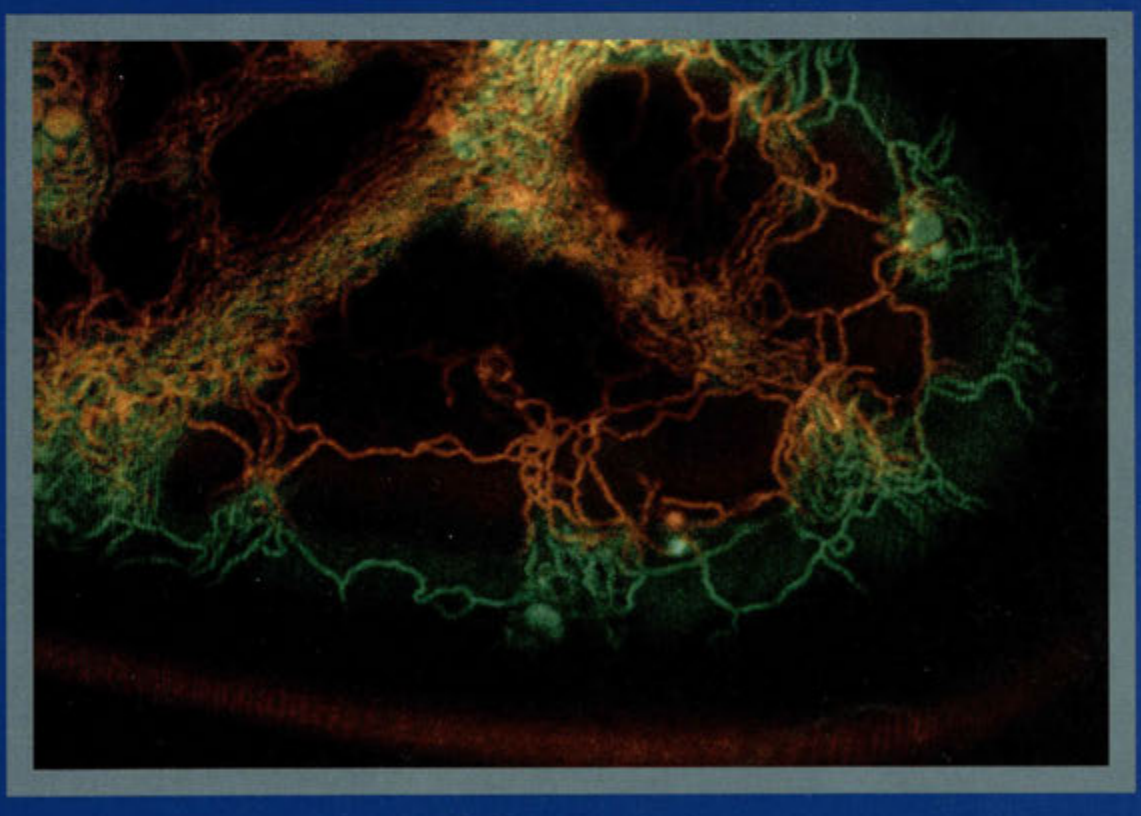
Scottish Crop Research Institute

Annual Report 1993



Cover photo :

Confocal laser scanning microscope image of a living onion epidermal cell. The endoplasmic reticulum was stained with a specific fluorescent probe and appears as a complex network of tubules.

Price £15

Scottish Crop Research Institute

Annual Report 1993

Governing Body

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

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

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

Lo SCRI e' uno dei maggiori centri internazionali nel campo della ricerca sulle colture agricole, orticole e industriali e sui meccanismi fondamentali comuni a tutte le piante. L'Istituto ha come obiettivo principale l'accrescimento del livello di conoscenza delle scienze biologiche fondamentali, il miglioramento della qualita' 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 capacita' scientifiche derivanti da ricerche di fisiologia e genetica ma anche di agronomica e fitopatologia supportate da prove di campo o in ambiente controllato.

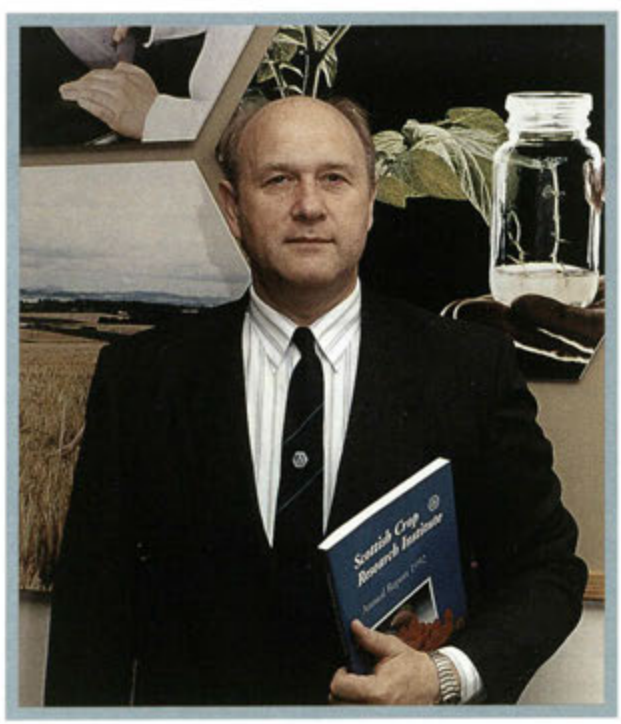
Contents

Contents

Report of the Director

John R. Hillman

Global perspectives influencing agricultural, biological and environmental sciences International trends in 1993, the fourth year of the post-Cold-War era, were associated with increasing political instability and a tendency for certain countries to disintegrate. The reasons were typically ethnic, religious, tribal or economic. Hopes of multilateral international co-operation through the auspices of the United Nations to solve disputes and avert human tragedies were not realised in countries such as the former Yugoslavia, Somalia, Haiti, and various nation-states in the former Soviet Union. In contrast, internal pressures and initiatives in long-standing problem areas such as South Africa and the Middle East generated optimism for their future. Economic insecurity and unemployment arising from a wide-spread recession, pressures on public spending, and disillusionment with established political structures, seemed to permeate much of the world. Scientific research, however, transcends political boundaries in a common pursuit of knowledge and understanding. Major research institutions such as SCRI sustain wide collaborative networks, allowing nations to benefit from shared projects.



The year began with the inauguration of an open internal market in the European Community (EC), whereby the 12 nations of c. 350m people began to implement the first phase of a complex series of changes leading to the free flow of goods, information, currency and people. Later in the year, the UK was the final member of the EC to ratify the Treaty on European Union (EU), the so-called Maastricht Treaty, which took effect on 1 November 1993, developing from the original 1958 Treaty of Rome. Expressions of severe concern over the Social Chapter and pervasive Euro-dirigisme were not confined to the UK. The EU, however, has become one of the main sources of competitive grants for SCRI, partially funding large co-operative projects with European partners.

Peter Sutherland, Director-General of the General Agreement on Tariffs and Trade (GATT), declared that the protracted Uruguay round of negotiations begun in 1986 had reached a successful conclusion, meeting the 15 December deadline largely as a result of the USA and EU eventually resolving their differences on agriculture. This is the most important trade pact in history, and finally brought agriculture under international trade rules for the first time. Since the mid-1980s, there have been substantial increases in the costs of supporting domestic agriculture in the EU and the USA. In addition to domestic political problems, general subsidies, import restrictions, and export subsidies for surpluses have also created trade disputes and trading disharmony between and within trading blocs. The GATT Agreement should be seen as building on the 1991 "Dunkel Text" and the 1992 USA-EC "Blair House Agreement", leading to the formation of a market-reg-

ulated trading system. Nonetheless, domestic agricultural policies were virtually excluded from the early stages in the lifetime of the new GATT Agreement, given the socio-economic importance of agriculture and horticulture. Moreover, it was generally agreed that a government could apply overriding recognised sanitary and phytosanitary controls which could affect trade in livestock and plants.

The formal signing of the GATT Agreement was scheduled to take place in Morocco in April 1994, and the Agreement would take effect on 1 July 1995, fundamentally influencing trade in the 117 participating nations. Other important trade issues dominating international relationships included the acceptance of the North American Free Trade Agreement involving the USA, Canada and Mexico. Policies relating to national food security are tending to evolve into strategies for trading-block food security, thereby influencing the future direction of relevant research in agricultural, biological and environmental sciences.

Preliminary estimates in the Quarterly Bulletin of Statistics issued by the Food and Agricultural Organization of the United Nations (FAO) point to a decline during 1993 in total agricultural production, total food production and per capita food production. Declines in production were noted for coarse grains, rice, oilseeds, cocoa, cotton, and dairy products, whereas wheat and sugar production were little changed. Market conditions in the Far East, especially with the freeing-up of markets in China, were buoyant and contrasted with confused market conditions in the former Soviet Union. Civil warfare and its aftermath posed a greater threat to food security in 1993 than weather perturbations, pests, weeds, and diseases. FAO also released a wide-ranging analysis of the current state of, and future prospects for, the global food situation - *Agriculture: Towards 2010.* This study noted slow but steady overall increase in global food production and per capita food supplies over the past 30 years, and a modest decline over the past 20 years in the number of people chronically undernourished. Basing their estimates on an annual world population growth of 1.5% occurring mainly in the less-developed countries (LDCs), FAO projected that serious hunger is likely to continue over the next two decades at least. Sub-Saharan Africa would remain the largest and fastest-growing concentration of undernourished humanity (around 300m). A further slowdown is anticipated in total world agricultural production, reflecting reduced needs for enlarging production in developed countries and several LDCs, and reduced effective demand in the poorest LDCs.

Development assistance, from members of the Organisation for Economic Co-operation and Development to developing countries fell sharply from $60.8 billion in 1992 to $54.8 billion in 1993. Compounding this, was a decrease in the overall ratio of development assistance to Gross National Product from 0.33% to 0.29%, the lowest figure since 1973. On the other hand, there was an increase in emergency aid and distress relief, high-profile short-term areas of funding.

One scenario worthy of attention highlighted by Professor I. Carruthers is that the industrially underdeveloped world will become the primary source of manufactured goods, effectively reversing the trend in trade established since the Industrial Revolution. The economies of most of the countries of the Pacific Rim are expanding, and in Asia there are several countries with sophisticated, urbanised workforces able to operate effectively and compliantly with relatively low incomes. Multinational trading, communication networks, and rapidly improving higher education in these countries will ensure that invention, intellectual property and entrepreneurial service industries will not be the preserve of the present developed world. Moreover, agriculture in developing countries is no longer regarded as their engine of economic growth - witness the pressures on the Consultative Group on International Agricultural Research. Most of the world's food and industrial crop production could eventually take place in the temperate zones in which are located most of the developed countries, but whether there would be the means to pay for the food is a moot point.

Demand for capital throughout the year started to apply pressure on longer-term interest rates despite generally low inflation. Now that Japan, Germany and oil-rich Arab states are no longer large providers of international capital, there is growing competition for funding, favouring rapid-growth areas such as the Pacific Rim, Latin America and Eastern Europe. Unless there is an offsetting rise in savings, investments in Europe and the USA, most notably in their peripheral regions, may well be adversely affected.

With regard to the food industry in the developed world, consumers sought value for money, causing a general decline in brand loyalty. There was market growth for well-presented convenience foods, fruit

and vegetables. Food labelling detailing compositional and nutritional details was a popular issue. Food and retail profits tended to decline in a highly competitive market-place. White-meat consumption increased at the expense of red meat, and the interest in vegetarianism continued. The incidence of food poisoning rose throughout the world, prompting re-appraisal of food irradiation as well as high-pressure technology for sterilisation and pasteurisation. Scientific interest also extended to fat substitutes; reduced fat, salt and low-calorie diets; increased dietary fibre; and clear (non-coloured) beverages. Modified-atmosphere packaging to enhance shelf-life and the use of "environmentally friendly" packaging remain priority areas of development.

Throughout the world, public-sector funding supports most scientific research at the strategic and fundamental levels. Recession, competing high-profile health and social welfare funding obligations, and general ignorance of matters scientific, have universally driven research into the applied camp, essentially towards product or process development, making it capable of delivering easily understood objectives to those who arrange funding. Underpinning research and development (R&D) for policy, statutory and regulatory activities have, with few exceptions, been subject to much greater analysis than hitherto. Scientists in all countries face major changes in organisational and funding arrangements, sensitivity over intellectual property, the implementation of various mechanisms to assess their performance, and redundancies.

United Kingdom perspectives Conventional agriculture, horticulture and forestry suffered in 1993 from pressures on profitability, which together with improved efficiency led to a continuing decline in the numbers directly employed, and an increase in the size of production units. These areas of activity have been paradigms of technology transfer to the point that planning targets and expectations of governments rarely allow for declining performance or catastrophe.

Since the 1960s, public-sector science and technology has been shaped by a tranche of policies aimed at improving efficiency and reassessing priorities. Key modifications in direction came with the 1965 Science and Technology Act, the 1968 Fulton Report, the 1971 Dainton Report, the 1972 Rothschild Report, the 1980s Financial Management Initiative, the development over the past five years of bureaucratically heavyweight ROAME (Rationale, Objectives, Appraisal, Monitoring, Evaluation) procedures, the 1989 Next Steps (Fairclough Guidelines) initiative and the formation of Executive Agencies, cessation of public funding to support near-market R&D, the Citizen's Charter, and piecemeal privatisation through Market Testing and Prior Options reviews, creation of the Office of Science and Technology, and operation of the Cabinet Office Efficiency Unit. Together with institutional amalgamations and closures, there are now unprecedented pressures on those organisations reliant to varying extents on public funding in the UK, with a constant need to scrutinise every activity and to adapt to policy reversals. Regardless of its role in quality of life, raising intellectual standards, creating wealth, upgrading the processes and resources of civilisation, and understanding the natural world, scientific research fits uneasily within the time-frames of conventional accountancy, legal systems and political priorities. Policy and re-organisational turbulence, the peculiarities of governmental annuality of funding with its associated cash limits and inflexible management practices pitched at nanolevel detail, a phenomenal range of auditing exercises, perpetual reviews and competition for shrinking resources collectively are discomforting to the point of hostility for public-sector research and development. Perhaps fortunately for those with responsibility for effecting change, the impacts of present-day decisions affecting R&D institutions will only become manifest in the years ahead. A life-time career in public-sector or private-sector science is no longer a proper expectation for most young graduates; perhaps this is strategically correct for there are not enough posts or resources to meet the rapid expansion in graduate output and employment demand. Trained scientists should be encouraged to pervade all sectors of society and industry. It is clear, though, that there is no room for under-achievers in respect of quality scientific output and ability to attract diverse sources of funding. Overhead costs will be driven down even further. Altogether, there is an increasingly high price to be paid in terms of lifestyle for accessing the public purse and for having scientific aspirations.

In the UK private sector, particularly the leading companies, R&D cannot thrive if there are inflationary-stoking expectations of short-term huge returns on R&D-related investments. Policies on company dividends, R&D taxation treatment, and Stock Exchange rules merit close examination and comparison with other countries, but realistically the likelihood of a change in culture is small at this juncture. R&D, and

ownership and exploitation of the consequent intellectual property, can be readily displaced outwith the UK.

SCRI is a special organisation for several reasons. Through its own efforts, it bridges the public and private sectors, it enjoys an unrivalled global reputation for its scientific quality and productivity, it has grown steadily in a competitive market whilst most similar bodies have contracted or merged, and wholly innovative lines of research cover nearly all our work which integrate laboratory and field experimentation. The mission of the Institute is to undertake research of the highest international scientific standards 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, to improve agricultural sustainability and diversification and to develop environmentally benign methods of protecting crops from depredation by pests, pathogens and weeds. A broad multidisciplinary approach is a special strength of the Institute, employing and fostering the disciplines of genetics and breeding, molecular and cellular biology; pathology (virology, bacteriology, mycology, nematology and entomology); physiology (metabolic, environmental and developmental); chemistry and biochemistry; agronomy; ecology (molecular ecology, vegetation dynamics, bioremediation); serology; physics; mathematics and statistics. The range of skills from fundamental studies on genetics and physiology, through agronomy and pathology to glasshouse and field trials with exploitation of genetic resources is unique within the UK research service. Close liaison with other research organisations, universities and colleges in the UK and abroad is an integral part of the scientific development of the Institute; such links are continually being developed and strengthened. SCRI actively seeks research contracts from Government Departments and agencies, levy boards, grower organisations, international agencies, commercial companies, local government and trusts.

Three pivotal documents directly influencing SCRI were released during 1993. Firstly, the White Paper *Realising our Potential: A Strategy for Science, Engineering and Technology* (Cm 2250, May 1993) heralded an enhanced role for the Office of Science and Technology and a series of initiatives that will alter the science landscape of the UK. Thus, the Research Councils were scheduled in April 1994 for restructuring with revised missions; Forward Looks were started; the Technology Foresight Programme began to take shape; and the ownership options for public-sector research establishments were to be subject to an Efficiency Unit scrutiny exercise in 1994. Implicit in the paper was the forging of a new partnership between industry, scientists, engineers, and science policy-makers. Secondly, the *Review of Allocation, Management and Use of Government Expenditure on Science and Technology* (The Levene/Stewart Report) was released at the same time.

Thirdly, the Scottish Office Agriculture and Fisheries Department (SOAFD) published in December its *Policy for Science and Technology*. This built on the successful framework of supporting agricultural, biological, aquatic, environmental and engineering science and technology, mainly in nine Scottish organisations (the "Scottish System"), and took into account the Science White Paper and the 1993 White Paper *Scotland in the Union: A Partnership for Good* (Cm 2225). The SOAFD paper signalled reduced core grants, a more arms-length relationship with contractors, greater monitoring, open competition, and a focusing on the importance of science and technology to wealth creation, all coupled to improved co-operation between the various constituent bodies. Regarded as timely and entirely consonant with the current situation, it was well received in the nine organisations (the five Scottish Agricultural and Biological Research Institutes, the Scottish Agricultural College, the Scottish Agricultural Science Agency, the Fisheries Research Service, and the Royal Botanic Garden, Edinburgh). The Scottish System is unique for its breadth and depth of science, horizontally and vertically integrated, engineering and technology carried out in internationally renowned bodies, rigorously overseen, encouraged and funded by SOAFD.

For SCRI it was reassuring to note that the conclusions of these three documents confirm the wisdom of the long-standing initiatives we have taken in redirecting our science, setting up effective technology transfer through the highly regarded Mylnefield Research Services Ltd. (MRS) and CAROS International Ltd., addressing the needs of industry and society, linking formally with universities and institutes at home and abroad, and becoming a widely recognised Centre of Excellence in international science. There are various mechanisms to ensure that the quality and standing of the science conducted in the UK Research Institutes are regularly and rigorously monitored. Additional to the internal reviews (operated at SCRI by individual scientists, Working Groups, Heads of Science Departments, the Director, and the Governing Body),

there are numerous external reviews. Foremost of these are the periodic Visiting Groups, reinforced by other frequent assessments carried out by research sponsors and government departments, associated scientific bodies, visiting scientists, international comparisons and peer evaluation of scientific submissions. In April 1993, SCRI received a Visiting Group ably organised by the Agriculture and Food Research Council (AFRC, now the Biotechnology and Biological Sciences Research Council) and forming part of the regular four-yearly inspections with intermediary two-yearly reports.

The Institute was visited from 19 to 23 April 1993 by a Group comprising Professor E C D Cocking FRS (Chairman), Professor N Atherton, Dr J T Braunholtz, Professor R Coffman, Professor E Griffiths, Dr M Hornung, Dr K Hummer, Professor J L Monteith FRS, Professor K Richards and Professor K Vickerman FRS. The Group was accompanied by Dr J N Wingfield, the late Dr I M Reid, Dr P Maplestone, Dr D Rawlins and Mrs M Pringle of the AFRC's secretariat, and by Dr K W Moore and Mrs L A D Turl (SOAFD). The Visiting Group conducted a scientific audit of the quality and conduct of research at SCRI. The emphasis of its audit was on the national and international standing of the research programme, its relevance to the remit of the Institute, progress made towards objectives, and the plans for future work. The Group also considered the scientific organisation and management of the Institute, the facilities and resources, interactions with other organisations nationally and internationally and links with industry.

The Group's assessment was based on discussions with the Director, the Deputy Director, Heads of Departments, and other scientific staff of the Institute. Although the Institute's programme is formulated as a series of ROAME projects, agreed with SOAFD, for the purposes of this visit, projects were grouped together into 28 scientific themes and the scientists involved in each were interviewed collectively. This allowed the Group to see the research programmes in cohesive units and to explore the interactions within research groupings.

The Visiting Group was provided with a comprehensive submission from the Institute. It was also provided with detailed comments from 50 eminent overseas referees who were asked to review aspects of the Institute's science programme, based on the information provided in the written submission. The referees were asked to comment on the: quality of science in relation to international standards; relevance to the Institute's remit; research output; and future direction of the programme. They were asked to assign the programme to one or more of the following categories: (i) internationally recognised science of the highest quality; (ii) good quality science worthy of continued support; (iii) research which is mundane or derivative of other, more innovative work, or which is unsatisfactory in its execution. The names of the referees were not disclosed to the Institute. During its visit the Group also met the non-science-group staff of the Institute; the post-graduate students, post-doctoral research fellows and visiting workers. It also toured the estate and laboratories, visited the Data Processing Unit and held discussions with representatives of MRS and CAROS International Ltd.

The Group reported that it was impressed by the high standard and productivity of science at SCRI, verified by the external referees, and by the quality and commitment of the staff and strong sense of community at the Institute. It was also impressed by the very high standard of the research facilities and instrumentation, reflecting the sustained support from SOAFD, and by the establishment of first-class groups carrying out innovative basic research alongside other scientists who were conducting applied research of more obvious application. It considered that the research programme was well designed to fulfil the Institute's remit. Several project teams and individuals were regarded at the international forefront of their area of science, confirming the views of other internal and external assessments. None of the work at SCRI was regarded as mundane, derivative, or unsatisfactory.

The Visiting Group recognised that the wide range of scientific expertise was crucial in maintaining the continuum of research. It was pleased to note that modern technologies were finding application throughout the research programme. SCRI was seen to have an excellent record in the commercial exploitation of science and to provide very good research training and supervision. Valuable links had been built up at home and abroad, attracting many eminent visiting scientists. Constructive recommendations were made aimed to strengthen our position and provide guidance for future development. In summary, the Visiting Group Report was outstanding; Professor Cocking and his Group members, and the accompanying AFRC and SOAFD staff are thanked for their penetrating, professional and courteous review.

