100 source, found in the cultivars Apex and Riviera, can show infection levels that were higher than expected. This contrasted with the L92 source, found in Atem and its derivatives. The SCRI marker can distinguish between them and is therefore of additional significance to breeders.

**Epiheterodendrin** Epiheterodndrin (EPH) is a cyanogenic glucoside produced during germination. Enzyme activity during mashing and fermentation releases a breakdown product that can, under certain conditions, react with ethanol in copper stills to form ethyl carbamate during whisky production. In cases

where this is a problem, the industry has utilised cultivars that have inherited an allele at the eph locus that appears to block formation of epiheterodendrin. In an HGCA and LINK funded project, SCRI identified SSRs that are linked to eph and can be used to select the non-producers. Whilst the linkage is close, a number of recombinants have been found, so the markers may not be diagnostic in all cases. They can still be used in MAS programmes, however, provided one knows the parental phenotypes, i.e. can identify one of them as a non-producer of EPH, and can also distinguish the parents genotypically by using the SSRs. In the longer term, we plan to identify the gene and develop a direct gene marker that will be diagnostic for non-producers. This could be adopted by plant breeders to ensure that there were sufficient cultivars that did not produce EPH to meet all the requirements of the distilling industry.

**Further Developments** As we acquire more knowledge about the barley genome, we will be able to identify more markers of value in selecting for economically important characters, particularly those relevant to stable yield and quality. Genomics initiatives such as the BBSRC Investigating Gene Function are expected to yield large amounts of potentially valuable information. The challenge is to connect such data to biologically relevant questions and then deploy it in a targeted manner to improve the enduser value of barley.

For further information on the above markers, contact: Jonathan Snape at Mylnefield Research Services, Invergowrie, Dundee DD2 5DA, UK.

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# Management of genes and organisms in the environment

### Geoff Squire, Glyn Bengough, Jim Duncan, Dave Marshall

The global summit in Johannesburg ensured that habitat protection and food security remained of international concern in 2002<sup>1</sup>. While interest is often attached to rainforests and coral reefs, the common arable soil and its biota are an essential part of the biosphere. If they cannot be sustained to yield food and raw materials in perpetuity, there is no possibility that other habitats can be left intact or restored. Locally, also, the choice is to exploit Scotland's fields to its detriment - "the land left dry as a rat-sucked swede<sup>2</sup>" - or to manage soil and its plant and animals with purpose to ensure their survival and productivity for millennia to come.

Disturbance is essential to the arable system. Hence, resilience – the ability of the system to reassert itself – is a most important quality. The scientific challenge is to define resilient states, because the arable soils and their associated plant and animal populations are surprisingly elusive. The controlling processes occur at small scales in fine soil pores, or are mediated by microscopic organisms, whose taxonomy and functions are often impossible to study outside the system. The new 'environment' theme at SCRI aims to probe these habitats using the most advanced technical and theoretical tools and to quantify resilient states towards which management can move the system. A crucial further contribution to define criteria for environmentally beneficial crop genotypes that confer resilience to the soil and food webs.

The Theme is organised into three inter-relating Programmes, concentrating on the plant and invertebrate food webs, soil-plant processes, host-parasite coevolution and the generic topics of bioinformatics and modelling. Plant genotype lies at the centre of each programme. We now profile one of the Programmes to illustrate how the science in Theme 3 links with other Themes at SCRI and with science and practice elsewhere. Longer articles follow, describing recent advances in probing soil processes, the arable seedbank, trait space, geneflow, and integrated pest management.

Co-evolution - the role and functioning of a new research programme The completely new programme - Host-Parasite Co-evolution - (?) is centred on interactions between plants and microbial pathogens, and nematode and arthropod (insects and mites) pathogens and pests, at the scale of field and ecosystem, rather than the molecular or genomic level. The main goal is not to determine the genetical or biochemical bases of host-parasite recognition but to predict the consequences of success and failure of host-parasite recognition in crops and natural ecosystems. Thus it complements the new Molecular Pathology (Plant-Pathogen Interactions) Programme in Theme 1. The two Programmes are now located in the same building, with the express purpose of maintaining, promoting and facilitating collaboration between them.

The 'raison d'etre' of 'Co-evolution', as the new Programme came quickly to be known, is the demand for 'sustainability' in modern agriculture. For sustainable agriculture to be successful, it will have to balance environmental and resource constraints with the overall food crop requirements of mankind. For the new programme, this essentially means maintaining crop yields while controlling diseases and pests and at the same time minimising or even eliminating the use of pesticides.

An important component of sustainable agriculture is host resistance to diseases and pests but one of the largest threats to pest such resistance is the ability of pests and parasites to overcome resistance, frequently with no prior warning. In natural ecosystems the coevolution of hosts and their parasites generally leads to a durable and sustainable balance. Understanding this balance, which has genetic interaction with the environment at its centre, will lead to manipulation of the key interactions in favour of stability and resilience in a agricultural systems which favours the crop.

Not only do parasites cause selection for resistance within host populations, but also resistant hosts apply selection to parasite populations to overcome that resistance. These selection pressures depend upon genetic, spatial and temporal factors, the expression of each being modulated in turn by environmental factors. In crops, we have some control on all but the environmental effects on gene expression in the host but we have no control over the parasite populations except through the host on which they are dependent. By manipulating host genetics of host resistance we can study the effects of this dependency which will determine which strategy is appropriate for achieving durable resistance in different types of host-parasite interactions.

The programme is focussed on this co-evolution of plant and parasite at the level of crops and natural populations of the parasite, and on variouson time scales. ranging from annual seasons to geological time (?). It covers various types and qualitative and quantitative expressions of resistance in the host (host, nonhost, mono-, oligo- or polygenic etc) and variation in the parasite. Fundamental studies on basic genetics, biochemistry and physiology of resistance and theoretical studies pursued in other programmes are being utilised to inform these studies. For example recent theoretical studies indicate that monogenic or specific resistance, which is not normally durable, reduces parasite fitness/aggressiveness, whilst polygenic resistance, often more durable, selects for increased fitness/aggressiveness. A sustainable strategy should therefore achieve an effective balance between these types of resistance and take into account environmental factors affecting their expression.

The longer a resistance can operate effectively against the challenge of the parasite, the more 'durable' it is. Some genes can be very effective, *e.g.* R-genes to late blight in potato, but they are not at all durable, hence potato growers have not abandoned fungicides in their favour. Durability cannot be determined because, by definition, it involves possible events in the future, but it might be possible to make useful predictions about it if there were enough knowledge about the genetics of crop and parasites, parasite migration and the stability of resistance in varying environments.

Work in the programme ranges from basic to applied but is largely strategic. Longer term. its aim is quite practical, namely to set particular and general ground rules for determining the potential benefits of various resistances and strategies for deploying them, for example to determine the relative value of resistances from several wild sources for future exploitation. A variety of plant-parasite systems are covered in the programme (see Table 1), although the same fundamental aim unites all of them, and no type of resistance is excluded from consideration. Thus pesticide resistance in the parasite and genetically modified resistance in the host are not excludedincluded.

Plant	Main areas of study
<b>Potato</b> Viruses Peach aphid Late blight Trichodorid nematodes Potato cyst nematode	Variation within, and resistance to, Tobacco rattle virus (TRV). Population dynamics/genetics and movement of M. persicae between potato and other crops. Population genetics and epidemiology of P. infestans pathogen and durability of host resistance. Variation within species and transmission of TRV. Population structure and virulence of G. pallida.
Barley	Population structure of Rhynchosporium secalis (leaf blotch) of barley in response to selection. Effect of host heterogeneity on pathogen population structure.
<b>Raspberry</b> Viruses Raspberry aphid <i>Phytophthora spp.</i>	Characterisation, detection, epidemiology and aetiology of virus diseases and resistance to them. Durability of resistance genes to Amphorophora idaei and transmission of raspberry viruses. Aetiology and control of, and resistance to, Raspberry root rot.
<b>Ribes</b> Viruses Ribes mites	Characterisation, detection, epidemiology and sources of resistance to Blackcurrant reversion and Gooseberry veinbanding viruses. Inter- and intraspecific variation in Cecidophyopsis species and their roles as pests and/or virus vectors.

 Table 1
 Main interactions studied in the Host-Parasite Co-Evolution Programme.

The scope and nature of the Programmes also implies a considerable amount of work on the aetiology and epidemiology of diseases and pests and of the ecology of the agro-ecosystem in which they are deployed. Much of the epidemiology and associated topics such as diagnostics are not core-funded but funded by contract with private bodies and other government agencies. This mix will be continued in future hopefully with even more participation of non-governmental bodies.

New opportunities and new funding The three other Programmes in Theme 3 – Ecosystem management and Biotechnology, the Plant-Soil Interface and Computational Biology - operate similarly across Themes and to a wide range of external collaborators and agencies. The Theme as a whole still has its base in the arable system but is extending its principles and techniques to a wide range of habitats including those in secondary succession and grassland and peri-urban environments. Within the Theme itself, new research will create synergies to tackle previously intractable problems. Major new laboratory and field experiments, operating across the Programmes, were put in place in 2002 to quantify self-organisation in systems resulting from perturbation and change in crop genotype. A range of major new externally-funded projects

also began in 2002. SCRI will lead a multi-institute collaboration on geneflow between crop fields (funded by DEFRA until 2006), is part of a European grouping examining the impacts of GM varieties on soil processes and organisms (EU funded) and leads internationally through new competitive funding for research and for collaborative networks on Phytophthoras. This new funding will allow SCRI to make major advances in genetic diagnostic techniques, spatial processes, mechanisms of pollen and gene movement and more generally heighten our capability to predict the consequences of change in environment and genotype. The grant-in aid from SEERAD provides a strong base and continuity while the present external, competitive grant income of more than £7M is a testament to the Theme's success and relevance to science and the modern world. SCRI is now a major and expanding European centre for research in environmental plant and microbial biology.

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<sup>&</sup>lt;sup>1</sup> World Summit on Sustainable Development, Johannesburg, South Africa, 26 August to 4 September 2002. http://:www.johannesburgsummit.org/html/documents/summit\_docs/2009\_keyoutcomes\_commitments

<sup>&</sup>lt;sup>2</sup> Lewis Grassic Gibbon. 1933. *Clay.* Republished 1993 as Booklet Number 1, The Grassic Gibbon Centre, Arbuthnot Community Enterprises.

# Probing the soil-plant system ?

R Wheatley, G Bengough, P Hallett, B Griffiths, T Daniell, B Marshall, GR Squire

Coil-plant based habitats are dynamic and constant-Oly evolving through many components that are intrinsically interconnected. A holistic approach is required in any research programme designed to study the integrity of such managed habitats. Solutions derived in isolation will inevitably present new problems. The programmes in Theme 3 integrate and extend research by combining advanced experimental techniques with theoretical modeling. The aims are to define the essential elements of the system and to devise and deploy plant genotypes that will maintain the system's resilience and productivity.

The arable system is driven by the primary production in crops, weeds and plants of hedgerows and field boundaries. Energy, from the sun, enters the biosphere, converting atmospheric carbon dioxide and soil water into sugars. In a complex of reactions, using nutrients obtained from the soil, plants produce many other chemicals and grow. Their vegetable matter is eaten, and used by animals to make their own tissues, or returned to the soil surface as litter. Respiration, by animals, plants and microorganisms returns carbon dioxide to the atmosphere directly. Decomposition of the dead remains of all these by microorganisms returns more of this carbon to the atmosphere, while some of the converted carbon dioxide remains as a pool of fixed carbon, as a standing crop of plants and animals or as soil organic matter (Fig. 1). These standing pools are dynamic and as some fixed carbon is gained, some is also lost; the two processes may not be in balance. Much of the organic material is used to provide energy to the primary decomposers (Fig. 2), leaving a surplus of nitrogen, as ammonium-nitrogen. So the carbon cycle is linked to the nitrogen cycle by the release of ammoniumnitrogen, which is then further transformed by a range of bacterial functions. By such pathways, the 'fixed carbon' is used as a source of energy, which is transferred through the biosphere.

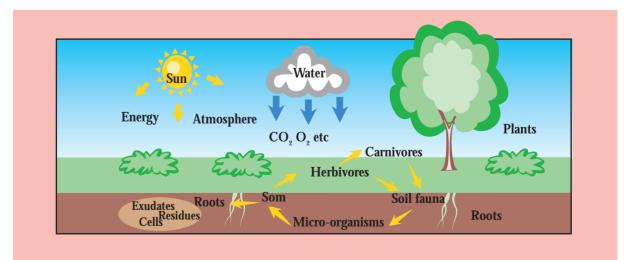
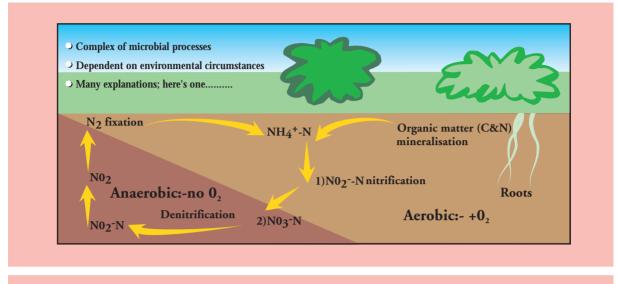


Figure 1 Carbon cycle



### Figure 2 Nitrogen cycle

These two cycles and the links between them are basic to the functioning of the system. They have to be maintained globally and locally to ensure continued production in the habitat and to avoid wider environmental problems. Soil must be enriched and stabilized, nitrogen kept within the system and not leaked away, atmospheric carbon dioxide levels prevented from rising and harmful organic compounds degraded within and not lost to cause damage elsewhere. Since regular offtake of grain or other materials is necessary, large disturbance is unavoidable, so factors conferring resilience to change are more important than factors



**Figure 3** The primary root of maize can shed >5000 border cells in a single hydration event. We are studying the role of these cells, and their associated exudates, in physical and biological interactions in the rhizosphere, using fluorescent reporter genes, measurement of root growth pressures, and kinetic analysis of nematode motion. Border cells both decrease the mechanical resistance to root penetration, and may act as biological decoys in the rhizosphere, influencing pathogens and biocontrol organisms.

conferring stability. It is often assumed, for instance, that resilience requires species diversity - but how much is required? And how much and of what form is required to maintain the system in good working order, as distinct from satisfying the aesthetic requirements of humans?

The difficulty in answering such questions is that most of the action occurs at very fine scales in soil structures that are currently impossible to observe *in situ*. Accordingly, we have developed a range of innovative concepts and techniques for probing the soilplant system so as to link the steps in Figs 1 and 2.



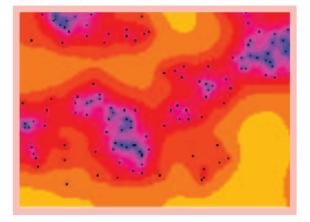
**Figure 4** A novel miniature infiltrometer probe designed to measure soil hydraulic properties at the rhizospherescale. The rate of wetting of soil by water and alcohol solutions indicates the nature of the organic coating on the surface of soil particles. The probe (0.4 mm radius) has successfully demonstrated differences in hydrophobicity between rhizosphere and bulk soil; effects which are species (root and microbial exudate) dependent.

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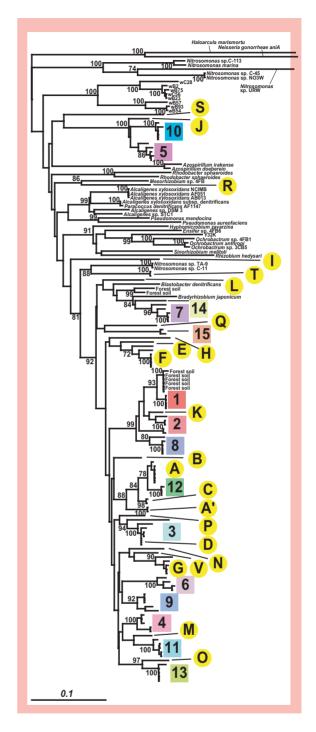


**Figure 5** We have developed the biological thin-section technique, and used it to study the spatial distribution of micro-organisms and pore-space in soil. This has been used to develop 3-dimensional models of organism distribution in relation to water and solute (including pesticide) transport

Highlights include thin sectioning and modeling techniques for quantifying pore structure and microbial distribution, elucidating the physical and biological roles of root exudates and border cells in providing carbon sources and signaling compounds for soil microbes, and molecular profiling of microbial communities. Figures 3 -9 gives examples of the methodologies and results and their role in elucidating biophysical and biological interactions.



**Figure 6** Mechanistic models of water and solute transport, and root uptake at scales from an individual root in a structured soil (horizontal section through 3-D lattice-Boltzmann model; LHS, above), to clustered distributions of roots from neighbouring plants (finite element model; RHS). Root function can be therefore be studied theoretically in field soils, with structures quantified using the thin section technique.



**Figure** 7 A phylogenetic tree showing relatedness between types of denitrifying bacteria isolated from an upland grass system. DNA was amplified from environmental samples taken from the Scottish borders. Sequences in black are markers from databases. Sequences in circles were isolated from mineral soil samples and sequences in boxes were amplified from plant roots (rhizoplane samples). The tree was produced using a neighbor-joining method and bootstrap support is shown where above 70%.

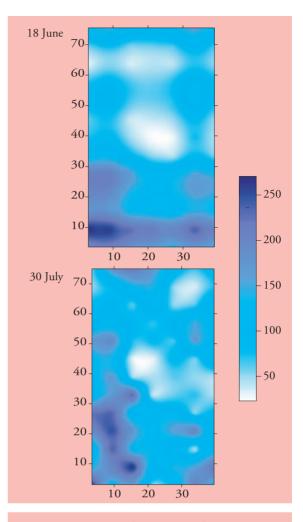
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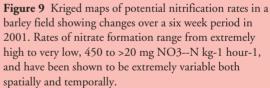
### Environment



**Figure 8** This bacterial feeding Caenorhabditis sp. Is characteristic of nutrient-rich conditions. The response of other soil nematodes to environmental changesis a bioindicator of soil health and function

We have, therefore, a suite of biophysical and molecular probes with which to assess the functioning of the soil-plant system. They will be combined with methods in plant and invertebrate population biology to prescribe criteria for a resilient and well functioning arable ecosystem, in which the flow of resource is well balanced between the crop and the wider food web, and losses from the system minimized. The capacity of our research to bridge disciplines of plant molecular sciences, genetics, physiology, microbiology and soil biophysics, will then be channeled into a major initiative on the genetic basis of root deposition through border cells, exudates and skeletal remains. Our aim is to guide plant geneticists and breeders as to the 'system-enhancing' traits that can be incorporated into new crop varieties.





# The arable seedbank as a source of biodiversity and a reliable indicator of field management

Cathy Hawes, Joyce McCluskey, Pete Ianetta, Milena Maule, Adele Parish, Gladys Wright, Mark Young, Geoff Squire

C eedbank-based plant populations have been a fea-Oture of arable fields since the beginning of farming. Many of the species that are now regarded as weeds were present at the last glaciation or else brought to the UK from Europe by migrant farmers in the Iron Age and later periods. These arable plants are primary producers, along with the crop and boundary vegetation. They retain structural and chemical diversity in canopies and root systems, and accordingly support many more species of herbivore, decomposer, predator and parasitoid than the crops themselves. Most of the species in these functional groups are not detrimental to crops and many of the predators and parasitoids attack crop pests. The arable plants also contribute to stabilising soil and retaining nutrients in the system at times when a crop cover is absent.

However, the disadvantage of arable plants as weeds has received more attention in the past 50 years than their benefits in stabilising

the carbon and nitrogen cycles. The balance of energy flux probably has swung so far towards the crop that some important properties of the arable system are in danger of being severely damaged. To define is\_\_\_an what acceptable balance between crop and

weed biomass requires a basic understand-

ing of the population dynamics of the arable flora and fauna. To this end, research in arable weed seedbanks at SCRI has intensified in recent years. Topics of interest include -

characteristics of the pre-herbicide (pre-1960s) seed-bank

the state of the arable seedbank in the late 20<sup>th</sup> century definition of resilient states in the seedbank community, defined by the number of functional types and their spatial distribution

indicators for assessing the impact on the system of new crop varieties and management, including genetic modification

mechanistic links between physiological traits and community properties.

Here, we report on progress in several of these topics and indicate recent initiatives.

**Pre- and post-intensification seedbanks** A steep rise in the intensification of field management occurred between the 1970s and 1990s. The area of the arable land sown with winter (autumn-sown) crops increased markedly, and though the area of cropped land sprayed with chemical herbicide stayed about the same, the practice and type of

> chemicals used both changed. More different types of active ingredient were used, more applications were made in both autumn and spring, and many of the new chemical affected more weed species than previously. Consequently, the light and nutrients that were available for the weed flora and the wider food web became severely reduced. Many author-

ities now consider that the seedbank, the weed flora, the invertebrates and the higher fauna have declined, not equally in all parts of the UK, but certainly over very large tracts of arable land. Despite this, the seedbank has resilience. Many of the commonest species in surveys of the seedbank in Scotland in the 1980s and 1990s were observed throughout the 20<sup>th</sup> century. Few new species have become established – perhaps only oilseed rape since the 1970s and some of the exotic willow herbs (e.g. *Epilobium ciliatum*). At the end of the 20<sup>th</sup> century, the seedbank still contained many species, particularly of the *Chenopodiaceae* and *Polygonacea*, that are nutritious and favoured as food for a wide range of invertebrate herbivores and birds. The opportunity is not yet lost therefore to find ways to balance what are perceived as the positive and negative aspects of the seedbank to the benefit of the arable food web. For this, adequate comparators are needed – objective criteria that define the state of the seedbank community and its change in response to physical factors and field management.



**Community indicators as guides to field management** Data on the seedbanks since 1915 have now been

probed to find such comparators. Few early authors gave information on the spatial sampling scheme they used. However, the rank-abundance curve (which can be derived simply from lists of species and their numbers) proved to be a useful indicator of the way the community as a whole changed in response to management (Fig. 1). The curve was similar in several preintensification studies, but fell systematically during periods of suppressive management (Fig. 1). Notably, the curve was also induced to rise again to near preintensification values by relaxing the management of the 1990s cereal rotations. The seedbank has therefore some resilience.

Knowledge of the numbers of each species and where they are found in a field allows a more useful descriptor to be calculated - the species-accumulation curve. This curve is derived from maps of spatial distributions by working out the average number of species in samples of two, three, four, etc., from the total sample pool. If the same field is sampled over time, say, before and after a change in field management the curve parameters give indications of the way the spatial distribution of floral diversity has been affected.

This method was used to answer the question as to

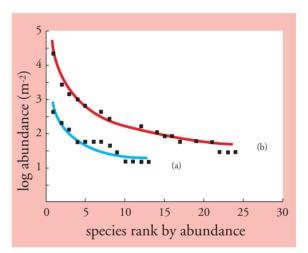


Figure 1 Species ranked by abundance in (a) an early seedbank subject to suppressive management for two years, and (b) a 1990s seedbank in a cereal rotation induced to expand over 6 years by having more overwinter fallow and using fewer herbicide sprays. The starting curve in (a) is similar to the ending curve in (b), showing resilience in the community.

whether seedbanks can be regenerated by treatments such as set aside. The result was consistent across five sites differing in their complement of species. Setaside increased the slope of the relation in a way that indicated uncommon and rare species had increased in abundance or new species had entered the seedbank at low abundance; there had been relatively little change to the total abundance of seed in the field (Fig. 2). The value of the species-accumulation curve was that it enabled quantitative comparisons between sites that had different complements of species.

The search for a balance in the seedbank Such comparative descriptors of the seedbank community are a

### Environment

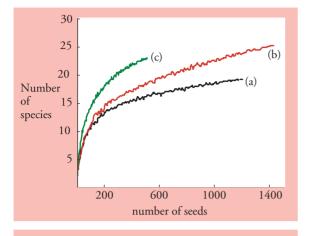
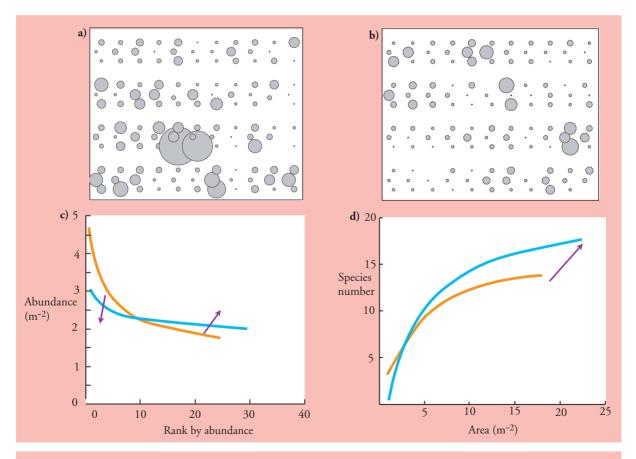
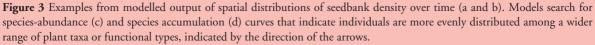


Figure 2 Species-accumulation curves at the end of cereal cropping (a), after five years in set-aside (b) and after return to arable cropping for two years (c).

unique baseline against which to compare developments in arable farming, specially in assessing new crop genotypes, including GM varieties. In this respect, SCRI's knowledge of seedbanks will contribute to the analysis of the Farm Scale Evaluations data, scheduled to be published in 2003. More generally, criteria for the seedbank and the soil habitat will be combined to provide a means for assessing the state and resilience of the arable system. The methodologies will be used to define characteristics of new crop varieties and methods of growing them that should enhance the soil and aerial systems.