SCRI acts as a parent body for the Scottish Agricultural Statistics Service (SASS) which operates as a unit of SCRI under the directorship of RA Kempton. SASS was reviewed separately by a Visiting Group in 1991 and received an excellent Report which regarded SASS as an extremely good organisation. The SASS Visiting Group Interim Report was considered by the AFRC Plants and Environment Research Committee in September 1993. With the advent of BBSRC, it was thought that it would be timely to reassess the potential to develop the highly successful SASS model south of the border.

This Annual Report details a small selection of the research achievements of the Institute and SASS, incorporating advances in fundamental and strategic science, contributions to the protection and understanding of the environment, and discoveries of both direct and indirect benefit to agriculture, horticulture, forestry and various biologically based industries. The outstanding performance, independently validated, of SCRI and SASS in respect of publications, reports, new cultivars, patents, contracts, reputation and influence, supervision of students, teaching, and value-for-money reflects not only the range of facilities and resource capacity of the Institute but most notably the dedicated commitment of talented staff in every department and section. Reports on the remarkable success of MRS and CAROS International Ltd. are included.

On behalf of the staff and Governing Body it is a pleasure to acknowledge with gratitude the staff of SOAFD for their continuing support of our research. They function as always at the highest professional standards of public service. Grants, contracts, donations and advice from the Scottish Society for Crop Research, governmental and non-governmental agencies, sister organisations, grower levy boards, local and regional authorities, commercial companies, farmers and other individuals are also warmly appreciated. In all respects, SCRI justifies its existence and flourishes.

People and Events

Retirements Two long-standing colleagues left during 1993. Dr A F Murant FRSE, UG6 in the Virology Department, retired in April after 33 years' service. His long and illustrious career in plant virology has provided sound biological, taxonomic and epidemiological foundations for many current molecular programmes. Never one to avoid a scientific challenge, he conducted a series of pioneering investigations of virus complexes in which the component viruses depend on one another for their ability to be spread by insects and to cause damage. He became an international authority in this area, and especially on the devastating groundnut rosette disease complex from Africa. This study attracted considerable funding from the ODA. His professional stature, reputation for precision, and the breadth of his contributions to the discipline of plant virology remain unparalleled. For many years he has edited the CMI/AAB series 'Descriptions of Plant Viruses' which is an invaluable resource to virologists throughout the world. He was elected a fellow of the Royal Society of Edinburgh in 1986.

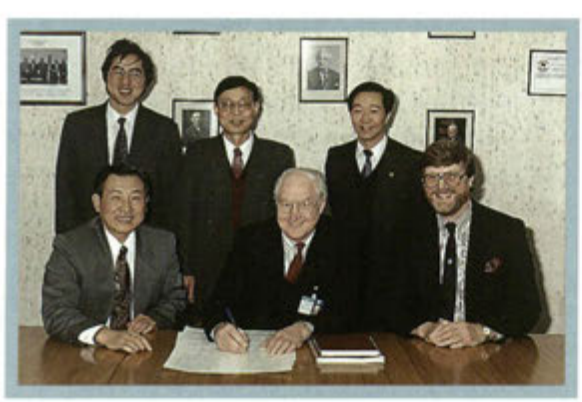
Pat Dashwood took early retirement in March after 27 years in the Mycology and Bacteriology Department. She was renowned for her experience in fungal diseases, and her expertise in the identification of microfungi, fungal ecology and blemish diseases of tubers will be missed throughout that sector of the scientific community.

Visitors In March 1993 the Virology Department welcomed 24 visitors from Africa, Asia, The Americas and Europe to a 2-week intensive training course, sponsored by the British Council, on modern techniques and approaches to plant virology. Members of the Virology Department also organised a

His Excellency Mr Ivan Stancioff, the Bulgarian Ambassador, and the Director, Professor J R Hillman.

2-day informal workshop on the topic of "Exploitation of plant viruses in biotechnology". This workshop, held in August just prior to the International Congress of Virology in Glasgow, was attended by approximately 140 plant molecular virologists and biotechnologists from around the world, many of whom also toured the facilities of the Institute. Both these events raised the international profile of the Virology Department and SCRI and are expected to stimulate future exchanges of scientists.

Dr Bernard Goodman, Head of the Director's Spectroscopy Group, was a member of the organising committee for the First International Conference on Oxygen and Environmental Stress in Plants. This Conference was held at the University of St Andrews under the auspices of the Society for Free Radical Research and the Royal Society of Edinburgh in September 1993. It was attended by over 100 scientists who took the opportunity to visit the Institute as part of the programme.



A Memorandum of Understanding between SCRI and Zhejiang Academy of Agricultural Science being signed.
Front l to r: Professor Yang Jizhong, Professor N L Innes, Professor T M A Wilson.
Back l to r: Mr Jianping Chen, Professor Chen Shengxiang, Professor Shen Pei Pei.

Professor Hillman was invited to present the prestigious Twentieth Bawden Lecture at the Brighton Crop Protection Conference in November. He chose as his theme 'Bio-engineering - Intellect, Enterprise and Opportunity'.

During the year many individual and groups of visitors from abroad and the UK were hosted at the Institute, ranging from eminent scientists, agricultural administrators and foreign delegations, through to student parties, members of the Scottish Society for Crop Research and local club organisations. As in 1992, there were several groups from the former Eastern Bloc countries, including trade missions from Czechoslovakia, Poland and Hungary.

Distinguished visitors included His Excellency the Ambassador Extraordinary and Plenipotentiary, Mr Ivan Stancioff, Bulgarian Ambassador to Britain. Mr Stancioff, accompanied by his wife, and two colleagues from the University of Sofia, visited SCRI on 17 August. Steps are being taken by the Ambassador and his colleagues from Sofia to draw up a Memorandum of Understanding between SCRI and an appropriate complementary Institute in Bulgaria.

A second ambassadorial visit took place on 3 September, when four members of the Commercial Office of the London Embassy of the People's Republic of China met the Director, and toured the Institute. In addition there were three delegations of senior scientists from China during the year, including a group from the Zhejiang Academy of Agricultural Science (ZAAS) in December. During this visit, Professor Yang, the Deputy Director of ZAAS, conferred an Honorary Professorship on Professor T M A Wilson, Head of Virology Department, and signed a Memorandum of Understanding to facilitate future applications for co-operative funding between the two Institutes.

In October, four new raspberries and a new strawberry were formally launched at Scotgrow, Scotland's National Horticultural Trade Exhibition held at Ingliston, Edinburgh. An agreement was signed with NSA Plants Ltd granting them the rights to propagate and sell the raspberry cultivars Glen Magna, Glen Ample, Glen Shee and Glen Rosa. An agreement was also signed with Commercial Fruit Plants Ltd, granting them the rights for the marketing of the new strawberry cultivar, Symphony.

The CGIAR system and links between SCRI and the International Agricultural Research Centres

N.L. Innes*

The Consultative Group on International Agricultural Research (CGIAR) is an informal organization that was formed in 1971[1] and now supports a global network of 18 international agricultural research centers (IARCs) (Table 1) that benefit from the voluntary pledges of over 40 donors which include industrialised countries, developing countries, regional development banks and private and public foundations.[2] The IARCs are scattered across the globe, yet are linked by a common purpose - the eradication of human hunger and poverty through research.

The mission of the CGIAR is:

"Through international research and related activities, and in partnership with national research systems, to contribute to sustainable improvements in the productivity of agriculture, forestry and fisheries in developing countries in ways that enhance nutrition and well-being, especially of low income people."

One of the earliest successes of the IARCs was generated by CIMMYT and IRRI in providing much of the germplasm to create the high yielding varieties (HYVs) of rice, wheat and maize[3,4] that formed the basis of what is popularly referred to as the green revolution. It has been calculated that the HYVs developed at the international centres led to an average annual increase between 1970 and 1983 of ca. 40 million tons of food, enough to meet the yearly food grain needs of 500 million people.[5] A number of important breakthroughs since the green revolution include an increase in the number of cultivars with host plant resistance to pests and diseases and integrated pest management schemes to reduce dependence on chemical pesticides. Among crops to benefit are rice, cassava and potato. Some of the recent successes of the IARCs are highlighted in the CGIAR Annual Report for 1992 which also lists the programmes of IARCs into six broad categories (Table 2). The CGIAR budget is relatively small, comprising well under 5% of total global resources devoted to agricultural research[6] and appears to be decreasing, as are budgets for national research programmes. In 1992, donor commitments for core budget totalled $247m, with a contribution of nearly $11m from the UK Government while in 1993, total pledges for core budget for 1994 were ca. $225m and final delivery could be lower.

The CGIAR has its headquarters, its chairman and secretariat at the World Bank, Washington DC, whilst a separate secretariat of the Group's Technical

Acronym	Name	Founded	Headquarters
CIAT	Centro Internacional de Agricultura Tropical	1967	Colombia
CIFOR	Center for International Forestry Research	1993	Indonesia
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo	1966	Mexico
CIP	Centro Internacional de la Papa	1970	Peru
ICARDA	International Center for Agricultural Research in the Dry Areas	1975	Syria
ICLARM	International Center for Living Aquatic Resources Management	1977	Philippines
ICRAF	International Centre for Research in Agroforestry	1977	Kenya
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics	1972	India
IFPRI	International Food Policy Research Institute	1975	USA
IIMI	International Irrigation Management Institute	1984	Sri Lanka
IITA	International Institute of Tropical Agriculture	1967	Nigeria
ILCA	International Livestock Centre for Africa	1974	Ethiopia
ILRAD	International Laboratory for Research on Animal Diseases	1973	Kenya
INIBAP	International Network for the Improvement of Banana and Plantain	1984	France
IPGRI	International Plant Genetic Resource Institute	1974	Italy
IRRI	International Rice Research Institute	1960	Philippines
ISNAR	International Service for National Agricultural Research	1979	The Netherlands
WARDA	West Africa Rice Development Association	1970	Ivory Coast

Table 1 International Agricultural Research Centres.

*Currently Chairman of the Board of Trustees of CIP and former Board member of ICRISAT.

Productivity Research	Creating or adopting new technologies (such as the 'dwarf' varieties of wheat and rice that brought about Asia's and Latin American's green revolution) to increase productivity on farmers' fields
Management of Natural Resources	Protecting and preserving the productivity of natural resources on which agriculture depends
Improving the Policy Environment	Assisting developing countries to formulate and carry out effective food, agriculture, and research policy
Institution Building	Strengthening national agricultural research systems in developing countries
Germplasm Conservation	Conserving germplasm and making it available to all regions and countries
Building Linkages	Helping to create or strengthen linkages between developing country institutions and other components of the global agricultural system

Table 2 Broad categorization of IARC programmes (CGIAR, 1993).

Advisory Committee (TAC) is housed at the Food and Agriculture Organization (FAO) of the United Nations in Rome. In addition to the World Bank and FAO, the United Nations Development Programme (UNDP) is a co-sponsor of the CGIAR. The Group is chaired by a vice-president of the World Bank and it meets annually in October at International Centres Week (ICW) in Washington. A Mid-Term Meeting (MTM) is usually held in May in a country that has offered to host the meeting. The MTM in 1994 will be held in New Delhi, India.

Technical advice on policy and strategy is provided by TAC which is made up of distinguished international scientists and administrators. Recently the formation of a CGIAR Finance Committee and a CGIAR Oversight Committee has served to strengthen the interactions among co-sponsors, donors and non-donor representatives of developing countries at a time when financial resources are scarce and the CGIAR is faced with a number of important challenges.

Each IARC is dependent on its core budget money from annual pledges by donors at ICW. Individual Centres produce a strategic plan, usually to cover a ten-year period. These plans are discussed by TAC and by the CGIAR and reflect CGIAR policy and guidelines. In addition, each Centre has an operational budget plan which looks forward over a 5-year period as well as a detailed budget plan for each year. In addition to "unrestricted" funding for the operation of the centres, there is also "complementary" funding, often of a bilateral nature, for special projects that appeal to particular donors. Centre plans are formally approved by the CGIAR. Moreover, there is a strong system of accountability whereby TAC and the CGIAR Secretariat arrange for external reviews at intervals of about five years by small teams of specialists to cover both scientific programmes and management. These reviews provide both donors and clients with information about the quality and relevance of the work of each centre, past and likely-future impact and the efficiency of centre management. Such reviews are sometimes extended beyond individual centres to cover commodities or specialised areas such as plant genetic resources, and intellectual property rights.

Figure 1 The Board of Trustees of the International Potato Centre.

Back (l to r) F. Winiger (Switzerland), K.L. Chadha (India), K. Raven (Vice-Chairman, Peru), P. Gregory (Deputy Director General, Research), D. Bateman (USA), N.L. Innes (Chairman, UK) H. Zandstra (Director General)

Front (l to r) A. Cerrate (Peru), S. Muturi (Kenya), T. Kajiwara (Japan), A. Brandolini (Italy), S. Sastrapradja (Indonesia), Shen Jinpu (China), J. Vallé-Riestra (Deputy Director General, Administration).

Each Centre is autonomous with its own Board of Trustees or Governing Board. Members of these Boards serve in their personal capacity; some are nominated by the CGIAR, and some are national (from the Centre's host country) representatives. Until recently, board members were mostly experienced research scientists or administrators but increasingly, members with financial and legal expertise, particularly from the private sector, are being sought. Each Director-General is a board member ex-officio. The board size varies, with bye-laws allowing as many as 20 members at some centres, and currently numbers range from eight to 17. It is rare to find two people of the same nationality on a Board at one time. Election is for a 3-year period in the first instance, with possible re-election for a further 3 years.

The CGIAR and IARCs are in the midst of radical change, partly as a consequence of financial constraints, partly because national agricultural systems in some countries have healthily developed considerable strengths of their own (e.g. China, India and Brazil) and partly because needs and priorities are changing and scientific opportunities are increasing. Previously, increased productivity per unit area has been the key contribution, largely sustained by utilization of the excellent germplasm collections of crop species and their relatives at many of the centres. These collections will continue to form the bedrock on which centres can build, but increasingly important issues such as sustainability, environmental degradation, global climate change, biodiversity, ecoregional research and the relationship of the IARCs with the emerging independent states in Eastern Europe and the former Soviet Union, are currently under debate and the outcome will undoubtedly influence the future shape and direction of the CGIAR and its centres.

Among recent changes that have been made, IPGRI (formerly IBPGR) has been entrusted with the responsibility of managing INIBAP's banana and plantain programmes. A Task Force has recently recommended the establishment of a new, single entity for livestock research in the CGIAR and the programmes of the existing livestock Centres in Africa, ILCA and ILRAD, will form the nucleus of the new, unified institution, the International Livestock Research Institute (ILRI).

The CGIAR system was created to address the world food problem and one of the roles of the IARCs is to provide bridges between institutes engaged in basic and strategic research in industrialised countries and the national agricultural research systems (NARS) of less developed countries. More specifically, IARCs help facilitate technology transfer, generate new technologies, undertake training, disseminate information, and fill important gaps in research in the international arena. As technology transfer is often location specific, close interaction between the IARCs and developing country NARS is essential for successful impact.

Links between CGIAR centres and SCRI. At SCRI, the multi- and inter-disciplinary approach to research, allied to its scientific strengths at a molecular level, have made close collaboration possible between the Institute and several IARCs on exotic crops or species, as well as on temperate crops which are part of SCRI's mandate. CIP and SCRI collaborate closely on potato research and both Institutes house potato gene banks, the World Potato Collection at CIP and the Commonwealth Potato Collection at SCRI. In a study for CIP on drought tolerance in potatoes, considerable diversity in rooting types was discovered at SCRI and the relative importance of the balance between root and shoot characters for the water economy of the plant highlighted. Further collaborative work has included research on resistance to potato leaf roll virus (PLRV) and has demonstrated that a form of plant resistance which is expressed as a restriction of virus multiplication was highly effective in reducing aphid-borne spread of PLRV from infected sources in the field. The resistance was found in several SCRI breeding clones, and was shown to be controlled by

Figure 2 Variation in tuber characteristics in the World Potato Collection at CIP.

two dominant, complementary genes. More recent work has revealed a synergistic effect on restricted virus multiplication when the PLRV coat protein gene was introduced into naturally resistant breeding lines. The combination of host- and transgene-mediated resistance offers the most promising method for control of PLRV. In further studies, the South American potato cv. Tomasa Condemayta, has been shown to inhibit virus replication within its leaves and, in addition, appears to be resistant to aphid vectors landing on its leaves.

The two potato cyst nematode species *Globodera rostochiensis* and *G. pallida* constitute the most important pest complex on potatoes in western Europe. South America is the ancestral home of both species and several of their pathotypes. Joint research with CIP has focused on developing resistant cultivars with an exchange of breeding clones and pathotype differentials, populations of *Globodera* and methods of testing for resistance. Currently, the DNA "fingerprints" of European and South American populations are being compared by nematologists at SCRI to identify relationships between pathotypes from the different countries.

Figure 3 Field collection of potato genotypes at the Huancayo field station of CIP.

A past contract with CIP to develop diagnostic tests for *Erwinia* spp. on seed potato tubers led to the application of a method in which numbers of bacteria on infected tubers can be determined. In addition, high resistance to blackleg in protoplast fusion products of *Solanum brevidens* x *S. tuberosum* was identified and the mechanism was found to be related mainly to resistance of cell walls to enzymatic degradation. The resistance has now been transferred within *S. tuberosum* by conventional crossing and its inheritance is being studied. Other collaborative projects with CIP include studies on somatic embryogenesis

Figure 4 Trials of groundnut cultivars at ICRISAT, India.

funded by ODA, and on the exploitation of linkage maps in potatoes funded by UNDP.

SCRI also has strong links also with ICRISAT, especially in virology and molecular genetics. In groundnuts there have been studies on the African groundnut rosette disease complex, on Indian peanut clump virus and on the transformation of groundnut to confer novel resistance *via* virus-derived sequences. The complex aetiology of groundnut rosette disease has been unravelled and the key role of a satellite RNA of the virus revealed. Diagnostic reagents for detection of five satellite RNAs have been produced and studies on their molecular biology begun. Methods for the transformation of groundnut tissue using *Agrobacterium tumefaciens*, and for the regeneration of groundnut plants from leaves or cotyledons have been established. Wild accessions of *Arachis* spp. constitute a valuable source of novel variation which has been successfully exploited by ICRISAT breeders to transfer pest and disease resistance genes from wild species into cultivated groundnut. Genetic fingerprinting techniques involving molecular and biochemical markers were developed at SCRI to examine biodiversity in groundnut, to study species relationships and to detect introgressed DNA carrying pest and disease resistance genes from wild species. Species specific markers have been identified which are likely to be exploited in ICRISAT's alien introgression breeding programmes. The close relationship between SCRI and ICRISAT virologists was reflected by SCRI organizing an ICRISAT-funded international meeting on groundnut viruses in Dundee in August 1993.

SCRI staff have collaborated closely and effectively with ICRISAT scientists in India and West Africa on the application of small-scale malting tests developed in barley to select for malting quality in sorghum and,

	Isozymes	RFLPs		RAPDS	STSs		
		Nuclear	Organellar		Microsatellites	CAPS	ESTs
Cocoa	✔	✔		✔			
Gliricidia		✔		✔	✔	✔	✔
Mahogany				✔			
Coffee	✔	✔		✔	✔	✔	✔
Tea	✔	✔		✔			
Groundnut	✔	✔	✔	✔			

Table 3 Genetic marker research on exotic crops at SCRI.

in conjunction with the Department of Plant and Soil Science at the University of Aberdeen, on cultivar differences in the root response of sorghum and chickpea to mechanical impedance during establishment. Over the last five years, SCRI has also provided advice to ICRISAT on electron microscopy which has culminated in joint publications.

There are regular exchange visits involving staff from SCRI, CIP, and ICRISAT, and SCRI has provided training for staff from both of the IARCs. Whenever possible, projects for visitors and overseas post-graduate students at SCRI concentrate on crops of importance in their own countries. SCRI is also collaborating with ICARDA in a project on salt tolerance in barley.

Other international collaboration SCRI's contribution to developing countries has not, however, been limited to collaborative projects with the IARCs. Studies on viruses of tomato, cassava, cotton and okra have also been done in association with national agricultural research groups and French aid organization (ORSTOM, CIAT and IITA). The sensitive ELISA and PCR-based virus detection methods developed at SCRI were used in a screening system for cassava clones supplied by CIAT to IITA, *via* Scotland to prevent the inadvertent introduction of South American cassava viruses to Africa. In addition, molecular genetic studies involving several countries supported by ODA and EC funding, as well as by levy boards, are currently in progress on cocoa, tea, coffee and tropical trees (Table 3).

Prior to the formation of SCRI through the merger of the Scottish Plant Breeding Station (SPBS) and the Scottish Horticultural Research Institute (SHRI) in 1981, potato seedlings from the SPBS breeding programmes were screened and selected in Africa and India for their adaptation to local conditions, providing improved cultivars with the prefixes Roslin in Kenya (eg. Roslin Eburu) and Kufri in India (eg. Kufri Jyoti).[7] The success of SPBS and SCRI potato cultivars has not, however, been limited to developing countries as these cultivars are also grown in Continental Europe and Mediterranean countries. Soft fruit cultivars from SHRI and SCRI are to be found as far afield as Chile, Australia, Scandinavia and South Africa, and SPBS/SCRI cultivars of forage brassicas are popular in New Zealand.

As constraints on funds for national and international research become greater, it is increasingly important to promote effective collaborative research between institutes on an international scale. The UK and its research establishments have contributed considerably to the well-being of international agriculture and will continue to do so.[8] Contributions could well be enhanced by the secondment of SCRI staff on short term assignments overseas. The comparative advantage that SCRI has in certain areas of biological and agricultural research has been put to good use in the past and the current and planned collaborative projects with international centres and national research programmes in different countries augers well for the future.

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Plant genetics

G.R. Mackay & R.J. McNicol

Research into conventional breeding methods and genetic studies of potatoes at the tetraploid level continue to be the principal route to the production of new improved cultivars, as evidenced by the listing in 1993 of three new potato cultivars and the submission of seven advanced clones to National List Trials, in association with our commercial partners.

The SCRI bred spring barley cv. Tyne, originally recommended for growing in Scotland in 1988, was also recommended for use throughout the UK in 1993. Similarly, the products of the now discontinued brassica genetics research programme at SCRI are appearing on the National List as new improved cultivars of swede with the support of commercial seed companies. In this latter case, research into breeding methodology and the mechanism of heterosis has also attracted sufficient funds to initiate a small, conventional swede breeding programme based on F_1 hybrids of SCRI elite germplasm. Nevertheless, research into novel techniques has progressed to the stage where they may usefully augment and improve on the conventional approach in the foreseeable future. For example, the production of potato cultivars using true botanic seed (TPS) rather than seed tubers may be enhanced by research which has shown that selection for more homogeneous, i.e. less variable TPS progenies, is achievable in the glasshouse and first clonal year stages of a breeding programme.

There have also been substantial improvements in the development of more efficient protocols for the regeneration of tissue culture explants in a wide range of potato genotypes and wild diploid and tetraploid *Solanum* spp. A range of somatic hybrids have been produced by electrofusion of protoplasts of cultivars such as Brodick, Record and Pentland Squire with several wild species from the Commonwealth Potato Collection (CPC) that are resistant to potato cyst nematode. This material is currently undergoing biochemical, molecular and morphological assessment in order to determine the most efficient means of incorporating these unique PCN resistance sources into future cultivars. The application of similar tissue culture-based methods to oilseed rape has resulted in a number of lines with enhanced oleic and linoleic fatty acid profiles.

In the grain legumes, advances have been made in *Agrobacterium*-mediated transformation techniques and protocols have been developed which dramatically improve rates of transient expression of the GUS reporter gene in seedling explants of *Vicia faba*. When stable transformation protocols are achieved, the means to produce new improved grain legumes and increase our understanding of the underlying

genetics and biochemistry of these important crop plants will be substantially enhanced.

Funds provided by the Scottish Society for Crop Research, Nickerson Seeds Ltd. and Dalgety plc enabled us to trial, for the first time, advanced lines of barley at Mylnefield at the same time as they were being trialled by Nickerson in England under the Consortium agreement with Nickerson and Dalgety. Genotype by environment interactions are complex and important in the breeding and selection of new cultivars and we have been concerned that trialling and selection of our advanced barley lines in England after initial selection in Scotland could be disadvantageous, leading to locally adapted material being discarded. In this first year, several lines that performed well in Scotland did not do so in England, providing evidence to support our concern. Further trials are scheduled for the next two seasons and it will be interesting should any of the SCRI lines prove sufficiently superior in Scotland, due to their local adaptation, to merit release as cultivars, when they would not have done so based on trials elsewhere in the UK or Europe.

G.R. Mackay, Head of the Crop Genetics Department

Basic research into barley genetics continues to add more markers to an already extensive linkage map and to reveal particular regions of the genome that are important in controlling economically important traits. A dipstick test ('Durotest' kit, Rhone Poulenc Diagnostics plc, Glasgow), has revealed a strong reaction between barley and the friabilin monoclonal antibody. Friabilin is a starch granule surface protein which conditions grain hardness in wheat and its presence in barley supports the view that diploid barley could be useful as a genetic model for hexaploid wheat in research into grain hardness.

The improved and adapted *Solanum phureja* 'gourmet' potatoes continue to attract much interest in the retail food industry, and two private companies have funded the independent production of additional populations of this material for evaluation and selection of clones suitable for their purposes. A colour brochure on the background to, and commercial potential of, *S. phureja* has now been produced and is available from MRS Ltd. It is hoped that sufficient industry funding will bridge the gap between SCRI's fundamental and basic research on wild species and primitive forms and the 'near market' research and development needed to bring these products into commerce, where they can be of benefit to the UK economy.

In 1993, Scottish Soft Fruit Growers Ltd, a co-operative owned and run by fruit growers and representing 95% of Scottish raspberry production, commissioned a research programme which will ensure the continuation of the raspberry breeding programme for at least six years. Emphasis has been placed on the release of cultivars that are capable of yielding uniformly over seasons and extending the fruiting season; and that are resistant to pests and diseases and suitable for hand and machine harvesting. A Littau raspberry harvester which operated on the beater principle has been in use for the past 15 years at SCRI. However, newer machines, notably the Korvan and Pattenden Harrier, operate on a vibrating vertical or horizontal drum principle have produced excellent samples from cultivars like Glen Moy that have previously been difficult to harvest mechanically. We have now acquired one of these machines for evaluating all selections in 10-25 plant plots and are testing a prototype hand-held single plant harvester which can be used to identify individual seedlings suitable for mechanical harvesting from within our unselected progeny plantations in their first fruiting year.

Pest resistance is particularly important in the blackcurrant and raspberry breeding programmes. Resistance to the large raspberry aphid (*Amphorophora idaei*) controls both aphid numbers and the viruses they transmit. However, the recent development and increased frequency of virulent aphid biotypes able to overcome the resistance gene A_1, present in Glen Moy and Glen Prosen, that has been in commercial use for over 40 years has caused considerable concern. The resistance gene A_{10} currently provides protection against all known *A. idaei* biotypes but its exposure to *A. idaei* populations has been limited and therefore the selection pressure on the aphid has not been extreme. At present we are screening for resistance to all four biotypes of *A. idaei* and, in collaboration with

the entomologists and chemists, we are studying leaf surface chemistry to identify components that interfere with settling behaviour of the aphid. The results will assist in the design of control strategies for using resistance genes which minimise selection pressure for pest virulence.

In blackcurrants, the need for commercially acceptable gall mite (*Cecidophyopsis ribis*) resistant cultivars is now acute. An extensive backcrossing programme has been in progress for a number of years to incorporate the resistance gene *Ce* from gooseberry into commercially acceptable cultivars with particular regard to juicing quality. Several selections are now approaching final testing prior to release.

Resistance to raspberry root rot (*Phytophthora fragariae* var. *rubi*), the most serious disease affecting raspberry plantations in Europe and North America today, is also important, and the production of cultivars with resistance to the disease has become a major objective in the breeding programme. However, to avoid the loss of potential cultivars without resistance, only seedlings surplus to normal planting requirements and progenies expected to possess resistance are screened. To date, 2,600 previously unselected seedlings have been field tested on a commercial farm severely affected by the disease. Twenty-four selections from the 600 survivors have been selected for further evaluation, both in terms of glasshouse testing for resistance and for agronomic value, and backcrossing to improve fruit quality. In addition, seedlings collected from 84 wild *Rubus idaeus* populations throughout Scotland by J. Luby (University of Minnesota, USA) were drenched with a zoospore suspension of the pathogen and seven populations showed some resistance which indicates that our indigenous species has unexploited potential as a source of resistance.

Molecular markers are being used in our breeding programmes as they provide a new and highly effective means of manipulating germplasm and raspberry, blackberry, blackcurrant, strawberry and sitka spruce have been fingerprinted. Phylogenetic analyses of closely related species are now being carried out to generate high density linkage maps and identify easily scorable markers for the indirect selection of agronomic traits which are difficult, expensive, or prolonged to score by conventional means.

Breeding potatoes for warm climates

G R Mackay, R L Wastie & Helen E Stewart

Introduction The European cultivated potato derives from introductions of the primitive form *Solanum tuberosum* ssp. *andigena* at about the time of the Spanish Conquest of South America. Andigena is a native of the high Andes of Peru. Once adapted to the long day summers of Europe, it spread around the rest of the world to become the fourth most important food crop after wheat, maize and rice. It is remarkable how adaptable tuberosum has proved to be in view of its probably narrow genetic base.

Historically, SCRI's breeding programmes were focused on the production of cultivars for use as ware in the UK, although cultivars such as Kufri Jyoti

Figure 1 The World Potato Collection at Huancayo (CIP), Peru, 3400m altitude in the Peruvian Andes.

(India) and Roslin Eburu (Kenya) were selected in these countries from clones produced in Scotland (at the former SPBS, Pentlandfield). During the 1980's, SCRI (then SPBS) was commissioned to breed cultivars to export as seed from the UK to countries such as Spain, Cyprus, Israel and Algeria. This work involved trialling a few of the most advanced selections overseas, but it also included more strategic research into some of the abiotic stress factors and diseases associated with hot climates.

Figure 2 A field of Roslin Eburu (SCRI clone 1521c3) on the outskirts of Nairobi, Kenya.

Although core funding for this research was subsequently withdrawn, the experience gained provided evidence of the opportunities awaiting development. Potato clones which might otherwise have been discarded were retained, and at least one cultivar with export potential (cv. Stirling) has been placed on the UK National List.

In reviewing SCRI involvement in breeding potatoes for warm climates, it is useful to consider the factors of importance in these different environments, and some of the results of our work in this area. Potatoes growing in such climates experience conditions quite alien to those in which they originated, and problems caused by high temperature and water shortage have severe implications for potato production. The semi-arid regions bordering the Mediterranean have rain in the cool season and a dry, warm summer in which potatoes can usually only be grown successfully under irrigation. In this climate there is no cold break between seasons, so the carry-over of pathogens between crops is not hindered as it is in more northerly regions. Other problems centre on those influenced by drought and the irrigation regimes designed to counteract it. Everywhere irrigation is used, the potential exists for encouraging late blight and early blight, both of which need a period of leaf wetness for infection to develop. Other diseases, such as Verticillium wilt and blackleg caused by *Erwinia chrysanthemi*, are directly favoured by warm temperatures, and the complex of post-harvest diseases is also influenced by the temperature at which the crop is grown and stored.

Applying protective chemicals to the soil before planting or to the foliage during growth, and employing judicious crop rotation, manuring and other cultural techniques are useful, albeit often expensive, measures; but it is to resistant or tolerant cultivars that the grower must ultimately turn to achieve long-term improvements in the economics of production and the quality of the crop.

Abiotic stress factors The most obvious abiotic stress on the growth of potatoes in hot climates is shortage of water, which has to be alleviated by irrigation. In the potato growing area around Valencia in Spain, water is supplied to fields by flood irrigation via a complex system of canals and ducts, but in Majorca and Cyprus it is supplied by overhead sprinklers and is drawn from underground aquifers. Unfortunately, this underground source is becoming more and more brackish. In Israel, where most water is obtained from the sea of Galilee, the demand for 'sweet' water is beginning to exceed supply. There are, however, substantial underground aquifers of brackish (salt) water under the desert, and Israeli irrigation experts have developed sophisticated systems to exploit this source. It is important that the salt in the irrigation water does not encrust the leaves nor accumulate in the rhizosphere. By 'flushing' the system with fresh water at the end of each irrigation cycle, these problems can be ameliorated. However, collaborative research between SCRI and Gilat Experiment Station demonstrated

Figure 3 SCRI clones harvested in the Negev desert showing 'resistance' and 'susceptibility' to chocolate spot.

that the use of brackish irrigation water greatly exacerbated the effects of diseases caused by *Verticillium* and *Alternaria*. The value of disease resistant or tolerant genotypes is therefore much greater in such conditions.

An important physiological defect which led to the demise of many otherwise promising clones is internal necrosis of the tuber flesh, termed 'chocolate spot' in Israel. The causes of this problem are still unclear, but calcium metabolism has been implicated and it is also associated with high soil temperatures during the growing season. Fortunately, there is a heritable component to this phenomenon and 'resistant' clones have been identified amongst SCRI germplasm.

Diseases Important pathogens infecting irrigated potato crops in the Negev desert of Israel include late blight (*Phytophthora infestans*), early blight (*Alternaria solani*) and *Erwinia* spp., particularly *E. chrysanthemi* and *E. carotovora* subsp. *carotovora*. Late blight is potentially serious on susceptible cultivars given overhead irrigation. The high daytime summer temperatures do not favour its spread, and resistant cultivars produced in the Institute's breeding programme are resistant also in the Negev.

Early blight Early blight occurs worldwide but is an important disease of hot climates where heavy dew at night favours infection and spore formation, dry windy days favour spore dispersal, and dry weather enables spores to survive from one growing season to the next on infected plant debris. Necrotic lesions develop on the leaves as the plant matures and becomes progressively more susceptible. These coalesce causing the leaves to drop, resulting in considerable loss in yield[1]. The disease is controlled using fungicides, but treatment is often only partly effective, achieving at best a reduction in disease severity, even in well sprayed crops[2]. Control through breeding

Figure 4 Symptoms of *Alternaria solani*, early blight on potato foliage in the Negev.

resistant cultivars is the only long-term solution to the problem. Collaboration with Gilat Experiment Station in the late 1980's enabled the resistance of SCRI clones to be assessed in the Negev. The cultivar Stirling was shown to be resistant to early blight, late

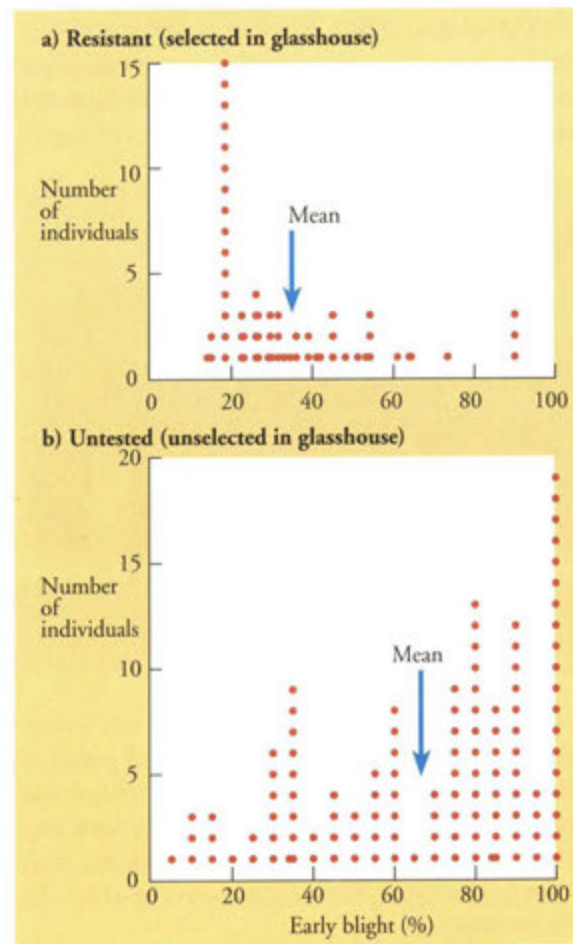

Figure 5 Early blight trial in the Negev of seedlings found resistant in a glasshouse test (a) and those not previously tested (b).

Figure 6 Alternaria infected plots, Negev, showing range of susceptibility and resistance in SCRI clones.

blight, soft rot and blackleg, as well as tolerant to *Verticillium*.

In order to breed for resistance, good parents need to be identified and the inheritance of resistance studied. This is facilitated by using glasshouse tests which enable large numbers of individuals to be assessed at the earliest possible stage in the breeding programme. The resistance detected in the glasshouse, however, must reflect field behaviour, and more recent collaboration at Gilat has shown that results of glasshouse tests developed at SCRI on eight cultivars of differing resistance to *A. solani* agreed well with field assessment in Israel[3]. The level of resistance can be assessed in the glasshouse using adult plants grown from tubers, enabling the screening of potential parents prior to field evaluation. A further trial at Gilat confirmed that it was possible to detect high levels of resistance in true seedlings inoculated in the glasshouse 55 days after sowing (Fig. 5), and that resistance can be selected effectively in the glasshouse within one year of crossing.

Blackleg Collaborative work at Gilat in the Negev and with Luis Matutano SA in Valencia has confirmed the effect of *Erwinia* on seed tubers on yield, irrespective of whether blackleg symptoms develop on the growing plants. In a trial of 15 cultivars near Valencia, tubers which had been inoculated with *Erwinia carotovora* subsp. *atroseptica* (Eca) had an average yield per plant that was 8% lower than uninoculated tubers planted whole, and 12% lower when they were cut into pieces before planting. Very little blackleg was observed in the trial.

Work in Israel has also indicated that plants grown from seed tubers heavily infected by Eca, which subsequently gives rise to latent stem infection, tend to be more susceptible to *Verticillium dahliae*[4] and hence have a shorter period of vegetative growth when the fungus is also present. Eca also has an effect on yield, particularly when the plants are infected with *A. solani* or *V. dahliae*[5]. In field trials in the Negev, cultivars have been categorised as resistant, intermediate or susceptible with respect to Eca and early blight, but the appearance of symptoms of *V. dahliae* coincided with the maturation of the foliage, so apparent susceptibility or resistance were confounded with senescence. Cultivars Pentland Squire, Baillie and Ailsa were among the cultivars whose yield was least affected by Eca and *A. solani*[2].

Conclusions By combining routine trialling of advanced potato clones as potential cultivars with investigative research, it has been possible to understand some of the factors which affect the adaptability of potatoes to warm climates. It has also provided the means whereby more purposive breeding strategies could be developed. However, the most tangible evidence of the benefits of this work has been to demonstrate that the genetically broadly based breeding programmes of SCRI can produce cultivars in the UK that are well suited for export to diverse warm climates. The cultivar Stirling is the most advanced product, and several other clones have performed very well in overseas trials run by SCRI's commercial partners.

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Endosperm cell walls - barriers to malting quality

J S Swanston, R P Ellis & J-L Molina-Cano

During malting and brewing, starch in the barley grain is converted to sugars by enzymes produced in the grain and the sugars are then fermented by yeast to produce alcohol. These steps are common to both beer brewing and whisky distillation, the latter requiring about one third of the malt utilised in the

Figure 1 A malt whisky distillery in the Scottish highlands.

UK. As all its distilleries (Fig. 1) are located in Scotland, the malt whisky industry constitutes a potentially major customer for Scottish malting barley. The suitability of a particular barley cultivar for brewing or distilling is controlled by the quantity and availability of starch contained in the endosperm. The endosperm forms two thirds of the total volume of the grain and it has a cellular structure with starch stored in granules in a matrix of protein.

Plant cells are surrounded by walls which, in the case of the barley endosperm, are composed mainly of β-glucan. The walls form an initial barrier to enzymes which degrade starch within the cells. Consequently, considerable research has been directed towards understanding cell wall structure, and breeding objectives have included selection for thinner walls which are quickly broken down. In addition, β-glucan, which survives malting and mashing, increases wort viscosity making filtration difficult. Brewers require the β-glucan to have been solubilised and degraded during malting, particularly as the enzymes which carry out this function are denatured quickly at the temperature required to bring the starch into solution.

The microscopic structure of barley endosperm from unmalted and malted grains is shown in Figure 2a-c. In the unmalted grain a network of cell walls is visible throughout the endosperm but, during the malting process, most of the cell walls are degraded.

In the unmalted grain β-glucan exists in two forms whose properties are determined by slight differences in molecular structure which controls their solubility in water, and they are referred to as soluble and insoluble β-glucan. The quantities and relative proportions of the two components are affected both by the cultivar and environment in which it is grown.

Malting barley cultivars from north-west Europe do not grow successfully in Spain, apart from a relatively small area near to the north coast, and breeders have produced cultivars specifically for the Spanish malting market. One approach has been to improve the malting properties of existing, high yielding cultivars by directed mutation. The cultivar, Troubadour, which

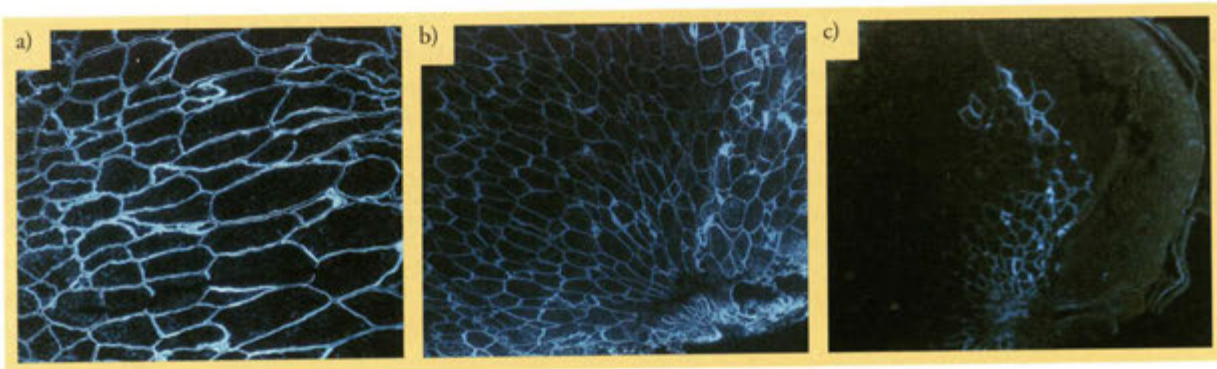

Figure 2 Transverse sections through barley grains with cell walls, to which a fluorescent dye is attached, observed by microscopy under UV light. (a) Unmalted (b) Malted 3 days (c) Malted 7 days.

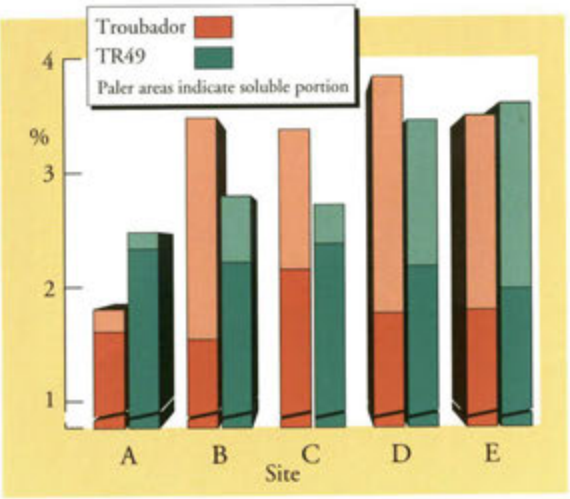

Figure 3 β-glucan contents of Troubador and TR49 grown at 5 sites in Spain.

both germinates and malts slowly, was treated with a chemical mutagen and mutants with the most rapid growth were selected by a germination test. One of these, TR49, with both improved agronomic and malting characteristics was identified and results from a range of sites in Spain showed it to have a higher proportion of insoluble β-glucan than its parent (Fig. 3). The levels of total β-glucan in Troubadour and TR49 varied between environments.

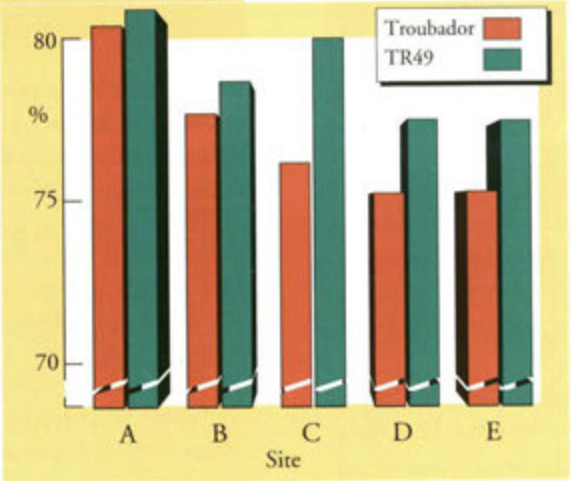

Figure 4 Hot water extracts of Troubador and TR49 grown at 5 sites in Spain.

TR49 had a higher level of hot water extract at all sites (Fig. 4), but it also had higher levels of milling energy which contrasted with the usual association of lower milling energy with improved extract. Grain of both genotypes from five sites were malted and samples taken each day during the malting process. After steeping and 1 day of germination, the milling energies of the TR49 samples remained higher than those of Troubadour (Fig. 5) but, by the third day of germination, TR49 samples from four of the sites gave lower values than those of Troubadour. Since a reduction in milling energy is associated with the breakdown, or modification, of the endosperm structure, the results suggested that modification was proceeding more rapidly in the mutant genotype.