What then is the ideal seedbank? Simple models of plant life cycle and seed dispersal are used to search for seedbank communities that contribute much to the food web but little to the weed burden (Fig. 3). The models lead to hypotheses that are being validated experimentally. To date, the communities have been characterised at species level, but the theoretical studies suggest that community-scale descriptors, based on individuals in small populations, should mimic those for species in a field, group of fields or even an agricultural region. If this is shown to be valid, then an individual-based approach to communities offers a possible means to reduce the scale of operations neces-





sary in future risk assessments of new biotechnology. New research at SCRI is measuring the genetic and physiological variation in selected species so that experimental populations can be constructed in order to explore the link between plant traits and community properties.

Acknowledgements SEERAD provides the core funding for the analysis of community-scale descriptors, for modelling and the new within-species studies. DEFRA funded a desk study on the importance of weeds to arable biodiversity, and field research through the Agricultural Development and Advisory Service, who managed the experiment from which Fig. 2 and Fig. 3 were taken. SCRI is a joint contractor with CEH, IACR and UAD on a modelling study of arable food webs funded by DEFRA.

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# **Outcrossing among crops and feral descendents - geneflow**

G R Squire, G Begg, J Crawford<sup>1</sup>, S Gordon<sup>2</sup>, C Hawes, C Johnstone, B Marshall, G Ramsay<sup>3</sup>, C Thompson<sup>3</sup>, G Wright, M Young

**ationale** Crops and wild plants have lived side by Riside and exchanged genes since the beginning of agriculture. Oilseed rape typifies these exchanges, having arisen from two wild species and being now a crop and a feral weed at the same time. Oilseed rape is also an outcrossing species: its crop and feral forms exchange genes freely, and they both cross at very low frequency with several wild arable plants (Fig. 1). The fields and feral populations form a shifting patchwork that changes spatially over time as crops are sown and harvested and as feral populations arise and die. The crop and ferals provide an interesting case study of evolution in fragmented and dynamic populations, but they have also brought to wider attention the issues of crop purity and integrity of habitat, specially following applications by seed companies to grow GM oilseed rape and other GM crops in Europe. Debate has intensified during the pre-



Figure 1 A field of oilseed rape contained the sown crop, probably feral descendents of previous crops grown in the same field, and occasionally a wild relative such as the Raphanus raphanistrum (white flowers) shown here.

sent Farm Scale Evaluations of herbicide-resistant GM crops. Particular issues are -

impurities in crop yield might result from outcrossing between nearby fields and feral populations left by an earlier crop

feral populations might become more competitive than species in the existing arable seedbank, and so change the composition of the seedbank assemblage, especially if they acquire GM traits such as herbicide resistance

Various authorities have set, or are considering, thresholds for GM impurities should GM crops be commercially grown. Quantitative and predictive approaches to geneflow and population dynamics are therefore sought.

**Methods of study** Research on geneflow and GM impact assessment is well networked internationally. Major projects are underway in North America,

Australia, France, Germany and the UK, among other countries. Within this subject of study, the Scottish Crop Research Institute and its collaborators have developed a major capability in geneflow research with an emphasis on regional processes (Table 1). Facilities and expertise include -

a study area of around 600 km<sup>2</sup> in Tayside and Angus in which the configurations of fields and ferals are used to examine regional dynamics

a range of molecular diagnostic techniques for detecting and estimating geneflow and paternity

techniques using male sterile 'bait' plants for estimating distance and frequency of geneflow from fields into the surrounding region (1 to10 km)

the study of physiological traits (e.g. induced dormancy) that differentiate crop varieties and encourage persistence

an extensive network of sites in the UK for tracking the population dynamics of feral weeds in the seedbank and their effect on the arable plant community,

predictive models of geneflow and persistence of

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<sup>&</sup>lt;sup>2</sup> Host-parasite Co-evolution Programme, SCRI

<sup>&</sup>lt;sup>3</sup> Genome Dynamics programme, SCRI

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Factors affecting cross-pollination between fields of different male fertility. (Development of advanced diagnostic and statistical methods for estimating and managing whole-field average cross pollination.) DEFRA. 2003-06. Consortium: SCRI, IACR, CEH, CSL, NIAB, ADAS. Contact: Geoff Squire, Scottish Crop Research Institute g.squire@scri.sari.ac.uk

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**Investigation of feral oilseed rape populations.** (Definitive study of the origin and persistence of feral populations.) DEFRA. 1993-96. Contact: g.squire@scri.sari.ac.uk

### Table 1. Research projects on geneflow in Scotland

crop-derived feral seed (relevant to management of GM residues)

advanced diagnostic and statistical sampling techniques for estimating whole-field average cross-pollination between fields, including GM crop fields

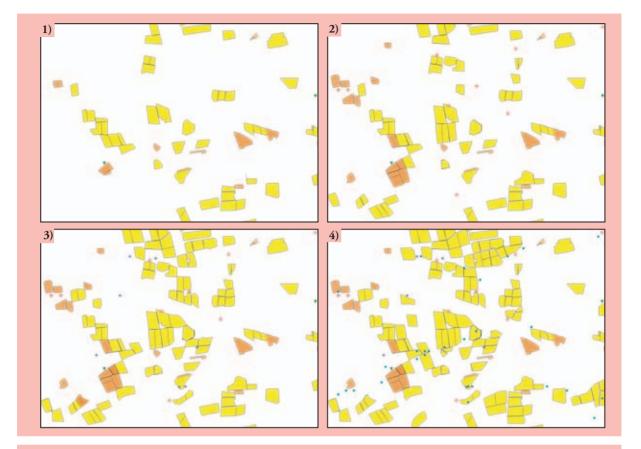
Work in the large study area is a particular strength of the programme. It provides a natural environment in which processes can be scaled between plant and landscape (Fig. 2).

**Persistence and role of ferals** Feral populations are established both in fields and along waysides and waste ground when seed from a crop drops to the soil during harvest or is moved around by farm machinery and vehicles. The populations decline rapidly during their first two years, but then persist as a residual at a typical density of 100 m<sup>-2</sup>. Oilseed rape is one of very few species to have entered the arable seedbank community in this way in the 20<sup>th</sup> century. It is now a widespread weed of middle to low rank in abundance. Factors that have caused its appearance are seed shatter at or before harvest, then inducible dormancy if conditions at the soil surface do not promote germination. Commercial varieties differ greatly in the fraction of seed that can be induced into dormancy by

factors such as low temperature or dryness. The trait is largely unobserved in conditions used for standard testing of uniformity in crop varieties.

Given that ferals commonly persist for more than five years, and that oilseed rape appears as a 'break' crop in a cereal rotation every two to four years, oilseed rape fields commonly consist of the sown crop and an assortment of ferals of different origin. This has caused little concern when the sown crop and ferals are of the same general oil type (e.g. both are foodquality varieties). If the ferals were of GM origin, and the sown crop not, then GM seed would be present in the harvested seed, though no GM-derived protein would be in the oil processed from the seed. Our understanding of the evidence on biosafety is that GM traits such as herbicide tolerance are neither more harmful nor beneficial to human or animal health than non-GM varieties. However, the presence or degree of GM impurity in harvested yield is an issue for many people. As a guide to management, therefore, mathematical models of GM feral persistence in the seedbank and in harvested yield have been constructed, based on research by SCRI and other groups, notably Peter Lutman and colleagues at Rothamsted Research. The models will be used to define forms of

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**Figure 2** A 5 x 7 km section of the study area, showing the cumulative area over four years (1, 2, 3, 4) occupied by winter (yellow) and spring (orange) oilseed rape and the occurrence of wayside feral populations (symbols) first observed in each of the four years. By the 4th year, oilseed rape had been grown in much of the arable land in the section

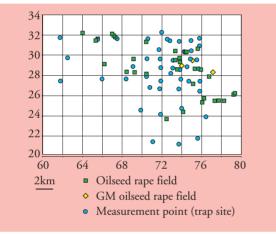
field management that would keep impurities arising through ferals below specified thresholds. At present, a level of impurity of less than 1% is feasible provided the most rigorous field management is applied. Impurities also arise through geneflow between fields however.

**Geneflow between and from crops** Research in the early 1990s at SCRI found that geneflow in oilseed rape was more extensive than was previously believed. Subsequent models of regional geneflow over several kilometers suggested cross-pollination at a receptor field or population should depend on the arrangement of donor fields in the landscape; moreover, the proportion of crossing from a particular type or variety of crop should depend on the proportion of this type among all donor fields.

A regional scale experiment was therefore put in place to test this hypothesis. A combination of male sterile bait plants (which are only fertilised by pollen from external sources), male fertile plants and molecular diagnostic techniques was used by Gavin Ramsay,

Caroline Thompson and colleagues to measure geneflow from normal commercial fields and a field that had unique markers. This experiment demonstrated that insects were important vectors (the previous model has incorporated only wind-borne pollen), that after declining very steeply over 50 m from a fieldedge, geneflow continued at low frequency for several kilometres; and that cross-pollination from different types of donor field was indeed in proportion to their area and configuration. From this research, and complementary studies by Jeremy Sweet and colleagues at the National Institute of Agricultural Botany (NIAB), workers in France and more recently in Australia, whole field cross-pollination was estimated to be typically less than 0.1%, i.e. 1 seed in one thousand set on the plants in a field would be from a pollen source outside the field.

How the values are influenced by local context is highly uncertain, however. Therefore work is now in progress to measure outcrossing from fields in Scotland which are sown with the GM herbicide-tolerant crops of oilseed rape used in the Farm Scale Evaluations (Fig. 3). The easily detectable markers in these crops should allow more accurate estimates off cross-pollination at low frequency than had been possible before. For wider applications, a consortium led by SCRI is developing advanced, high throughput diagnostic techniques for measuring geneflow at low frequency among non-GM fields. Together these studies will also quantify the pollination efficiency of insects, such as bumble bees, hive bees and pollen beetles that contribute to crossing, quantify the spatial patterns of crossing in fields, and develop the sampling protocols necessary to estimate whole-field crossing accurately.



**Figure 3** Plan of experimental study area in 2002, showing oilseed rape crops (green), GM oilseed rape crops in the Farm Scale Evaluations (yellow) and stations for measurement of geneflow and pollinators (dots).

Impact on habitat integrity At present, feral oilseed rape is less abundant than the main cruciferous weeds such as charlock (Sinapis arvensis) but more frequent than most of the wild and feral relatives with which it might potentially cross (albeit at very low frequency). Very substantial records now exist on the 'position' of feral oilseed rape in the arable plant community. It has not risen to dominance in weed populations despite the high seed drop that occurs at each harvest, nor has it invaded semi-natural habitats around farmland. Even in field management regimes that allowed many other species to increase in population, feral oilseed rape has remained a small component of the seedbank. It might be abundant enough to cause impurities in harvested yield, but not enough to cause even moderate shifts in the seedbank community. Whether it will increase or die out is uncertain, however. Other weed species have risen or declined over time, but the weed community has remained a resilient assemblage, admitting new species only occasionally. It is very

unlikely therefore that a single new weed species could cause major disruption to the habitat.

Concerns that feral oilseed rape possessing GM herbicide-tolerance traits would increase in abundance to the point of having major effects on the habitat are also unfounded. Herbicide tolerance confers no advantage, at least in the absence of use of the herbicide to which it is tolerant. Herbicide tolerant ferals would increase (or decrease more slowly) if the herbicide was used with later crops of oilseed rape. Many other options exist to control such GM feral plants, and again, no major disruption of the habitat is likely to be caused by the plants themselves.

**Conclusions.** Geneflow in oilseed rape occurs at low frequency over several km, mediated by a range of insect vectors and wind-borne pollen.

geneflow is a regional process depending on the configuration of fields in a locality

best current estimates indicate cross pollination between nearby fields is 1 in 1000 or less; the values might be much higher to fields of partial male fertility (as in some modern varieties) or from fields to small feral populations

ferals persist in the arable seedbank and can contribute more (i.e. 1 in a 100) to impurities in crops than does geneflow by pollen movement from other crops

impurities cannot be prevented in the harvested yield of outcrossing crops or crops that give rise to feral populations, but could be reduced to around 1% by rigorous field management and regional segregation of crop types

herbicide-tolerant feral populations should have no selective advantage except where the specific herbicide is used

geneflow and feral persistence in oilseed rape makes an interesting 'model' for studying the links between physiological process and regional meta-population, but the ecological risk of ferals, herbicide tolerant or otherwise, is presently small.

Present effort is directed in two topics, including basic science and potential practical applications (Table 1). The first is to obtain more certain estimates of geneflow to small populations of feral plants and its impact on their evolution: oilseed rape is again used as a 'model' system for an outcrossing species. The second topic is to refine diagnostic techniques for detecting geneflow between fields, and then to develop high throughput methodologies for assessing the level of impurity in crop yields.

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# Functional diversity in vegetation – the role of the individual

Results from a Co-ordinated Programme in Vegetation Dynamics G S Begg, J W McNicol, BioSS G R Squire, B Marshall, G Wright, ,Scottish Crop Research Institute Peter Millard, Ursula Bausenwein, Macaulay Institute, Aberdeen George Marshall, Scottish Agricultural College, Ayr Jim Bown, John Crawford, SIMBIOS Centre, University of Abertay Dundee

Debate in the 1990s among SEERAD-funded ecologists identified the need for a robust set of concepts and methods to link scientific theory and practice in vegetation management. The first step was to form a co-ordinated research programme among the organisations so as to combine their skills, infrastructure and experience. The second step was to petition SEERAD to fund a central project that would raise and integrate the organisations' capability

in important generic topics such as advanced statistics, nitrogen dynamics, genetic fingerprinting and mathematical modelling. The project concentrated on a 'model' system species-rich grassland, a widespread form of vegetation that supports sheep and cattle, holds a rich source of biodiversity and contributes notably to the landscape.

The main scientific aim of the project was to make progress on the question of 'the individual' in 'the system'. The physiological processes of individual plants determine their survival and fecundity, which in turn govern the continuous selective and adaptive changes in vegetation. However, the vegetation is measured, defined and managed by system-scale variables that include biomass, nutrient retention, and the abundance and distribution of species. Uncertainties in grassland management arise from knowing too little about the link between the individual-scale and system-scale properties of grassland. If the proportions of individuals change, or if some die out - how is the system affected? If the community is managed in a specific way - which individuals coexist within it? Such questions have been considered at the relatively crude level of the species or assemblage (e.g. suppress these species, conserve those). Yet great variation

might exist among individuals of a species and contribute to ecosystem function. The aim of the project was to measure variation among the individuals of species and assess the importance of such variation to habitat functioning.

A particularly formative step was the construction of a community dynamics model, which when populated with individuals made up from the measured

traits, produced emergent, systemscale, properties (such as species abundance curves) that reproduced structures normally found at much larger scales among species in habitats. As among species, the reason why many genetic types coexist in a small area of land seems to depend on optimisation of tasks. Plants can never do all

tasks, such as intercepting resource,

reproducing, dispersing, to the maximum. Here, stable modelled communities were composed of individuals having

trade-offs between traits. ABERTAY DUNDEE

Also, by producing emergent properties from the traits of one species, the modelling showed that within-species variation, at least of the major species, should be considered when assessing the diversity of swards. Two swards

of similar species complement could be functionally very dif-





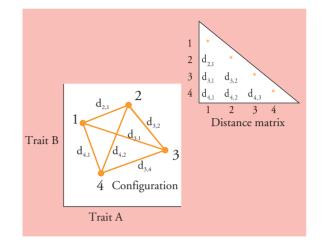


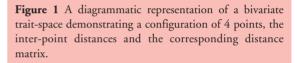


ferent if their main species have different levels of variability but the more detailed analysis is still under way. Some results from the project, including initial descriptions of the model, have now been published (see Bibliography). However, much of the detailed analysis is still in progress. Here we report on a statistical approach to biodiversity that is complementary to the spatial dynamics modelling and which can use measured data to define diversity at the scale of the individual.

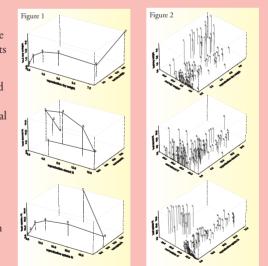
**Trait-space** In studying ecosystem structure and function the predominant approach is to adopt a taxonomic classification of organisms and to describe the mean behaviour of the resulting groups. Yet in many instances there may be no mechanistic relationship linking the ecological process under consideration to the taxonomic classification of the organisms involved. When ecosystems are composed of variable populations the failure of taxonomic classification in this way sets a limit on our understanding. In response to this problem we take an approach that is both functional and individual based in which we define individuals with respect to a set of key functional traits (phenotype) and recognise the individual as the fundamental unit of diversity.

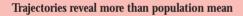
Individual organisms are considered to exist within a multidimensional space, the 'trait-space', the dimen-





sions of which represent the key functional traits. At any one time an individual is represented as a point in trait-space and a group of individuals or a population can be envisaged as a 'cloud' of points. The distribution of individuals in trait-space provides insight into the structure of a population or community in functional rather than taxonomic terms and may be used to address issues such as the recognition of functional types and the quantification of functional diversity.

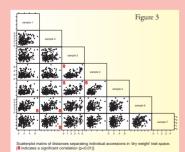




The growth and reproduction of Rumex acetosa are dictated by the uptake and use of nitrogen within periods defined by grazing events and the length of the growing season.

The mean values of 20 accessions (genotypes) measured ex situ and sampled on 7 dates over one year showed changes in biomass and nitrogen in different plant parts that were characteristic of perennial life-history processes (figure 1).

However, the means masked considerable variation between the accessions (figure 2).



Significant positive correlation occurred between distances during peak reproduction (Sample 3-5) and in a comparison of trait

positions before and after reproduction. Further examination of the data suggests that these were due to individual differences in the timing of reproduction and the storage capacity of the roots (figure 3).

**Box 1.** As an example the non-parametric statistical method for the detection of individual differences in trait-space trajectories has been applied to a study of the life-cycle of the perennial grassland species Rumex acetosa.

To infer structure from data or to simulate communities with known structural properties requires the distribution of individuals in trait-space to be modelled. At its simplest, the distribution of individuals may be modelled as a single multivariate probability density function. However, more complicated probability models such as mixture or a hierarchical models may be necessary to provide a realistic characterisation of community structure.

To this point we have taken a static view of traitspace, yet in many cases the trait values characteristic of an individual change over time so that an individual follows a trajectory through trait-space. In general terms a trajectory may be modelled as  $y_t = f(x, \theta_t)$ . Here, the vector of trait values for an individual at time t,  $y_t$ , is determined by  $f(\cdot)$  a function of the variables x with the parameters  $\theta_r$ . Variation in parameter values is representative of variation between individual trajectories. Therefore, assuming that the form taken by  $f(\cdot)$  is the same for all individuals, the distribution of the parameter values reflects the distribution of the dynamic attributes of a community. By treating the parameters themselves as continuous random variables their distribution may be modelled using the same approach applied to the distribution of to the traitvalues in the static case.

Modelling the distribution of individual points and trajectories in trait-space in this way provides a novel viewpoint from which to consider key issues such as the quantification of diversity, the identification of functional groups, and the importance of variation between individuals. It is in relation to the latter point that the modelling described has been used as a basis for developing a non-parametric statistical method to identify differences in the trait-space trajectories of individuals. To illustrate we return to the geometric representation of trait-space. The distance matrix, i.e. the set of distances between each pair of points defines the configuration of points in trait-space at that time (fig.1). The similarity between configurations at two times can be measured by the correlation between their distance matrices. It can be shown that this correlation is dependent on the values taken by the parameters of the function  $f(\cdot)$  and consequently it is possible to use the correlation between the distance matrices to provide a measure of the extent to which trajectories vary among individuals. This method has been successfully applied to the analysis of plant life-history data (Box 1). Notably, the analysis showed that individual plants moved towards a seasonal end-point (e.g. an amount of nitrogen stored in a tap root) but did so in different ways that would not have been detected by taking a series of population averages. Knowing how individuals 'behaved' differently enabled better prediction of how the community might change in response to external forcers such as grazing intensity and the first frost at the onset of winter

Next steps This project was among the first of the SEER-AD co-ordinated research programmes, bringing together a diverse range of skills in ecophysiology, modelling, molecular ecology, statistics and habitat management. Scientific progress was possible that could not have been made by any partner alone. From commissioning to completion, the project was designed and executed to explore basic scientific problems in vegetation. For practical benefit to be realised, further coordinated effort is required to test the importance of within-species diversity to the resilience of vegetation. The partners have at their disposal a range of unique field sites and facilities in which to carry out these studies. The results will be of significance to agri-ecological regions throughout northern Europe and beyond. The concepts outlined here, and the theoretical work in collaboration with the University of Abertay Dundee, are being extended to a other vegetation types, notably the habitats of arable and early successional vegetation.

This article is adapted from presentations at a workshop organised by SAC Hill and Mountain Research Centre, Crianlarich, 9-10 October 2001.

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# **Developing sustainable pest management strategies for a changing future:**

A.N.E. Birch, G. Begg, R. Brennan, B. Fenton, S.C. Gordon, B. Griffiths, D.W. Griffiths, J. Hillier, G. Malloch, G. Squire & R. Wheatley

Worldwide, agricultural pests destroy 30-40% of crops before the harvested food reaches human consumers or our livestock. These huge losses continue each year, despite \$6 billion being spent globally on synthetic pesticides in an attempt to control pest problems on crops. It is estimated that each year insects consume the quantity of crops which would feed one billion people. The impact of crop losses due to pests is exacerbated by the rapid increase in human populations and the depletion of natural resources globally.

## Plant breeding for pest-

resistant crops Breeding for host plant resistance to insects has been a successful strategy deployed by SCRI's plant breeders and entomologists together with other research centres over 40 years Breeding resistance into crops has reduced, and must continue to reduce, the need for insect control based on synthetic agrochemicals. However, breeding is a long-term approach (e.g. 10-15 years for new pest-resistant varieties to reach farmers), throughout which pest insects and mites are constantly adapting and overcoming plant resistance genes in a co-evolutionary "arms race". This interactive process between insects and plants has been occurring for thousands of years, but has speeded up due to intensification of agriculture, e.g. use of fewer crop varieties and genotypes being deployed, grown in monoculture rather than as a mix of genetically variable landraces. This means the pest resis-

andraces. This means the pest resistance 'targets' for plant breeders and biotechnologists are constantly moving, allowing no time to rest on past laurels and successes. Recent examples of pests' counter-adaptations to introduced pest resistance genes are seen in SCRI's crop varieties. These include red raspberry varieties which were formerly resistant to the main aphid and virus vector pest, Amphorophora idaei (the large

raspberry aphid), and most recently in blackcurrant varieties resistant to galling and virustransmitting *Cecidophyopsis* mites (e.g. blackcurrant gall mite). In both cases, many years of careful plant breeding is being overturned by rapid counter-adaptation in genetically variable pest populations see fig 1. This process typically occurs through co-evolution



**Fig 1** The use of pest-resistant crop varieties by growers to help control insects and mites inevitably creates selection pressure for counter-adaptation in the pest populations. SCRI scientists a new research programme are studying this "co-evolutionary battle" in (Host Parasite Co-evolution), using virus-transmitting blackcurrant gall mites and raspberry aphids as examples. The aim is to maximise the durability of pest resistance genes used in SCRI's current and future crop varieties.

between pest and plant, particularly if the crop is put under intense selection pressure from the target pest (e.g. subjected to very high pest densities). This pest adaptation process may take as little as one third of the time taken to breed a pest-resistant variety, so the "arms race" is generally skewed in favour of the counter-adapting (-) pest, not the plant breeder.

Moving targets and fewer weapons against pests Consumers, environmentalists, farmers, supermarkets and government policy makers are demanding more sustainable and ecologically-friendly approaches to crop protection. As a result, many of the older and broader spectrum pesticides are currently being withdrawn in the U.K. and in Europe. For example, three years ago, UK brassica growers had nine insecticides available to control cabbage root fly on vegetable crops. Soon growers will be left with only one pesticide to control root flies, which cause at least £25 million damage per year with the current level of pesticide-based control (HDC News, June 2002). Farmers are increasingly being faced with gaps in their armoury of approved insecticidal weapons against crop pests. In addition, pests under strong selection pressure from existing pesticides are constantly counter-adapting to widely used synthetic chemicals, as well as to pest-resistant crop varieties. For example, some formally very effective insecticides are now much less effective against important UK pests like the peach-potato aphid, Myzus persicae. This aphid, which causes more than £100 million losses to UK agriculture each year, now has three different types of resistance (i.e. metabolic over-production of certain carboxylesterase enzymes; and target site - MACE and kdr) to several commonly used insecticides, including pirimicarb, aldicarb and deltamethrin. Perhaps the time is now right to start applying more widely the many years of IPM-related ecological research SCRI and other UK institutes have been developing.

**Can pest-resistant GM crops help?** Biotechnology has the potential to offer new solutions to agricultural crop protection, in the form of pest-resistant GM crops (for background see SCRI Annual Report for 1996/7, p68). Several types of genes from plants and microbes (e.g. *Bacillus thuringiensis* toxins, lectins, enzyme inhibitors, anti-metabolites) can now be routinely expressed in a wide range of crops to confer added pest resistance and thus reduce pesticide applications. However, until various environmental and health-related regulatory questions have been addressed and the public's concerns are answered, it is unlikely that these solutions will be widely available to

UK and EU farmers in the near future. If GM crop varieties are approved for use in the UK and Europe it will be important to maximise their durability, since pests have the ability to rapidly adapt to resistance genes unless they are used in a carefully planned way as part of an integrated approach to pest management.