Although TR49 also showed higher levels of β-glucanase activity than Troubadour at all sites, there was no obvious association with milling energy loss. In addition, no association between β-glucanase and extract was found and it was concluded that the differences between the genotypes related to the endosperm structure rather than the activities of the enzymes disrupting that structure during malting.

Comparisons of malting properties between unrelated cultivars are often of limited use since differences exist in so many genetic factors that it is difficult to identify those of most importance. Troubadour and TR49 are closely related genotypes and have similar genetic backgrounds. However, they clearly differed for a genetic factor which affected the structure of the endosperm cell walls. All of the preliminary data on malting properties was derived from Spanish sites and a comparison of grain grown in Spain and Scotland gave the opportunity to observe the effects of contrasting environments. In the first year, the results indicated that differences between environments were much greater than those between genotypes, with strong evidence of a genotype x environment interaction for some characters. The most noticeable effect was that milling energy of the unmalt-

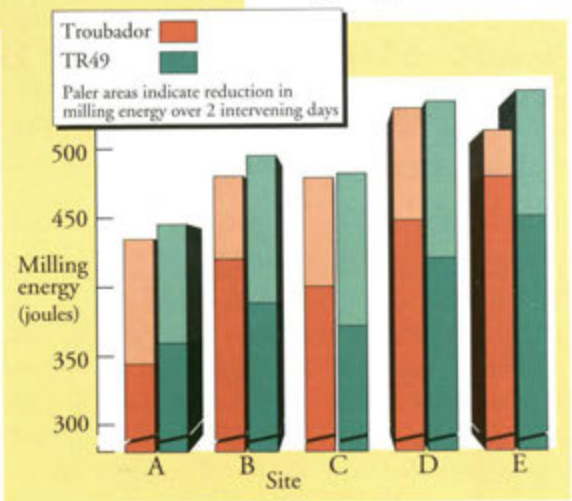

Figure 5 Milling energies after 1 and 3 days germination during malting of Troubador and TR49 grown at 5 sites in Spain.

ed grain grown in Scotland was lower in TR49 than in Troubadour, the reverse of the result from Spanish grain.

When the genotypes were malted, however, the Spanish-grown samples of both cultivars modified more rapidly than the Scottish-grown as demonstrated both by the reduction in milling energy, and the extent of cell wall breakdown detected using a fluorescence technique. A plot of cell wall modification (logarithmic scale) against milling energy reduction showed a linear relationship (Fig. 6). Samples were taken after 4 and 5 days germination during malting and after 4 days, the Spanish-grown samples of both genotypes had modified more extensively than the Scottish-grown after 5 days. Samples collected earlier in the malting process had demonstrated higher levels of both total and soluble β-glucan in the Scottish-grown samples compared to their Spanish-grown equivalents.

Although the effects of environments differed from those of previous experiments, the importance of cell wall structure and breakdown to the malting properties of the two cultivars remained consistent. Preliminary results from the second year established that differences between sites in the relative quantities and proportions of cell wall components were also associated with the differences in water uptake during steeping.

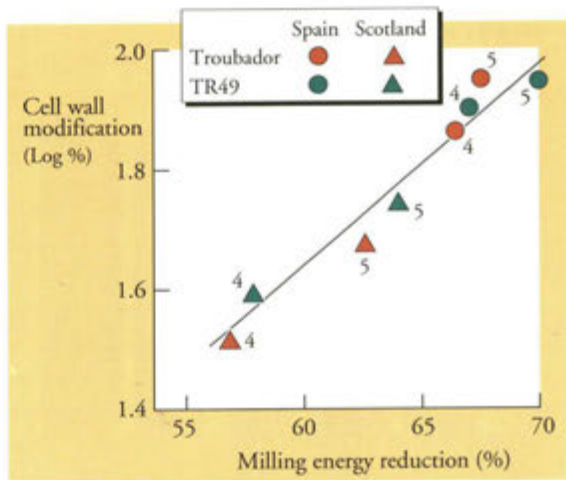

Figure 6 Relationship between cell wall modification and milling energy reduction during malting in Troubadour and TR49 grown in Spain and Scotland. Numbers indicate days of germination during malting.

Experiments done previously (*Annual Report*, 1990, pp. 16-20) suggested that there was a relationship between milling energy reduction and cell wall modification during malting which varied between cultivars of differing malting quality. Both that research and the joint work carried out with our Spanish colleagues have emphasised the importance of rapid and extensive cell wall modification to the attainment of good malting quality.

Case studies in the investigation of potential industrial oil crops

S. Millam, A. Craig & W.W. Christie

Oilseeds were among the first crops domesticated by man, with fossil evidence indicating the use of linseed from about 8000 years ago. Oil crops continued to be important for a range of uses up until the early twentieth century when the availability of large amounts of cheap mineral oil derived from fossil fuels resulted in a decline in the use of vegetable oils for non-food applications. Currently, the commercial production of seed oils is primarily concerned with their utilisation as an edible component of the human diet. The worldwide market for vegetable oils has increased by 300% since 1960, driven by the growth in population, increased production and export of oil crops from tropical countries, and the increase in the standard of living in the western world. An interesting factor to be considered in this context is that the world market share supplied by animal fats fell from 39% of the total in 1960 to 26% in 1990, probably due to 'lifestyle' changes, particularly the increased awareness of dietary factors. A great deal of conventional breeding, i.e. years of crossing, selection and backcrossing, has been applied to produce and

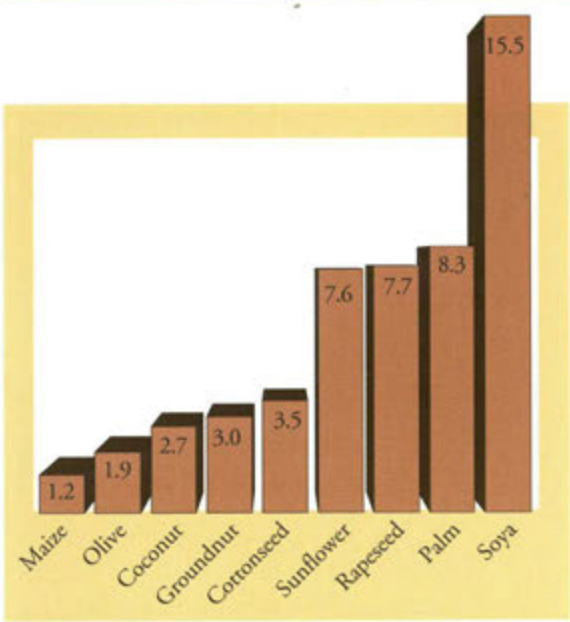

Figure 1 World production of major vegetable oils (Mt) (Source: Oil World Annual, 1988).

improve today's vegetable oil crops, and the principal world vegetable oil production pattern is shown in Figure 1.

Of these figures, only 18% of the total are designated for non-food usage. However, recent economic forecasts have predicted that the markets for plant oils for industrial application have potential for significant expansion (Oil World Forecast, 1988). A possible area for increased consumption is the use of short-chain fatty acids in the manufacture of soaps and detergents. An important component in their manufacture is lauric acid, normally derived from imports of palm kernel oil with a value of £120 million p.a. in the UK alone. Another area is the use of long-chain fatty acids as lubricants, solvents for pesticides and sprays, and in the huge and expanding market for industrial plastics, such as in car manufacturing.

Considerable biodiversity exists among plants for such traits as novel oils and proteins and high-value secondary metabolites. Since seed oils with unusual fatty acid profiles tend to be nutritionally less valuable, indigestible or even toxic, they have not been represented among the traditional oil crops grown in world agriculture. It is only recently that such

Figure 2 *Coriandrum sativum*, the common herb 'Coriander'

undomesticated plants have generated interest, not only for scientific reasons, but also as potential replacements for mineral oil derived products.

There are three fundamental strategies for the development of crops for industrial oils.

1. To search, evaluate, select and domesticate wild plant species.
2. To evaluate and improve forms of existing under-developed or under-utilised plant species.
3. To modify existing crops by means such as genetic transformation.

At SCRI we have adopted the latter two strategies and will illustrate these approaches by two case studies.

Coriander. *Coriandrum sativum*, though widely grown in the middle east and North Africa, also has a history in the UK as a minor seed and leaf crop. Among the uses for its seeds are as a herb or aromatic in curries and as a flavouring for gin. The seeds con-

Short Chain	(<C12)	Nylon, plastics, lubricants, detergents
Medium chain	(C12-C18)	Surfactants, emulsifiers, soaps, adhesives, cosmetics
Long chain	(C18-C22)	Inks, coatings, plastic additives, fabric softeners
Very long chain	(>C22)	'Non-fat' nutritional factors, high value lubricants

Table 1 Potential industrial usage of fatty acids, and their derivatives.

tain 13-18% dry weight of oil which we have shown to be composed of up to 82% petroselenic acid, an isomeric form of the common C18:1 oleic acid . This acid could be oxidatively cleaved to form a C12 lauric acid (with industrial usage in soaps and detergents) and also a C6 dicarboxylic acid for use as a feedstock in the manufacture of nylon. Considerable effort was made at SCRI to develop an analytical method for the determination of petroselenic acid as it was difficult to distinguish it from its isomer oleic acid[1] using GC techniques. In addition, preliminary plant tissue regeneration systems have been developed to facilitate genetic transformation of the crop. However, potential problems precluding the widespread adoption of genetic transformation approaches for the alteration of the fatty acid content of plants are not necessarily scientific or technical constraints but focus on environmental and ethical issues associated with the release of genetically engineered organisms into the environment. To circumvent such problems and also to offer an alternative approach towards the modification of the existing crops of oilseed rape, the Ministry of Agriculture Fisheries and Food are funding a three-year project at SCRI to investigate the potential of applying plant tissue culture techniques to alter the oil profile of oilseed rape.

Oilseed rape *Brassica napus* L. has been cultivated in northern temperate areas for several centuries although its oil was largely replaced by imports of other oils which became available in the late 19th century. However, the worldwide shortage of vegetable oils that followed the second world war restored interest in rapeseed as an oil crop. In Britain, the area grown has risen from 2,000 ha in 1972 to 300,000 ha in 1992. The reasons for this expansion are mainly that it replaced the use of imported tropical oils and, more importantly, the EC provided subsidies for oil crops to ensure that Europe would not become reliant on imports of oil or be subject to the vagaries of world trade price fluctuations.

Most oilseed rape is used to produce a relatively nutritious but low-value cooking oil. However, whilst *B. napus* is of fairly recent origin, with no truly wild forms, there exists great genetic diversity within its constituent diploid progenitors which may be accessed and exploited for the manipulation of its oil content.

Conventional breeding can slowly introduce some of the characteristics of some of this material, but only if the crossing species are sexually compatible. This is a major constraint as traits of interest that occur in wild

Figure 3 The triangle of 'U' - *Brassica napus* (front centre) and its diploid ancestors *Brassica rapa* (front left) and *Brassica oleracea* (front right). There are also cytogenetic relationships with the other species, *Brassica nigra* (back left) commonly known as Black Mustard, *Brassica juncea* (back centre) known as Indian Mustard and *Brassica carinata* (back left) known as Ethiopian Mustard.

or non-related species cannot be introgressed readily into the crop plant if the species are sexually incompatible. However, *Brassica napus* has been shown to be amenable to all aspects of plant tissue culture[2,3,4] and it is the exploitation of two aspects of this science that forms the basis for our research.

Somaclonal variation is the phenotypic variation observed from plants derived from any form of plant cell culture using somatic cells. Plant tissue is exposed

Figure 4 Regeneration of shoots from internodal explants of *Brassica napus*.

	Oleic	Linoleic	Linolenic	Erucic	Nervonic
Barbarea intermedia	16.7	14.5	10.5	40.9	1.0
Crambe abyssinica	19.2	11.6	6.5	47.1	0.7
Lunaria biennis	25.9	6.7	1.1	44.9	16.5
Thlaspi montanum	13.9	19.9	4.9	37.9	3.4

Table 2 Fatty acid profiles of a range of wild cruciferous species. Means of three samples, % total.

to an artificial environment in culture, and may also be subjected to mutagenic plant growth regulators. Thus, some genetic change is inevitable and, although this will be deleterious in most cases, in others it can generate advantageous characteristics. In our work, a number of experiments were initiated to regenerate oilseed rape plants via tissue culture with the aim of inducing changes in fatty acid profile via somaclonal variation.

The regenerant products were examined in a replicated glasshouse trial and morphological assessments were made, such as plant height, leaf colour, and number of seeds, for use as potential markers of genetic variation. The seeds were analysed by extracting the fatty acids as methyl esters and both the content and profile of the fatty acids present were determined by gas chromatography.

Figure 5 Replicated glasshouse trial of seed-derived, micropropagated and somaclonally-derived *Brassica napus* cv. Topas.

The variability of percentage oleic acid content as a 'marker' for diversification within a population of seed-derived, micropropagated and somaclonally derived *B. napus* plants was investigated. Both the micropropagated and somaclonally derived material had a higher mean level of oleic acid compared with the seed-derived population and up to 13% of the total population of somaclones had significantly altered levels of oleic and other C18 fatty acids.

Protoplast fusion involving two somatic plant cells from different genotypes to form a hybrid plant containing a random mix of the two parental genomes has great potential to circumvent the barrier of sexual incompatibility. There are two principal methods for fusing protoplasts, (1) using chemicals such as polyethylene glycol, or (2) electrofusion, the method in principal use at SCRI. Protoplast regeneration protocols have been developed at SCRI for a range of commercial spring, winter and high erucic acid rapeseed (HEAR) lines. A range of potential fusion partners for oilseed rape were screened using GC techniques, and the results are presented in Table 2.

A programme of protoplast fusion to create hybrids between *B. napus* and *Lunaria biennis* has been initiated. There have been no previous reports of tissue culture of *Lunaria*. However, techniques have now been developed in our research for the isolation and fusion of protoplasts of this genus.

In summary, our work indicates that there are several tissue culture-based approaches that can, under defined conditions, produce material of great interest for the incorporation of novel plant material into genetic improvement programmes. These approaches will allow us to circumvent some of the difficulties in handling and integrating the products of *Agrobacterium*-mediated transformation and related techniques involving genetically modified organisms (GMOs) into crop improvement programmes used elsewhere.

This work was supported by the Ministry of Agriculture, Fisheries and Food.

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New potato cultivars

Cramond

Buchan

Brodie

Cramond

A high yielding, early maincrop cultivar placed on the National List in 1992. It produces an attractive sample of exceptionally uniform and regularly oval-shaped tubers with a white skin which is well suited to the prepack trade. It has good cooking qualities with good dry matter levels, though not high enough for processing. It has high resistance to foliage and tuber blight, gangrene, PVY and PVX and moderate resistance to common scab and PLRV.

Buchan

A maincrop cultivar consistently returning significantly higher yields than the National List control cultivars, Desirée and Pentland Crown; added to the National List in 1993. The white skinned, oval tubers have moderate dry matter and good cooking qualities. This cultivar combines high levels of resistance to blackleg and leafroll virus, with moderate resistance to tuber blight, common scab and external damage. It is also resistant to Ro1 potato cyst nematode (*G. rostochiensis*).

Brodie

A high yielding maincrop cultivar placed on the National List in 1993. It produces attractive, uniform, parti-coloured (pink splashed) tubers with excellent table cooking qualities of intermediate dry matter with a high 'baking' fraction. It is very resistant to blackleg and tuber blight, with moderate resistance to common scab and damage. It is also resistant to Ro1 potato cyst nematode (*Globodera rostochiensis*).

	Cramond	Buchan	Brodie
Origin	7683a12 x10333ab32	Croft x Cara	Croft x Cara
Year of cross	1978	1981	1981
Maturity	Early maincrop	Early maincrop	Early maincrop
Wart	Field immune - RG1	Field immune - RG1	Field immune - RG1
Late blight foliage	6	4	5
tuber	8	7	8
Gangrene	7	5	5
Dry rot	6	6	5
Skin spot	3.5	-	4
Common scab	6	6	6
Virus: PVY	7	Under test	Under test
PLRV	3	Under test	Under test
PCN: G. rost.	Susc.	9	9
G. pall.	Susc.	Susc.	Susc.
Blackleg	7	8	9

New Swede Cultivars

Airlie
Airlie was placed on the National List in 1991. It is a general purpose swede with very high fresh yields of low dry matter content resulting in average yields of dry matter. It has purple skinned, intermediate shaped roots with cream coloured flesh. It has good resistance to powdery mildew. It is being marketed by Nickerson Seeds Limited.

Airlie

Brora
Brora was placed on the National List in 1993. It was selected as a shopping swede, although it can be fed to livestock. It has the attractive deep purple skin colour and globe shape of the traditional shopping cultivar, Acme, but with the advantage of its skin not becoming corky. It is yellow fleshed and gives average yields of dry matter at a low to medium dry matter content. It is being marketed by Sharpes International Seeds Limited.

Brora

Kenmore
Kenmore was placed on the National List in 1994. It gives very high yields of both fresh and dry matter at a low to medium dry matter content. As a white fleshed swede, it could be used for processing, but it is primarily intended for feeding to livestock. It is bronze skinned and has good powdery mildew resistance. It is being marketed by Nickerson Seeds Limited.

Kenmore

New soft fruit cultivars

Raspberries

Glen Rosa (7815A12), derived from a complex cross involving Glen Prosen and Meeker and provides a combination of high yield and outstanding quality. The canes are spine-free, upright and of good vigour. Internodal distances on floricanes are short and fruiting laterals are erect, giving excellent fruit presentation. Fruit is bright and weighs between 3-5.5 g. Plants have genes H, A_{10} and B_u which confer resistance to all five biotypes of *A. idaei* together with resistance to spur blight, cane botrytis and the common strain of raspberry bushy dwarf virus (RBDV). Importantly, at a time of increasing use of mechanical harvesters, Glen Rosa provided good machine harvested samples some 8 days later than Glen Clova.

Glen Ample (7815B8) is a sister seedling to Glen Rosa, is higher yielding, but has slightly inferior fruit setting ability under cool conditions. Canes are very upright, vigorous and spine-free. It has a similar season to Glen Rosa but has a considerably longer harvest period than Glen Clova.

Glen Shee (8044C9) was raised in 1980 from a complex cross of SCRI breeding lines. Although not suitable for machine harvesting because of its relatively brittle pedicels, the large, pale and firm textured fruit have provided excellent canned, frozen and fresh samples. Its 50% pick date is 9 days later than Glen Clova and it has an exceptionally long season of some 37 days. The primocanes are also upright, vigorous and spine-free.

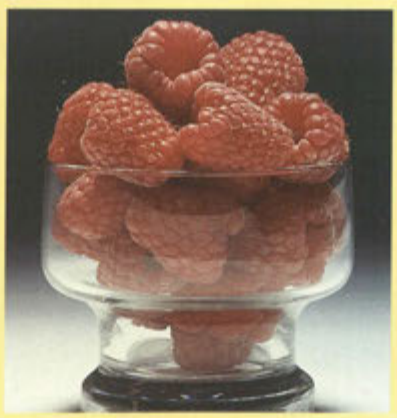
Glen Magna (8032A3) has a fruit season about 17 days later than Glen Clova. The canes are sparsely spined, upright, vigorous and have very well presented laterals. The very large dark fruit, which have frequently been weighed at over 9 g have an excellent flavour and appearance. It is expected that this cultivar will be particularly suited to the amateur and PYO markets.

Symphony

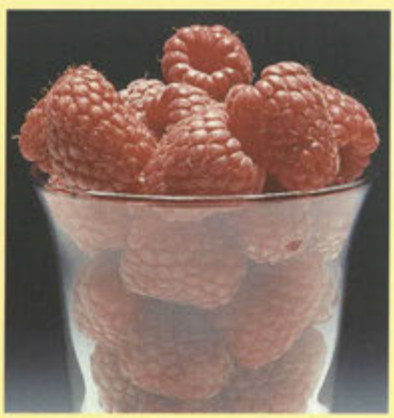
Strawberry

The new strawberry, **Symphony**, was raised from a cross between Rhapsody and Holiday. It has produced higher yields than standard trial cultivars with a very low percentage of unmarketable fruit. The season is 8-10 days later than Elsanta to which the fruit are similar in many respects in that they have a bright glossy appearance, excellent shelf-life, and are juicy with a very good flavour. The cultivar is regarded as being more suited to the fresh fruit market than for the processing trade. It runners exceptionally well and has field resistance to red core.

Glen Rosa



Glen Shee



Glen Magna

Molecular biology

B.P. Forster

In 1988 the international Human Genome project was initiated, this and subsequent developments in genetic fingerprinting and gene therapy have generated considerable interest in molecular biology. Public interest was further stimulated this year with the release of Spielberg's blockbuster film 'Jurassic Park' in which scientists recreated dinosaurs from preserved DNA fragments. Whilst 'Jurassic Park' remains science fiction it has focused attention on the rapid pace at which molecular genetics is proceeding. The potential of DNA technology to improve the quality of life is now being realised with the development of new methods in disease control, the production of improved foodstuffs and the development of novel products from transgenic crops. Such products are beginning to be marketed and the so called 'Genetic Revolution' is now responsible for the fastest growing industry in several countries, including the UK. With widespread media coverage, public interest, debate on moral and environmental issues, commercial exploitation and above all scientific achievement, it is an exciting time to be involved in molecular biology.

At SCRI molecular biology is applied to various studies of plants, particularly crop plants, and their associated pests and diseases. The work is carried out increasingly at the international level with scientific exchange being a key element to our success. There is a continual turnover of postgraduate students, postdoctorate researchers and visiting scientists who contribute significantly to our science and create a dynamic research environment. One feature of SCRI which helps to attract visiting workers is the multi-disciplinary research base which provides greater opportunities for visiting workers to exploit their research findings. Our international connections are further enhanced by SCRI scientists working abroad and learning new techniques. Another important factor which has accelerated research in molecular genetics is the increasing automation of basic techniques.

Many opportunities now exist for the exploitation of genetic markers in crop improvement programmes. Central to this challenge is the requirement to locate markers to specific regions of the genome which are important in controlling the expression of traits of economic or biological significance (see following articles on salt tolerance and $\delta^{13}C$ values in barley). The identification of major genes and genetic factors influencing quantitative traits has been greatly facilitated by the development of genetic maps, e.g. for barley (*Ann. Rep. 1991*, 31-33). These maps have become more detailed as new marker techniques have evolved.

The majority of maps are based on restriction fragment length polymorphisms (RFLPs), but the application of the polymerase chain reaction (PCR) has significantly increased the number of markers which can be applied to genome analysis. One PCR-based method which produces randomly amplified polymorphic DNA (RAPDs) markers has continued to be used successfully by the Plant Genome Analysis Group. For example, RAPDs have been used in genetic diversity studies of a number of crop species and have been used in conjunction with pooled DNA samples to detect genes of special interest, e.g. to reveal two loci controlling green plant formation in barley microspore culture. One of the most useful techniques currently being developed is the analysis of simple sequence repeats, or microsatellites. These repeated sequences have been shown to be highly variable and widely distributed in genomes and are therefore of great interest. The application of microsatellite technology in molecular genetic research at SCRI is described on p35 below.

Dr. B.P. Forster, Acting Head of the Cell & Molecular Genetics Department

One exciting aspect of molecular biology is that certain fundamental knowledge and technologies are now developed to such an extent that they can be applied more generally. The development of genetic linkage maps and transformation techniques are two areas which can now be exploited for a number of studies with the targeting of specific genes being pre-eminent. This is illustrated in articles in this report on the use of transformation in characterising the S-adenosylmethionine decarboxylase (SAMDC) gene of potato, and the use of transgenics in the analysis of plant spliceosomal proteins.

The study of specific genes is often stimulated by industrial interest, one such programme is the study of cold temperature sweetening in potato by the Carbohydrate Metabolism Group. This programme has industrial and EC sponsorship and is aimed at modulating levels of invertase in tubers to reduce hexose accumulation during cold storage. Five invertase (ß-fructofuranosidase) genes from potato have now been characterised. Two are arranged in tandem on potato chromosome IX separated by 1.75 kb. The development of a combined reverse transcriptase-PCR assay has demonstrated tissue and developmental specificity in their expression. Transgenic plants are being used to determine the wider biological function of these enzymes in the carbohydrate metabolism of different tissues throughout the plant. In addition, invertase promoter function, the significance of a miniature 9 base-pair exon, and the targeting signals determining localisation of potato invertases are being examined.

RNA processing is not only essential as a fundamental aspect of plant gene expression, but also illustrates the complex interactions between molecules which is the basis of gene transcription, mRNA splicing, polyadenylation and transport, and translation into proteins. Research within the RNA Processing Group has led to the first demonstration of a requirement for a branchpoint in plant intron splicing (*Ann. Rep. 1991*, 42-44). The isolation of genes of two highly related spliceosomal proteins, U1A and U2B", is extremely relevant to studies of RNA binding proteins (*Ann. Rep. 1990*, 28-30). Human U1A and U2B" provide a model for RNA binding specificity and the evolutionary comparisons now possible with the cloning of plant analogues have identified amino acids which may be involved in binding specificity. The development of an epitope tag system based on a monoclonal antibody produced by Dr L. Torrance in the Virology Department will be invaluable in analysing the sub-cellular localisation of proteins involved in RNA processing. Finally, the knowledge of plant splicing and ribosomal RNA processing and their components is being channelled into applied biotechnology programmes in the development of novel vectors and identification of novel promoter sequences.

Development of a generic microsatellite-based PCR assay for the detection of genetic variation

W. Powell, J. Provan, K.J. Chalmers & R. Waugh

The detection of differences in the sequence of DNA molecules is the basis of modern genetic analysis and DNA profiling is established as an essential feature of both theoretical and experimental biology. Initially, profiles were revealed by a hybridisation-type assay but the recent development of Polymerase Chain Reaction (PCR) technology has significantly increased the variety of approaches available for the detection of sequence variation. As an alternative, one such method which has found widespread application is Random Amplified Polymorphic DNA or RAPDs. The advantage of RAPD analysis is that it is immediately applicable to any organism as no prior sequence information is required to generate the amplified DNA profiles. However, RAPDs generally behave as dominant markers and are often difficult to transfer from one population to another. In addition, the sensitivity of RAPDs to small changes in either the reaction conditions or components is a negative feature of the assay. We have been exploring ways of exploiting characteristic sequences known as 'microsatellites' to develop generic, multi-locus PCR-based assays for DNA profiling. Microsatellites are short stretches of DNA which consist of a repeated array of simple di-, tri- or tetra-nucleotide repeats such as $(CA)_n$, $(CAC)_n$ or $(GACA)_n$. The number of repeats at a microsatellite locus has been shown to be highly variable and, as such, microsatellites represent a rich source of allelic diversity which can be exploited in many types of genetical analysis. In addition, microsatellites are both abundant and distributed throughout eukaryotic genomes. However their successful application requires an initial development phase involving cloning microsatellites and characterising their flanking sequences for primer design which may hinder their immediate and wider application. This report summarises some preliminary findings on the use of modified simple sequence repeat, containing primers directly in PCRs for the generation of specific and robust DNA profiles.

The concept behind the approach is that a single, repeat-containing primer should promote inter- as opposed to intra-microsatellite DNA amplification if two microsatellites are closely linked in the genome. Initially, we tested a selection of di-, tri- and tetrameric repeat sequence primers on a range of plant DNAs. Our general observation was that discrete amplification products were generated more frequently in the larger repeat containing primers. The di-nucleotide repeat primers generated a large number of products which resulted in a characteristic smear after electrophoresis. We therefore considered increasing the specificity of the di-nucleotide repeat primers by adding unique, non-repeat sequences to the primer ends. A number of primers consisting of $(CA)_8$ or $(GA)_8$ were synthesised with a four base-pair GC-rich

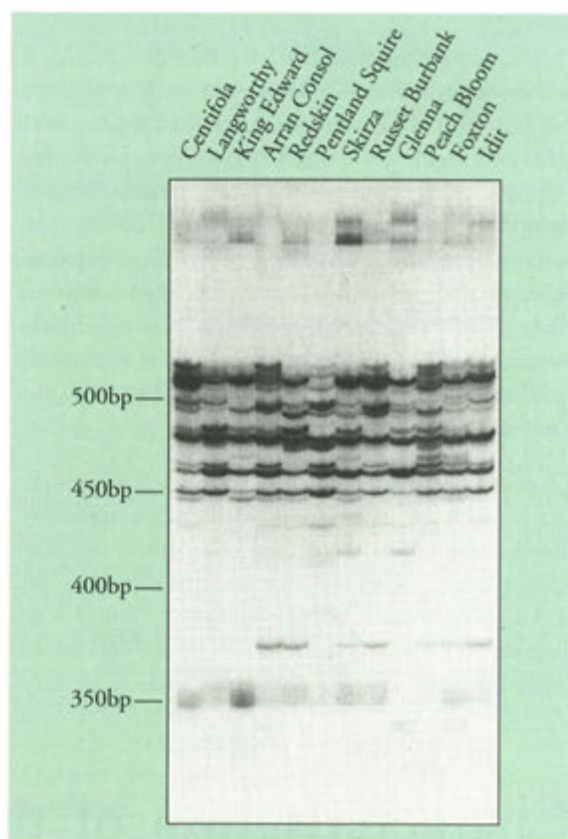


Figure 1 Potato cultivar-specific genetic fingerprints generated by PCR amplification of total genomic DNA with a single 5'-anchored di-nucleotide repeat primer. This single primer is able to differentiate all of the cultivars examined in this experiment. The polymorphisms were detected by separating radio-labelled amplification products on a polyacrylamide gel followed by autoradiography.

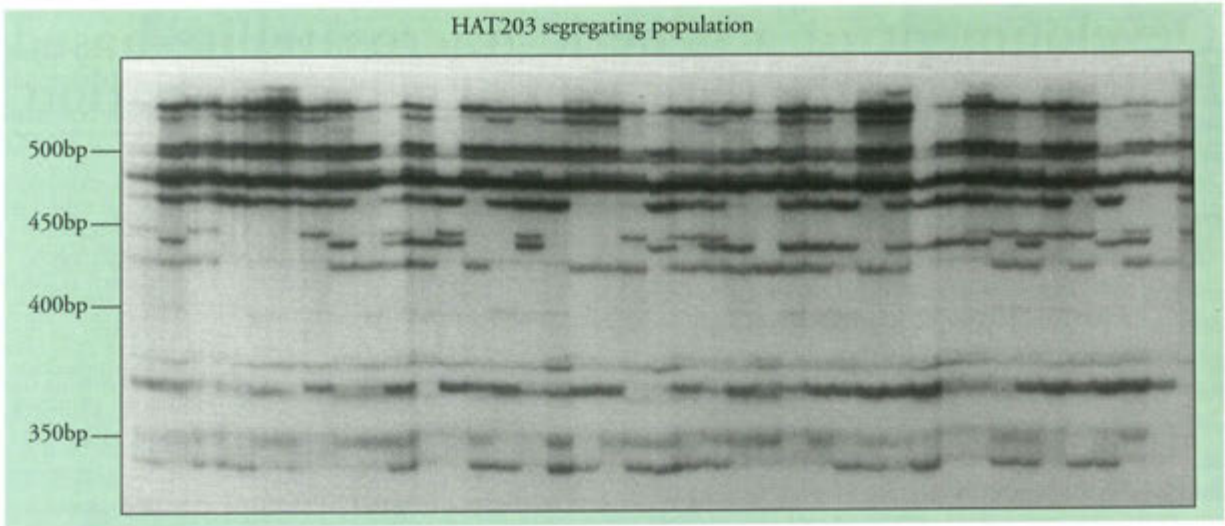


Figure 2 Amplification products generated by a single anchored di-nucleotide repeat primer from the DNA of a number of individuals from a segregating potato population. A number of amplification products clearly segregated in the potato population and these have been located to specific potato chromosomes. Products were separated on a polyacrylamide gel and revealed by autoradiography.

anchor at their 5' end. Adding the anchor sequence had the effect of reducing the number of products and generated highly polymorphic, genotype-specific fingerprints. Figure 1 shows the type of results obtained from this type of analysis on a range of potato cultivars. Importantly, changing the anchor sequence changed the profile of the amplification products. These results suggested that a large number of polymorphic products could be generated by a relatively small number of anchored simple sequence oligonucleotide primers.

To determine both the nature of the polymorphisms and their usefulness as genetic markers, we monitored their inheritance in segregating populations of barley and potatoes. Using two oligonucleotide primers and product separation on agarose gels, 12 loci were mapped to specific regions of the barley and potato genomes. However, separating the products on acrylamide gels provided a marked increase in resolution and increased the number of segregating polymorphic bands which could be scored in the populations. An example of the products generated from one of the primers in a selection of progenies is shown in Figure 2. To explore whether the approach would be of general value, DNA from a wide range of genetic material including plants, animals, insects and a fungus was examined. Amplification products were generated from every DNA examined and the levels of variation within and between plant species was high. Thus, although these results are preliminary, they clearly demonstrate that anchored simple sequence repeat-containing primers are effective for revealing high levels of variation and therefore represent an addition to the arsenal of tools which are available for detailed genetic analysis. We are currently examining alternative modifications of simple sequence repeats to refine and adapt the outlined approach.

Characterisation of the S-adenosylmethionine decarboxylase (SAMDC) gene of potato

A. Kumar, S.A. Mad Arif, M.A. Taylor & M.J.R. Stark*

S-adenosylmethionine decarboxylase (SAMDC: EC 4.1.1.50) is a key enzyme in the biosynthesis of the polyamines, spermidine and spermine. The substrate for this enzyme is S-adenosylmethionine (SAM) and the product is decarboxylated SAM which provides the aminopropyl moiety required for spermidine and spermine biosynthesis from putrescine[1] (Fig. 1). SAMDC activity has been shown to be rate limiting

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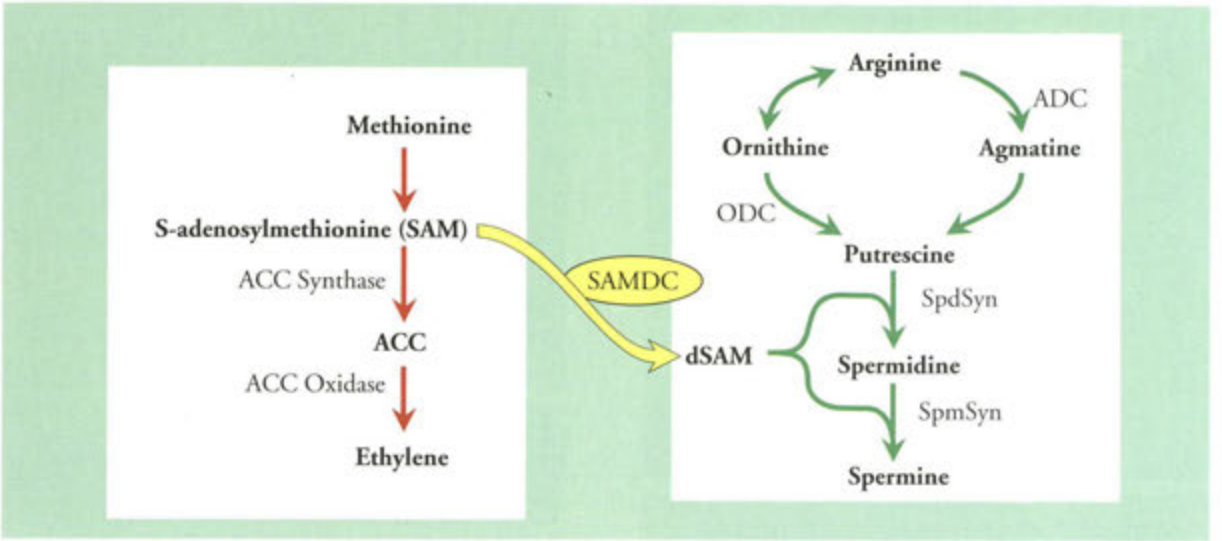

Figure 1 Polyamine and ethylene biosynthetic pathways and their inter-relationships.

for the biosynthesis of these polyamines. Recently, a cDNA clone of a putative SAMDC gene from potato was isolated by differential screening of a swelling stolon tip library[2]. The degree of similarity at the level of deduced amino acid sequence between the potato cDNA and the other eukaryotic SAMDC genes was only 35%.

In the absence of any cloned SAMDC gene from plants and the low degree of sequence similarity between the potato and the other eukaryotic SAMDC genes, it was necessary to confirm that the putative cDNA clone of the potato SAMDC gene does encode SAMDC. To do this, its ability to complement a yeast *spe2* gene (i.e. SAMDC gene) was investigated. This *spe2* null mutant (yeast strain Y342, *MAT∝ ura3-52 leu2 spe2Δ :: LEU2* was kindly provided by Dr Herbert Tabor, National Institute of Health, Bethesda, USA) was generated by a deletion-insertion mutation in the SAMDC gene using a one-step disruption technique in which the coding sequence was replaced by the LEU2 gene[3]. The *spe2* null mutant

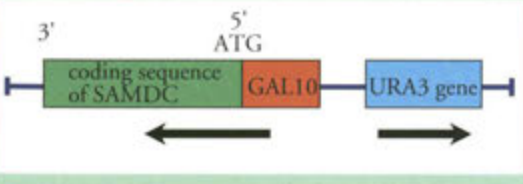

Figure 2 Construction of chimaeric SAMDC gene under the control of a yeast *GAL10* promoter (*YCpGAL10::SAMDC*) in yeast transformation vector YCplac33.

has no detectable SAMDC activity, endogenous spermidine and spermine, and has an absolute requirement for exogenous spermidine or spermine for growth. To express a full-length cDNA clone of the SAMDC gene of potato in yeast, a plasmid (YCpGAL) containing the galactose inducible promoter (GAL10) was used to construct an expression vector, *YCpGAL10 :: SAMDC,* as shown in Fig. 2. This vector was then subcloned into a yeast transformation vector YCplac33 and introduced into the yeast mutant strain by the lithium acetate transformation method. Complementation of the *ura3* mutation in the mutant by the functional gene (URA3) on the vector allowed selection of transformants on medium without uracil. The presence of the SAMDC cDNA sequence in the transformants was confirmed by PCR analysis using a pair of primers specific to the potato SAMDC gene. To test if the expression of the chimaeric SAMDC gene (*GAL10 :: SAMDC*) in the transformed mutant strains was able to complement for the efficiency of SAMDC (thereby restoring the biosynthesis of spermidine and spermine), the transformants were grown in media without exogenous spermidine. The transformed line with the vector containing the chimaeric SAMDC gene grew at a normal rate in the absence of exogenous spermidine (Fig. 3) confirming that the cDNA clone is a *bona fide* SAMDC gene of potato. In contrast, the mutant line transformed with the same vector but lacking the chimaeric SAMDC gene was unable to grow in the absence of any exogenous spermidine (Fig. 3). Furthermore, activity of SAMDC in the transformed yeast line with the chimaeric SAMDC gene was

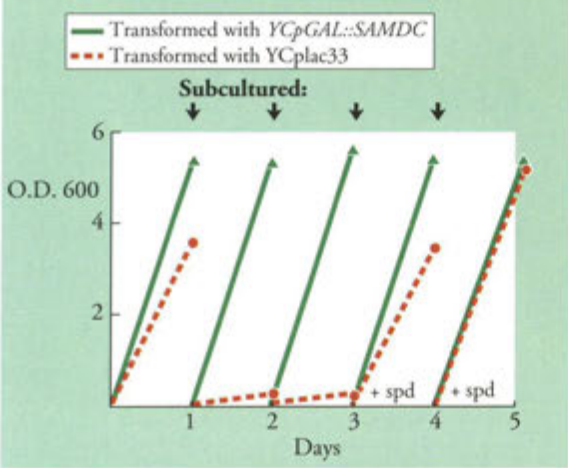

Figure 3 Yeast cell growth measurements of the transformed Y342 mutant line with YCplac33 vector or with *YCpGAL10::SAMDC* vector. Growth was measured by determining the optical density at 600nm in a Beckman spectrophotometer. Transformed yeast lines were grown with or without 1μM spermidine for 24h and then subcultured into fresh medium.
Arrows represent each subcultured stage and +spd represent the addition of spermidine at the time of subculture.

detectable. No SAMDC activity was observed in the original mutant strain and in a transformed line lacking the chimaeric *SAMDC* gene. The results suggested that despite the low level of nucleotide sequence similarity between the SAMDC gene of potato and yeast, functional similarity exists at the enzyme level. This is consistent with some functionally important domains being conserved between potato and yeast, e.g. residues surrounding the cleavage site of the pro-enzyme (LSE'SSLFV). The conservation of the cleavage site suggests that a processing mechanism similar to that shown for other SAMDC pro-enzymes may be operating for the potato enzyme.

Expression of the SAMDC gene was high in the young and actively dividing tissues and low in the mature and non-dividing tissues of both vegetative (*Ann. Rep. 1992*, 44) and reproductive organs (Fig. 4). The highest level of SAMDC transcript was observed in the young flower buds, a lower level could be detected in the mature flowers and in the mature pollen a very low level of the transcript could be seen. This is consistent with the many reports of maximum SAMDC activity and polyamine in rapidly dividing and differentiating tissues.

The physiological functions of polyamines remain unclear despite their ubiquitous presence in all living organisms. In plants, very little is known about either

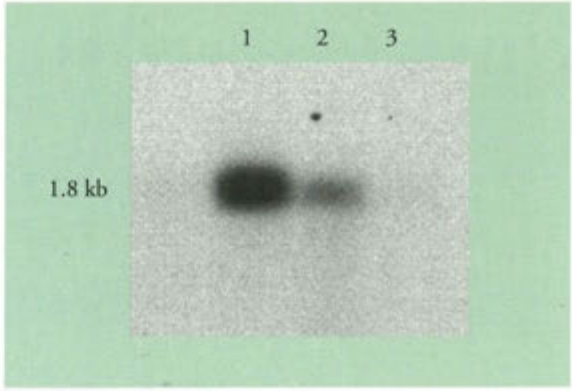

Figure 4 RNA-blot showing the expression levels of the SAMDC gene in (1) buds, (2) flowers and (3) mature pollen of potato.

the mechanisms that regulate polyamine biosynthesis or their subcellular localisation which are both critical to understanding their functions in plant cell proliferation and morphogenesis. Previous studies based on various inhibitors have provided some useful insight into the role played by polyamines in plant cell proliferation and morphogenesis[1]. However, because the inhibitors are non-specific and not well characterised for their permeability and stability in cells, the interpretations of the results are not precise. With the SAMDC gene now available, it will be possible to manipulate polyamine biosynthesis more precisely using cellular and molecular techniques. We have transformed potato with engineered SAMDC genes in both anti-sense and sense orientations to both down-regulate and over-express the SAMDC transcript in transgenic plants. This will allow precise manipulation of the biosynthesis of polyamines and consequently, should provide valuable insight into the functions of polyamines in plant developmental processes. Additionally, any modulations in the expression of the *SAMDC* gene are likely to not only affect the biosynthesis of polyamines but should also affect the biosynthesis of ethylene since SAM is a common precursor (Fig. 1) in both biosynthetic pathways.

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Genetic basis of water use efficiency discovered for barley

B.P. Forster, L.L. Handley, E. Nevo* & J.A. Raven†

There now exists a theoretical and mechanistic basis for relating carbon discrimination in whole tissues of plants with potential water use efficiency[1]. Carbon isotope discrimination (measured as $\delta^{13}C$) provides an integrated record of the photosynthetic and water use history of the tissues sampled. This, in turn, provides an integrated record of all of the plant physiological processes regulating growth. Combined with manipulative experimentation, this is a powerful new tool for use in crop research and crop selection.

When cultivars or landraces are grown simultaneously under equal and equable conditions, plant $\delta^{13}C$ variations can be attributed to genetic differences. Hence, landraces and cultivars of cereals can be assessed rapidly for their growth characteristics and grain yield without the necessity of growing plants for a full season. Additionally, the analytical method is quick, easy and reliable.

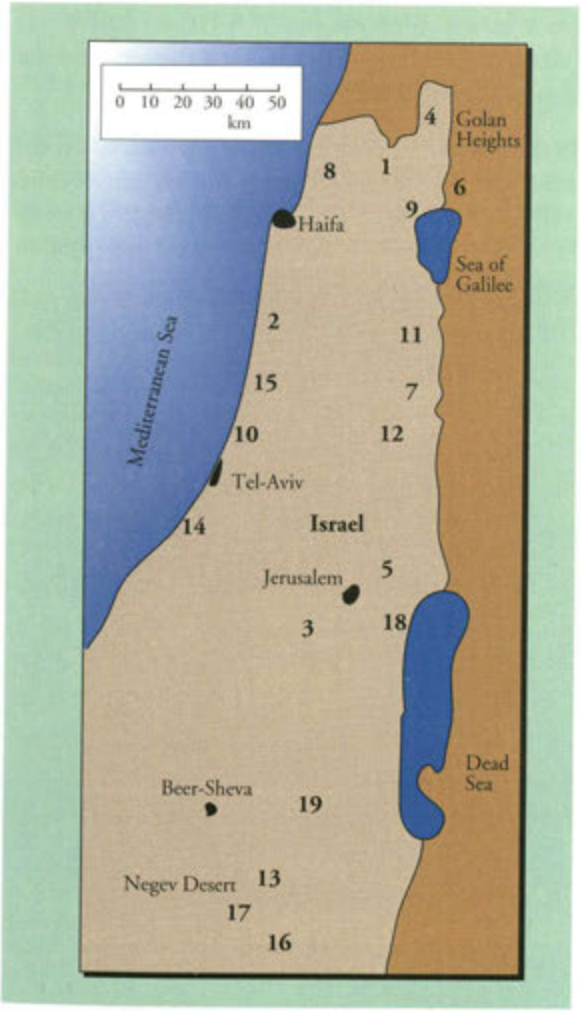

Figure 1 Origins of 19 wild barley populations.