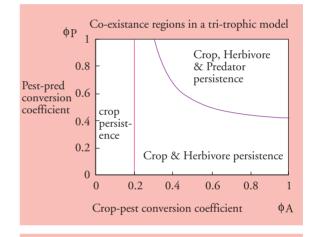
Selecting complementary strategies to fill the armoury gap What then are the viable options for European farmers in the short to medium term (i.e. the next 10 years), faced with fewer pesticides, fewer pest-resistant crop varieties and few immediately usable biotechnology solutions? Organisations including 'LEAF' (Linking Environment and Farming - see www.leafuk.org for details) and 'DEFRA' (Department for Environment, Food and Rural Affairs) are actively promoting Integrated Farm Management (IFM) systems These give increased emphasis to the consideration of environmental factors, through minimising the reliance on synthetic crop protection chemicals (pesticides). Unlike organic farming codes of practice (e.g. the organic codes of practice under UKROFS, the UK Register of Organic Food Standards and by EU legislation), IFM allows careful use of more selective pesticides, while at the same time promoting careful selection and deployment of any available resistant (or partially resistant) crop varieties in carefully planned crop rotation systems. IFM is promoted by LEAF and other similar organisations as a "win-win" solution for farmers and consumers and aims for affordable, quality produce achieved with responsibility to the environment. During 2002, SCRI scientists have initiated research projects with LEAF, to develop these ideas scientifically, bringing in new research findings for testing on the experimental farm at SCRI and then around the U.K. on leaf demonstration farms.

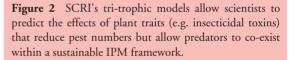
**Organic farming – good for consumers and pests?** Potentially, organic farming systems have certain advantages that could be more fully researched and compared with IFM and high input, intensive farming. A recent 21 year comparative study by Swiss scientists (*Science* **296**, May 2002) shows that although mean yields of organic crops are lower than conventional farming systems, there are net energy gains because of greatly reduced inputs of fertilizers and pesticides, as well as increased biodiversity on organic farms. In this recent study, average activity density of beneficial carabids, staphylinids and spiders (important natural predators of insect pests) were almost twice that of the conventional treatments. In the EU, only 3% of farms are currently organic, but numbers

are increasing by 25% each year. Surveys indicate that many consumers in the EU are willing to pay 10-30% more for organic produce. However, organic agriculture still produces food for a limited niche market, that can only be sustained by a majority of more conventional farmers in the larger regional landscape. Pest populations on organic farms are, to an uncertain degree, suppressed indirectly, by use of insecticides and other pest control strategies on the surrounding conventional farms. It is unclear therefore, whether pest problems would be controllable if Organic farming were the only or the dominant form of farming in a region. Moreover, in perennial crops, such as raspberry, the option for Organic production may be more difficult, due to the build up over several years of the pest burden and disease inoculum. Whatever the furture of organic and GM farming in the UK and EU, there will be increased need for research to support IFM-based systems over at least the next ten to twenty years. How will the UK agricultural landscape look and function ecologically in the future, if we have a patchwork of organic, IPM-based and conventional fields and farms (with future GM crops potentially adding to the complexity)? This question requires a knowledge of how different fields and farming systems interact at a landscape scale, which is a challenge mathematical modellers are currently starting to address.

Understanding agro-ecosystems - the key to sustainable and 'greener' farming Concerns about the environmental impact of pest-resistant GM crops have necessitated novel methods of risk assessment. In addition, accessibility to more powerful computers in recent years has allowed the employment of computationally intensive individual-based modelling methods. At SCRI we have developed a tri-trophic, individualbased, mathematical model with which we can explore the possible impacts of pest-resistant crops (including conventional and GM crops) on their associated arthropod community. Individual insects in the model have common traits but are parameterised differently, to allow for different strategies regarding resource foraging and resource acquisition, dispersal, temperature responses, and reproductive strategies. Different pestresistant GM crops act in different ways on target and non-target pests (e.g. lectins as distinct from Bt toxins). Such differences at the plant-pest interface and consequences for the pest-natural enemy interface can be incorporated in to the model, to explore their effects on system properties, such as crop yield (efficacy), arthropod diversity (community structure), and sustainability of deployment (pest counter-adaptation).

Our models have recently been used to predict the time taken for pests to counter-adapt to the introduction of a new crop variety having conventional or GM resistance to the pest. The ability of a pest to get round the plant's resistance genes occurs naturally in a pest population but in extremely low frequency, for example 1 individual in a million. We have moved beyond existing models to include factors such as disturbance in arable and horticultural systems, variation in crop growth rate, and the arrangement of resistant and susceptible varieties in different planting configurations (see also the article on canopy heterogeneity). The model, funded by DEFRA, is now available with a 'user-interface' to enable other researchers to explore counter adaptation and tri-trophic interactions.





Chemical ecology of pest-plant interactions – fundamental knowledge leading to future crop protection tools Practical developments in pest control can also be achieved through understanding the subtle plant chemical signals (semiochemicals) that insects use to select or reject a plant as a host for feeding or egg laying. Three SCRI studies based on the chemical ecology of pest-plant interactions have been developed from fundamental research through technology transfer to a stage where successful and end-user application is possible in the near future.

**Raspberry beetle attractants from host flowers** Several years of research at SCRI have led to the development of chemically-enhanced traps for monitoring and trapping raspberry beetles (see SCRI Annual Report for 1995, pp144-148). These traps release specific flower volatiles and can enhance the visual com-



**Figure 3** Insects use specific plant-produced chemicals (e.g. plant odours) to locate suitable host plants to attack. Chemical ecology studies involving SCRI scientists and international collaborators identified the key flower volatile chemicals and reflected wavelengths from raspberry flowers that raspberry beetles are attracted to when searching for egg-laying and feedng sites. Specially enhanced white traps are now being evaluated in the U.K. and U.S.A for their potential as monitoring tools in Integrated Crop Management. Such traps could allow growers to reduce synthetic pesticide applications in conventional soft fruit production and may also have potential in organic production.

ponent (white colour, mimicking raspberry flowers) by a factor of x10 or more. These traps have now been field-tested in several countries under EU CRAFT funding (see fig 3). Most recently, the Horticultural Development Council have funded a new 3 year PhD project at SCRI, to develop the practical deployment of these traps for UK growers and to study how they affect other pests and beneficial insects. The chemically-enhanced traps are also being evaluated against N. American pests of raspberry by collaborating partners.

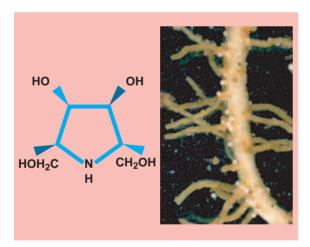


Figure 4 A plant-derived chemical called DMDP, which was isolated from tropical legume seeds, was found by scientists from SCRI and the Royal Botanic Gardens Kew to have systemic activities against root-feeding nematodes. This technology is now patented in key territories worldwide. The British Technology Group (BTG) have recently undertaken commercial evaluations of this natural plant product on a range of tropical, sub-tropical and temperate crops, including coffee, bananas, soybeans, tomato and potatoes. Commercial licences are also currently being sought by BTG to exploit this discovery.

**Pesticidal plant compounds from South American legumes** Similar approaches, based on the chemical ecology of pest-plant interactions are leading to other new ideas for environmentally-benign pest control. We have developed a plant-derived pesticide from tropical legumes in collaboration with with the Royal Botanic Gardens, Kew and InBio in Costa Rica (see fig 4). This research was funded and patented by the British Technology Group. The plant natural product devel-



**Figure 5** Cabbage and turnip root flies are major pests of vegetable and forage brassica crops. Pesticides which are currently used to control root flies are being withdrawn, due to environmental hazards. SCRI scientists working with collaborators in Switzerland have identified the key plant chemicals on the leaf surfaces of crop and wild crucifers which either stimulate or deter these pests from laying their eggs. These plant-derived chemicals could provide valuable components of alternative control strategies in the future, replacing the increasing list of banned pesticides for these crops in the U.K. and Europe.

oped in this collaborative project is now undergoing final field trials in several countries, as a new antinematode treatment for tropical and temperate crops including coffee and potato. This SCRI discovery is already generating income for SCRI's commercial arm, MRS Ltd through a successful technology transfer.

Stimulants and deterrents affecting egg laying by the cabbage root fly from wild crucifers In previous studies at SCRI with Swiss collaborators (see SCRI Annual Report 1993; *Grower* March 28, 2002, pp 20-21) we discovered that specific leaf surface chemicals triggered the sequential egg laying behaviour of the cabbage root fly (see fig 5). Some wild (non-cultivated) crucifer species related to the normal host plants of this pest (cabbages, swedes, turnips, broccoli) were later

**IOBC and EU funded GM Crop Biosafety Guidelines Projects initiated during 2002** The GMO Guidelines Project was launched in 2002 by a group of scientists, the Global Working group on Transgenic Organisms in IPM and Biocontrol. The project is funded by the Swiss Federal Agency for Development and Cooperation and organised by the International Organisation for Biological Control (IOBC). The project aims to 'establish international, scientifically sound, conclusive and acceptable guidelines for assessing the environmental risks posed by GMOs.'

The Project's first workshop was held in Nairobi in November 2002 and concentrated on Bt maize in Kenya as a case study. The workshop was attended by around forty scientists, half from Africa. The aim was to draft scientific guidelines for the assessment of risk posed by GMO cultivation in Kenya, using insect-resistant (maize stem borer species) *Bt* maize as the case study.

SCRI staff (Nick Birch, Ron Wheatley) led workshops on Non-target impacts of *Bt* maize and on Soil ecosystem function. Other international scientists from ICIPE Kenya, KARI Kenya , University of Minnesota USA, Ohio State University USA, Swiss Geobotanical Institute, CSIRO Australia led linked workshops on Plant characterisation, Gene flow, Resistance management and on African agricultural systems. Finally, workshop outputs were presented at a Public Day, attended by Kenyan stake holders, policy makers and press agents. The next workshop will be held in Brazil and will be hosted by EMBRA-PA.

Further information on the IOBC GMO Guidelines Project can be obtained at <u>www.gmo-guidelines.info</u>

SCRI staff (Bryan Griffiths, Nick Birch) are also involved in a second new project, 'ECOGEN', which is funded by the European Union. This project is investigating ecological and economic impacts of using *Bt* and herbicidetolerant GM crops on soil organisms and functions at

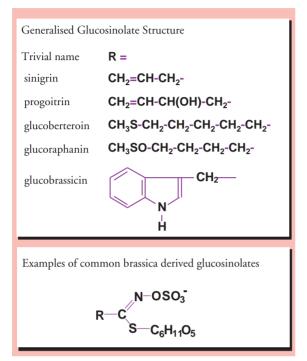
found to be avoided as sites for egg laying. Recent chemical analyses of leaf surface extracts from 18 wild Crucifer species, which varied in their attractiveness to this pest, showed a wide range of glucosinolate structures (characteristic plant secondary compounds from Cruciferae) on their leaf surfaces (see text box). A clear relationship was detected between different classes of leaf surface glucosinolates and egg laying preference by the pest. This study also confirmed the presence of other classes of chemical deterrents to egg laying in leaf surface extracts from wild Crucifers. Such discoveries open up new possibilities to push the pest away from crops using natural deterrents and also attract the pest to trap crop areas sprayed with the stimulant compounds. This pest control strategy, based on selective use of behaviourally-active natural compounds, is

various experimental scales (laboratory to field). It involves scientific partners from Denmark, France, Netherlands and Slovinia and also includes various endusers from the biotechnology industry and from European policy-making organisations. Further information is available from www.ecogen.dk



**Figure 6** Pest-resistant GM crops could offer potential economic and ecological benefits to farmers in developing countries, where pest problems can be severe and food shortages are common. However, some GM traits could cause adverse environmental effects (e.g. on non-target insects which are beneficial or on the soil ecosystem). Each GM variety needs to be assessed on a "case-by-case" basis, to measure potential risks and benefits to local agricultural systems. SCRI scientists recently co-led IOBC workshop sessions in Kenya which evaluated the potential ecological impacts of Bt maize. This GM trait protects the crop from several important stem borer pests. Further IOBC workshops are planned in Brazil and S.E Asia during 2003.

know as the 'stimuli-deterrent' or 'push-pull' strategy and has already been used successful to reduce insect attack in African millet and maize.



**International Guidelines for GM crops** SCRI's expertise in integrated management and modelling is finding a range of applications throughout the world, notably where GM technology is being developed to solve problems of crop production in severe environments. In 2002, SCRI staff were involved in setting up the GMO Guidelines Project and contributing to its first workshop, which was held in Nairobi, Kenya (See text box opposite and fig 6).

# Biomathematics and Statistics Scotland

**Rob Kempton & Jim McNicol** 

Biomathematics & Statistics Scotland (BioSS) is devoted to the application of statistics and mathematics in agriculture, the environment and human health. Its core remit is to support the SEERAD programme of research, which is carried out within the SABRIs, SAC and RBGE. This is achieved through a dispersed group of statisticians, mathematicians and computing experts based at BioSS centres in Edinburgh, Dundee, Aberdeen and Ayr. A successful bid for increased core funding in 2001 allowed BioSS to expand its work in bioinformatics and systems modelling. A Bioinformatics Task Force was established to bring together BioSS researchers, consultants and trainers to address more effectively the problems created by the wealth of data emerging from new molecular technologies. This group will link with specialist bioinformatics staff being recruited in the SABRIs and contribute to a Scottish Bioinformatics Network involving Dundee, Edinburgh and Glasgow Universities. Extra resources are also being devoted to a new research theme, modelling complex systems and risk, which will integrate knowledge on the system sub-processes to predict whole system outputs. The theme will focus on how variability and uncertainty within the sub-processes affects system outputs and how results can be presented in a form more immediately usable by decision makers.

At SCRI there have been two developments in our genetic linkage work. An investigation was carried out into the effects of genotyping errors, missing values and segregation distortion in molecular marker data on the construction of linkage maps. Three locus-ordering criteria, weighted least squares, maximum likelihood and minimum sum of recombination fractions, were compared using a simulated doubled haploid population of size 150. Maximum likelihood was the most successful at ordering loci correctly but generated substantially inflated map lengths in the presence of typing errors. In general missing values created shorter map lengths for more widely spaced markers. Segregation distortion had little effect. The second development was to make publicly available the software for our tetraploid linkage map methodology. This is called TETRAPLOIDMAP and can be found at the BioSS ftp site. In molecular sequence analysis, we have improved an earlier Hidden Markov model used to detect evidence of recombination in DNA sequence alignments of four sequences. Our approach explicitly models the sequence of phylogenetic tree topologies along a multiple sequence alignment. Inference under this model is done in a Bayesian way, using Markov chain Monte Carlo (MCMC). The algorithm returns the sitedependent posterior probability of each tree topology, which is used for detecting recombinant regions and locating their breakpoints. The algorithm has been programmed in C++. Work is in progress to develop a graphical user interface.

In ecosystem structures we have been examining the potential of grouping individuals according to their trait values rather than by their traditional taxonomic classification. In particular, comparisons over time of configurations of individuals looks to be a promising method of defining functional groups and the similarity of these configurations is conveniently quantified by the Mantel test.

The SEERAD funded Micronet project seeks to establish the nature of any spatial structure among soil microbial communities below an area of unimproved grazed grassland in the Scottish Borders. The inter sample distances ranged from 0.1 to 12m and a wide range of microbiological and chemical properties was measured. Geostatistical analysis revealed spatial dependence for a relatively small number of characters, but in particular for total C, total N, total P and microbial biomass C. The statistical analyses also suggested that much of the variation in microbial variables was present below the minimum scale of measurement of 10cm.

# Modelling weather data

D.J. Allcroft<sup>1</sup>, C.A. Glasbey<sup>1</sup> & M. Durban<sup>1</sup>

Meteorological variables are essential inputs to many models in agriculture and hydrology. In particular, crop models need them because crop growth is substantially affected by weather. Frequently though, weather data will not be available in the required form. For instance, the available data may not be for exactly the right location, for the right time period or at the right scale, or simply not enough data may have been collected. However by developing appropriate mathematical models from the available data, we can simulate data of the right form and quantity.

Typical requirements for crop models are long series of daily data, but these are rarely available in the quantity needed. For example, 50 years of data might be required to encompass the range of weather patterns for model prediction. Alternatively, we might have data from a restricted network of weather stations, but want to simulate realistic data for the whole area containing the stations. Two examples are dis-

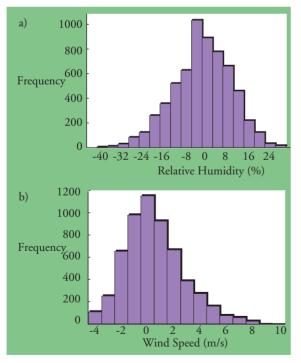
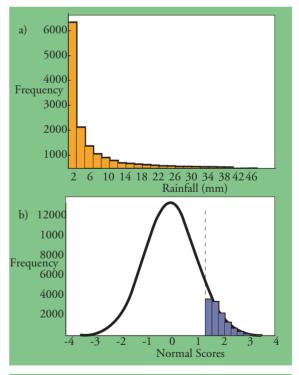


Figure 1 An illustration of two weather variables that are approximately Gaussian distributed: histograms of a) relative humidity and b) wind speed, after subtraction of seasonal trend.

<sup>1</sup>Biomathematics & Statistics Scotland, Edinburgh

cussed below in some detail. In the first, we build a model to simulate time series of many weather variables simultaneously, taking account of the dependencies between variables. The second is a rainfall disaggregation problem, the aim being to produce a realistic pattern of rainfall at a finer spatial scale than that recorded. First we discuss some general issues about weather variables.

Weather variables Most weather variables can be assumed to be normally distributed, i.e. to have come from a Gaussian distribution. Consider, for example, daily wind speed and relative humidity. Figure 1 shows histograms of 17 years of daily data collected at Mylnefield (SCRI), after cyclic seasonal trends have been removed. Though some skewness is evident, the distributions are approximately Gaussian. Other weather variables that are approximately Gaussian include air pressure and daily maximum and mini-



**Figure 2** An illustration that rainfall is not Gaussian distributed, but can be transformed to a censored Gaussian variable: a) histogram of hourly rainfall, with zero values omitted; b) histogram of rainfall after a normalising transformation, with superimposed Gaussian distribution (—) and zero rainfall threshold (- -).

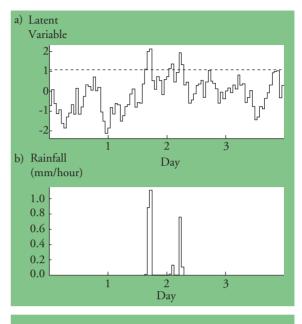
mum temperatures. Rainfall, in contrast, is highly non-Gaussian, most periods having no rain at all and even restricting to wet periods, the distribution of rainfall is still very non-Gaussian. For example, Figure 2a shows the distribution for hourly rainfall in the Arkansas-Red Basin River area, USA. This histogram omits the 91% of hours that had no rain at all and shows that of the wet hours, most have little rainfall but the long tail of the distribution allows for the occasional hours which have very heavy rainfall.

For rainfall simulation, multi-stage approaches have previously been used, e.g. first simulating a rain/no rain process and then simulating the amount of rain for the wet periods from some skewed distribution. However, there are many advantages of working in a Gaussian framework. Most importantly, there is much well-developed theory for Gaussian processes, hence we can build models based on well-established methodology. A further advantage of Gaussian variables is that they are closed under scaling and addition. So for example, if the distribution of daily totals of a variable is Gaussian, then so is the distribution of weekly totals, and so is the distribution of differences between the daily totals and an overall daily average.

Hence for modelling rainfall, we seek a transformation to normality, enabling us to use established models for Gaussian processes. The method we have developed is to define a transformation such that for wet periods, rainfall values are converted to values distributed as the upper part of a Gaussian distribution, and for dry periods, zero rainfall corresponds to censored values from the lower part of the same distribution. Figure 2b shows how hourly rainfall values are transformed to match the upper tail of a Gaussian distribution, whereas the lower part of the distribution corresponds to the 91% of dry hours. Thus, a latent Gaussian process can be thought to have generated the rainfall data: for values below the threshold, the period is dry and for values above the threshold the period is wet, with a transformation applied to generate the actual rainfall value. Figure 3 illustrates this, showing both a realisation of the latent process (Fig. 3a) and the resulting rainfall (Fig. 3b).

## Example 1: A weather generator for several weather variables

The aim is to generate daily values at a single site for several weather variables simultaneously, namely minimum and maximum temperatures, radiation levels, humidity, wind speed and rainfall. Previous approaches have either first simulated rainfall and

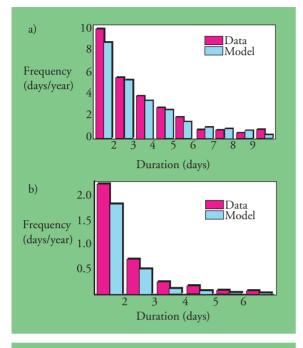


**Figure 3** An illustration of the relationship between the latent Gaussian process and rainfall: a) simulation of three days of hourly data from a Gaussian process (--), together with the zero-rainfall threshold (---); b) the resulting rainfall sequence.

then conditionally simulated the other variables or, conversely, simulated the other variables and then conditionally simulated the rainfall. By using the approach described above to transform rainfall, and applying a simple log-transformation to the radiation level, all six variables can be assumed Gaussian. Hence we can use a standard multivariate Gaussian model to generate values for all variables simultaneously, taking into account the dependencies between the variables.

The data we model consist of 17 years of daily values of the six variables recorded at Mylnefield (SCRI). All exhibited annual cyclic patterns, which were accounted for by fitting finite Fourier series. This effectively removes the cyclic aspect of the data and we assume the resulting series are stationary, a feature assumed in all basic time series models.

ARMA (auto-regressive moving average) processes are well known statistical models for time series. They model the value of the variable at time t as a function of the values of the variable at the previous few time points, plus a Gaussian-distributed random error. The steps in model fitting are usually: estimate the auto-correlations of the time series, i.e. the correlation of the variable with itself at given time-lags apart; from these identify the appropriate ARMA model to use; and finally estimate the ARMA parameters by



**Figure 4** An illustration of the agreement between Mylnefield data and simulations from our model for two summary statistics: a) frequency of wet periods; b) frequency of warm, humid periods with duration of two or more days.

maximum likelihood.

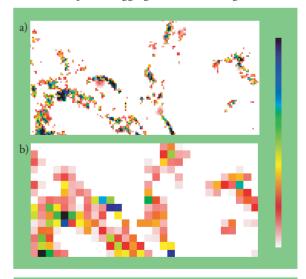
Simple ARMA processes model a single variable; VARMA (vector ARMA) processes model a vector of variables instead, i.e. model several variables simultaneously. To fit these models, as well as the auto-correlation of each variable, we need to estimate the cross-correlations between the variables, i.e. the correlation between one variable and another at a given time-lag apart. Because the transformed rainfall variable is censored, the model fitting procedure is slightly more complex than usual. Firstly, estimation of the auto-correlation for the rainfall variable, and for the cross-correlations involving the rainfall, have to take into account that the value of the latent Gaussian variable is unknown on dry days. Therefore, we must substitute instead an integral over the range within which it can fall. Secondly, in estimating the parameters, the usual maximum likelihood approach is unavailable due to the data being censored. Instead, we use a simple least squares approach, minimising the sum of squares of differences between the estimated correlations and those predicted by the model.

After fitting the model, we can then simulate from it. Here we simulated 100 runs of 17 years of data and calculated various statistics to compare with the observed data. These included monthly means of weather variables, numbers of wet days and total amount of rain per month. In addition, we compared run lengths of rainy days and of warm, humid days. Reasonable consistency was seen in all cases, hence this model can be used to make predictions about the frequency, duration, etc. of various types of weather conditions. For example, conditions that result in the onset of potato blight have been summarised as "a temperature in excess of 10°C and relative humidity above 90% for 11 or more hours in each of two or more consecutive days". Figure 4 shows how well the model captures the distribution of both this particular combination of conditions (Fig. 4b) and wet periods (Fig. 4a).

#### Example 2: Rainfall disaggregation

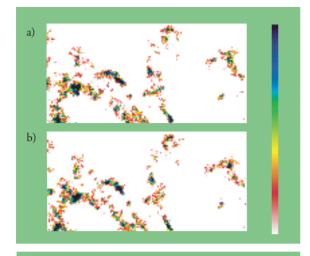
Rainfall data are frequently collected at coarser spatial scales than required. Methods are, therefore, needed for simulation of realistic patterns of rainfall at finer scales. We use the same approach described above to transform the rainfall to a thresholded Gaussian variable, though now we are in a space-time framework and hence each measurement of rainfall corresponds to a given area over a given time.

We apply our model to 12 hours of hourly data from the Arkansas-Red Basin River. We model the data at 8km x 8km resolution, aggregate to  $5 \times 5$  blocks and then disaggregate from here, so allowing an assessment of how well the disaggregation procedure works, since we can compare disaggregations to the original data at



**Figure 5** The spatial distribution of rainfall in the Arkansas-Red Basin River area for one hour, at a) fine and b) coarse spatial scales. White indicates zero rainfall, through to black indicating the highest.





**Figure 6** An illustration of how a latent Gaussian Markov random field can be used to disaggregate the coarse-scale rainfall data in Figure 5b: two simulations of a disaggregation.

fine scale. Figure 5 shows one hour of rainfall data, both at the fine scale we wish to disaggregate to (Fig. 5a), and the coarse, aggregated scale (Fig. 5b).

As in the first example, we need to estimate the correlation of the underlying Gaussian variable, using methods which take into account that the values are censored at dry locations/times. As the problem here is spatio-temporal, we need to estimate the correlation over all combinations of lags in two-dimensional space and time, i.e. in three dimensions. The pattern of correlation was judged to be similar in all directions in space, i.e. the process is spatially isotropic.