In the past few years this model has been extended to studies of cereal crops, and, in particular, to choosing cultivars suitable for extreme growth conditions such as drought, soil salinity, or desirability for early flowering. This work was done mainly in conjunction with traditional breeding practices. Rather than relying on traditional breeding practices, we combined whole shoot $\delta^{13}C$ with genetic procedures to: (1) assess the natural genetic variability in wild populations of barley from 19 geographically diverse environments and (2) to identify the chromosome responsible for regulating carbon fixation and water requirement (potential water use efficiency) in barley. This is the first time that a chromosome affecting water use efficiency in barley has been identified.

From previous work[2], we reported on the genetic diversity of wild barley *(Hordeum spontaneum)* and its correlation with eco-geographical data (*Ann. Rep.* 1992, 32-35). In new studies, these findings were tested against measurements of whole shoot $\delta^{13}C$. Nineteen populations of wild barley were selected to represent a wide range of original habitats in Israel (Fig. 1) and grown under equal conditions of ample water, light and nutrients. The plants were harvested

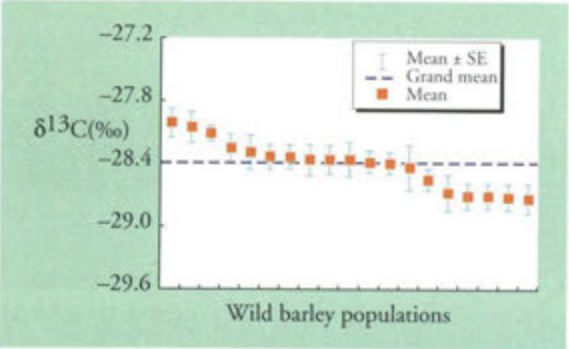

Figure 2 $\delta^{13}C$ of whole shoots of 19 wild barley populations ranked by means.

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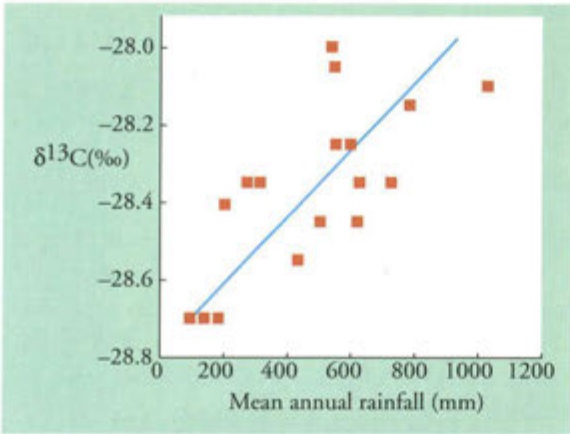

Figure 3 Correlation of $\delta^{13}C$ of 19 wild barley populations with annual rainfall.

after six weeks, before flowering, and then analysed for $\delta^{13}C$ using continuous flow mass spectrometry (CF-IRMS). Figure 2 shows the $\delta^{13}C$ of these 19 populations ranked in decreasing order. Whole shoot $\delta^{13}C$ was highly correlated (Fig. 3) with site-of-origin water availability, measured as annual rainfall. The four populations with the most negative $\delta^{13}C$ originated in the most arid environments, three of them from the Negev Desert. Relatively negative $\delta^{13}C$ indicated low potential water use efficiency and identified these populations as opportunistic desert ephemerals adapted for quick growth.

Simultaneously, we tested whether a single barley chromosome was responsible for modulation of $\delta^{13}C$ and, hence, potential water use efficiency. In this experiment, wheat/barley chromosome addition lines, in which individual barley chromosomes are added to the genome of wheat were assessed for $\delta^{13}C$. Thus,

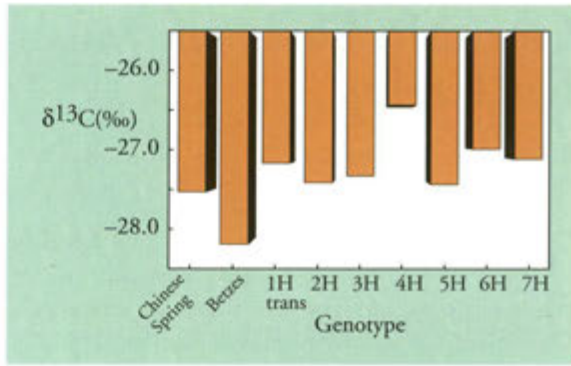

Figure 4 $\delta^{13}C$ of wheat/barley chromosome addition lines compared to wheat (Chinese Spring) and barley (Betzes).

the separate effects of each of the seven chromosomes of barley could be measured. The $\delta^{13}C$ of wheat, barley and the wheat/barley chromosome addition lines showed that only barley chromosome 4(4H) had an effect on shoot $\delta^{13}C$ (Fig. 4). Additionally, this effect was highly significant. These results support previous work in which chromosome 4(4H) of barley was found to carry genes important to adaptation for droughted environments[2].

In the previous study, over 78% of the variation at the *Bmy1* locus on the long arm of chromosome 4(4HL) was found to be restricted to specific climatic regions. Research continues to characterise genes on chromosome 4 more precisely.

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A salt tolerant mutation in barley

H. Pakniyat, M. Macaulay & B.P. Forster

Saline soils are a problem for agriculture in many parts of the world, especially in arid and semi-arid regions where low precipitation, irrigation with brackish water and poor drainage bring about saline conditions. At present there are about 950 x 10^6 ha of saline soils in the world and the area is increasing due to poor management.

The main effect of salinity is inhibition of crop growth leading to poor yields. The majority of crop

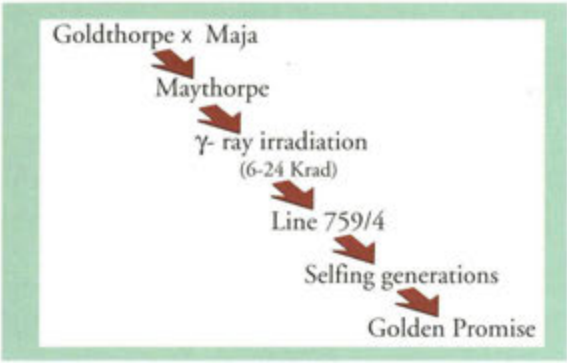

Figure 1 Pedigree of Golden Promise.

species are extremely susceptible to salt and most are unable to tolerate concentrations higher than 100 mol m^{-3} NaCl. A non-biological method for managing saline soils is to apply large scale irrigation and drainage schemes along with chemical soil treatment. However, these methods are slow and costly, especially for developing countries, and alternative strategies are needed. One biological approach is to develop salt-tolerant crop species and barley (*Hordeum vulgare*) is the most salt-tolerant of the cereal crops.

Our work on barley has showed that a number of genes are involved in determining salt tolerance and they are located on at least five of the seven chromosomes. In preliminary work on one aspect of salt tolerance, the ability to limit the amount of sodium (Na^+) taken up by the barley shoots, it was noted that lines carrying a mutant gene, *GPert*, generally accumulated less salt in their shoots than other non-*GPert* genotypes. The *GPert* mutation was produced by γ-ray irradiation of the cultivar Maythorpe (a tall, malting quality barley). One of the derived mutants was developed into the cultivar Golden Promise (a short, malting quality barley) which became the dominant barley cultivar in Scotland in the 1970s and mid 1980s (Fig. 1).

The *GPert* mutation which is located on chromosome 7(5H) is the only known mutation in Golden Promise and has effects on a number of developmental traits (Fig. 2) as well as salt tolerance. This is an interesting finding as other developmental genes, notably, the vernalisation (Vrn) and photoperiodic (Ppd) genes, also have pleiotropic effects on sodium uptake in cereal species.

The sodium content of shoots of Golden Promise were compared with its parent line (Maythorpe) and its grandparents (Maja and Goldthorpe) when grown in hydroculture experiments with either no NaCl or 150 mol m^{-3} NaCl added to the nutrient solution. In saline conditions Golden Promise was found to have significantly less sodium in its shoots compared to

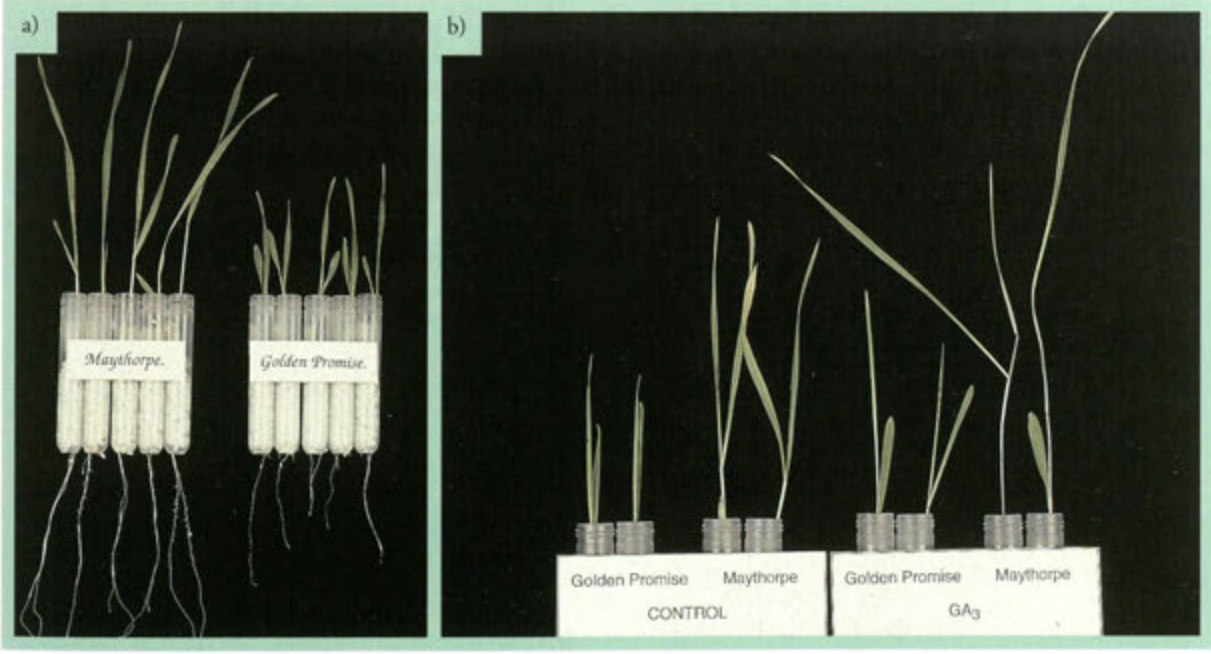

Figure 2 Effects of *GPert* on seedling growth. (a) Seedlings of Golden Promise and Maythorpe showing the reduced root and shoot lengths produced by *GPert*. (b) Seedlings of Golden Promise and Maythorpe grown in the presence of the plant growth hormone, gibberellic acid. Note that Golden Promise is relatively unaffected by the gibberellic acid treatment, the short stature of *GPert* carrying lines may therefore be the result of a lack of response to endogenous growth hormones.

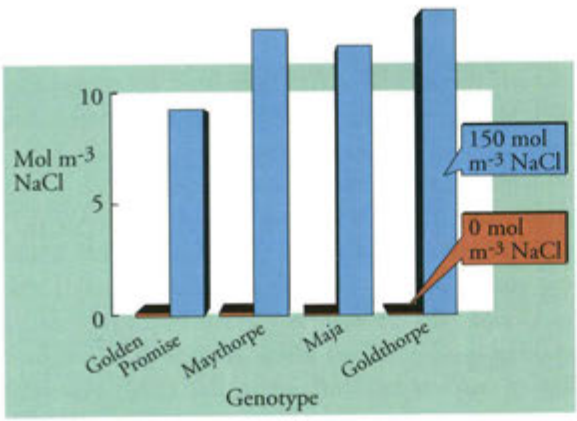

Figure 3 Sodium content of shoots of Golden Promise, its parent Maythorpe and grandparents Maja and Goldthorpe when grown in nutrient solution containing no NaCl and containing 150 mol m^{-3} NaCl.

Maythorpe, Maja or Goldthorpe (Fig. 3). Since the only differences between Golden Promise and Maythorpe are due to irradiation, the lowered shoot Na^+ content of Golden Promise must be a product of the γ-ray treatment and probably a pleiotropic effect of the *GPert* mutation. In addition to *GPert*, several beneficial developmental mutants have been produced in barley which have been incorporated into cultivars. We are currently testing the effect these mutants have on a range of environmental stresses.

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PCR methods for the analysis of expression from plant multigene families

G.C. Machray, P.E. Hedley, C.G. Simpson, R. Waugh & J.W.S. Brown

Analysis of the regulation of plant gene expression requires accurate determination of gene products at the protein or RNA level. Measurement at the protein level can be achieved by biochemical, enzymatic or immunological assay, all of which are subject to problems of recovery and stability. In many cases an assay of steady state mRNA levels as a more direct measure of gene expression is preferred. Northern analysis has been widely used for this purpose, but it requires microgram amounts of RNA and is therefore not suitable for low abundance transcripts and, in addition, cannot readily distinguish between expression from closely related genes within a multigene family. The application of polymerase chain reaction (PCR) methods, utilising cDNA, synthesised by reverse transcriptase (RT) from mRNA, as a template[1] (Fig. 1), has provided a rapid and sensitive method for the assay of steady state mRNA levels which can also be rendered quantitative by the use of appropriate conditions and controls. We describe here further developments of RT-PCR to the analysis of complex multigene families in plants which allow expression from individual genes within families of closely related sequences to be measured.

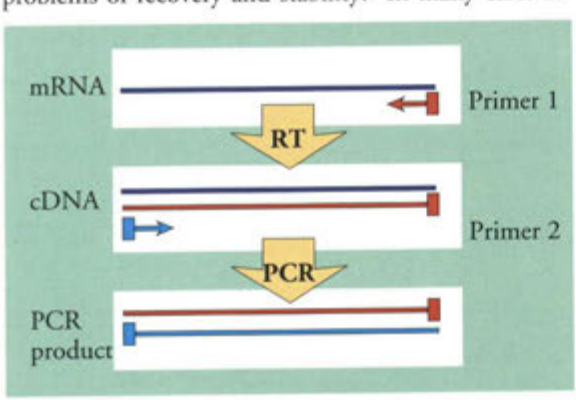

Figure 1 RT-PCR (reverse transcriptase-polymerase chain reaction). cDNA is prepared from the target mRNA present in a population using reverse transcriptase and a specific primer. A second specific primer is added to achieve amplification of the cDNA in a polymerase chain reaction which yields a product of known size for gel characterisation.

Where sufficient dissimilarity exists between members of a gene family, RT-PCR reactions, carried out on total RNA and using a common end-labelled primer in combination with unlabelled gene-specific oligonucleotide primers, can be designed to produce message-specific amplification products with small size

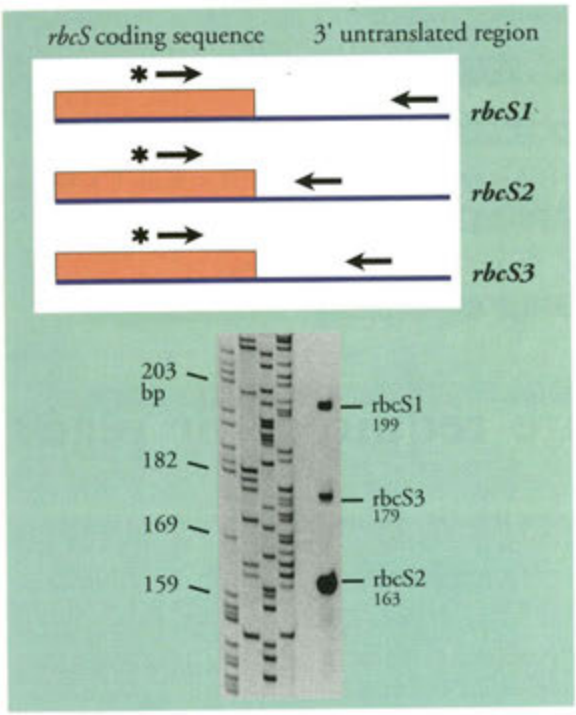

Figure 2 Schematic representation of rbcS mRNA transcripts showing positions of common end-labelled (*) primer and the three gene-specific primers, and RT-PCR analysis of total light-grown leaf RNA from *Phaseolus vulgaris* L. Sizes of PCR products corresponding to the transcripts are given.

variations[2]. For example, discrimination between the expression of the three genes encoding the small subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (*rbcS*), which have identical coding sequences, can be achieved by making use of differences in the 3' untranslated sequences for gene-specific primer design (Fig. 2).

Where sufficient regions of sequence divergence are not present, or available sequences are unsuitable (e.g. contain a high AT/GC ratio), an alternative strategy can be employed in the RT-PCR which uses primers to sequences conserved in all the genes under study[3]. Amplification products of identical size are produced from each message, but these can be resolved on the basis of sequence variation within the amplified region in a single-strand conformational polymorphism (SSCP) gel assay. This assay relies on conformational differences, resulting from sequence variation, being preserved in a non-denaturing gel matrix allowing isomers to be resolved. An example is shown in Figure 3 where expression from two invertase genes yields products of identical size which differ at seven positions within the 65 bp region between the PCR primers. Denaturing gel electrophoresis cannot resolve these but the SSCP assay reveals tissue- and developmental-specific differences when compared to controls. In another approach, discrimination between equally-sized amplification products is made by restriction enzyme digestion, but the SSCP gel assay has the benefit of requiring neither sequence polymorphism for a restriction enzyme nor full knowledge of the sequence variation within the gene family. It is also likely to reveal previously uncharacterised members of gene families which become available for subsequent cloning and characterisation.

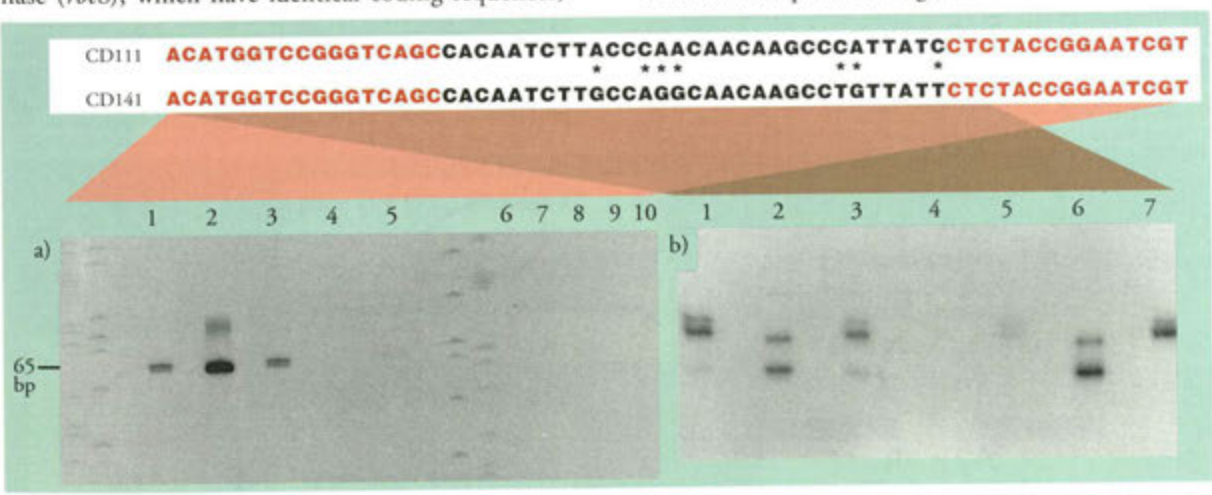

Figure 3 DNA sequence of two invertase genes (CD111 and CD141) with common sequences used for primer design in red, and RT-PCR products using these primers and total RNA from mature leaf (1), developing leaf (2), stem (3), root (4) and tuber (5) of potato analysed under denaturing (a) and SSCP (b) gel analysis. Control reactions for the denaturing gel (6-10) omit reverse transcriptase (using total RNA as in 1-5 respectively). For the SSCP gel, controls (6 and 7) show PCR products from reactions using plasmids carrying the invertase genes (pCD111 and pCD141 respectively) as template.

These RT-PCR methods offer many advantages over traditional technologies such as Northern, S1-mapping and RNase A/T_1 analyses. They are time-saving and less laborious, allow accurate determination of size or conformation in comparison to controls, and can dissect expression from plant multigene families in a quantitative manner.

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Branchpoint sequences are required for plant pre-mRNA splicing

C.G. Simpson, G. Clark, D. Davidson & J.W.S. Brown

The majority of eukaryotic protein coding genes contain intervening sequences (introns) which interrupt segments of coding sequences (exons). Intron removal or pre-mRNA splicing follows a two-step reaction which firstly cleaves the intron at the 5' intron/exon border (5' splice site) and ligates the 5' end of the intron to an adenosine nucleotide within the intron (branchpoint) to form a lariat structure. Secondly, the intron is cleaved at the 3' intron/exon border (3' splice site) and the exons are ligated together releasing the intron lariat and the spliced exon sequences (Fig. 1). The mRNA is subsequently transported to the cytoplasm and translated. The splicing reaction is mediated by the assembly of a large ribonucleoprotein complex, called the spliceosome which catalyses the precise recognition and cleavage of the intron from sequence information in the pre-mRNA transcript.

The formation of an intron lariat at the branchpoint is a fundamental aspect of splicing in animal and yeast systems and is involved in both steps of the splicing reaction. While intron signals and many spliceosomal components appear to be similar between plants and animals, the mechanism of splicing in plants and the formation of an intron lariat remain to be demonstrated. Early plant splicing analyses suggested that conserved branchpoint sequences may not be necessary in plant splicing and the higher content of adenosine (A) and uridine (U) nucleotides may define the extent of a plant intron, implying novel mechanisms in plant splicing. Signals such as the transition from AU-rich introns to the relatively higher GC content of the exon sequence (AU/GC borders) and the presence of AU-rich 'islands' throughout the length of the intron have both been implicated. However, the splicing patterns of some plant introns do not appear to be

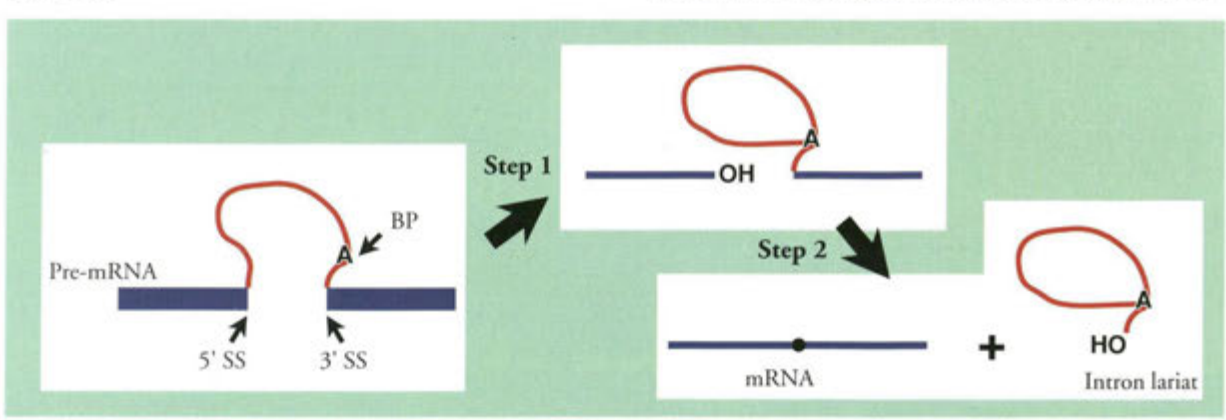

Figure 1 Splicing of pre-mRNA. Splicing follows a two-step reaction which firstly cleaves at the 5' splice site (5' SS) and forms an intron lariat at the branchpoint (BP) adenosine (A). The second step involves cleavage at the 3' splice site (3'SS) and ligation of the two exons to form the fully spliced product (mRNA) and release of the intron lariat.

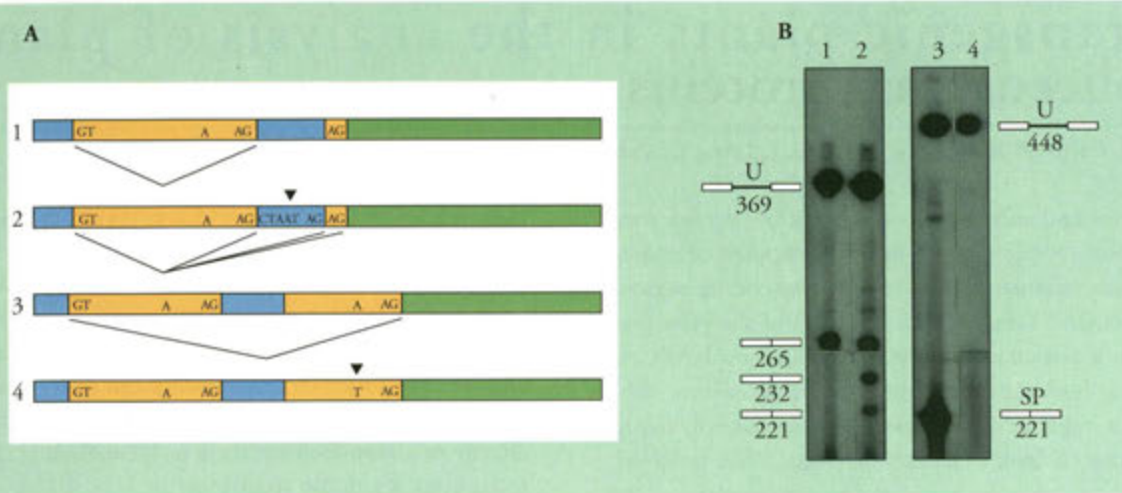

Figure 2 Branchpoint consensus insertions and deletions alter splicing patterns in tobacco.
A Schematic diagram of intron constructs and mutations to investigate branchpoint involvement. The splicing events determined by RT-PCR (B) are shown by the lines between 5' splice site (GT) and 3' splice site (AG).
Mutations are indicated by arrowheads.
B RT-PCR analysis of splicing of branchpoint mutants in tobacco protoplasts. In intron A1, the upstream 3' splice site AG- is selected (lane 1) generating the 265 bp product. Insertion of a second branchpoint upstream of the second 3' splice site AG- (A2) results in utilisation of three 3' splice site AGs generating the 265 bp, 232 bp and 221 bp products (lane 2). Mutation of the branchpoint adenosine of intron A3 to make intron A4 resulted in complete abolition of splicing as envisaged by the absence of the 221 bp product (compare lanes 3 and 4). U - Unspliced. SP - Spliced product.

consistent with models of intron definition solely on the basis of AU content. In animal systems, one of many factors involved in 3' splice site selection is a conserved branchpoint sequence and lariat formation, and the subsequent scanning to select the first 3' splice site downstream from the branchpoint[1]. In an attempt to determine whether a branchpoint is involved in splicing in plants, point mutations were made to potential plant branchpoints, and branchpoint consensus sequences were inserted upstream of inactive 3' splice sites (Fig. 2A). The insertion of a branchpoint resulted in the activation of the downstream 3' splice site and a cryptic 3' splice site as well as the normally selected 3' splice sites (Fig. 2B, lanes 1 and 2). A single point mutation of the branchpoint adenosine in a second construct abolished splicing completely in this intron (Fig. 2B, lanes 3 and 4). These results indicate for the first time that branchpoint sequences are required for splicing of, at least, some plant introns and that changes to plant branchpoint consensus sequences can alter 3' splice site selection patterns. The demonstration of the importance of branchpoints in plant splicing suggests that, despite the differences in AU content between plant and animal introns, the basic mechanism of splicing is likely to be very similar, and the importance of AU sequences may be in stabilising splicing components and the spliceosome instead of directly defining intron borders. This information may aid the prediction of splicing patterns of intron-containing transgenes.

Reference

[1] Smith, C.W.J., Porro, E.B., Patton, J.G. & Nadal-Ginard, B. (1989). *Nature* **342**, 243-247.

Transgenic plants in the analysis of plant spliceosomal proteins

A.D. Turnbull-Ross, G.G. Simpson, J. Lyon, J. Watters, G. Clark, P. Shaw[1] & J.W.S. Brown

Plant and animal genes which encode the information for proteins are usually composed of coding regions (exons) interrupted by non-coding regions (introns) . Transcription (copying) of the gene produces a precursor-messenger RNA (pre-mRNA) containing both the coding and non-coding regions. The introns must be removed and the exons correctly joined together to form a mature messenger RNA (mRNA), which can then be exported from the nucleus to the cytoplasm, and translated into protein (*Ann. Rep. 1991*, 42-44). The essential and complex process of excising intron sequences from pre-mRNA and ligating together the exons, termed pre-mRNA splicing, occurs in large RNA-protein complexes (spliceosomes). Spliceosomes are dynamic structures which assemble on a pre-mRNA, splice out an intron, dissociate and reform on a new pre-mRNA. The main components of the spliceosome are four small nuclear ribonucleoprotein particles (snRNPs) each of which is composed of one or two small nuclear RNAs (snRNAs; U1, U2, U5, U4/U6) and a number of common core proteins, snRNP-specific proteins (*Ann. Rep. 1990*, 28-30), and proteins which transiently associate with the spliceosome during assembly, splicing or dissociation/recycling.

The characterisation of plant spliceosomal proteins can be analysed by producing transgenic plants in which modified versions of the proteins are expressed or the genes are inactivated by antisense RNA. Genes for the plant spliceosomal proteins, U1A and U2B", have been cloned from potato (*Ann. Rep. 1990*, 28-30) which has allowed evolutionary comparisons to be made between plant and animal U1A and U2B" proteins and their interactions with snRNAs. U1A and U2B" both contain two regions (RNP motifs) which can interact with RNA, separated by a central domain. U1A binds directly to a stem-loop structure in U1snRNA; U2B" recognises a similar stem-loop in U2snRNA but requires an additional protein, U2A', for efficient binding to the RNA. U1A and U2B" are models for RNA-protein interactions in animal systems but their function in pre-mRNA splicing remains unknown.

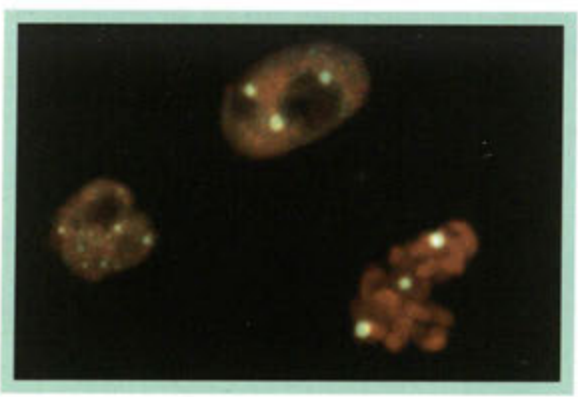

Figure 1 Nuclear localisation of U2B" protein in pea roots visualised using a monoclonal antibody which recognises human U2B" (mAb4G3) and confocal fluorescence microscopy. U2B" (green) localised to interchromatin fibres and nuclear bodies or foci; chromatin is counterstained red.

Transgenic plants may provide a means of studying the *in vivo* function of such proteins. Initial experiments looked at the distribution of U2B" in normal plant nuclei. This work, performed in collaboration with Peter Shaw (JII), found that U2B" was present in discrete foci (Fig. 1); a result consistent with localisation to interchromatin granules and coiled bodies in animal cells. Modified U2B" clones have been produced that contain a few additional amino acids (epitope tag) which are recognised by a monoclonal antibody. The epitope tag allows the nuclear location of only the introduced version of U2B" to be detected in transgenic plants. Transgenic plants have been made which carry the full-length epitope tagged U2B" and the tagged protein has been localised to foci. This system is now being used to analyse the distribution of other nuclear proteins for which genes have been isolated, for example, an RNA helicase of unknown function (*Ann. Rep. 1992*, 37).

Transgenic plants have also been made which express antisense U2B" RNA. These plants can be used to examine the effect of inactivating U2B" on snRNP formation, stability and splicing efficiency. The expression of antisense RNA and accumulation of unspliced pre-mRNA is being assessed using sensitive reverse transcription and polymerase chain reaction (RT-PCR) methodology (*Ann. Rep. 1991*, 42-44). To determine the function of specific regions/motifs of U2B" and U1A, mutants are being constructed which can be overexpressed in transgenic plants and the effect on snRNP and spliceosome formation, and pre-mRNA splicing assessed.

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Cellular and environmental physiology

H.V. Davies

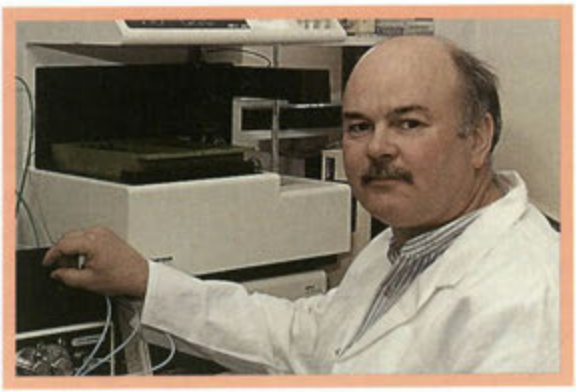
The breadth of activity and interdisciplinary nature of programmes in Cellular and Environmental Physiology continues to reflect those of the Institute as a whole. This is a major strength, facilitated by the desire of scientific managers and researchers alike to form unique problem solving teams. The new programme on vegetation dynamics falls perfectly into such a category, with links already developed with weed ecology, soil-plant dynamics, physiology and genetic and theoretical biology groups. This has also been a very encouraging year for the Department in terms of excellent reports from the recent visiting group and in terms of grant income generated. Last, but not least, significant scientific progress has been made on several fronts, opening up new opportunities and niches.

It is appropriate to start this overview by announcing a new initiative in Vegetation Dynamics. A more extensive introduction to the topic is provided on p. 58. This area of strategic research will concentrate initially on quantifying the flow of environmental resources e.g. solar radiation, water and nutrients, through individuals in mixed communities, including managed and semi-natural vegetation. The programme will forge working links with applied R&D in a range of managed ecosystems.

Last year's Annual Report announced a new initiative on the use of the natural abundance of stable isotopes in physiology, biochemistry and ecophysiology. By exploiting the natural difference in ^{13}C that exists among C3 and C4 plants, CO_2 released by roots of a C4 species (Bermuda grass, *Cynodon dactylon*) has been distinguished from that originating from the decomposition of organic C from the residues of C3 crops. Microbial respiration in soil occurs at a constant rate, over short or long time scales. An AFRC

funded project in collaboration with the universities of Dundee and York on the impact of elevated CO_2 in the atmosphere on rhizosphere processes has begun. Methods have been developed to separate inorganic N species from soil solutions and to determine their $\delta^{15}N$ signatures. Already, a 'carry-over' effect of roots of plants grown at elevated CO_2 on the subsequent decomposition of organic matter following the removal of those roots from the soil has been demonstrated.

In the area of water uptake a technique has been refined to provide single roots in soil with $H_2{}^{18}O$ which is then trapped and measured in the plant's transpiration stream to provide $\delta^{18}O$ as an indicator of the rate of uptake by the root. In collaboration with geneticists, ^{13}C discrimination techniques are being used to assess 20 landraces of barley for differences in water use efficiency. The landraces showed more genetic variation in $\delta^{13}C$ within, than between populations. However, insertion of barley chromosome 4 into wheat strongly depressed the $\delta^{13}C$ value. The wheat and barley lines used are already genetically mapped, and offer the possibility of locating the genetic elements associated with ^{13}C discrimination during photosynthesis and transpiration. Carbon isotope discrimination is also being used to study water use efficiency in potato. Analysis of the response of potato to drought has demonstrated that in field-grown plants, this stress has no significant effect on the functioning of Photosystem II. Maintained electron transport in droughted plants suggests that photosynthetic electrons are being used in photochemical processes despite stomatal limitation of photosynthesis.



Dr. H.V. Davies Head of the Cellular & Environmental Physiology Department

In studies on salt tolerance, a comparison of the response of *Solanum tuberosum* and *S. chacoense* to long term exposure to salinity showed that the species did not differ in tolerance. Another experiment examined the response of long term exposure to salinity in populations previously selected for tolerance using a seedling selection. Although salt depressed yields in populations of *S. tuberosum*, the populations did not differ in response to salt stress. The data cast doubt on the value of seedling based tests of salt tolerance and emphasise the need to understand the effects of salt on the physiology of 'normal' plants.

The nature and relative amounts of volatile organic compounds produced by a selection of bacteria and fungi grown under controlled conditions have been shown to be remarkably reproducible and may represent a specific 'fingerprint' for a particular organism grown under a defined set of conditions. Similarly, consistent patterns of volatile products from incubated soils were identified during assays for activity such as nitrification and denitrification. The possibility that the amount and types of product can be linked to activity levels is under scrutiny. Methods which permit the broad scale assessment of the community structure of the soil microbial biomass, based on the analysis of DNA extracted from entire soil communities, have been devised and further refined. A suite of complementary molecular techniques is now available to provide information on the extent of shifts in overall soil community structure.

Fungal colonies have been grown in circumstances where a detailed mapping of the distribution of hyphae across many size scales (<mm to cms) can be made. Theoretical studies are also exploited to isolate the genetic and phenotypic parameters involved in cellular morphogenesis. It has been possible to reproduce the complex growth characteristics of fungal colonies by invoking simple mechanisms, and factors which may play a major part in linking morphogenesis in higher and lower fungi have been identified.

A full characterisation of soil structural heterogeneity has facilitated an understanding of the relationship between soil structure and hydrology and this complimented earlier work which examined the influence of structure on the diffusive properties of soil. The results have shown how the accepted methodologies of soil physics must be revised to accommodate the crucial role of structure.

There has been significant success in driving the growth of the potato crop with foliar applied N whilst reducing residual soil nitrate and hence the risk of nitrate leaching. A recent EC grant, obtained in collaboration with St Andrews University, will focus on

the effects of manipulating patterns of nitrate assimilation in potato using genetic engineering techniques.

In an ongoing collaborative project with soft fruit geneticists differential hybridisation has identified several blackcurrant cDNA clones potentially related to fruit ripening. Seven clones were differentially expressed during ripening. Two of the clones were expressed with a high degree of specificity in the fruit only and offer substantial potential in the search for a fruit-specific promoter. A newly initiated programme with geneticists is aimed at understanding the control of ripening and shelf life in raspberry fruit.

Studies on the molecular basis of tuberisation in potato funded by the EC Biotechnology programme continue to concentrate on the isolation of differentially expressed genes using PCR-based subtractive hybridisation. The programme is also concentrating on the isolation of different tubulin genes to determine their individual expression profiles. Two different β-tubulin genes have been isolated from a tuberising stolon tip cDNA library using a maize β-tubulin probe. The sequences of these genes differ by only 4 nucleotides and their individual expression profiles are currently being analysed. Another area of interest is the promoters of genes that are induced at tuberisation. The S-adenosylmethionine decarboxylase gene is induced on tuberisation and a genomic clone has been isolated and its primary structure determined.

In studies on cell physiology, two alkaline invertases have been identified in sugar beet taproots and one of the proteins purified. Antibodies raised against the alkaline invertase are currently being used to screen a cDNA library prepared from sugar beet taproots. Alkaline invertase is the predominant invertase in mature taproots and there is no evidence that acid invertase increases during post-harvest storage. Studies on the effect of gamma irradiation on the carbohydrate metabolism of potato tubers have demonstrated that starch biosynthesis is inhibited and glucose accumulation is stimulated. A combined biochemical and theoretical approach has demonstrated the influence of feedback and coupling in a model system of glycolysis. This raises the possibility of complex dynamical behaviour in metabolism, including chaos and hysteresis, for non-equilibrium conditions.

In August 1993, a BIO-RAD confocal laser scanning microscope (CSLM), was acquired, the first of its type to be installed in the world, and it will be used in several projects throughout the Institute. Plasmodesmatal structure and function are under scrutiny, and xenobiotic uptake has been examined both qualitatively with the CLSM and quantitatively using spectrofluorimetric methods. The latter has shown that when carboxyfluorescein was fed via the transpiration stream, or applied to an abraded area of leaf, it was excluded from the epidermal layers and its movement out of the xylem or from the phloem was limited by its sequestration by the surrounding mesophyll cells. Application of the drug probenecid significantly reduced the uptake of xenobiotics by leaf discs and eliminated the pattern of dye accumulation characteristic of sequestration.

In weed ecology studies undertaken jointly with Queens University, Belfast, cumulative effects of imposing a predominantly winter cropping regime on an initially spring germinating arable weed flora have been demonstrated to be more important than herbicide choice or dose. This indicated that rotational strategies for preventing the build-up of weed populations to levels requiring intensive use of herbicides should avoid continuous winter or spring cropping. The decline in natural seedbanks of five arable species in the absence of seed return has also been studied jointly with IACR, Long Ashton in a succession of autumn-sown crops in fields ploughed annually for 3-4 years. This data will be used to predict longer term consequences of poor control of weeds in individual crops. Funding was awarded by MAFF during the year for a further four years' research into seedbank and nematode population changes and diversity during and after set-aside.

In a joint project with crop geneticists on feral rape, vegetation and seedbank composition have been assessed in a range of locations where non-arable populations of oilseed rape were found during a local survey. The objective is to identify habitats suitable for colonisation by feral rape, using wild plant species or associations as indicators. Preliminary results suggest that the cruciferous species *Sisymbrium officinale* might serve this purpose.

Plasmodesmata: an open and shut case

K.J. Oparka & D.A.M. Prior

Most higher plant cells are interconnected across their walls by extremely small (~50 nm diam) pores known as plasmodesmata. These fine channels function as important conduits for solutes, signalling molecules and systemic virus infections. Plasmodesmata form an integral part of the **symplast**, a term used to refer to the cytoplasmic continuum which occurs between higher plant cells.

Until recently, plasmodesmata were viewed as rather static pores, exerting little control over the molecules which pass through them. However, the picture which is now emerging depicts plasmodesmata as much more dynamic structures than previously thought, with the capacity to 'gate' in response to a variety of internal and external stimuli, and thus exert control on the transport of materials between cells[1]. In our laboratory we have been interested in the developmental and physiological regulation of the molecular size exclusion limit (MSEL) of plasmodesmata, and how this might be modified between different cell types in order to determine the nature of molecules which are allowed to pass from one cell to another.

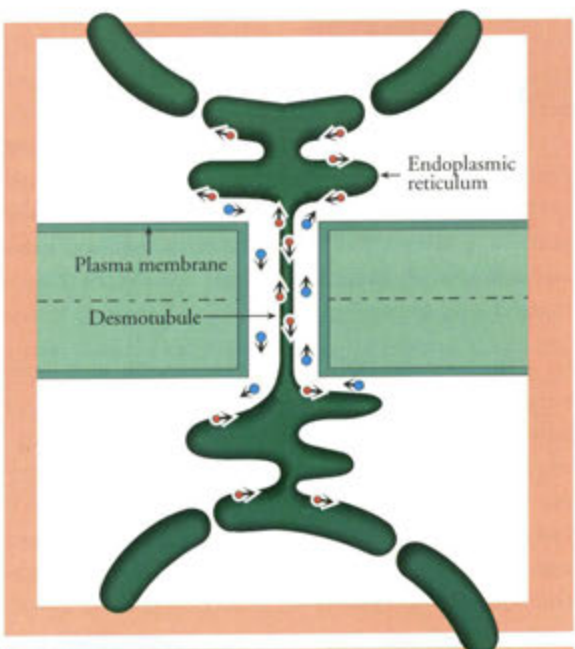


Figure 1 Diagram of a single plasmodesma. The plasma membrane is continuous from cell to cell, as is the endoplasmic reticulum via the central desmotubule. Molecules may move between cells along the ER membranes (⇝) or via the cytoplasmic sleeve separating the desmotubule from the PM. (⇝)

Plasmodesmata are complex structures. In the electron microscope they are often seen, in longitudinal section, as plasma membrane (PM)-lined pores, each containing a central strand of endoplasmic reticulum (ER) known as the desmotubule. Both PM and ER are continuous between adjacent cells. This basic structure is shown, much simplified, in Figure 1. Two potential pathways are available for transport between cells; across the ER membranes via the central desmotubule (shown recently to be a functional pathway for lipids and lipid-signalling molecules) or via the so-called cytoplasmic sleeve which separates the desmotubule from the PM (Fig. 1). Our work has been concerned predominantly with the second of these pathways and how it is regulated *in vivo*.