We model the correlations as a Gaussian Markov random field (GMRF), 'random field' just being the term given to a random variable in several dimensions. A Markov process in time is one in which the observation at time t depends only on the immediately preceding observation(s) and is conditionally independent of those occurring earlier. The Markov random field is the higher dimensional version of this, so an observation at a certain point in space and time depends on the values at a (small) neighbourhood of points around it, but is conditionally independent of values at locations further away, both in space and time. Parameters for the GMRF are estimated in a similar way as for the ARMA process in the previous example. Here weighted least squares are used to minimise the sum of squares of differences between the estimated correlations and those predicted by the fitted model.

Simulation of the fitted process is carried out using Gibbs sampling, the procedure being to start from some initial configuration, here the aggregated picture of Figure 5b, and then simulate updates for all the values, conditional on the values at neighbouring points. This is easily done, as the conditional distributions are multivariate normal, and computationally fast to simulate. We update  $5 \ge 5$  blocks in turn, constraining the total rainfall in the block to be consistent with the observed total for that block. The updating of all sites forms a complete update – many complete updates are run and, after each, statistics calculated in order to judge when the procedure has reached equilibrium. Once this is achieved, any of the realisations produced can be regarded as a candidate disaggregation.

Figure 6 shows two simulations of a disaggregation. Inspection of these and other realisations shows them to be visually more similar to the original data than has been achieved by previous methods, and comparison of quantities such as lag 1 auto-correlations in space and time and proportions of wet pixels also showed good agreement.

#### Conclusion

We have seen, *via* two particular examples, that the modelling of rainfall using a latent Gaussian process is both mathematically convenient and effective. Simulations from the fitted models show that realisations are similar to the data and summary statistics generally show better agreement than previous, less elegant models.

# Research services

# **Analytical facilities**

C.M. Scrimgeour

aboratory Accreditation Within SCRI, the Gas Chromatography-Mass Spectrometry Laboratories, Stable Isotopes Facility and Lipid Analysis Unit of MRS Ltd, operate a formal Quality System certified to BS EN ISO 9001 by SGS Yarsely International Certification Services Ltd. The certification standard was upgraded from ISO 9002 to ISO 9001 in August 1999, and now includes the design and conduct of research within its scope. A generic Quality System operates in other parts of the Institute and this is summarised in the SCRI Quality Plan, a copy of which is included in the Institute's Corporate Plan. The measures required for implementation of the generic SCRI Quality System are described in a Code of Practice document, a copy of which is issued to all members of staff. The Code of Practice is also reproduced at the end of the latest version of the SCRI Laboratory Notebook. The generic system is based on the correct maintenance of work records in which specially designed hardback notebooks comprise the primary record, with other data recording systems, archival procedures etc. as secondary records. The preparation of written methods or protocols (Standard Operating Procedures) and the correct use of equipment and facilities are strongly encouraged. The plan ensures full compliance with all safety regulations, and demands high standards of laboratory hygiene. If required, the Quality System can be readily upgraded to the standard required for formal certification within any activity or area. An archive facility is located within Building S and this is used for long-term storage of data as hard copy and in electronic format. Archival of data on electronic media is based on the use of compact disc writers (CD-R format) installed in several personal computers. Data can be transferred over the network or from a portable high capacity data storage disc to the computer's hard disc, and then to CD. Each CD can hold up to 650 Mbytes of data. Two copies are made, one for the owner of the data and one for the archive.

**Stable Isotope Facility** Stable isotopes are widely used for the study of plant physiology, crop genetics, ecology and food webs. Valuable information comes

both from studying natural variation in stable isotope composition and from following the fate of added isotopic tracers. SCRI is equipped with a comprehensive range of modern instrumentation for stable isotope analysis of the biologically important light elements, <sup>13</sup>C, <sup>15</sup>N, <sup>18</sup>O and <sup>34</sup>S, in a wide range of solid, liquid and gas samples.

All the instrumentation is based on continuous-flow isotope-ratio-mass spectrometers that are fully automated and operated through computer data systems. Automation allows a high through-put of samples, essential for many biological experiments where large data sets are required. For solid samples, the Europa Scientific Tracermass and 20-20 mass spectrometers are interfaced to Roboprep CN and ANCA-NT SL combustion sample converters. A Roboprep G+ gas purification unit is used for gas analysis. Plant samples of one to five milligrams are used, containing 25 to 100µg of the element of interest. Where possible, analytical protocols are devised to minimise sample preparation and fully exploit the automation.

SCRI also has expertise and resources for sample preparation from a wide range of matrices. These include plant sample drying and grinding, freeze drying and weighing facilities. Research support is aimed at developing new methods to assist the Institute's research programme.

**Organic Mass Spectrometry** The Institute's three state-of-the-art mass spectrometers, which are devoted to structural analysis of organic compounds, continue to yield valuable information on a diverse range of materials pertinent to the research remit of the institute. The core instrument is a Hewlett Packard 5989B MS ENGINE research-grade quadrupole instrument with electron impact, chemical (positive/negative) ionisation modes and a mass range of 2000 amu. Distributed processing software permits off-line data processing and reduces analysis times. This instrument can provide mass and structural data on a wide range of organic compounds.

A further bench top instrument is dedicated principally to the analysis of naturally occurring volatile com-

pounds. This consists of a Perkin Elmer automated thermal desorption system (ATD) linked to a VG TRIO-1000 quadrupole gas chromatograph-mass spectrometer and permits detailed characterisation of the profiles of organic volatiles generated by biological systems.

A Finnigan SSQ 710C dedicated liquid chromatography- MS instrument, with atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI) interfaces, is also available. This is suitable for samples whose high molecular weight, lack of volatility or polarity, make HPLC the preferred separation method. APCI and ESI are soft ionisation techniques and generally only produce molecular ions, e.g. [M-H] + or MH + , but the multicharge ionization mechanism of electrospray can extend the basic 2000 mass range of the instrument by a factor of about 20, giving a mass range of greater than 40,000 amu. This permits accurate mass determination of peptides, proteins and nucleic acids to within 0.1%, compared to the 5.0% error usually expected from SDS-PAGE determination.

Mass spectrometric analysis at SCRI covers a broad spectrum of chemical investigations generated by the research programme of the Institute. A wide range of plant metabolites has been analysed, both in the native form and as derivatives, including sterols, monoterpenes, sesquiterpenes, pentacyclic triterpenes, dimeric forms of phenolic acids, glucosinolates, longchain wax esters, peptides, essential oils, carbohydrates, polychlorinated biphenyls and lipids, including fatty acids. The facilities are operated by experienced and expert staff, ready to tackle and solve most structural problems.

During 2001, SCRI took delivery of two new mass spectrometers to substantially increase its capabilities and sample turnover in both high throughput metabolic profiling and proteomics. The first of these is a ThermoQuest LCQ-DECA, an ultra sensitive ion-trap LC-MS<sup>n</sup> system.. It is capable of many more scan functions than the existing SSQ710C spectrometer, including data-dependent full scan MS/MS, a tool of great utility in high throughput profiling. The same is true for protein/peptide sequencing, where rapid repeats of ion isolation and fragmentation generate



The new GC-MS and LC-MS system

sequence tags, which are searchable via on-board databases.

The other new instrument is a ThermoQuest TEM-PUS-TOF, an innovative GC-MS system capable of rapid detection, characterisation and quantification in fast GC separations. The benefits of the design provide parallel mass analysis with a short duty cycle at high transmission. This delivers rapid acquisition and fast sampling of narrow peaks at high sensitivity with high sample throughput.

A fundamental requirement of successful gas chromatographic mass spectrometric investigation is the development of robust chromatographic separations. Together with the MRS Lipid Analysis Unit, there are six gas chromatographs for high throughput analysis and the development of new separation protocols.

## Media Kitchen

### W. Ridley

The Media Kitchen was established in 1996 to provide a wide range of sterile microbiological, mycological and plant tissue culture, media and disposable plasticware for the Institute's numerous laboratories. The advantage of centralized buying in bulk has meant huge savings for the Institute. Prices for disposable plasticware have actually fallen as the volume of orders has steadily increased over the last five years.

The Media Kitchen operates as a research facility under the central administration overhead, to minimise bureaucracy, with each user site being 'shadowtolled' for its throughput of consumables etc. It is staffed by two full-time and one part-time members of staff. The facility is supported by the efforts of one full-time and one part-time worker who were initially recruited from The Helm Project in Dundee.

Orders are delivered daily to 13 pick-up and drop-off locations around the site. At the same time, used Media Kitchen glassware is collected to be washed and re-cycled. Agar plates and any other specific media can be ordered by telephone, E-mail or by visiting the Media Kitchen itself. These requests are usually met within 24 hours. The support workers, in addition to delivering the orders, filling tip boxes and

Eppendorf pots, also collect, autoclave and dispose of waste microbiological materials.

The Media Kitchen quickly outgrew its original premises and moved to much larger premises at the end of 2000. This much improved working environment has given us greater diversity i.e. the pouring by hand of larger and smaller plates and slopes etc. We can also carry larger stocks of the most popular media/buffers which relieves the pressure of holiday and sick leave. A lot of progress has been made during the year with regard to achieving accreditation according to ISO 9000, hopefully in 2002. This will prove invaluable as regards future grants and contracts.

Figure 1 shows the output of the Media Kitchen, year by year since the first full year in 1997. From these figures, it would appear that 'saturation point' has been reached. However, the departmental changes that are ongoing are likely to have had some effect on the demand for media.

The work that is carried out by the Media Kitchen staff frees the innovative scientists, visiting workers, trainee students and support technicians from the



repetitive and time-consuming tasks associated with media preparation. It also secures a standardised quality of service throughout the Institute, saves the additional expense of each department maintaining a sterilisation and media preparation facility and saves money by bulk buying. The facility and service the Media Kitchen offers is appreciated and often envied by visiting scientists and students. With the extra

staff to be transferred from Dundee University due to arrive in mid-2002, the provision of a standardised, quality-assured media and sterile disposable-ware facility, with its daily delivery service and daily removal of waste microbiological materials will continue to be invaluable both to researchers and to those monitoring costs and assessing value for money.

	1997	1998	1999	2000	2001	
Boxes of tips (100/box)	13,933	14,300	19,738	19,653	20,609	
Eppendorf tubes (c. 200/pot)	2,600	2,620	4,211	4,279	4,130	
Agar plates	37,011	43,600	56,084	52,064	51,349	
Other items *	24,654	45,080	47,928	51,850	43,389	
* Item = anything bottled and capped.						

Figure 1 Media kitchen output.

# **Division of Finance and Administration**

### **Douglas Watt**

The Division is responsible for the provision of the 'non scientific' services to the Institute and encompasses the Units of Engineering and Maintenance, Estate, Glasshouse & Field Research, Finance and Human Resources, Information Technology and Scientific Liaison and Information Services, including Health & Safety, employing a total of 76 staff.

The Division provides a comprehensive service to the scientific community to ensure that they have the resources and ability to carry out the research commissioned by the various bodies, and that the infrastructure within which they work meets all the requirements in terms statutory legislation and health

and safety requirements. The variety and sophistication of the work carried out at the Institute continues to increase and the staff within the various units have responded by working closely with the science staff, adapting working patterns, learning new skills and taking on additional responsibilities.

### The Division is an integral

part of the Institute and the staff interact very closely with the scientific staff, often providing a breadth and depth of practical experience that is not available elsewhere. The Estates and Glasshouse Unit provide a service ranging from the planting and monitoring of a wide range of agricultural and horticultural trials on the Institute's 400 acres of farmland to the provision of sophisticated facilities in both glasshouses and controlled environment facilities. In doing so they produce consistent, high quality results for scientists whose work requires increasingly detailed and accurate results, despite the vagaries of the Scottish climate.

Similarly the Engineering Unit has to maintain the basic infrastructure of the Institute whilst having to adapt it to meet the needs of increasingly sophisticated (and expensive) equipment required by the science programmes. The Institute was very successful in attracting £2.5 million of capital grant from the

Scottish Executive Department of Environment and Rural Affairs, which was extremely welcome but had to be managed by the Unit, within an already tight staffing and finance budget. Much of the new equipment also requires more sophisticated support and maintenance, and the staff have become extremely adaptable and knowledgeable in the provision these services, whilst continuing to maintain a wide breadth of facilities within a structure that, other than glasshouses, has not changed substantially in the last ten years.

The Institute is increasingly reliant on its computer systems for the management of internal communication, the manipulation of massive databases and scien-

> tific information, the Internet and its administrative and financial systems. As such, the IT Unit and its development is central to the Institute's activities. New staff have been appointed and, building on the investment in the IT infrastructure in earlier years, the Institute plans to invest in an Institute wide information management system to allow it to manage the ever

increasing flow of information, provide an effective and efficient environment for staff to manage their work and enable staff to develop the Institute web site, to allow them to disseminate their work to an ever larger and information hungry 'customer' base.

In this, staff are also assisted by the Scientific Liaison and Information Services Unit who are tasked with promoting the science of the Institute to as wide an audience as possible, with particular emphasis on schools. This has been assisted by the appointment of a part time Education Officer, supported by the Mylnefield Trust. The quality of the displays, posters and presentation is remarkable given the size and resources of the Unit and they are expected to be able to answer questions and provide detailed information on all aspects of the Institute's work, usually at very short notice. In anticipation of Dr Bill Macfarlane Smith's retirement in April 2002, Mr Ian Kelly was appointed as provisional Head of SLIS in January 2002, combining this position with the new role of Development Manager, which will encompass the management of the proposed development of a Science Park within the Institute grounds as part of the proposed 'Western Gateway' development for Dundee. This proposal is in line with the Institute's aim of developing relevant science and working with end users and is seen a critical part of the Institute's strategy to become one of Europe's leading plant research facilities.

The provision of a safe and healthy working environment has always been one of the priorities of the Institute and the Mike De,Maine, the Institute's Safety Coordinator works closely with the Engineering Unit and all other departments within the Institute to ensure all requirements are adhered to and that a culture of safe working is promoted throughout the Institute by the development of detailed safety assessments and the implementation of good working practices. This will be further developed in the coming year with the increased implementation of procedures under the ISO 9001 standards.

Underpinning all of this is the Finance and Human Resources Unit, which works to ensure that all the administrative processes run as smoothly as possible and that the Institute operates within the available funds by providing relevant and timely financial and management information. Similarly, the HR staff support the Institute staff in all aspects of their work, training and personal development. An important part of this has been the accreditation under the Investors in People initiative, which was awarded in October 2001 and is a reflection of the commitment of the staff and the development of the Institute. Employment legislation and statutory requirements continue to increase and the HR Unit will require to develop its resources and abilities to keep pace with such developments.

The Division has to carry out its work within tight financial constraints but the staff approach their work with an enthusiasm and dedication which demonstrates their commitment to the work of the Institute.

# **Development and Scientific Liaison and Information Services**

Ian Kelly

The Institute year of 2001/2002 marked the start of a significant process of change in the Scientific Liaison and Information Services Unit. The planned retirement of Dr. Bill Macfarlane Smith and the subsequent resignation of Tim Heilbronn (to work full time in his own craft business) coincided with the introduction of different roles and different priorities for the SLIS Unit. That change of focus will be fully operational by the end of the 2002/2003 annual report period and so, in many ways, this is an interim report.

The clear wish of the Institute's Senior Management Group is that the SLIS Unit should focus on developing key corporate priorities and that press activities should be focussed on promoting the positioning and the profile of the Institute. Therefore, we have moved towards the concept of developing a media and marketing plan that will guide a range of proactive PR activities. Furthermore, our current approach is to seek an even greater direct involvement of the Institute's key scientists in the provision of information, press notes, public lectures and short talks for visitors.

However, as indicated above the roles of SLIS have been reviewed and the priorities now include:

- development of the Institute's proposed Science Park and associated land management

- assisting with corporate planning and corporate change

- promoting good business practice and providing additional private sector business advice within the Group

- development of more effective corporate links with the EU

- assisting with the enhancement of the Institutes positioning and profiling

- development of Visual Aids, Library, Visitor Management and Education/Press activities

The proposed Science Park, with a potential development value of around £100m, is the largest and the most important development ever promoted by the Institute. Successful implementation will enable and assist in the spin out of companies based on our science and will attract UK and international companies who will wish to work with our science. It will be a national level economic development project and in the role of Development Manager, the Head of SLIS is leading this project with support from Scottish Enterprise Tayside and the two Councils of Dundee City and Perth and Kinross.

As such, it is proposed to concentrate the Institute's research activities at an expanded Mylnefield site and to seek a change of use of Gourdie (to housing and business uses) in order to generate development value to invest in the Science Park. The Park will occupy some 34 hectares at the North Bullionfield area of the Mylnefield site. Key aspects of the project that have been put in place include an outline planning application in respect of the Science Park, an outline planning application for the change of use at Gourdie and a submission to SEERAD seeking the transfer of all existing land and assets to the SCRI Group. Subject to agreeing a final funding package it is hoped that the first buildings in the Park will be occupied in 2005.

This critical project is now integral to the Institute's corporate and scientific objectives as set out in the Corporate Plan. That Plan now also incorporates a clear expression of the Institute's vision and the route map by which that vision will be secured.

In terms of basic press and visitor related activities the Institute has:

- explained its research to biological science students

- given 33 public lectures

- participated in 4 science festivals, garden shows, etc. to take our science to the general public

- hosted 1,322 individual and groups of visitors

- welcomed local teachers and their pupils to the Institute

- produced 288 refereed and 184 non-refereed papers and articles in the past year

One measure of our impact is the 190 plus press references to SCRI's work during the year 2001/2002.

## Research services

As in previous years Politicians have appreciated the importance of SCRI's research, and the Institute has been visited by a range of MPs, MSPs, MEPs and local council representatives. We continue to liase with the Information Officers from the other SABRI's, SAC and RBGE and to contribute to reSEARCH, the newsletter of the Agricultural and Biological Research Group of SEERAD.



In the Visual Aids section, the SCRI web site has been maintained and the basis of a new site reflecting the new Institute structure has been put in place. Digital photography has virtually replaced the use of film, allowing a faster, more tailored service for customers. We now also have a facility for digital video editing. We designed, constructed and mounted a series of exhibits during the year, including a gold medal winner at the Dundee Flower Show for the best display from a public body. As more presentations are made using computer projectors, the time that was devoted to preparing lecture slides in the past is increasingly being spent on specialist graphics, and on technical graphic and photographic support for scientists.

The SCRI Librarian has played a leading role in RESCOLINC (the Research Council Libraries and

Information Consortium). The Consortium has been negotiating with publishers to supply electronic access to their scientific journals. The organisational models employed by the publishers to licence online access to their scientific journals have had little relevance for Research Institutes and consequently a great deal of time and patience has been required to reach agreement. Nevertheless, access to titles from Blackwell Publishing, Nature Publishing and Elsevier Science has been arranged and negotiations with Science are ongoing. Questions still remain about the integrity of the archive and our continuing access to it.

The vast majority of journals are still taken in print format and are now well accommodated in the newly refurbished library. The new shelves are more sturdy than those they replace and, with new lighting, the stock is more easily used.

The end-users in agriculture and horticulture continue to take a great interest in the Institute's research and, indeed, support aspects of it through their membership of SSCR. Information is disseminated through the Annual Report and through Crop Walks for soft fruit and potatoes (the latter in conjunction with SAC and BPC) that attracted 50 and 300 visitors respectively in the year 2001.

In the future the work of SLIS will continue to develop and evolve in three related areas:

- firstly, to develop and deliver key strategic change aspects such as the Science Park

- secondly, to assist in the creation of a suite of enterprises, based around our science, as envisaged in our Corporate Plan Vision

- thirdly, to provide our internal and external customers with the highest quality and Institute relevant visual aids, library, media and information activities

# **Information Technology**

Bruce Marshall and Scott Clark

The installation of the new network infrastructure to Cat 6 class was completed early in the year and on-time by Computer Cable Networks. The system has run smoothly from day one and eliminated the problems associated with the old and increasingly overloaded network. The success of the operation was due to a concerted team effort by all involved, CCN, IT, Engineering and Maintenance and all staff at institute who had to vacate offices and labs for periods during the installation.

Louise Davidson has taken over responsibility for Systems Administration of the Novell network with many new challenges on the horizon. We congratulate her on her promotion. Lesley McGregor was then appointed as PC specialist. Lesley joined us from the commercial sector with strong customer relations experience and excellent organisational skills. We also congratulate Lesley on her major achievement of an Honours Degree in Computer Science with the Open University. Staff compliment remains at 4.5 persons.

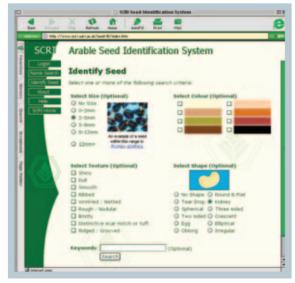
As in all parts of the IT sector, security and the threat from computer viruses and hackers is a growing concern. We have now completed the roll-out of new virus software which is continually updated. Messages and advice are periodically posted on our intranet raising staff awareness of the problem, which is also communicated through our IT Interface Group. Security has been further enhanced by placing the Institutes network behind a firewall. There have been no significant security issues, but all staff remain vigilant.

The IT department has long maintained contacts with other IT groups on the system and network administration side, both in the local universities, especially Dundee, our sister institutes in Scotland and the University of Edinburgh. This year we have started up new collaborative projects with the University of Abertay which takes IT into new areas. Two examples are the setting up of DRASTIC, Database Resource for the Analysis of Signal Transduction in Cells, and ASIS, Arable Seed Identification System. DRASTIC is a research project which is addressing the recent explosion of data-generation in the molecular biology of host-pathogen interactions. Our understanding of plant disease resistance at the molecular level has increased particularly through studying expression data. But, there is a real danger of being swamped by the sheer volume of data. It is apparent that alternative strategies to represent signal transduc-

DRASTIC

tion cascades need to be considered. A data-based approach with appropriate interfaces between stored data and interactive graphical outputs offers a resolution to these problems (http://www.drastic.org.uk/). ASIS is the result of a new venture with Computing Students in the their final Honours year. Buried seeds, known as seedbanks, have a dominant role in the ecology of many types of vegetation. In arable land, seedbanks are strong indicators of plant biodiversity and of long term economic weed problems. SCRI has a quarter of a century of experience in experimentation, measurement and modelling of weed seed dynamics, and close interactions with industry and government departments. At the heart of this work is the skill to identify the diverse range of seeds present in the soil of arable and neighbouring land throughout the UK. ASIS was created to aid





researchers in identifying these seeds and act as a training tool (http://www.scri.sari.ac.uk/asis/). During the summer months we also employed a student from Abertay to carry out an audit of PC hard-

ware and software. The information collected is stored and maintained on database constructed specifically for the purpose. This provided vital information with which to plan new policies in software and hardware management.

Finally, while striving to provide a robust and reliable service to our customers and implementing the activities described above, IT also spent a significant amount of time planning and preparing for future developments. These include SIMS (SCRI Information Management System), migration to Netware 5, the refurbishment of the main IT user area and special provisions for the integration of staff from the University of Dundee who opened offices and laboratories at SCRI during 2002. Much planning has taken place in all these areas in the current year and in the case of SIMS a final specification was completed and put out to tender at the end of the year. The next annual report will provide an update on the progress of these new initiatives.

# **Finance and Human Resources Unit**

### N. G. Hattersley & Alison Cartwright

Dr N G Hattersley joined the Institute in September 2001, as Financial Controller, replacing Ian Harrington who left to join West Lothian College of Further Education.

The Finance and Human Resources Unit (FHR), comprises 16 staff covering accounts, contract management, administration, payroll and human resources.

The Institute employs approximately 350 staff (plus visiting workers and postgraduate students) and has income and expenditure of approximately £14 million with an additional capital grant this year of £2.48 million. The Unit is responsible not only for the financial regulation of the Institute's activities but also for providing training and a human resources service for all staff.



As such, the HR section within the Unit plays an important part in the development of staff and the provision of a positive working environment, providing support and guidance in all areas of staff welfare. The section played an important part, along with internal staff committees, in the attainment of the Institute's Investors in People accreditation (which was awarded in October 2001), and administers the training budget on behalf of the Institute Training Committee, in addition to collating training requests from staff and arranging training with the appropriate training providers. The section co-ordinates the recruitment of Institute staff (during the year 50 posts were advertised, attracting 435 applications from external and internal sources), and carries out the administrative induction for new members of staff, as well as supervising probation procedures during their first year of employment. HR are also involved in monitoring the progress of the 24 research students based at the Institute, and in providing assistance to new staff, visitors and students with accommodation and other welfare related matters.

The accounts staff process over 17,000 purchase invoices per annum, 95% of which are paid within 30 days, utilising the BACS payment system to reduce the number of cheque payments. Over 1,000 sales invoices are raised in addition to the claims on grant aided projects. In addition to the 'core' research projects funded by the Scottish Executive Environment and Rural Affairs Department, additional income is sought from other external grant awarding bodies and from industry and the Institute carries out research on about 200 projects which are monitored and supported by the accounts team, particularly where the Institute acts as the co-ordinating partner for collaborative projects. The team also maintains over 2,500 items on its fixed asset register, ranging from personal computers to laboratory buildings.

The Institute is reliant on the funding from external bodies to maintain the resources and facilities of the Institute, therefore the development of staff, the monitoring of the finances and the control of the overhead expenditure is critical to the management of the Institute and its ability to produce world class science in an increasingly competitive research environment, both in terms of funding and output. This year has seen a reduction in research funding by commercial organisations and the role of budgeting and forecasting has become increasingly important to maintaining the financial integrity of the Institute, to ensure that the Institute can respond to the changing demands of the sponsors and research programmes.

## Estate, Glasshouse & Field Research Unit

### G. Wood

The Estate, Glasshouse and Field Research Unit fulfils the fundamental and crucial rôles of producing and maintaining plant material for the Institute's scientific research programmes and contractual undertakings. A wide variety and large number of plants are made available throughout the year for work both in contained/controlled environments and in a broad range of field trials.

The Unit provides a fully equipped and professionally expert service to fulfil the requirements of its clients with regard to the preparation of land, growing medium, sowing, drilling, planting, propagation, plant maintenance, environment control, harvest and clearance of residues for the Institute's field and glasshouse research objectives. It may have responsibility for an entire package from start to finish, or can provide prepared land and/or controlled environment régimes for inputs to be undertaken in varying degrees by scientific clients.

Staff development, recruitment and retention continue to be vital aspects of support and service provision, especially in these specialist areas of glasshouse plant production and field trialling operations. Quality, to a high standard within a framework that allows this to be achievable consistently, is of paramount importance. The service/support provision, and the necessary resources to achieve it, must keep pace with or pre-empt the demands of dynamically developing research programmes.

A structured training and development programme is well supported by the Institute's Training Committee, and staff in the EGFR Unit undertake essential skill/competency training and certification to nationally recognised standards. Several members of staff are now progressing to vocational and further academic studies that serve to equip them with a wider range of expertise and a greater level of underpinning knowledge relevant to improving the service and support they provide. The staff in the Unit are organised operationally in small sub-teams but not on an inflexible or restricted basis so that the service provision is effective and efficient.

During the year, stringent control measures were implemented throughout the entire glasshouse and controlled environment facilities due to an outbreak of a particular insect pest. This involved the glasshouse staff in a lot of extra work and restrictions on access, but with the co-operation of clients the projects were not significantly disrupted and the whole exercise was very effective. Hygiene levels as a whole have benefited tremendously throughout the facilities, and general standards have risen accordingly.

The new research glasshouse complex came through its first full season of use: there were some teething problems but these were addressed and the facility was well used with many essential research projects being undertaken that could not previously have been catered for without such well controlled, containment glasshouses.

Controlled environment facilities were subject to ongoing review by the Institute's Glasshouse and Growth Cabinet Organisation Committee. As well as seeking to improve the maintenance of existing structures, a rolling facility replacement programme has been drafted which allows a co-ordinated approach to be taken to the replacement of old or outmoded facilities in line with developments in the research and other programmes.

A new site was found, adjacent to the Institute, which allows us to produce seed potatoes of common origin for breeding and other research projects. The previous location for this was south-west of Edinburgh but it was not cost-effective to continue at that site and all operations at the new site were taken on board by the current field trials team. A new cold store for holding the seed potatoes following harvest was built within one of the existing crop-handling buildings and even though planting was delayed (due to foot and mouth restrictions) in this first season at the new site, there has been an immediate gain in operational efficiency.

The Institute was selected as one of several sites throughout the UK for a biomass/biofuel perennial grass trial. The field trial started in May 2001 and will continue through four seasons, during which time the suitability of American-switch, reed canary, and elephant grasses will be assessed for genotype x environment interactions and yield.

In the late spring of 2002, a similar trial was commenced but with American and UK cultivars of



oilseed rape and mustard. These will be assessed for not only biofuel (oil) production but also for wholecrop exploitation and, potentially, extraction of other important chemical components.

Just as with other resources and facilities, field trialling and land use have to match the needs of the new research themes and programmes and be capable of coping with the additional needs of contractual undertakings, as well as other enterprise initiatives. Local Council and Regional development plans may affect the Institute's land resource and these very important issues are being considered by the new internal Farm Strategy Group, whose remit is to be pro-active (rather that reactive) in assessing needs and various options. Again, this is a mechanism to improve the quality of resource utilisation, facility provision, strategic planning, and decision-making in an integrated and co-ordinated manner.

David Pugh retired on 30 August 2001 after almost 26 years service to the Institute. He joined SHRI (as it was then) from a farming background and progressed to become a senior field trials officer within this Unit, liaising directly with numerous clients and always attentive to the quality aspect of fulfilling their needs. His contribution has had a highly significant impact on the success of field trialling at SCRI and we wish him well for the future.

# **Engineering and Maintenance Department**

## S. Petrie

The Engineering and Maintenance Unit within SCRI has a wide-ranging remit regarding site services and facilities.

The unit consists of fifteen engineering/technical posts along with six ancillary posts covering site security, stores and administration.

The unit has a reputation for providing quality work and this has resulted in its role evolving into not only one of dealing with maintenance and repairs but also managing and carrying out refurbishment projects. The unit is also involved in the installation and servicing of new equipment and undertook a large number of such projects following the provision of £2.48 million capital funding from SEERAD. It is also the policy of the Institute to allocate a significant proportion of these funds to maintain the infrastructure and basic facilities of the Institute and the unit is involved in developing, costing and implementing long-term strategies to maintain and develop the resources of the Institute.

Refurbishment projects undertaken during the year included the complete refurbishment of seven laboratory areas plus refurbishment of twelve offices. These projects were carried out mainly by our in-house staff who provided the electrical, heating, plumbing, data/telephony cabling, painting and joinery work. Where external contractors were required for other disciplines these resources were procured and thereafter managed by the Unit.

Laboratory Equipment Services form a major part of the work of the unit. Although as much as possible of this is carried out in-house the ever increasing sophistication of major pieces of equipment requires the services of external engineers. Again these resources are managed within the unit which also negotiates service contract costs and conditions.

With good quality craft and engineering skills becoming increasingly difficult to find the importance of carrying out sizeable projects using the skills base available within the unit have become ever more critical. The unit liases with the scientific staff to assess their requirements and thereafter effectively plans and manages the project through to completion.

The site continues to become more sophisticated in terms of the systems required to ensure it operates effectively and safely and the unit works closely with the Safety Co-ordinator and the Health, Safety and Welfare Committee to ensure that the Institute is able to provide a safe work place and promote safe working practices.



The on site systems that are managed and maintained by the unit include those for automatic fire detection, intruder alarm, closed circuit television, telephone exchange, door access, heating controls (including computerised glasshouse controls) and data networking throughout the site. The fire detection and alarm systems were significantly upgraded during the year as part of the continuous programme of improvement and have been linked to the new door access system to provide an enhanced control over what is a quite dispersed site.

The unit must also, through its farm workshop section, provide a repair and maintenance service to the institutes estate unit in order to keep its large fleet of farm vehicles and machinery in good order

## Mylnefield Research Services Ltd., The Mylnefield Trust and Mylnefield Holdings Ltd.

N. W. Kerby and J. B. Snape

**Introduction** Mylnefield Research Services (MRS) Ltd., the wholly owned subsidiary of the Scottish Crop Research Institute (SCRI), was established in 1989 as the commercial arm of SCRI to enhance competitiveness, understand and fulfill the needs of the life sciences and "agro" industries.

MRS aims to match the outstanding quality of science at SCRI with excellence in technology transfer and commercialisation.

MRS works to help SCRI fulfill its mission by supporting SCRI's Corporate Plan and the SEERAD Strategy for Agriculture, Biological and Related Research (1999-2003). It does this by providing links between end-users and the Institute, providing funds that can be reinvested in strategic areas of research and by marketing the activities of the Institute internationally. The Mission Statement of MRS is:

"To develop commercially the Scottish Crop Research Institute's scientific expertise, resources and intellectual property, and to improve the quality of services to achieve new standards of excellence".

Our overall intention is to be a significant part of the creation of the knowledge economy in Scotland and in terms of the local economy, to create the base from which new, innovative technology-based businesses will be established, and reinforce the success of existing companies.

The business of MRS can be divided into six complementary activities, namely:

Contracts Office Project Management Contract Research Analytical Services Sale of Products Licensing and IP management

**Finances** From the time of incorporation, MRS has been self-sufficient in providing its own accommodation and staffing, achieved without Government startup funding, subsidy or venture capital. Turnover increased from £0.7 million in 1992/1993 to more than £2.5 million in 1999/2000 which was maintained in 2000/2001. The turnover for financial year 2001/2002 was £1.94 million. The percentage of income derived from contract research was down compared with the previous year, whereas royalty income continued to grow. The drop in turnover was attributed to four main causes: (i) public perception of GM crops causing several customers and many potential customers to discontinue research in this area; (ii) restructuring of all the major life science companies involving first consolidation and then divesting of ag-biotech activities, a phenomenon especially pronounced in the UK; (iii) low profitability in the whole of the agricultural sector, especially in the UK and; (iv) revaluation of technology stocks and lack of R&D investment in technology.

Despite the drop in turnover, MRS still managed to gift  $\pm 138$ k to SCRI and  $\pm 20$ k to the Mylnefield Trust. A further drop in turnover is predicted in 2002/2003 due to continuing difficulties in the agriculture and ag-bio industries.

The Lipid Unit continues to be profitable and is currently adapting its procedures so that it is compliant ith Good Laboratory Practice (GLP). In 2002, MRS signed a three-year contract with Laxdale Ltd of Stirling to provide serum lipid analysis services. The Unit continues to offer a wide range of specialist analytical services, offers training courses and undertakes contract research. The Unit also successfully tendered for a Food Standards Agency project on maize oil authenticity.

Position	Variety	Crop
1	Symphony	Strawberry
2	Ben Hope	Blackcurrant
3	Ben Alder	Blackcurrant
4	Glen Ample	Raspberry
5	Ben Gairn	Blackcurrant
6	Caledonian	Kale
7	Loch Ness	Blackberry
8	Ben Tirran	Blackcurrant
9	Spey	Potato
10	Claret	Potato

Figure 1 MRS royalty earners.

Royalty income grew from £200k in 2000/2001 to over £220k in 2001/2002. Figure 1 shows the top ten royalty earners for MRS in 2001/2002.Sales of Symphony remained more-or-less constant in the UK but new licenses granted in the rest of Europe started to generate income. The real success story of the year was the new blackcurrant varieties, Ben Hope and Ben Gairn, bred by Rex Brennan for Glaxo SmithKline. The demand outstripped supply and the varieties attracted considerable interest throughout Europe and beyond. The raspberry variety Glen Ample became established as the industry standard in

Territory	Title	Inventors	Application No	Status	Priority Date	International Filing date
European	PHYTOPHTHORA PCR PRIMERS	A Dolan EP96303105.9 J M Duncan D E L Cooke		Undergoing Examination	4.5.95	2.5.96
USA Japan EU Canada Australia New Zealand	METHOD (PHAGE Typing)	B Hyman I Toth	PCT/GB99/01363	Proceed to Grant Pending Pending Pending Pending Pending	2.5.98	2.5.99
PCT Eu Australia Canada New Zealand USA	METHOD OF PRODUCING CHIMERIC PROTEINS	S N Chapman S P Santa-Cruz K J Oparka T M A Wilson	PCT9501443 EP95934228.8 AU36598/95 CA2202761 NZ294014 US844045	Pending Granted Granted Granted Granted (6232099)	18.10.95	18.10.95
USA EU	TRANSPORTER PROTEIN	M E Talianski E V Riabov D J Robinson T M A Wilson	PCT/GB99/02424	Pending	29.8.98	29.8.99
РСТ	GENE SILENCING SUPPRESSOR	M E Talianski E V Riabov S A MacFarlane B Reavy	0101513.0	Pending	19.1.01	19.1.02
GB	NUCLEOTIDE Pyrophosphatase	R Viola R Hancock H Ross C Simpson A De Matteo	0219931.3	Provisional Filing	28.8.02	28.8.03
РСТ	PROTECTION AGAINST GENE SILENCING	M E Talianski E V Riabov S A MacFarlane B Reavy	0101505.6	Pending	19.1.01	19.1.02
PCT (Biosource Genetics Corporation)	EXPRESSION OF FOREIGN GENES FROM PLANT VIRUS VECTORS (IRES)	S P Santa Cruz G P Pogue R L Toth S N Chapman F Carr	PCT09/758,962	Pending	08.1.01	08.1.02
PCT (Norsk Hydro)	AGRICULTURAL COMPOSITION AND METHOD FOR TREATMENT OF PLANTS THEREWITH	A C Newton G Lyon K Holmes W Smith K Osnes	PCT/NO01/00322	Pending	28.7.00	28.7.01

Table 1SCRI IP portfolio.

Agbiotech Market Bayer's acquisition of AventisCrop Sciences means that in 2002 there were just six agrochemical multinationals with sales of more than \$1,000 million compared with 11 in 1995. The recent wave of consolidation left the second tier of agrochemical majors even further behind the market leaders. The deployment of enabling genomic, proteomic, and metabolomic technologies across the agricultural and pharmaceutical sectors to form global life science companies was no longer accepted by investors and analysts - the life sciences concept of the late 1990s has been truly buried. The payoff and profits are very different for the two sectors. The pressure to consolidate business interests is still strong and agricultural activities are being consolidated into separate free-standing companies (e.g. Monsanto, Syngenta and Bayer Crop Science).

About 85% of the revenue of the agriculture market is generated by sales of pesticides and other crop protection products. The remaining revenue comes from seed and intellectual property.

The seven largest agrochemical companies accounted for approximately 80% of global sales (Table 2). The current market leader Syngenta experienced the largest decline in sales among the majors in 2001. Syngenta shut 10 of its manufacturing and supply facilities and 6 of its technology centres following a world wide review of its capacities. Bayer and Aventis recorded the greatest sales increase from

the UK and Caledonian kale continued to gain market share, primarily for game cover use.

Our current patent portfolio consists of 9 patents (either applied for or granted), and in excess of 45 cultivars protected by Plant Variety Rights (or rights applied for) (see Table 1)

Following its launch in January 2001, the Management Advisory Package for Potato (MAPP) has attracted considerable interest, both in the UK and overseas. This interactive computer package, which helps with critical decisions related to optimizing financial returns from potato production, has been purchased by growers, agronomists, food processors and for educational use. MAPP has a critical role to play in transferring knowledge from the science base to the end-user to increase productivity and maintain margins. existing business. Bayer and Aventis were out performed only by BASF and Dow AgroSciences, who grew mainly due to the acquisition of Cyanamid and Rhom and Haas's agrochemical business respectively. Monsanto agrochemical sales suffered from lower sales of Roundup. However, seed and genomics increased from \$1,608m to \$1,707m an increase of approximately 6%. Monsanto reduced its earning forecast for 2002 by 30% due to a slow start to the season in the US and worsening economic problems in Latin America. Monsanto and BASF both posted double-digit sales declines in the first half of 2002.

Rat	ing		2000 Sales	2001 Sales	%
2000	2001	Company	(\$M)	(\$M)	Change
1	1	Syngenta	5,888	5,385	- 8.5
3	2	Aventis	3,659	3,842	+ 5
2	3	Monsanto	5,493	5,462	- 0.6
6	4	BASF	2,228	3,105	+ 20.0
4	5	Dow	2,346	2,612	+ 11.3
4	6	Bayer	2,252	2,418	+7.4
7	7	DuPont	2,009	1,917	- 4.6

**Table 2**Agrochemical Sales of the Leading Companies(Agrow 29 March 2002).

The customer base for contract research in aggenomics and sale of proprietary intellectual property is dominated by 6 (note merger of Bayer and Aventis) major ag-bio players.

To consolidate and grow MRS from its stable foundations, our strategy can be summarised as follows:

Continue to build relationships with key customers to maximize value.

Win new contracts, diversify the funding base and increase financial returns to SCRI.

Identify, build and exploit innovative platform technologies and IP.

Develop new products and capture greater value.

Deliver high quality, profitable services and consultancies.

Create strategic alliances, partnerships, joint ventures and businesses.

Ensure MRS offers value for money both for SCRI and its customers.

To implement our strategy, we will satisfy customers; attract new business; invest in people; reward success;

deliver quality services; conduct technology audits and implement technology foresight; and manage risk.

**New Appointments** New appointments in 2001/2002 include Ruth Razzo in the lipid analysis unit, Derek Coyle (potato breeding) and Nikki Wood, Tiina Martila and Velia-Matti Rokka (externally-funded projects). MRS would like to congratulate Lesley Beaton on achieving her MBA at the University of Abertay Dundee.

Acknowledgements MRS gratefully acknowledges the cooperation of SCRI scientists, administrative and field and glasshouse staff for their contribution to the success of the company. MRS would also like to thank all the companies and other sponsors of work at SCRI for their continued support.

#### The Mylnefield Trust

The Mylnefield Trust was registered in 2000 as a charity with objectives to: - promote research and scientific work in the life, environmental and related sciences, in particular production of agricultural, horticultural and forestry crops, methods of limiting or eradicating pests and diseases, wood sciences and biomathematics, methods of increasing production or growth, improving cultivation and research into improved cultivars; - promote the dissemination of such research. The Trust, as of 1 April 2002 has funds in excess of £225,000.



Nigel Kerby discussing MAPP with the Rt Hon Michael Meacher MP at the Royal Show 2002.

The Trust has recently supported the following: An Incentive Fund to provide further support for scientists actively winning external contracts.

Funds to support an Education Officer at SCRI

A hardship fund for an overseas PhD student

### Mylnefield Holdings Ltd.

In February 2000, Mylnefield Holdings Ltd. was incorporated as a wholly owned subsidiary of The Mylnefield Trust. A new company PhyGene Ltd. was also incorporated as a wholly owned subsidiary of Mylnefield Holdings, but has yet to trade. Mylnefield Holdings was established to facilitate the creation of new ventures such as "start-up" and spin-out companies.

## **Scottish Society for Crop Research**

## D.L.Hood

Trustees: -

Mr A G M Forbes Mr I E Ivory Mr A Pattullo (Resigned) Mr J S Whitehead

Chairman: -

Vice Chairman: -

Members of Committee of Management: -

Mr T D Gray Mr A Logan Mr L M Porter Mr G Rennie Dr S Wale

Secretary and Treasurer: - Mr D L Hood

Registered Office: - c/o Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA

Membership Numbers: - 261

The Scottish Society for Crop Research is a registered Friendly Society formed in 1981 by the amalgamation of the Scottish Society for Research in Plant Breeding and the Scottish Horticultural Research Association.

The Society provides a link between the Scottish Crop Research Institute and farmers, processors and other interested bodies: -

by organising field walks and meetings for the exchange of information

by financing science based publications for the benefit of the membership

through the formation of crop-based sub-committees which maintain contact with members on specialised topics.

The Society continued to support projects initiated in previous years such as "Malting Quality Analysis in Winter Barley Mixtures" with results of interest to the brewing industry.

Grower Trials for the management of Potato Cyst Nematode and Aspects of Physiological age of Potato and suitability for specific market outlets were two additional projects undertaken during the year. A contribution towards Mylnefield Research Services Ltd Raspberry Breeding Programme was repeated.

The XVI<sup>th</sup> Eucarpia Congress "*Plant Breeding: Sustaining the Future*" was held in Edinburgh and the Society was pleased to offer support.

A grant from the Thyne Bequest was awarded to Dr D J Robinson to attend a meeting of the European Association for Potato Research in the Czech Republic.

These projects, together with the award of travel grants to enable Institute members of staff to attend conferences and seminars overseas to discuss their research, promote both the Institute and Society. Reports from those awarded travel grants are available on request from the Secretary.

Dr Roger Turner, Chief Executive of the British Society of Plant Breeders took as his topic, "The Future of Plant Breeding", at the Annual General Meeting of the Society.



The Chairman of the Society, Mr. J.S. Whitehead presents the Peter Massalski Prize for 2002 to Dr. J. Jones, who was congratulated also by Professor David Hughes, University of London.

No Society Soft Fruit Walk was held during the year because of staff involvement in the 8<sup>th</sup> International *Rubus* and *Ribes* Symposium in July.

"Potatoes in Practice", the Society Potato event, in conjunction with the British Potato Council, the Scottish Agricultural College, and the Institute, was held in August and proved of interest to many members from all sections of the industry.

The Crop Sub-Committees including Cereals met throughout the year and informed the Management Committee of their approved projects for the coming season.

The Management Committee met twice during the year, in May and November, and was particularly concerned about the future of the raspberry breeding programme at the Institute.

Mr A Pattullo MC resigned as a Trustee at the Annual General Meeting. A Member of the Board of Directors of the Scottish Society for Research in Plant Breeding since 1973, he was elected a Trustee of the Scottish Society for Crop Research on its inauguration in 1982. His wise counsel and wide experience was noted and the Chairman thanked him warmly for his contribution to the Society. Society membership continues to decline due to retirement and contraction within the industry. Farming controversies and countryside matters exercise the minds of politicians who consider a ban on hunting with dogs in Scotland a priority in the legislative calendar.

The Management Committee welcomes suggestions for research topics and comment from members and others and urges them to contribute to the Society by joining one of the Crop Sub-Committees or indeed the Management Committee.

The Newsletter of the Society languishes and will not make an appearance in the immediate future. Nevertheless, contributed articles together with photographs of interest should be forwarded for the attention of the Secretary and Treasurer who will attempt to collate them into some form of publication.

# Health and Safety

## MJ De, Maine

The Institute is committed to the provision of a safe and effective workplace and the promotion of a culture of safe working. The Safety Co-ordinator presents regular reports to the Institute's Governing Body and Senior Management Group, and a member of the Governing Body is responsible for maintaining regular contact with Institute staff in this area.

Staff regularly reviewed all working areas and appropriate operating procedures are drawn up to ensure safe working practices. It is intended that these procedures will be integrated into the proposed development of the ISO 9001 quality accreditation system.

Regular surveys are undertaken by the Institute in conjunction with the other Scottish Institutes and the BBSRC, and the SCRI Health & Safety Committee which is made up of staff and Union representatives is charged with ensuring that the appropriate Health and Safety policies are implemented and legislation is adhered to.

Surveys are also undertaken by external bodies and the improvements in fire safety, which were recommended by Tayside Fire Brigade and carried out over the last five years, have now been completed. The recommendations were made as a result of two surveys by the Brigade from the time when the Institute ceased to be covered by Crown Immunity with regard to the Fire Regulations. These have included the installation of extra fire doors, ensuring that safe paths out of buildings are adequate, the improvement of the emergency lighting system and the carrying out of fire risk assessments. In addition the fire detection and alarm systems have been radically upgraded so that the fire alarm is now raised by means of automated voice announcements over the tannoy system. In addition, the newly installed door access security system is fully integrated with the fire alarm system so that it is now possible to obtain accurate listings at any time of the occupants of a building where a fire alarm has been

triggered. The system works by means of swipe cards individually programmed for each member of staff, which are presented to a card-reader at the door of each building. A computer printout can then be generated as soon as the fire alarm is triggered for a building.

A Health and Safety survey was carried out by our insurers and they recommended improvements in machinery guarding, the bunding of fuel tanks, minor repairs to some fire doors and refresher training of staff in the erection of scaffolding platforms. All the recommendations have been complied with or are in progress.

Training in fire safety for users of laminar flow cabinets has continued. This training appears to have reduced the occurrence of fire-related incidents in general and in the last nineteen months there has been only one such incident, where smoke was generated by a hot plate that had been left on.

Douglas Watt, Company Secretary, was appointed as Chairman of the SCRI Health, Safety and Welfare Committee, vice Professor Wayne Powell. Xm Services was appointed as occupational health adviser to the Institute, vice Dr Ann Simpson. Dr David Hewitt of the University of Dundee was appointed as Radiation Protection Adviser vice Dr Jock Forrest who has now retired. The Committee recorded its thanks to Professor Powell, Dr Simpson and Dr Forrest for their services. Six new first-aiders were trained to HSE standard by our in-house HSEapproved trainers and refresher training was held for six qualified first-aiders.

There was one RIDDOR-reportable incident this year in which a member of staff fell on an internal staircase and tore a ligament in her leg resulting in an absence from work of more than three days.

## **Staff Association**

#### Jane Fairlie

The staff Association of the Scottish Crop Research Institute is now in its 9<sup>th</sup> year, and to date has raised almost £7000 for charities, both locally and nationally. There are approximately 200 members, and the primary aim of the association is to raise funds for a nominated charity through raffles, prize draws, functions and donations from exhibiting companies.

At the Annual General Meeting, members nominate a different charity each year. In 2001 the organisation chosen to benefit was DEBRA which raises funds for children with a rare skin disorder. Vice-Chairman Jim Wilde presented a cheque for £1444 to Robin Hood from DEBRA, on 10 April 2002 in the staff restaurant.



The Staff Association organises events for staff such as golf and angling outings and competitions, curling 'taster' evenings and ten-pin bowling fun nights. Currently, there is a weekly yoga class and there are netball, volleyball, badminton and football teams, who are provided with equipment and strips from Association funds.

In late June, the Summer BBQ is hosted by the Association for staff and their families. In December, a Ceilidh, a staff Disco, and children's party are held annually. A highly successful Institute Christmas lunch took place in 2001 when eight members of staff entertained us with Scottish music, followed by our own piper prior to lunch being served.

Every member has the opportunity to win cinema tickets or a meal for two each month, and periodically there are prize draws held where local companies have donated prizes. All the proceeds from the prize draws are donated to the nominated charity. The Association holds a corporate membership for the National Trust for Scotland and four cards are available for staff use. Also discount schemes are organised with local retailers, hairdressing salons, and seed and plant companies. Corporate Sports membership at Dundee University Sports Union for staff families is also organised by the Association, and 'Which' magazine is provided for the Institute library.

Membership fees continue to be  $\pounds 1.50$  per month and the office bearers and committee are elected annually at the AGM. The charity for the year 2002/2003 is Ninewells Cancer Campaign.

Publications

## **Publications**

Publications are classified in the following manner:

- J Papers describing original research in refereed journals.
- R Critical reviews in journals, book chapters and reviews in books providing each has been edited externally.
- P Published proceedings of contributions to conferences or learned societies (including published abstracts).
- T Technical reports, other publications.
- O Popular articles, other publications.

Armstrong, M., Whisson, S.C. & Birch, P.R.J. 2002. Cloning of avirulence genes from *Phytophthora infestans*. *Molecular Biology of Fungal Pathogens XIII*, Gregynog, Wales 17-19 July, 2002. P

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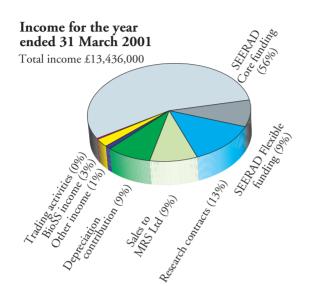
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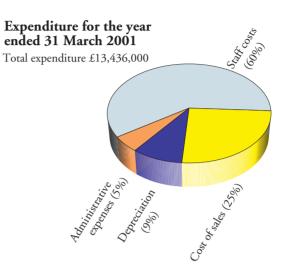
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## Summary of the Accounts

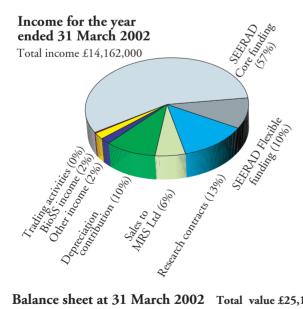


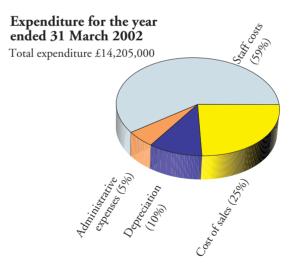


#### Balance sheet at 31 March 2001 Total value £21,451,000

Assets		
Fixed assets Stocks Debtors	92 % 0 % 8 %	

Capital reserve	91 %
Income & expenditure account	3 %
Current liabilities	5%





#### Balance sheet at 31 March 2002 Total value £25,195,000

Assets		Liabilities		
Fixed assets Stocks Debtors	92 % 0 % 8 %	Capital reserve87 %Income & expenditure account3 %Current liabilities10 %		

# Statement of health & safety policy

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

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

# Statement of quality policy

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 our customers and ourselves.

## Statement of environmental policy

The Institute will regularly assess the impact of its operations on the environment and take measures to reduce or eliminate negative effects. It aims to reduce waste of all types, increase the recycling of materials and have a benign or beneficial influence on the locality in which it is situated. Employees will be made aware of these aims and training will take place where necessary in order to achieve them. The Institute will work with statutory agencies in order to comply with legislation concerning the environment and related issues. It will carry out regular audits of its operations to ensure compliance with the policy.

## Statement of data protection policy

The Scottish Crop Research Institute\* will manage data in accordance with the requirements of the Data Protection Act (1998) and the BBSRC Staff Code.

It will retain only such personal data that are required for the conduct of staff administration. The data will be maintained securely to avoid unauthorised access and processing. Access to data is restricted to those who require it in order to efficiently administer the workforce. Data which are requested by a data subject will be confined to data which apply to that person. Data not referring to the data subject will be withheld or made unreadable.

Processing of personal data will be restricted to that required for the administration of the SCRI workforce

There will be periodic reviews of personal data and those which are obsolete or no longer necessary for staff administration will be destroyed. The review will take place annually (usually in March).

\* For the purposes of data protection administration The Scottish CropResearch Institute includes MRS and BIOSS.

### **The Governing Body**

**Chairman: J.E.Godfrey, B.Sc., F.R.Ag.S.**, gained his degree in agriculture from the University of Reading. He is a Director of family farming companies in Lincolnshire and Yorkshire. He is Chairman of Willisham Group plc, and is a member or adviser to numerous committees including the Royal Agricultural Society of England. He is a Trustee of the International Potato Center (CIP) in Peru and a Director of World Potato Congress Inc. He was appointed to the Governing Body of SCRI in 1991, became Vice Chairman in 1997 and Chairman in 1999.

**E.** Angus, MBE, M.Sc., Fio.D., has been actively involved in the start-up of several knowledge economy companies since retiring from Napier University in 1999, where he held the post of Business Director for the University and Managing Director of Napier University Ventures Limited. His degree in corporate leadership was gained after studying business incubation systems and processes in the US, UK, the Continent and Scandinavia. His strategic management experience at Board level in food, textiles and distribution companies, span a period of 25 years and he was awarded the honour of an MBE for his contribution to exporting in 1977. He was appointed to the Governing Body of SCRI in 2000.

**Professor J.J.F. Belch, M.B., Ch.B., F.R.C.P., M.D.**, is Professor of Vascular Medicine at the University of Dundee, where she is interested in the causes, manifestations and treatment of disease of the blood vessels and circulation. Additionally she is a member of the Medical Research Council Advisory Board, a member of the Scottish Office Acute Services Review Sub-Committe on Peripheral Arterial Disease, and UK Chairman of the Forum on Angiology. Her interests in terms of crop research relate to the antioxidant content of food, specific fatty acid types within oils, and the relationships of these to vascular disease. She was appointed to the Governing Body of SCRI in 1998.

**Professor R.J. Cogdell, B.Sc., Ph.D., F.R.S.E.**, was awarded his two degrees by Bristol University, and completed his post-doctoral research in the USA. He joined the Botany Department of Glasgow University (now the Institute of Biomedical and Life Sciences) in 1975, and currently holds the Hooker Chair of Botany there. He was awarded a Humbolt Research Prize in 1995. He was appointed to the Governing

Body of SCRI in 1997, and was recently re-appointed. He is a member of the Chairman's Committee, and Chairs the Science Sub-Committee. He is a Director of MRS and a Trustee of the new Mylnefield Trust.

Dr K. Dawson, B.Sc., Ph.D., D.I.C.P., is Technical Director of CSC CropCare, the largest privately owned crop consultancy service in the North of the UK. He trained as an agricultural and environmental scientist and was awarded his degrees by the University of Newcastle-Upon-Tyne and the University of Reading. He joined the Scottish Agricultural College in 1982 and, after a spell as Northern Technical Advisory Manager for BASF(UK) Ltd, formed CSC CropCare in 1987. He is an elected director of BASIS(UK) Ltd and a member of the Government's Pesticide Forum. He also has been closely associated with the Scottish Natural Heritage TIBRE programme, utilising new technology for agronomic and environmental benefit. His main interests are in crop protection and Integrated Farming Management. He was appointed to the Governing Body of SCRI in 2000.

**Dr M. Eddie, B.Agr., Ph.D.**, gained both his degrees from The Queen's University, Belfast. After employment as a research scientist by the Ministry of Agriculture, Northern Ireland for 4 years, he joined Unilever plc where he spent 25 years mainly in their agribusiness operations, eventually becoming Chairman, in sequence, of two agribusiness companies. The first was based in Scotland and the second in Malaysia. After retirement from Unilever in 1999, he was appointed to the Governing Body of SCRI in March 2000.

**Professor M.J. Emes, B.Sc., Ph.D.**, is Director of the Research and Graduate School in the Faculty of Biological Sciences, University of Manchester, where he is responsible for over 120 academic staff and the training of 400 postgraduate students. His own research activities are focused on understanding the control of plant metabolism, particularly mechanisms of regulating starch synthesis in cereals. He has extensive experience of BBSRC grants committees and is a member of the Governing Council of the John Innes Centre. He is also an editor of the Journal of Experimental Botany. He was appointed to the Governing Body of SCRI in 2000.

**Professor J. Evans, OBE, B.Sc., Ph.D., D.Sc., F.I.C.For.**, is Professor in Tropical Forestry (part time) at Imperial College, London, and was formerly Chief Research Officer (S) with the UK Forestry Commission from 1989 to 1997. He is Chairman of the Commonwealth Forestry Association and is Chair of DfID's Programme Advisory Committee for Forestry Research. Professor Evans also holds an honorary Chair of Forestry at the University of North Wales, Bangor. He is the author of eight technical books, including the newly published Forests Handbook and the standard text on tropical forest plantations. Professor Evans owns and manages his own small woodland. He was appointed to the Governing Body of SCRI in 1998.

Wendy Goldstraw, B.Sc., P.G.Dip.B.A., M.C.I.P.D., gained her degrees from the University of Edinburgh, before joining the Post Office as a management trainee. After a number of roles in human resources and line management, she was latterly General Manager for Post Office Counters Ltd for Scotland and Northern Ireland, with responsibility for 2800 Post Offices. She was an executive member of both the Scottish and Northern Ireland Post Office Boards, and served as a Director of Edinburgh Chamber of Commerce and also on the Scottish Committee of the Institute of Directors. She has been a member of the Accounts Commission for Scotland since 1994. She was appointed to the Governing Body of SCRI in 2000.

K. Hopkins, F.C.A., joined Reeves & Neylan, Chartered Accountants, in Canterbury, Kent, in 1971, from a farming background. He moved to open the Scottish Practice in 1978 and was appointed a partner in 1981. 'The Scottish Partnership' (a separate business since April 1996) acts for over 500 farmers in Scotland, and specialises in the establishment of farmer-led agricultural cooperatives. His firm now has three offices, Forfar, Perth and Dundee, and employs over 60 staff. Mr Hopkins specialises in capital taxes, agricultural law and cooperatives, development and expansion of business, writes for the agricultural press, and lectures throughout Scotland. He is Treasurer for District 1010 of Rotary, a member of the Institute of Directors, and Chairman of the charity Childlink Scotland. He was appointed to the Governing Body of SCRI in 1997.

**Professor B. King, M.Sc., Ph.D., F.I.W.Sc., C.Biol., F.I.Biol.**, is Principal and Vice-Chancellor, University of Abertay Dundee, having joined it in 1992 from the Robert Gordon University, Aberdeen, where he was Assistant Principal and Dean of the Faculty of Health and Food. He is a Non-Executive Trustee of Tayside Primary Care NHS Trust, Board Member of Scottish Enterprise Tayside, Governor of the Unicorn Preservation Society, and a member of the Institute for Learning and Teaching in Higher Education. He is a member of the International Research Group on Wood Preservation and of the Biodeterioration and British Mycological Societies. He was appointed to the Governing Body of SCRI in 1998.

I. McLaren, S.D.A., is a partner in a family owned farming business, specialising in potato and cereal production. He is also a partner in a retail dairy business, a garage business, and a visitors' centre. He is Chairman of a leisure complex, the Dewar's Centre in Perth, and a member of the Perth & Kinross Agricultural Forum, and was a member of the Home-Grown Cereals Authority from 1988 to 1997. He was appointed to the Governing Body of SCRI in 2000.

Emeritus Professor Sir John S. Marsh, C.B.E., M.A., P.G. Dip. Ag. Econ., F.R.A.S.E., F.R.Ag.S., C.Biol., F.I.Biol., was Professor of Agricultural Economics, University of Aberdeen, from 1977-1984, then Professor of Agricultural Economics, University of Reading from 1984-1997. He is a Former Director of the Centre of Agricultural Strategy and Chairman of the Agricultural Wages Board, and is currently Chairman of RURAL Council, Governor of the Royal Agricultural College, and Member of the Agriculture, Horticulture and Forestry Foresight Panel. He was made a Knight Bachelor in the Queen's Birthday Honours List in 1999 for his wide-ranging contributions to agriculture and agricultural research. He was appointed to the Governing Body of SCRI in 1998.

**Professor A.R. Slabas, B.Sc., D.Phil.**, is Head of Plant Molecular Biology Research in the Department of Biological Sciences, University of Durham, where he leads a team involved in various aspects of lipid metabolism ranging from novel gene identification to structural studies. His more recent interests are in proteomics and the plant cell wall. He has extensive collaboration with Industry, including Biogemma, Zeneca, Linnaeus and Unilever. He has served as a panel member of the UK Technology Foresight Programme 'Crops for Food and Industrial Use'; the Eukaryotic Cell Link Management Committee; and the BBSRC Inovative Manufacturing Committee. He joined the Governing Body in 1995.

**P. Whitworth** retired from United Biscuits plc as Technical Director, Snacks. During his 38 years with the company, he was associated with all aspects of the development and production of biscuits, potato crisps and savoury snacks. He joined the board of the European Snacks Association (ESA) in 1988, and served as President of the Association from 1994 to 1996. He was a founder Director of ECSA Research Ltd (ERL), which through an ECLAIR funded project sought to improve the quality of crisping potatoes using genetic manipulation. A major part of this work has been carried out at SCRI. Having retired from the board of ERL, he was appointed to the Governing Body of SCRI in 1997 and is now a member of the Chairman's Committee and a Director of MRS.



Director	Professor J.R. Hillman, B.Sc., PhD., D.Sc., F.I.S., C.Biol., F.I.Biol., F.C.M.I., F.I.Hort., F.R.S.A., F.R.S.E. 2,3,4	Band 1
Deputy Director	Professor W. Powell, B.Sc., M.Sc., Ph.D., D.Sc. <sup>5,6,7,20</sup>	Band 2
Company Secretary	D. Watt, L.L.B., C.A.	Band 3
Assistant to Director	T.J.W. Alphey, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 4

#### Theme 1 – Mechanisms and Processes

Theme Co-Ordinator: G.C. Machray, B.Sc., Ph.D.<sup>1</sup> Band 3

#### Programme 1 – Gene Expression (GE)

Programme Leader: J.W.S. Brown, B.Sc., Ph.D. <sup>9</sup>	Band 3 (IMP)
Associate Programme Leader:	
S.A. MacFarlane B.Sc., Ph.D.	Band 4
G.C. Machray, B.Sc., Ph.D. <sup>1</sup>	Band 3
M.A. Mayo, B.Sc., Ph.D. <sup>6</sup>	Band 3 (IMP)
P.F. Palukaitis, B.Sc., Ph.D. <sup>6,12,13,22</sup>	Band 3
H Barker, B.Sc., Ph.D.	Band 4
J.W.S. Forrest, B.Sc., Ph.D.	Band 4
M. Taliansky, Ph.D, DSc. <sup>23</sup>	Band 4
P. Hedley, B.Sc., Ph.D.	Band 5 (SPD)
B. Reavy, B.Sc., Ph.D.	Band 5 (SPD)
C.G. Simpson, B.Sc., Ph.D.	Band 5 (SPD)
S.Millam, B.Sc., Ph.D. <sup>9</sup>	Band 5
T Canto, B.Sc., Ph.D.	Band 6 (PD)
G Thow, B.Sc., Ph.D.	Band 6 (PD)
M.M. Swanson, B.Sc., Ph.D.	Band 6
G. Clark, H.N.C., B.Sc.	Band 7
S.M.S. Dawson, H.C.	Band 7 (P/T)
B. Harrower, H.N.D., B.Sc., M.Sc.	Band 7
K.D. McGeachy, H.N.C.	Band 7
J. Middlefell-Williams, H.N.C.	Band 7
D. Davidson	Band 8 (P/T)

G.L. Fraser
J. Morris, H.N.D., B.Sc.
J.D. Fuller
DNA Sequencing / Genotyping Facility
C. Booth, B.Sc.
Media Kitchen
W. Ridley
E. Warden, O.N.C.
W. Burry
M Burton
J. McMillan
Administrators
E.L. Stewart
F. Watt
Leverhulme
S.H. Kim, B.Sc., Ph.D.
EU
J.S. Miller, B.Sc., Ph.D.
K. Harper, B.Sc., Ph.D.
M. Liney

#### Band 8 (P/T) Band 8 Band 9

Band 7

Band 7 Band 9 Band 11 (HELM) Band 11 (P/T) Band 11 (P/T) (HELM)

Band 8

Band 6 (PD)

Band 6 (PD) Band 7 (P/T) Band 7 (P/T)

#### Programme 2 – Cell-to-Cell Communications (CCC)

Programme Leader: K.J. Oparka, B.Sc., Ph.D. <sup>6</sup>	Band 3 (IMP)	F. Carr	Band 8
Associate Programme Leader: A.G. Roberts, B.Sc., Ph.D. <sup>9</sup>	Band 6 (PD)	K. Hrubikova, B.Sc. Administrators	Band 8
I.M. Roberts, H.N.C. Dip.R.M.S.	Band 4	E.L. Stewart	Band 8
P.Boevink, B.Sc., Ph.D. G.H. Duncan, H.N.C.	Band 5 (SPD) Band 5	F. Watt SEERAD FF	-
C. Lacomme, B.Sc., Ph.D. K.M. Wright, M.A., Ph.D.	Band 5 (SPD) Band 6 (PD)	K.M. Nurkiyanova, M.Sc., Ph.D. J. Moir,	Band 6 (PD) Band 7

#### Programme 3 – Plant-Pathogen Interactions (PPI)

Programme Leader: L. Torrance, B.Sc., Ph.D.9	Band 4	Administrators	
Associate Programme Leader: P.R.J. Birch, B.Sc., Ph.D.	Band 5 (SPD)	M. Murray F. Watt	Band 8
G.D. Lyon, B.Sc., M.Sc., Ph.D., D.I.C. <sup>9</sup> M.S. Phillips, B.Sc. B. Williamson, B.Sc., M.Sc., Ph.D., D.Sc. <sup>2</sup>	Band 4 Band 4 Band 4	S. Whisson, B.Sc., Ph.D.	Band 5 (SPD)
V. Blok, B.Sc., M.Sc., Ph.D. J.T. Jones, B.Sc., Ph.D.	Band 5 (SPD) Band 5 (SPD)	<b>SEERAD FF</b> M. Armstrong, B.Sc., Ph.D.	Band 6 (PD)
A. Kumar, B.Sc., Ph.D. I.K. Toth, B.Sc., Ph.D. <sup>15</sup>	Band 5 (SPD) Band 5 (SPD)	A. Avrova, B.Sc., PhD. K. Bell, B.Sc., Ph.D.	Band 6 (PD) Band 6 (PD)
A. Ziegler, B.Sc., Ph.D. L.J. Hyman, B.A., M.Sc.	Band 5 (SPD) Band 6	L. Castelli, B.Sc., M.Sc. EU	Band 7
G.H. Cowan, H.N.C., M.Sc. J. Heilbronn, H.N.C., B.Sc.	Band 7 Band 7	B. Banks, B.Sc., Ph.D. J. Wishart, B.Sc., Ph.D.	Band 6 (PD) Band 6 (PD)
A. Smith, B.Sc. A.M. Holt A.J. Paterson, H.N.D.	Band 7 (P/T) Band 8 (P/T) Band 8 (P/T)	G. Ellis, B.Sc. H. Grant, B.Sc.	Band 7 (P/T) Band 7 Band 8
A.J. Falcison, n.N.D.	Daliu o $(r/1)$	J.A. Stewart, H.N.D., B.Sc.	Dana 8

- <sup>1</sup> Visiting Professor in the University of Strathclyde

- <sup>2</sup> Visiting Professor in the University of Dundee <sup>3</sup> Visiting Professor in the University of Edinburgh <sup>4</sup> Visiting Professor in the University of Glasgow <sup>5</sup> Honorary Senior Lecturer in the University of St. Andrews
- <sup>6</sup> Honorary Senior Lecturer in the University of Our Andal
   <sup>6</sup> Honorary Senior Lecturer in the University of Dundee
   <sup>7</sup> Honorary Professor, Oregon State University
- <sup>8</sup> Professor, Universities of Cordoba and Malaga
- 9 Honorary Lecturer in the University of Dundee <sup>10</sup> Honorary Lecturer in the University of Glasgow
   <sup>11</sup> Associate Professor, University of Parma
   <sup>12</sup> Adjunct Professor, Cornell University
- <sup>12</sup> Adjunct Professor, Corneil University
   <sup>13</sup> Visiting Professor, Agricultural University of Athens
   <sup>14</sup> Visiting Professor, University of Zhejang, China
   <sup>15</sup> Honorary Lecturer in the University of Aberdeen
   <sup>16</sup> Honorary Research Fellow in the University of Dundee

- 17 Honorary Professor of Botany, Florida International University
- <sup>47</sup> Honorary Professor of Botany, Florida International Univ <sup>18</sup> Honorary Fellow in the University of Edinburgh
   <sup>19</sup> Honorary Lecturer in the University of Strathclyde
   <sup>20</sup> Honorary Professor, Herior-Watt University, Edinburgh
   <sup>21</sup> Visiting Professor, University of Naples, Italy
   <sup>22</sup> Honorary Professor, Seoul Women's University
   <sup>23</sup> Adjunct Professor, Moscow State University

#### Theme 2 – Genes to Products

Theme Co-Ordinator: H.V. Davies, B.Sc., Ph.D., C.Biol., F.I.Biol<sup>6,8</sup> Band 3

#### Programme 4 – Quality, Health and Nutrition (QHN)

Programme Leader: H.V. Davies, B.Sc., Ph.D., C.Biol., F.I.Biol <sup>6,8</sup>	Band 3	C. Torrie	Band 11 (P/T) (HELM)
Associate Programme Leader:		Administrator	P 10
R. Viola, B.Sc., Ph.D. <sup>11,21</sup>	Band 4	E.L. Stewart	Band 8
D.W. Griffiths, M.A., Ph.D., C.Chem., M.R.S.C.	Band 4	SEERAD FF	D 17
G.J. McDougall, B.Sc., Ph.D.	Band 4(Prom. Jul 01)	A. Blake, B.Sc.	Band 7
D. Stewart, B.Sc., Ph.D.	Band 4	DEFRA	
M.A. Taylor, B.Sc., Ph.D. <sup>10</sup>	Band 4	R.D. Hancock, B.Sc., Ph.D.	Band 6 (PD)
N. Deighton, B.Sc., Ph.D., C.Chem., M.R.S.C.	Band 4	C. Jorna	Band 10
G. Dobson, B.Sc., Ph.D.	Band 4	FSA	
B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C.	Band 4	M. Kutuzov, B.Sc., Ph.D.	Band 6 (PD)
J.S. Swanston, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 5 (SPD)	P. Dibdin, B.Sc.	Band 8
S. Glidewell, M.A., Ms.C., Ph.D.	Band 6 (PD)	P. Neave	Band 10
H.A. Ross, H.N.C., Ph.D., C.Biol., M.I.Biol.	Band 6 (PD)	EU	
T. Shepherd, B.Sc., Ph.D.	Band 6 (PD)	P. Baumgartner, B.Sc., M.Sc.	Band 6 (PD)
R.A. Marshall, B.Sc., Ph.D.	Band 7	O. Faivre-Rampant, B.Sc., Ph.D.	Band 6 (PD)
W.L. Morris, B.Sc.	Band 7	E. Pascual, B.Sc., Ph.D.	Band 6 (PD)
J.A. Sungurtas, H.N.D.	Band 7	M.L. Ruiz Del Castillo, B.Sc., Ph.D.	Band 6 (PD)
S.R. Verrall, H.N.C.	Band 7	L.V.T. Shepherd, B.Sc., M.Sc., Ph.D.	Band 6 (PT)
F. Falconer, H.N.C.	Band 8	P. Fordyce	Band 10
D. McRae, O.N.C.	Band 8	Zenecca	
P. Dobson	Band 10 (P/T)	P.P.M. Iannetta, B.Sc., Ph.D.	Band 11
J.F. Wilkie,	Band 10		

#### Programme 5 – Genome Dynamics (GD)

Associate Programme Leader:           J.E. Bradshaw, M.A., M.Sc., Ph.D.         Bassociate Programme Leader:           J.E. Bradshaw, M.A., M.Sc., Ph.D., D.Sc. <sup>5,6,7,20</sup> Bassociate Programme Leader:           W. Powell, B.Sc., M.Sc., C.Biol., D.Sc. <sup>5,6,7,20</sup> Bassociate Programme Leader:           G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol. <sup>6</sup> Bassociate Programme Leader:           R.M. Brennan, B.Sc., Ph.D.         Bassociate Programme Leader:           M.F.B. Dale, B.Sc., Ph.D.         Bassociate Programme Leader:           R.P. Ellis, B.Sc., Ph.D.         Bassociate Programme Leader:           J. Graham, B.Sc., Ph.D.         Bassociate Programme Leader:           G. Bryan, B.Sc., Ph.D.         Bassociate Programme Leader:           G. Ramsay, B.Sc., Ph.D.         Bassociate Programme Leader:           J. Russell, B.Sc., Ph.D.         Bassociate Programme Leader:           J. Russell, B.Sc., Ph.D.         Bassociate Programme Leader:           J. Russell, B.Sc., Ph.D.         Bassociate Programme Leader:           J. Caldwell, B.A.         Bassociate Programme Leader:           J. Lyoon         Bassociate Programme Leader:           M. Maccallum, B.Sc.         Bassociate Programme Bassociate Programe Bassociate Programme Bassociate Programe Bassoci	and 3 (IMP)(Prom. Jul 01) and 4 and 2 and 3 and 4 and 4 and 4 and 4 and 4 and 4 and 4 and 4 and 4 and 5 (SPD) and 5 (SPD) and 5 (SPD) and 5 (SPD) and 6 (PD) and 7 and 8 and 8	R. Keith P.E. Lawrence H. Matthews S. Mudie K. Smith, Dip.H.E. A.M.S. McInroy M. Myles, O.N.C. S.J. Neilson, Dip.Biol.Sci. G. Wilde Administrators S. Forsyth C. Goldmann, M.A. SEERAD FF A. Purvis, B.Sc., Ph.D. C. Thompson, B.Sc., Ph.D. C. Thompson, B.Sc., Ph.D. H. Liu, M.Sc. S. Williamson, B.Sc. S.F. Blackie, B.Sc. J.N. Anderson, B.Sc. F. Blackie, B.Sc. J.N. Anderson, B.Sc. <b>EU</b> I. Tierney, B.Sc., M.Sc. J. Brown G. Dargie <b>FSA</b> A. Ibrahim, B.Sc., Ph.D. <b>Glaxo SmithKline</b> L. Jorgensen, H.N.D.	Band 8 Band 8 Band 8 Band 8 Band 9 Band 9 Band 9 Band 9 Band 9 Band 9 Band 8 Band 8 Band 8 (P/T) Band 6 (PD) Band 6 (PD) Band 7 Band 7 Band 7 Band 7 Band 7 Band 8 Band 9 Band 6 Band 11 Band 5 (SPD) Band 5 (SPD)
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#### Theme 3 – Management of Genes and Organisms in the Environment

Theme Co-Ordinator – G.R. Squire, B.A., Ph.D. Band 3

#### Programme 6 - Ecosystem Management and Biotechnology (EMB)

Programme Leader: G.R. Squire, B.A., Ph.D.	Band 3
Associate Programme Leader:	
D.L. Trudgill, B.Sc., Ph.D., C.Biol., F.I.Biol., F.S.O.N. <sup>5</sup>	Band 3
D.K.L. MacKerron, B.Sc., Ph.D.	Band 4
C. Hawes, B.Sc., Ph.D.	Band 6 (PD)
G. Wright, H.N.C.	Band 7
Administrator	
S. Inglis	Band 8
SEERAD FF	
G.S. Begg, B.Sc., Ph.D.	Band 6 (PD)

J. Hillier, B.Sc., Ph.D. S.N. Humphris, B.Sc. J. McCluskey, B.Sc. P. Torr, B.Sc. **DETR** M. Yourng, H.N.D., M.Sc., Pg.Dip.I.T. A. Cocker, B.Sc. J. Reay, B.Sc. G. Banks, M.Sc.

DEFRA

Band 6 (PD) Band 10 Band 10 Band 10

Band 6 (PD) Band 7 Band 7 Band 7

#### Programme 7 – Plant-Soil Interface (PSI)

#### Associate Programme Leader: A.G. Bengough, B.Sc., Ph.D.

B.Boag, B.Sc., Ph.D.
B.S. Griffiths, B.Sc., Ph.D.
L.L. Handley, B.A., B.Ed., M.Sc., Ph.D.
L.L. Wheatley, B.Sc., Ph.D.
T. Daniell, B.Sc., Ph.D.
P.D. Hallett, B.Sc., Ph.D.
C.M. Scrimgeour, B.Sc., Ph.D.
C.M. Scrimgeour, B.Sc., Ph.D.
C.Gordon, H.N.C.
W.M. Stein, H.N.C., B.Sc.
S. Caul, H.N.C.
K. Harris, B.Sc.

Band 4(Prom. Jul 01) Band 4 Band 4 Band 4 Band 5 Band 5 (SPD) Band 5 (SPD) Band 5 (SPD) Band 6 Band 6 Band 7 Band 8 Administrator S. Inglis SEERAD FF L. Deeks, B.Sc., Ph.D. X. Zhang, B.Sc., Ph.D. J. Squires, B.Sc., Ph.D. DTI LINK N. Nunan, B.Sc., Ph.D. K. Wu, B.Sc., Ph.D. BBSRC V. Stubbs, B.Sc., Ph.D. K. Zhang, B.Sc., Ph.D. EU C. Fernie, B.Sc.

Band 8

#### Band 6 (PD) Band 6 (PD) Band 7

Band 6 (PD) Band 6 (PD)

Band 6 (PD) Band 6 (PD)

Band 6

#### Programme 8 – Host-Parasite Co-Evolution (HPC)

Programme Leader: J.M. Duncan, B.Sc., Ph.D. <sup>6</sup>	Band 3		
Associate Programme Leader:	Band 4	G. Malloch, D.C.R., B.Sc., Ph.D.	Band 7
A.C. Newton, B.Sc., Ph.D.		D.C. Guy, H.N.D.	Band 7
<ul> <li>A.T. Jones, B.Sc., Ph.D.<sup>6</sup></li> <li>A.N.E. Birch, B.Sc., Ph.D., C.Biol., M.I.Biol., F.R.E.S.</li> <li>D.J.F. Brown, B.A., Ph.D., D.Sc., C.Biol., F.I.Biol., F.R.</li> <li>D.J. Robinson, M.A., Ph.D., P.S., C.Biol., F.I.Biol., F.R.</li> <li>D.J. Robinson, M.A., Ph.D., P.I.<sup>4</sup></li> <li>J.A.T. Woodford, B.A., M.A., Ph.D., F.R.E.S.<sup>9</sup></li> <li>B. Fenton, B.Sc., Ph.D., C.Biol, M.I.Biol.</li> <li>D.E.L. Cooke, B.Sc., Ph.D.</li> <li>A.K. Lees, B.Sc., Ph.D.</li> <li>OII</li> <li>S.C. Gordon, H.N.C.</li> <li>R. Neilson, H.N.C, M.Sc., Ph.D.</li> <li>H.E. Stewart, C.Biol, M.I.Biol.</li> <li>R. Lowe</li> <li>R.M. Solomon-Blackburn, B.A., M.Sc.</li> <li>W.J. McGavin, B.Sc.</li> </ul>	Band 4 Band 4 Band 5 (SPD) Band 5 (SPD) Band 5 (SPD) (Prom. Jul Band 5 Band 6 (PD) Band 6 Band 6 Band 7	S.S. Lamond L. Sullivan, B.Sc. Administrators M. Murray F. Watt SEERAD FF M.R. MacLeod, B.Sc., Ph.D. V. Young, B.Sc. J.A. Sinclair, M.Sc. SEERAD / BPC P. Van de Graaf, B.Sc., M.Sc., Ph.D. DEFRA D. Cullen, B.Sc., Ph.D. Y. Pitkin, B.Tec, HND.	Band 7 Band 8 Band 8 - Band 6 (PD) Band 7 Band 8 Band 6 (PD) Band 6 (PD) Band 6 (PD) Band 9 (P/T)
A. Dolan, H.N.C.	Band 7 (P/T)	<b>BPC</b>	Band 7
N.A. Williams, H.N.C.	Band 7	M. Elliott, B.Sc.	

#### Programme 9 – Computational Biology (CB)

Programme Leader: D.F. Marshall, B.Sc., Ph.D.	Band 4	J. Liu, B.Sc., M.Sc., Ph.D.	Band 5 (SPD)
Associate Programme Leader:	Band 4	L. Cardle, B.Sc., Ph.D.	Band 6 (PD)
B. Marshall, B.Sc., A.R.C.S. Ph.D. <sup>16</sup>		P.D. Shaw, M.Sc.	Band 7



#### **Division of Finance and Administration**

Head: J.D. Watt, L.L.B., C.A.

Band 3

#### Unit of Finance and Human Resources (FHR)

Financial Controller: N.G. Hattersley,	Band 4	P.C. Duridari	D10
Assistant Secretary: D.L. Hood, B.Admin., Dip.Ed., L.T.I., A.I.I.M.	Band 6	P. Duncan	Band 8 Band 8 Band 8
Personnel Officer: I. Paxton, H.N.C., M.Sc., M.I.P.D.	Band 6		Band 8
Director's Secretary: A. Pack	Band 7	B.V. Gunn	Band 8
C. Skelly	Band 7	S. Phillip, B.A.	Band 8
D.L. Beharrie, Dip.Ed.	Band 8	L Fiddes	Band 10
S. Bell	Band 8	J.Keith	Band 10

#### Unit of Scientific Liaison and Information Services (SLIS)

Head: W.H. Macfarlane Smith, B.Sc., Ph.D., C.Biol., M.I.Biol., F.I.Mgt. Deputy Head: T.D. Heilbronn, B.Sc., M.Sc.	Band 4 Band 6	·····	Band 6 Band 6
I. Kelly, B.Sc., Dip. T.P. M.R.T.P.I <sup>9</sup> . T.G. Geoghegan, A.B.I.P.P., A.M.P.A. I.E. Geoghegan, M.Sc.	Band 4 Band 5 Band 6 (P/T)	U.M. McKean, M.A., Dip.Lib. S.F. Malecki, A.B.I.P.P. <b>Safety Coordinator:</b> M.J. De,Maine, B.Sc., M.Phil.	Band 7 Band 7 Band 5

#### Unit of Information Technology (IT)

Head: B. Marshall, B.Sc., A.R.C.S., Ph.D. <sup>16</sup>	Band 4	L.H. Davidson, B.A.	Band 7
Operations Manager: S. Clark, H.N.C., M.Sc.	Band 5	L.A. McGregor, B.Sc.	Band 8
P. Smith, B.Sc.	Band 6		

#### Unit of Engineering and Maintenance (EM)

0 0	. ,		
Head: S. Petrie, B.Sc.	Band 4	W. Scott	Band 9
D. Gray, H.N.C.	Band 6	C. Conejo	Band 10
A. Low	Band 7	D.J. Redford	Band 10
I.C. McNaughton, H.N.C.	Band 7	J. Rowe	Band 10
K.A. Henry	Band 8	M.J. Soutar	Band 10
R.D. McLean	Band 8	J. Lawrence	Band 11 (P/T)
G.C. Roberts	Band 8	E. Millar	Band11 (P/T)
R. White	Band 8	F. Mitchell	Band 11 (P/T)
J. Anderson	Band 9	W. Pollock	Band 11 (P/T)
D. Byrne	Band 9	G. Pugh	Band 11 (P/T)
J.Flight	Band 9	V. Tait	Band 11 (P/T)
E. Lawrence	Band 9	Administrator	
C.G. Milne	Band 9	W.A. Patterson, H.N.D.	Band 8
D.L.K. Robertson	Band 9		Durid U

#### Estate, Glasshouse and Field Research (EGFR)

Head: G. Wood, B.Sc., Ph.D., F.E.T.C.	Band 4		
Glasshouse Manager: P.A. Gill, H.N.D., N.E.B.O.S.H.	Band 5	I. Fleming	Band 10
G.R. Pitkin, H.N.D.	Band 6	A.C. Fuller	Band 10
J.R.K. Bennett	Band 7	M. Grassie, H.N.C., B.Ed.	Band 10
E. Caldwell	Band 7	D.J. Harkins	Band 10
W.D.J. Jack, B.Sc.	Band 7	P. Heffell, O.N.C.	Band 10
D.S. Petrie	Band 7	J. Mason	Band 10
A.W. Mills	Band 8	T.A. Mason, N.E.B.S.M.	Band 10
R. Ogg	Band 8	A.D. Munro, H.N.D.	Band 10
G Pugh	Band 8	J.K. Wilde	Band 10
A.M. Thain, H.N.C.	Band 8	J. Abernethy	Band 11 (P/T) (HELM)
J.T. Bennett	Band 9	J-M. Ford	Band 11 (P/T)
A. Dobson, H.N.C., H.N.D.	Band 9	T. Mundt	Band 11
B. Fleming	Band 9	M. Torrie	Band 11 (P/T) (HELM)
D.I. Matthew, B.Sc.	Band 9	Administrator	
P. Baird	Band 10	W.A. Patterson, H.N.D.	Band 8
C. Cuthill, N.C.	Band 10		



#### Biomathematics and Statistics Scotland (BioSS)

King's Buildings, University of Edinburgh	
Director: R.A. Kempton, M.A., B.Phil., C.Stat, FRSE.14	<sup>8</sup> Band 3
C.A. Glasbey,	
M.A., Dip. Math. Stats., Ph.D., D.Sc., M.I.S.I. <sup>18,19</sup>	Band 3 (IMP)
E.A. Hunter, B.Sc., M.Phil. <sup>18</sup>	Band 4
D. Husmeier, B.Sc., Ph.D.	Band 5 (SPD)
I.J. McKendrick, B.Sc., Ph.D.	Band 5 (SPD) (P/T)
G.R. Marion, B.Sc., M.Sc., Ph.D.	Band 5 (SPD)
J. Sales, B.Sc., M.Sc.	Band 5 (SPD)
J.M. Dickson, B.Sc.	Band 5 (P/T)
A.M. Roberts, B.Sc., M.Sc.	Band 5
D.J. Allcroft, B.Sc., M.Sc., Ph.D.	Band 6 (PD)
K.M. MacKenzie, B.Sc., M.Sc., Ph.D.	Band 6 (PD)
A.D. Mann, B.Sc.	Band 6
J.C. Wood, B.Sc.	Band 6
M.A.M. Kirkwood, D.A.	Band 7
Administration Officer: E.M. Heyburn, M.A.	Band 7
J. Clabby	Band 8 (P/T)
D. Glancy	Band 10 (P/T)
A.G. Stewart	Band 10 (P/T)

West of Scotland Unit, Hannah Research Institute Head: S. Brocklehurst, B.Sc., Ph.D. I.M. Nevison, M.A.

Aberdeen Unit, Rowett Research Institute Head: G.W. Horgan, B.A. M.Sc., Ph.D. C-D. Mayer, M.Sc., Ph.D. G. Zuur, M.Sc., Ph.D.

 Environmental Modelling Unit, The Macaulay Institute

 Head: D.A. Elston, B.A., M.Sc., Ph.D.

 M.J. Brewer, B.Sc., Ph.D.

 D.M. Walker, B.Sc., M.Sc., Ph.D.

 J.M. Potts, B.Sc., M.Sc., Ph.D.

 E.I. Duff, B.Sc.

Dundee Unit, Scottish Crop Research Institute Head: J.W. McNicol, B.Sc., M.Sc. C.A. Hackett, B.A. Dip. Math. Stats., Ph.D. K. MacKenzie, B.Sc., Ph.D. F.G. Wright, B.Sc., M.Sc., Ph.D. Band 5 (SPD) Band 6 (PD)

Band 4 Band 5 (SPD) Band 6 (PD) (P/T)

Band 4 Band 5 (SPD) Band 5 (SPD) Band 6 (PD) Band 6

Band 4 Band 4 (Prom. Jul 01) Band 6 (PD) Band 5 (SPD)

#### Mylnefield Research Services (MRS)

Managing Director: N.W. Kerby, B.Sc., Ph.D., C.Biol., F.I. Biol. Commercial Manager: J.B. Snape, M.A., M.Sc., Ph.D., C.Biol., M.I.Biol. Administrative Executive Officer: A. Ross, H.N.C., C.P.P. Administrative Assistant: L. Beaton, H.N.C., D.M.S. Personal Secretary/Administrative Assistant: H. Wilson.

E. Campbell, M.Sc. S. Chapman, B.Sc., Ph.D. D. Coyle. N. Escobar, B.Sc., Ph.D. J.E. Fairlie, O.N.C., B.Sc. T. Gillespie, B.Sc. S. Haupt, Dip. Biol. I. Hein, M.Sc. S.N. Jennings, B.Sc. M. Jones, B.Sc., M.Sc., Ph.D. S. Mitchell, B.Sc. R. Razzo, C.M. Reid, B.Sc. V-M. Rokka, Ph.D. S. Rowbottom, O.N.C., H.N.C. L. Smolenska, B.Sc. R. Toth, B.Sc., Ph.D. T. Valentine, B.Sc., Ph.D.

### Resignations

Name	Unit	Band	Month
A. Blair	VS	8	May 01
L.B. Broadfoot	BioSS	6	March 02
I.M. Carey	BioSS	6 PD	September 01
P. Davie	GEN	8	April 01
M. Del Castillo	QHN	6 PD	March 02
I.F. Harrington	FHR	4	August 01
M. Maule	SPD	6 PD	December 01
D. Milbourne	GEN	6 PD	December 01
A. Prior	PPI	7	February 02
K. Ritz	SPD	4	January 02

## **Staff Retirements**

Name	Unit	Band	Month
D. Pugh	EGFR	7	August 01
R.J. McNicol	AG	3	May 01

## Honorary Research Professors Honorary Research Fellows

Professor P. Broda, M.A., M.Sc., Ph.D., D.Sc., Hon.D.Sc.

Professor M.C.R. Davies, BSc., Dip. Theol., M.Phil., Ph.D., C.Eng., M.I.C.E. F.T.G. Professor G.M. Gadd, B.Sc., Ph.D., D.Sc., F.I. Biol. Professor J. Raven, Ph.D., F.R.S.E., F.R.S.

A. Blackwell, B.Sc., Ph.D., M.R.C.V.S.

F. Bransby, B.A., M.A., Ph.D.

- J. Brown, B.Sc., Ph.D. W. Christie, M.B.E., B.Sc., Ph.D., D.Sc., C.Chem., F.R.S.E., F.R.S.C. J. McColl, M.B.E., S.H.M., N.D.H., S.D.H.
- I.M. Morrison, B.Sc., Ph.D.
- T. Newson, B.Sc., Ph.D.

W. Robertson., H.N.C., F.L.S

N. White, B.Sc., Ph.D., C.Biol., M.I.Biol.

### Postgraduate Students

Name	Unit	Subject
H. Al-Meniae	GD	Salt tolerance in barley in Kuwait.
M.A.Y.Akhond	GE	Gene targeting in crop plants.
G. Banks	EMB	Spatio-temporal variations in potential nitrification rates in soils under a barley ( <i>Hordeum vulgare L.</i> ) crop.
S. Bierman	BioSS	Spatio-temporal models in ecology
I. Krishnarajah	BioSS	Analytic approximations to non-linear stochastic models in epidemic processes
K. Binnie	PSI	Visualisation of interactions between soil physical conditions and fungi.
Q. Chen	PPI	Protein based approaches to the study of <i>Xiphinema</i> nematodes.
J. Bolandandam	GE	Study of resistance mechanisms in potato leafroll virus in diploid and tetraploid potato.
K. Boutsika	HPC	Molecular identification and phylogenies of virus and non-virus vectors trichidorid nematodes.
K. S. Caldwell	GD	Grain hardness: linkage disequilibrium in barley.
L. Castelli	PPI	Novel sources of resistance to potato cyst nematodes (Globodera rostochiensis & G.pallida) in wild potato species
		held in the Commonwealth Potato Collection.
C. Furlanetto	PPI	Genes encoding oesophageal gland secreted proteins involved in host-parasite and/or nematode-virus interactions
		of Xiphinema index.
E. Gilroy	PPI	The role of plant defence genes in the hypersensitive response.
C.M. Goncalves De Oliveira	HPC	Development of polymerase chain reaction diagnostics (PCRDs) for Xiphinema species (Nematoda: Longidoridae)
		occurring in Brazil.
J. Heilbronn	PPI	Characterisation of signalling genes induced by <i>erwinia</i> in potato.
I. Hein	PPI	Discovering resistance pathways in response to elicitors and pathogen attack.
M. Holeva	PPI	A study of the pathogenicity and molecular biolgy of <i>Erwinia carotovora</i> subsp. <i>Atroseptica</i> .
R. Holeva	HPC	Molecular diagnostics of trichodorid nematodes and tobacco rattle virus.
S. Humphris	PSI	Biological control as part of an environmentally friendly future for the eradication of dry rot from buildings.
S. Hussain	HPC	Diagnostics and epidemiology of <i>phytophthora</i> .
E. Isidore	GD	Construction and application of a multifunctional ultra-dense genetic map of potato.
V. Ivandic	GD	Simple sequence repeats in relation to adaptation in wild barley.
H. L Kuan	PSI	What is the link between microbial diversity and soil resilience?
H.Liu	GD	Molecular analysis of grain development in barley.
H. McGovern	PSI	A national soil vulnerability based framework for provision of farm-specific guidance on the management of soil
		structure.
L.E. MacKinnon	GE	Transformation of Cannabis <i>sativa</i> L.: a multipurpose crop.
E. Pachepsky	PSI	Modelling phenotypic interaction in species-rich grassland.
B. Pande	GD	The genetic analysis of traits of economic importance in the principal cultivated potato <i>Solanum tuberosum</i> spp.
K. Stamati	GD	Biodiversity of sub-artic willow.
K.Stewart	HPC	Breakdown of Mlo resistance under stress.
N. Taleb	HPC	Determination of the molecular basis of genetic resistance to <i>Phytophthora infestans</i> in potato.

## Short-Term Workers and Visitors

Name	Country of origin	Programme	Month/yr of arrival	Length of stay
				,
D. Allan	UK	GD	Jul 01	3 months
R. Bacon	USA	GD	Sep 01	6 months
J. Beaton	UK	EMB	Apr 01	8 months
K. Beaton	UK	EMB	Jul 01	3 months
M. Berodier	France	EMB	Jun 01	2 months
A. Blair	UK	EMB	Apr 01	1 month
O. Borras-Hidalgo	Cuba	PPI	Oct 01	6 months
J. Brown	UK	PSI	Jan 02	6 weeks
A. Campbell	UK	EMB	Jun 01	5 months
Y. Chen	UK	PSI	Dec 00	4 months
Y. Choeng	Malasia	GD	Jan 02	1 month
S. Clark	UK	GD	Jan 01	4 months
C. Cl?che	France	EMB	Jun 01	2 months
A. Complainville	France	CCC	Nov 01	6 weeks
J. Copeland	UK	HPC	Jun 01	4 months
C. Daume	Germany	PSI	Feb 01	6 months
D. Davies	UK	HPC	Jun 01	2 months
E. Dekkers	Netherlands	PPI	Jan 02	1 month
P. Dibdin	UK	EMB	Jun 01	3 months
H. El-Meniae	Kuwait	GD	Jul 01	2 months
I. Ember	Hungary	HPC	Oct 01	3 weeks
T. Frenzel	Germany	QHN	Apr 02	6 weeks
J. Gardiner	UK	EMB	Apr 01	5 months
J. Gustafsson	Sweden	BioSS	Jan 02	2 weeks
S. Hameed	Pakistan	CCC	Jun 01	3 months
N. Kalinina	Russia	HPC/GE	Jul 01	6 months
M. Kimber	UK	PPI	Jun 01	2 weeks
M. Kvarnstrom	Sweden	BioSS	Oct 01	2 weeks
A. Lund	USA	GD	Jun 01	2 months
A. Lyles	UK	GD	Jul 01	3 months
M. McKinlay	UK	EMB	Apr 01	8 months
C. Macmurran	UK	HPC	Jun 01	4 months
A. Masoudi-Nejad	Iran	GD	Feb 02	1 month
A. Moore	UK	GE	Jul 01	6 weeks
K. Norvez Le-Ny	France	EMB	Jun 01	2 months
K. Nyerges	Hungary	HPC	May 01	1 month
D. O'Halloran	Ireland	PPI	Jan 02	1 month
V. Okrslar	Slovenia	GE	Mar 01	3 weeks
J. Oparka	UK	QHN	May 01	10 months
J. Page	Greece	BioSS	Aug 01	2 weeks
B. Pillay	South Africa	PPI	Aug 01	2 weeks
G. Quirk	UK	GD	Jul 01	3 months
M. Rakhimova	Kazakhstan	PPI	Nov 01	2 months
S. Ranomenjanahary	Madagascar	HPC	Feb 02	6 months
G. Scrim	UK	GE	Apr 01	4 months
R. Shaik	South Africa	PPI	Aug 01	2 months
I. Snowball	UK	PSI	Feb 01	3 months
I. Soutar	UK	EMB	May 01	4 months
R. Stadler	Germany	CCC	Feb 02	2 weeks
K. Stamati	Greece	GE	Jun 01	3 months
S. Stetner Klemsdal	Norway	GD	Aug 01	2 weeks
P. Torr	UK	EMB	Mar 02	8 months
M. Usoltseva	Sweden	HPC	Feb 02	2 weeks
G. Valdivieso	Spain	QHN	Aug 01	3 months
E. Venter	South Africa	PPI	Jul 01	3 months
K. Walton	UK	EMB	Jun 01	3 months
A. Ward	UK	HPC	Jun 01	3 months
J. Zandleven	Netherlands	GE	Jun 01	3 months
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## Editorial Duties

Name	Position	Journal Title
H. Barker	Editor	Annals of Applied Biology
A.G. Bengough	Editor	Annals of Botany
A.N.E. Birch	Editor	IOBC Bulletin for Working Gourp 'Breeding for Resistance to Insects and Mites
M.F.B. Dale	Editor	Annals of Applied Biology
J.M. Duncan	Associate Editor	Journal of Horticultural Science & Biotechnology
S.C. Gordon	Member of Editorial Board	Proceedings of the 4 <sup>th</sup> International Strawberry Symposium Acta Horticulturae (ISHS) No. 567
C.A. Glasbey	Associate Editor	Journal of Royal Statistical Society, Series B
J. Graham	Associate Editor	Journal of Horticultural Science and Biotechnology
B.S. Griffiths	Editorial Board	Pedobiologia
J.R. Hillman	Publication Committee	Journal of Horticultural Science
,	Editorial Board	Agricultural Systems
	Editorial Board	Journal of Agricultural Science
G.W. Horgan	Statistical Editor	British Journal of Nutrition
D. Husmeier	Referee	Bioinformatics and Neural Networks
A.T. Jones	Editor	Annals of Applied Biology – Description of Plant Viruses
D.K.L MacKerron	Associate Editor	Journal of Horticultural Science & Biotechnology
	Member Editorial Board	Euphytica
J.W. McNicol	Statistical Editor	Annals of Applied Biology
B. Marshall	Editor	European Journal of Agronomy
A.C. Newton	Member of Editorial Board	Plant Pathology
	Editor	Cereal Rusts and Powdery Mildews Bulletin
K.J. Oparka	Editor	Plant Physiology
K.J. Oparka	International Advisory Board	Journal of Experimental Botany
	Member	Faculty of 1000
P.F. Palukaitis	Editorial Board	Virology
	Editorial Board	Journal of General Virology
	Editorial Advisory Board	Plant Pathology Journal
W. Powell	Associate Editor	Molecular Ecology
D.J. Robinson	Editorial Board	Journal of Virological Methods
M. Taliansky	Editorial Board	Journal of General Virology
I. Toth	Assistant Editor	Molecular Plant Microbe Interactions
D.L. Trudgill	Advisory Board	European Journal of Plant Pathology
D.L. Hudgin	Editorial Board	Nematology
R. Viola	Book Reviewer	Experimental Agriculture
R. Waugh	Editor	Plant Biotechnology
R. Waugn B. Williamson		
D. WIIIIailisoii	Deputy Chairman Editor	Annals of Applied Biology
E C Which t	Editor Member of Editorial Board	AAB Descriptions of Plant Viruses
F.G. Wright	Member of Editorial Board	Heredity

### Service on External Committees or Organisations

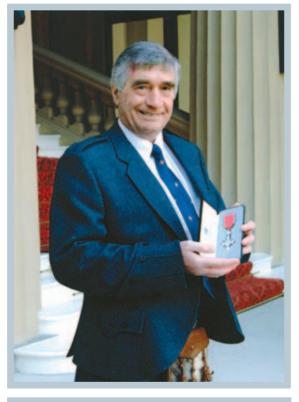
Name	Position	Committee or Organisation
T.J.W. Alphey	Secretary	Committee of Heads of Agricultural and Biological Organisations in Scotland
	Secretary	Scottish Management Advisory Committee
A.G. Bengough	Co-Chair March en of Stearing Course	EC COST 631 Working Group – Modelling Plant-Soil Interactions in the Rhizosphere
A.N.E. Birch	Member of Steering Group Convenor	IOBC Global Working Group on Biosafety Guidelines
	EU Commission	IOBC Working Group on Breeding for Resistance to Insects and Mites Expert evaluator (two panels)
B. Boag	Committee Member	BRISC
J.E. Bradshaw	Chairman	Potato Section, EUCARPIA
,	Committee Member	BBSRC Brassic IGF Steering Committee
R.M. Brennan	Secretary	Organising Committee for 8 <sup>th</sup> International <i>Rubus-Ribes</i> Symposium, July 2001
	Associate Editor	Journal of Horticultural Science & Biotechnology
S. Brocklehurst	Member	HRI Ethical Review Committee
D.E.L. Cooke	Management Committee	EU COST 853 'Agricultural Biomarkers for Array-Technology'
	Chair	International Society of Plant Pathology, Phytophthora Committee
M.F.B. Dale	Member	Association of Applied Biologists – Plant Physiology & Crop Improvement Group
M.J. De,Maine	Member	BBSRC Joint Committee on Health & Safety
LL D	Member	SABRI Safety Officers' Group
J.M. Duncan		GILB (Global Initiative on Late Blight)
R.P. Ellis	Member	BSPB Cereal Crop Group
	Representative of BSPB	SAC Cereal Recommended List Consultative Committee
	Representative of BSPB UK Representative	GENRES CT108 EU Research Project European Co-operative Programme/Plant Genetic Resources
	Chairman	ECP/PGR Barley Working Party
	Co-ordinator	Barley chromosome organisation
D.A. Elston	Member	ITE Biometrics Network
	Member	RRS Highland Local Group Committee
	Member	Statistical and Technical Working Group, UK Environmental Change Network
B.P. Forster	Chairman	COST Action 851
	Co-ordinator	Barley chromosome organisation
C.A. Glasbey	Member	EPSRC Peer Review College
	Chairman	RSS Statistical Image Analysis and Processing study group
	Member	RSS2001 Conference Committee
	Member	Management committee of SAC-BioSS CT Scanning Unit
	Member	Management group of Scottish Centre for Genomic Technology and Informatics
	Member	RAE2001 User Panel for Statistics and OR
C.A. Hackett	Member Member	Conference Advisory Committee for International Biometric Society
C.A. Hackett	Member	Editorial Board, Heredity International Biometric Society (British Region) Committee
P.D. Hallett	Meetings Secretary	Scottish Soils Discussion Group, British Society of Soil Science
J.R. Hillman	Chairman	SCRI/SASA/SAC Liaison Group
,,	Chairman	Tayside Biocentre Group
	Deputy Chairman	Board of Directors, Mylnefield Research Services Ltd
	Member	Board of the Mylnefield Trust
	Member	Board of Mylnefield Holdings Ltd
	Chairman	Committee of Heads of Agricultural and Biological Organisations in Scotland
	President	Agriculture and Food Section, the British Association for the Advancement of Science
	Member	ECRR Board of Management
	Member	SNSA Adviser to Committee
	Member	Court of University of Abertay Dundee and its Audit Committee
	Member	Senate, University of Dundee
	Member Member	University of Strathclyde Sub-Board for the Degree of B.Sc. in Horticulture
	Member	Tayside Economic Forum Perth & Kinross Agricultural Forum
	Member	Board of Directors, BioIndustry Association
	Adviser	International Foundation for Science, Stockholm
	Member	House of Lords Rural Economy Group
	Member	Forum for Representation of Industrial and Environmental Biotechnology Suppliers (Department
		of Trade and Industry)
D.L. Hood	Secretary & Treasurer	Scottish Society for Crop Research
G. W. Horgan	Member	RSS Highland Local Group Committee
E.A. Hunter	Member	RSS local committee
	Member	Editorial Board of Food Quality & Preference
	Member	SAC – Edinburgh, Animal Experiments Committee
	Member	Scientific Committee of the ASU conference, Lille, France, Dec 2001/ Jan 2002
	Member	Inter-departmental Statisticians Group (IDSG)
	Member	Herbage VCU group
A.T. Jones	Member	Scientific & Editorial Committee of ISHS Rubus/Ribes Symposium
J.T. Jones	Elected Member of Council	British Society for Parasitology

Staff

Name	Position	Committee or Organisation
	D	
I Kelly	Director	Dundee Science Centre
	Member Member	Council of the Royal Scottish Geographical Society
R.A. Kempton	Member	Research Committee of the Royal Scottish Geographical Society International Biometric Society Awards Fund Committee
K.A. Kempton	Member	International Biometric Society Awards Fund Committee
	Member	Fisher Memorial Committee
	Member	Steering Group, Biometric Network for Sub-Saharan Africa
	Member	Edinburgh Centre for Rural Research
	Member	Advisory Committee on Forest Research
	Member	Working Group on Risk Assessment for Mixtures of pesticides, Food Standards Agency
A.K. Lees	Member	Board of Directors, British Society of Plant Pathology
G.R. Mackay	President	EUCARPIA – until Oct. 01
,	Chairman	Organising Committee of XVIth Congress of EUCARPIA (Sep.'01)
D.K.L. MacKerron	Secretary	SSCR Potato Crop Sub-committee
	Chairman	Section Physiology, EAPR
U.M. McKean	Joint Chair	Scottish Agricultural Librarians' Group
I.J. McKendrick	Member	The Forest of Bowland Louping III Eradication Project
	Member	MRI Animal Experimentation and Ethics Committee
A.C. Newton	Board Member	European and Mediterranean Cereal Rusts Foundation
	Committee Member	United Kingdom Cereal Pathogen Virulence Survey
	Organising Committee	Crop Protection in Northern Britain
K.J. Oparka	Member	International Organising Committee, Plasmodesma 2001, Cape Town, South Africa
P.F. Palukaitis	Member	ICTV Satellites Study Group
W. Powell	Managing Director	International Triticeae Mapping Initiative Management Office (SCRI)
	Member Member	BBSRC Initiative on Gene Function (IGF) Genetical Society Committee (Quantitative Genetics), British Society of Plant Breeders, Working
	Member	Party on Biotechnology
	Member	Advisory Board for Scottish Informatics Mathematics Biology & Statistics (SIMBIOS) Centre (2001)
	Member	External Review Team for ICARDIA (Syria), IITA (Nigeria), VIB (Belgium) and CIP (Peru)
	External examiner	Genetics (B.Sc.) and M.Sc. at UCW Aberystwyth
	External examiner	Genetics (M.Sc.) at the University of Birmingham
	Committee Member	DEFRA Sustainable Arable Link Programme
G. Ramsay	Member	UK Potato Quarantine Unit review committee
,	Member	UK Plant Genetic Resources Group
A.G. Roberts	Member	Royal Microscopical Society, Cell Biology & Histochemistry Committee
A.M.I. Roberts	Secretary	Statisticians Group, UK Plant Varieties and Seeds Committee
	Member	Technical Working Party on Automation and Computing Programs, International Union for the Protection of Plant Varieties
	Member	Potato VCU group, Plant Varieties and Seeds Committee
	Member	Vegetable DUS group, Plant Varieties and Seeds Committee
	Member	SASA Ethical Review Committee
D.J. Robinson	Member	ICTV Tobamovirus & Tobravirus Study Group
	Member	ICTV Umbravirus Study Group
S.E. Stephens	Joint Chair	Scottish Agricultural Librarians' Group
	Member	Information Services Group – Scottish Library Association Committee
	Member	Tayside and Fife Library and Information Network
	Working Group Chair	British Research Institutes Serials Consortium (BRISC)
	Secretary	Research Councils Library and Information Consortium (RESCOLINC)
	Chair	BBSRC Research Institutes Librarians Committee (BRILCOM)
M. Taliansky	Chairman	Umbravirus Study Group of International Committee on Taxonomy of Viruses
I. Toth	Director Committee Member	British Society for Plant Pathology
	Committee Member	Crop Protection in Northern Britain BSPP President's Meeting
J.D. Watt	Member	Scottish Management Advisory Committee
j	Company Secretary	Mylnefield Research Services (MRS) Ltd.
	Secretary	Mylnefield Trust
	SABRI Representative	BBSRC Joint Negotiating and Consultative Committee
R. Waugh	Board Member	SEERAD Genomics Task Force
R. Walliamson	Treasurer	Association for Crop Protection in Northern Britain
D. WIIIIaiiisofi	Chairman & Treasurer	8 <sup>th</sup> International <i>Rubus &amp; Ribes</i> Symposium
J.A.T. Woodford	Member	Insecticide Resistance Action Group

## Awards and Distinctions

Name	Programme	Degree/Award/Distinction/Appointment	
D.J. Allcroft	BioSS	Ph.D., University of Edinburgh	
N. Aziz	GE	Ph.D., University of Dundee	
G. Banks	PSI	M.Sc., University of Dundee	
B.P. Forster	GD	Chairman of COST Action 851	
E. Isidore	GD	Ph.D., University of Dundee	
J.R.Hillman	Director	Dr Hardie Memorial Prize, VTSC, 2001	
V. Ivandic	GD	Ph.D., University of St Andrews	
L.A. McGregor	IT	B.Sc., Open University	
G.R. Mackay	GD	M.B.E. (July 2001)	
G. Malloch	HPC	Ph.D., University of Dundee	
K. Stanley	HPC	Ph.D., University of Dundee	
M. Taliansky	GE	Adjunct Professor, Moscow State University	
E. Vellios	GE	Ph.D., University of Dundee	
C.J. Zhang	GE	Ph.D., University of Abertay, Dundee	



George Mackay, M.B.E.

## SCRI Research Programme

SEERAD funded research programme showing: SEERAD project number; Title (prefixed ROA for ROAMEd corefunded projects; FF for Flexible Fund projects); Scientific Project Leader. In addition to this list, there are research projects undertaken on behalf of various bodies, including other governmental bodies, commerce and levy boards.

SCR/513/98	ROA Gene expression and manipulation in barley	Machray G C
SCR/525/99	ROA Interactions between the structure of soil habitats and biological processes	Bengough G
SCR/526/99	ROA Integrative mapping of the long arm of barley chromosome 5H	Thomas W T B
SCR/527/99	ROA Development of a graphical database for the visualisation of genotypic and phenotypic data in barley	Marshall D M
SCR/528/99	ROA Use of an accelerated marker assisted selection scheme to introgress novel variation for economically important traits into cultivated barley	Thomas W T B
SCR/533/99	ROA Molecular and genetic studies of the basis of virulence/avirulence in plant parasitic nematodes	Phillips M S
SCR/536/00	ROA Development and application of chemical strategies to facilitate genetic and molecular marker studies of factors affecting quality traits in potatoes	Davies H V
SCR/537/00	ROA Biochemical approaches to define novel targets for the genetic improvement of malting barley	Davies H V
SCR/538/00	ROA Optimising production and biodiversity of arable plants and invertebrates at patch and landscape scales	Squire G
SCR/539/00	ROA Self organisation of plant and canopy architecture in barley and feral brassicas: trade offs between production and defense	Squire G
SCR/540/00	ROA Genetics of cultivated potatoes	Bradshaw J E
SCR/541/00	ROA Genetic approaches to evaluation and utilisation of soft fruit germplasm	Bradshaw J E
SCR/542/00	ROA Consequences of soil biodiversity for the functioning and health of agricultural soils in relation to C cycling dynamics and resilience	Griffiths B S
SCR/544/00	ROA Consequences of soil biological diversity for the functioning and health of agricultural soils in relation to N cycling processes	Ritz K
SCR/545/00	ROA Detection, diversity and epidemiology of important viruses and their vectors in berryfruit crops and strategies for their effective control	Jones A T
SCR/546/00	ROA Development and use of molecular markers to study the epidemiology of late blight ( <i>Phytophthora infestans</i> ) of potato in Scotland	Cooke D
SCR/547/00	ROA Biodiversity in the antioxidant status and composition of Rubus and other soft fruit germplasm	Stewart D
SCR/549/00	ROA Characterisation of molecular interactions between soft rot erwinias and potato	Lyon G D
SCR/550/00	ROA Control of meristematic activity in plants: dormancy in potato tubers as the model system	Viola R

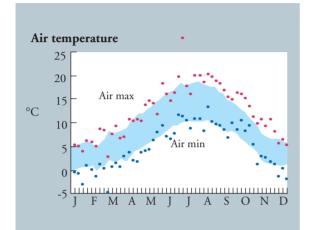
### Research Projects

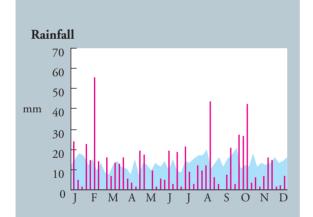
SCR/551/00	ROA Post-transcriptional control of gene function	Brown J W S
SCR/552/00	ROA Barley 'deletion' mutation grid	Waugh R
SCR/553/00	ROA Characterising plant responses to viral infection	Palukaitis P
SCR/554/00	ROA Protein-protein interactions and the role of virus proteins in disease processes	Torrance L
SCR/557/01	Targeted long-distance transport of macromolecules in plants	Oparka K
SCR/558/01	Resistance to potato viruses: exploitation of host gene resistance and transgenic resistance to study resistance mechanisms and to develop resistant germplasm	Barker H
SCR/559/01	Molecular biology of potato leafroll virus: aphid transmission and the establishment of infection in host plants	Mayo M A
SCR/560/01	Molecular bases of resistance and susceptibility in potato and barley	Birch P R J
SCR/561/01	Molecular bases of pathogenicity in potato cyst nematodes, <i>Xiphinema index</i> and <i>Phytophthora infestans</i>	Jones J
SCR/562/01	Genetics of seedling root traits in barley	Forster B
SCR/563/01	Conservation and utilisation of the Commonwealth Potato Collection	Mackay G R
SCR/564/01	A gene map of the interval between GP21 and GP179 on potato linkage group V	Bryan G
SCR/565/01	Identification and characterisation of bacterial artificial chromosome (BAC) clones from gene rich regions of the barley genome	Waugh R
SCR/566/01	Produce and maintain pathogen-tested stocks of Rubus, Ribes and Fragaria germplasm and index for infection material imported into SCRI	Jones A T
SCR/571/01	OC Ecological management and biotechnology	Squire G
SCR/572/01	OC Computational biology	Marshall D F
SCR/573/01	OC Functional analysis of novel genes from potato and barley	Oparka K J
SCR/574/01	OC Development and application of metabolic profiling technologies to enhance the understanding of metabolic and developmental processess in plants	Davies H V
SCR/575/01	OC Enhancing food quality and nutritional value through multidisciplinary approaches which exploit genetic and molecular diversity	Davies H V
SCR/576/01	OC Sequence diversity and horizontal genomics (targeted gene discovery)	Waugh R
SCR/577/01	OC Molecular plant diversity and germplasm resources	Waugh R
SCR/578/01	OC Parallel gene expression technologies supporting the discovery of plant and pathogen genes important to agriculture and biotechnology	Machray G C
SCR/505/97	FF Molecular approaches to manipulate the development and composition of strawberry fruit	Davies H V
SCR/516/97	FF Genetic mapping and molecular cloning of novel sources of resistance to <i>Globodera pallida</i>	Waugh R
SCR/522/98	FF Development of Rubus genotypes with transgenic resistance to raspberry bushy dwarf virus	Jones A T

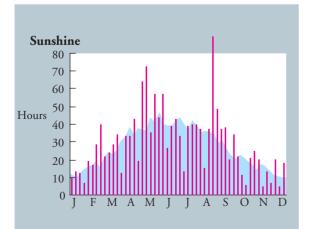
SCR/523/98	FF Investigation of the mechanisms of disease induction and host- specificity in major bacterial and fungal potato pathogens	Birch P R J
SCR/524/98	FF Unravelling the pathways of protein transport in plant and animal cells using virus-based vectors	Oparka K J
SCR/535/99	FF Impacts of a conventional and an organic crop insecticide spray treatment on life history traits of two-spot ladybirds	Birch A N E
SCR/555/00	FF Cereal transcriptome resources	Waugh R
SCR/556/00	FF Comparison of the molecular bases of pathogenicity in the model oomycetes Peronospora parasitica and Phytophthora infestans through a genomics approach	Birch P R J
SCR/567/00	Appraisal of options for aphid monitoring and control to manage virus transmission in Scottish seed potato crops	Woodford J A T
SCR/568/00	Significance and mechanisms of landscape-scale gene flow	Ramsay G
SCR/569/00	Phytophthora diseases of soft fruit: determining their prevalence and the source of new outbreaks in Scotland	Duncan J M
SCR/570/00	Mechanical properties of primary cell walls by micro-stretching in vivo	Bengough A G
SCR/579/01	Development of robust, broad basedQTL maps to improve barley breeding	Thomas W T B
SCR/582/01	Cloning of avirulence genes from the oomycete plant pathogens Peronospora parasitica and Phytophthora infestans	Birch P R J
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. + Phase 2.	Ritz K
SCR/816/95	FF Phenotypic and genotypic bases of population dynamics in heterogeneous, species-rich grassland.	Squire G
SCR/818/95	FF Genetic engineering of crop plants for resistance to insect and nematode pests: effects of transgene expression on animal nutrition and the environment	Jones A T
SCR/823/97	FF Significance of physical heterogeneity for scaling of solute chemistry in soils from fine scale to subcatchment	Bengough G
SCR/824/97	FF Efficacy studies on a plant virus-based expression system and on alternative delivery routes for peptides and proteins with pharmaceutical, therapeutic and related uses for improving animal health, nutrition and welfare	Brown J W S
SCR/832/99	FF Identification and assessment of nutritional relevance of antioxidant compounds from soft fruit species	Davies H V
SCR/833/00	Microsatellites as population genetic markers	Powell W
SCR/834/01	Assessment of plant germplasm for bioactive molecules	Ramsay G
SCR/835/01	Genomic sequencing and proteomic analyses of the potato pathogenErwinia carotovora subsp. Atroseptica (Eca) and the animal pathogen Chlamydophila abortus (Ca)	Toth I
SCR/837/01	Biodiversity: taxonomy, genetics and ecology of Sub-arctic willow Scrub	Russell J

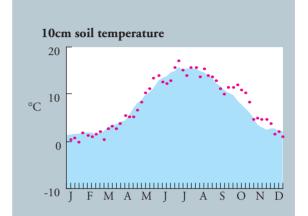
## Meteorological Records

G. Wood Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 2001 and the long term average for 1961-1990(\_\_\_\_\_).

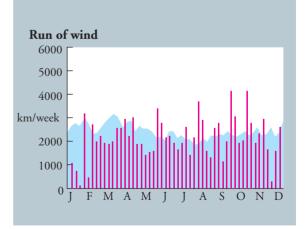












# Institutes supported by the Biotechnology and Biological Sciences Research Council

BBSRC Office **BBSRC Bioscience IT Services** Babraham Institute Laboratory of Molecular Signalling Institute for Animal Health Compton Laboratory Pirbright Laboratory BBSRC & MRC Neuropathogenesis Unit Institute of Arable Crops Research Rothamsted Broom's Barr Institute of Food Research Institute of Grassland and Environmental Research Aberystwyth Research Centre North Wyke Research Station Bronvdd Mawr Research Station Trawsgoed Research Farm Iohn Innes Centre Roslin Institute

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Polaris House, North Star Avenue, Swindon, Wilts SN2 1UH
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01793-413200 01582-714900 01223-496000 01223-496406

01635-578411 01483-232441 0131-667-5204

01582-763133 01284-812200 01603-255000

01970-823000 01837-883500 01874-636480 01974-261615 01603-450000 0131-527-4200 01525-860000

01732-843833 01789-470382

## Scottish Agricultural and **Biological Research Institutes**

Hannah Research Institute The Macaulay Institute Moredun Research Institute Rowett Research Institute Scottish Crop Research Institute Biomathematics and Statistics Scotland (Administered by SCRI) University of Edinburgh, James Clerk Maxwell Building,

#### Ayr, Scotland KA6 5HL

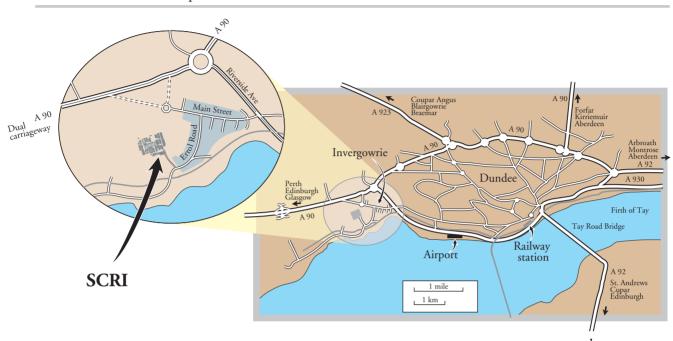
Craigiebuckler, Aberdeen AB9 2QJ Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ Greenburn Road, Bucksburn, Aberdeen AB21 9SB Invergowrie, Dundee DD2 5DA King's Buildings, Mayfield Road, Edinburgh EH9 3JZ

01292-674000 01224-318611 0131-445-5111 01224-712751 01382-562731 0131-650-4900

## List of Abbreviations

AAB	Association of Applied Biologists	IOBC	International Organisation for Biological Control
ACRE	Advisory Committee on Releases to the Environment	IMP	Individual Merit Promotion
ADAS	Agricultural Development and Advisory Service	ISHS	International Society for Horticultural Science
BBSRC	Biotechnology & Biological Sciences Research Council	ISPP	International Society for Plant Pathology
BCPC	British Crop Protection Council	IVEM	Institute of Virology and Environmental Microbiology
BioSS	Biomathematics and Statistics Scotland	MAFF	Ministry of Agriculture Fisheries and Food
BPC	British Potato Council	MLURI	Macaulay Land Use Research Institute (now the Macaulay Institute)
BSPB	British Society of Plant Breeders	MRI	Moredun Research Institute
BTG	British Technology Group	NERC	National Environmental Research Council
CAPS	Cleaved Amplified Polymorphic Sequence	NFT	National Fruit Trials
CEC	Commission of the European Communities	NFU	National Farmers Union
CHABOS	Committee of Heads of Agricultural and Biological	NIR	Near Infra-Red
	Organisations in Scotland	NMR	Nuclear Magnetic Resonance
CIP	International Potato Centre - Peru	NPTC	National Proficiency Test Council
COST	European Co-operation in the field of Scientific and	ORSTOM	Organisation for research in science and technology overseas
	Technical Research	PCR	Polymerase Chain Reaction
DEFRA	Department for Environment, Food and Rural Affairs	PD	Post-doctorate
DfID	Department for International Development	PVRO	Plant Variety Rights Office
EAPR	European Association for Potato Research	RAPD	Randomly Amplified Polymorphic DNA
ECRR	Edinburgh Centre for Rural Research	RFLP	Restriction Fragment Length Polymorphism
ECSA	European Chips and Snacks Association	RNAi	RNA interference
EHF	Experimental Husbandry Farm	RRI	Rowett Research Institute
ELISA	Enzyme linked immunosorbent assay	SABRI	Scottish Agricultural and Biological Research Institutes
EPPO	European Plant Protection Organisation	SAC	Scottish Agricultural College
ESTs	Expressed Sequence Tagged Sites	SASA	Scottish Agricultural Science Agency
FF	Flexible Funding (SEERAD)	SCRI	Scottish Crop Research Institute
FLAIR	Food-Linked Agro-Industrial Research	SEB	Society for Experimental Biology
GILB	Global Initiative on Late Blight	SEERAD	Scottish Executive Environment and Rural Affairs Department
GIUS	Glasshouse Investigational Unit for Scotland	SET	Scottish Enterprise Tayside
H-GCA	Home-Grown Cereals Authority	SNSA	Scottish Nuclear Stocks Association
HDC	Horticultural Development Council	SPD	Senior Post-doctorate
HPLC	High Performance Liquid Chromatography	SSCR	Scottish Society for Crop Research
HRI	Hannah Research Institute	STS	Sequence Tagged Sites
IACR	Institute of Arable Crops Research	UNDP	United Nations Development Programme
ICTV	International Committee for the Taxonomy of Viruses	WHO	World Health Organisation
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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 London City, and scheduled services operate from many domestic and international destinations to Edinburgh and Glasgow.