Much of our current understanding of the function of plasmodesmata is derived from microinjection studies in which non-toxic, membrane-impermeant, fluorescent probes are introduced directly into the cytosol of living cells. The subsequent intercellular spread of such probes is then 'tracked' using fluorescence microscopy. In this respect, the development of a novel micropressure injection system in our laboratory has greatly facilitated *in vivo* studies of intercellular communication. Early work on the microinjection of fluorescent probes gave rise to a general consensus that the MSEL of plasmodesmata was less than M_r 800, very similar to that of the mammalian gap junction. Thus, it was thought that only relatively small molecules could pass freely between cells. However, this now seems unlikely to be the case, and values considerably above (and below) this value are beginning to be reported.

Opening the gate

Increase in the MSEL during virus infection An increase in the MSEL of plasmodesmata occurs during the systemic movement of certain viruses and previous work at SCRI (*Ann. Rep., 1990, 86*) was instrumental in unravelling the changes which occur to the MSEL during the early stages of virus infection. Much work, worldwide, is now concentrating on viral-encoded 'movement proteins' which are able to modify the

structure and function relationships of plasmodesmata. Some of our recent work, however, has been aimed at studying non-viral modifications of the MSEL of plasmodesmata.

The sieve element-companion cell complex The solute conducting cells of the phloem, the sieve elements, are enucleate at maturity and intimately connected, via plasmodesmata, to their nucleate partners, the companion cells. It has long been suspected that macromolecules, particularly proteins, might be able to pass between these cell types in order to maintain the continued functioning of the sieve element, but a direct demonstration of a modified MSEL between these cells has only recently been forthcoming. In joint work with the University of Utrecht (R. Kempers and Dr. A.J.E. van Bel), we injected fluorescent dextrans and proteins into both companion cells and sieve elements of *Cucurbita maxima* and demonstrated that molecules of at least M_r 3000 are able to exchange freely between these cell types, the first demonstration of a significantly increased MSEL in the absence of virus infection[2]. Recent studies are beginning to suggest that such 'up regulation' of the MSEL may occur also at sites where high solute fluxes occur between cells[3]. Joint work with Dr. D.B. Fisher (Washington State University; currently on sabbatical at SCRI) is exploring such an increase in the MSEL, and its effects on the transport of solutes into the wheat grain.

Closing the gate

Pressure-generated closure of plasmodesmata By inserting the pressure probe into living cells of leaf trichomes of *Nicotiana clevelandii*, we were able to change the turgor pressure of individual cells relative to their neighbours and thus induce a turgor differential (ΔP) across the end wall between cells. A ΔP of approximately 200 kPa was sufficient to close the plasmodesmata between cells, preventing the movement of microinjected fluorescent probes (Fig. 2). These observations provided a clear demonstration that plasmodesmata may function as pressure-sensitive 'valves', opening or closing in response to pressure gradients between cells. In a second set of experiments, trichome cells were allowed to lose their turgor pressure by controlled puncturing with the pressure probe. In this case, a large pressure differential (and predicted plasmodesmatal closure) was induced at the junction of the punctured cell with its turgid neighbour. These findings have obvious implications for cell wounding (whether mechanical or induced by pathogens) and suggest that one of the first responses during wounding is the pressure-generated closure of plasmodesmata, isolating the wounded cell from its neighbours and preventing further intercellular communication[4].

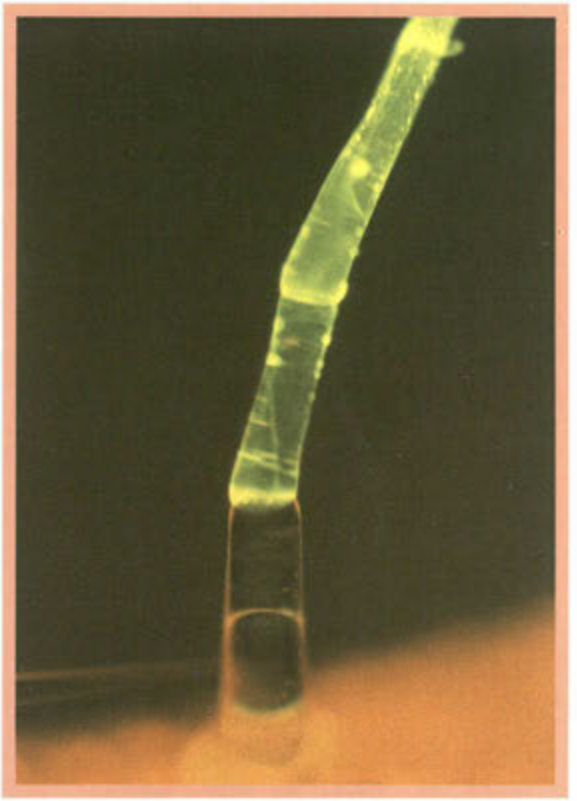


Figure 2. Pressure generated closure of plasmodesmata. Lucifer yellow, injected into the apical region of the leaf trichome of *Nicotiana clevelandii*, is impeded from entering a cell whose turgor pressure has been 'clamped' at 200kPa above its normal pressure.

Closure during osmotic stress Under severe water stress, plant cells may undergo plasmolysis. During this phenomenon, the plasma membrane separates from the cell wall, initially at discrete locations, but later over its entire surface as the protoplast 'rounds up'. We have been interested in the behaviour of plasmodesmata during such osmotic trauma, since plasmolysis will clearly have profound effects on intercellular communication and its successful resumption following deplasmolysis. The literature suggests that plasmolysis induces the breakage of plasmodesmata. However, we have shown recently that during the rigours of plasmolysis, plasmodesmata have their delicate substructure preserved by a quite remarkable continuity of both ER and PM through the plasmodesmatal pore[5]. 'Hechtian' strands (fine tubular extensions of the PM), emanating from the surface

of the contracted protoplast, provide continuity between the protoplast surface and the cortical ER/plasmodesmata complexes near the cell wall (Fig. 3). In

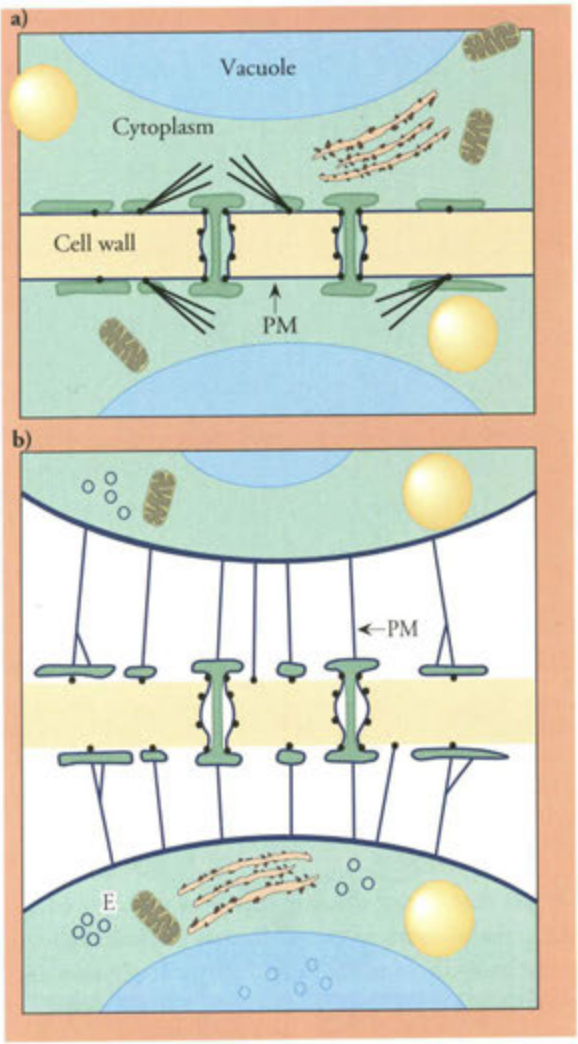


Figure 3 Conservation of plasmodesmatal structure during osmotic shock. In the unplasmolysed cell (a) the plasma membrane lines the wall and is continuous from cell to cell via plasmodesmata (see also Fig. 1). In the plasmolysed cell (b) the anchored cortical endoplasmic reticulum network becomes encased by the plasma membrane (PM) as it retracts from the wall. Membrane surface area is conserved by 'Hechtian strands' which connect the surface of the contracted protoplast with anchoring sites at the cell wall. The PM is continuous between the surfaces of the contracted protoplasts and is anchored along the lining of the plasmodesmatal pores. Cessation of intercellular transport during plasmolysis is brought about by the close contact of PM and ER membranes near the neck region of the plasmodesmata. During deplasmolysis, the Hechtian strands are reincorporated into the main body of the expanding protoplast and the PM returns to its original position lining the wall.

August 1993, SCRI acquired a BIO-RAD MRC 1000 laser confocal scanning microscope (CLSM), the first of its generation to be installed in the world. Using this form of microscopy, we successfully demonstrated the nature of the complex membrane interactions which occur during plasmolysis, and used the information to generate a new model for the behaviour of cell membranes during osmotic shock. In the model, the PM is shown to be continuous through the plasmodesmata (although the functional pathway is inoperational) and membrane surface area is conserved by fractal branching of the PM. During deplasmolysis, functional continuity is restored as the PM returns to its original position lining the wall (Fig. 3).

Symplast 'domains' In some instances plasmodesmata may be rare, or absent, between different cell types, causing an interruption in the symplastic pathway. Such cells, or groups of cells, may be in communication within a given 'domain', but the domain may be isolated from surrounding cells by an absence of plasmodesmata. Such symplast domains frequently occur where neighbouring cells become specialised for quite different functions. Examples are guard cells, which are connected to neighbouring cells during differentiation but become symplastically isolated at maturity. In the sieve element-companion cell complex in stems, the sieve element is intimately connected to the companion cell (see above) but the complex is completely isolated from surrounding parenchyma cells. In some instances, entire tissues may become symplastically isolated, e.g. the leaf epidermis of *N. clevelandii*, which lacks symplastic connections with the underlying mesophyll cells.

In the above examples, plasmodesmata appear to be absent between the symplast domain and surrounding cells. Recently, however, we have focused our attention on a second type of symplast domain; one in which plasmodesmata appear to be present, but intercellular communication is still lacking.

Symplast domains in *Arabidopsis*

The root hair Root hairs are extensions of single epidermal cells and play a major role in the acquisition of water and nutrients by higher plants. It is generally believed that the symplast of the root hair is continuous with the underlying cortex via plasmodesmata at the base of the root hair. In joint work with the John Innes Institute (Dr K. Duckett and Professor K. Roberts) we have examined intercellular communication between the root hair and the underlying cortex. Although plasmodesmata are present between these

Figure 4 Symplastic isolation of the *Arabidopsis* root hair. A fluorescent probe was ester-loaded into the cytoplasm of successive root hairs but no subsequent intercellular communication was observed out of the fully mature root hairs.

types, microinjected fluorescent probes were unable to leave the cytosol of the root hair and enter the cortex. In a second set of experiments, fluorescent probes were loaded into the root-hair cytoplasm as their ester derivatives, in order to overcome the problems of microinjection and the possibility of turgor alteration during impalement. The roots were subsequently examined using the CLSM. Using this non-invasive loading method, the cytosol of the root hair fluoresced intensely, but again no movement occurred into the cortex (Fig. 4). The results suggest strongly, and in conflict with published dogma, that the mature root hair is symplastically isolated from the rest of the plant. These observations indicate, therefore, that most major nutrients taken up by the root hair must be transported across the plasma membrane at the base of the hair before they can enter the cortex.

The root/shoot junction In further experiments with *Arabidopsis* we found that fluorescent probes, when introduced into the parenchyma cells of the cotyledons, were able to move freely via plasmodesmata. However, a major barrier to the movement of fluorescent probes occurred at the junction between the hypocotyl and the root (Fig. 5). On the other hand, if fluorescent probes were successfully loaded into the phloem, and translocated out of the cotyledon, they freely entered the main root and were successfully unloaded at the root tip (Fig. 6). The significance of these major symplast domains in plant development, and also in root-to-shoot signalling, are currently being explored further.

Figure 5 Symplastic 'domain' in *Arabidopsis*. A major barrier to the symplastic transport of a fluorescent probe occurs at the junction between the hypocotyl and root.

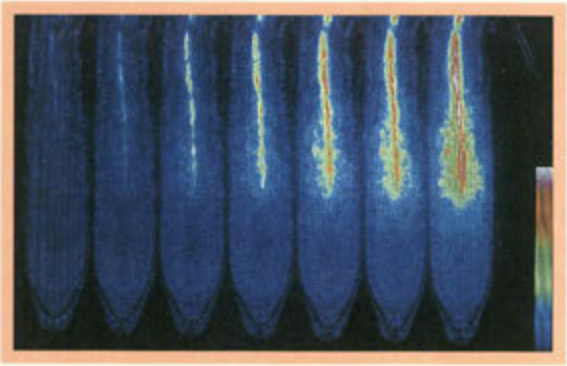


Figure 6 When the same probe is successfully loaded into the phloem of the cotyledon, the root/shoot barrier is bypassed and the probe is successfully translocated to the root. In the root tip the probe is unloaded into the zone of elongation and moves intercellularly via plasmodesmata. These false colour images (taken at 6 min intervals) show the same root being monitored, non-invasively, using the CLSM.

Future directions A major thrust of our future work will be aimed at understanding how plasmodesmata are programmed and regulated during plant development in order to produce the intricate (and complex) patterns of intercellular communication which become established in the mature plant. In particular, emphasis will be placed on the structure and function relationships of plasmodesmata with the aim of understanding how the *in vivo* regulation of intercellular communication is achieved.

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Fungal growth in heterogeneous environments

Karl Ritz

Soil microorganisms, whilst comprising only a tiny fraction of the organic material in soils, play a crucial role in the functioning of all terrestrial ecosystems. The microbial biomass mediates the bulk of biological and biochemical reactions which occur in soil, including the nutrient cycling phenomena which are fundamental to life on earth. Fungi may comprise more than half the microbial biomass in mineral soils and are implicated in a plethora of roles, encompassing physical, chemical and biological interactions (Fig. 1).

All fungi possess enzymes capable of degrading their food bases; some have the ability to decompose recalcitrant materials like lignins and humic acids; others grow rapidly on readily assimilable substrates such as root exudates, and many species degrade material of intermediate nutritional status. Such decomposition results in the mineralisation of organic matter and provision of nutrients for plant growth.

Fungi strongly influence transport processes in soils. For example, they may disperse nutrients through the soil following colonisation of a resource. The resultant mycelium may extend through the soil matrix, transporting nutrients away from a locally high concentration centred on a resource base, such as a piece of plant debris, a dead insect or faecal pellet, or even a fertiliser granule. When such mycelia eventually decompose, nutrients which were once localised in the proximity of the resource will have effectively been spread through a larger volume of soil and may ultimately be more available to plant roots.

Mycorrhizal fungi form symbiotic associations with most plants. Carbon they derive from their autotrophic partners is used to build extensive mycelia which effectively amplify the volume of soil occupied by their host's roots. This enhances the nutrient uptake ability of the plant and affects its ability to compete with neighbouring plants. Mycorrhizal networks may also link the roots of plants of the same or different species, providing the possibility of direct nutrient transport between plants along such networks. There is increasing evidence that mycorrhizas play an important role in governing vegetation dynamics. Soil-borne fungal pathogens, such as take-all which infects cereal roots, can be very virulent because of the rapid rates of extension of hyphae along host roots, while, in contrast, pathogenic bacteria can rarely colonise at a rate commensurate with root extension.

Fungal mycelia also exert a strong effect on soil structure, through acting as binding agents, meshing together and strengthening soil aggregates like iron bars in reinforced concrete.

A fundamental characteristic of the filamentous (eucarpic) fungi is their growth-form, which is based on an indeterminate tube, or hypha, which by extension and branching permeates the environment in which they grow. The collection of such hyphae is termed a mycelium (Fig. 2), which is essentially an interconnected network of tubes along which materials can be transported from zone to zone. Mycelia can

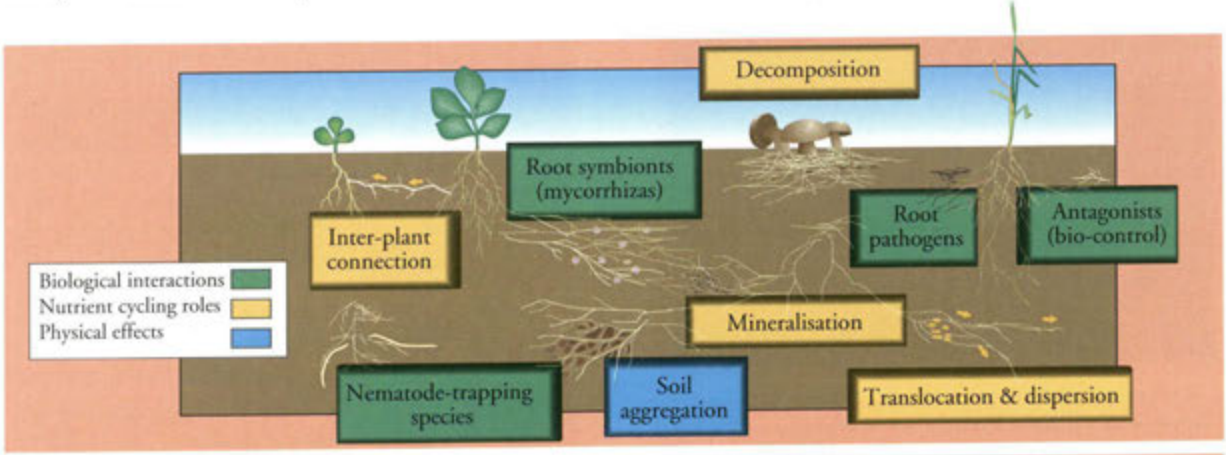


Figure 1 Conceptual diagram of the roles eucarpic fungi play in mineral soils.

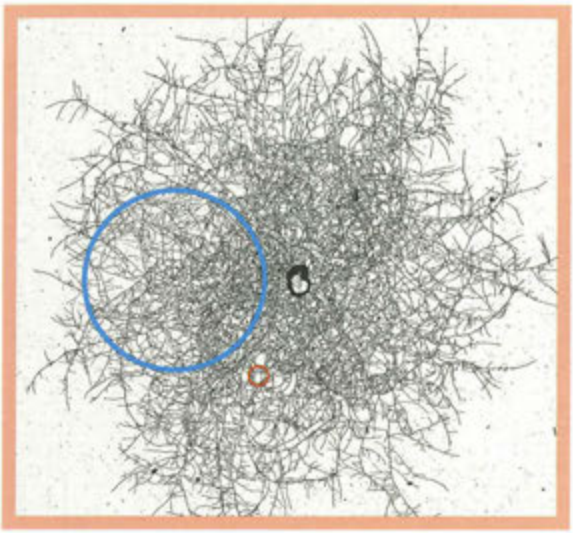


Figure 2 Mycelium of *Alternaria alternata*, visualised by growing the fungus on a transparent membrane overlaying agar gel, and enlarging the colony directly within a photographic enlarger onto high-contrast film. The coloured spots show the maximum field of view attainable with a compound microscope (red) or stereo microscope (blue).

vary in size from a fraction of a millimetre to several kilometres in extent. The degree of interconnection, or anastomosis, varies between species, and may depend on the age of the mycelium. The extent of intra-hyphal transport also varies between species, and appears to be governed by many anatomical, physiological and environmental factors.

It is these characteristics of being able to grow great distances (relative to the diameter of their hypha), to fill space so effectively by means of a ramifying network, and to transport substances within the mycelium, which reflect the roles fungi play in soils. Such characteristics are in sharp contrast to the way bacteria colonise soils and reflect the different roles these organisms play in this environment.

Soils are highly heterogeneous environments in space and time, and fungal mycelia, being indeterminate space filling structures, are particularly suited to growth in such non-uniform circumstances.

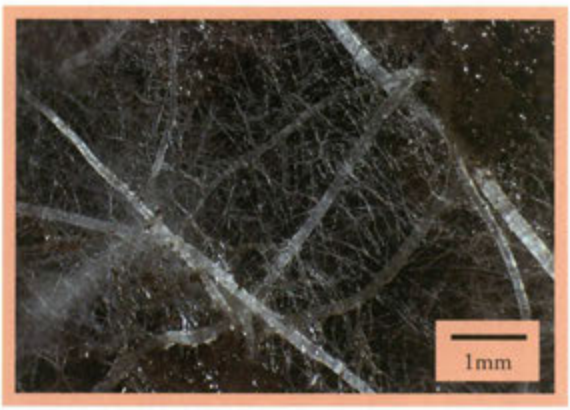


Figure 3 Macrophotograph of grass roots and associated fungal hyphae growing in soil.

Surprisingly little is known about the detailed responses of fungal mycelia to heterogeneity in their environment. Whilst it is known that mineral soils typically contain several hundred metres of fungal hyphae per gram, with much greater lengths in grassland and woodland soils, almost nothing is known about how these hyphae are arranged in space.

Such lack of knowledge relates in part to the inherent opacity of soils, and relatively large volumes hyphae may occupy. Normally only a small part of a mycelium growing in soil can be visualised by microscopy (Fig. 3) and special techniques must be employed for detailed visualisation of mycelia grown *in vitro* that are greater than a few millimetres in size (Fig. 2).

Most studies of fungal growth to date have been based on observations *in vitro*, with fungi grown in *uniform* culture media, either at the macroscopic scale (such as colony diameter, area or mass) or at the microscopic scale (such as hyphal length, extension rate or number of branches). Little consideration has been given to the responses of fungi to heterogeneity in their nutritional or physical environments. At SCRI, research is under way to study the responses of fungi to experimentally controlled heterogeneity, at a variety of scales, with the aim of understanding the consequences of such responses in relation to the functional roles fungi play in soils.

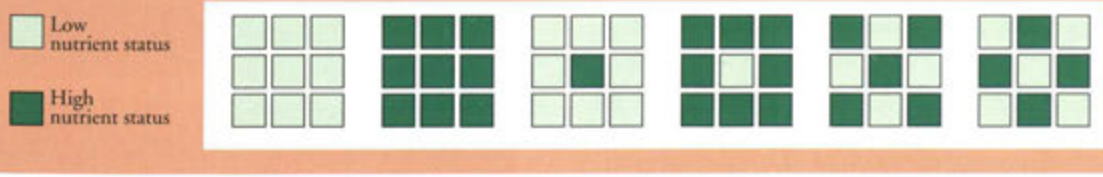


Figure 4 Patterns of agar tiles used to study fungal responses to heterogeneously distributed nutrients.

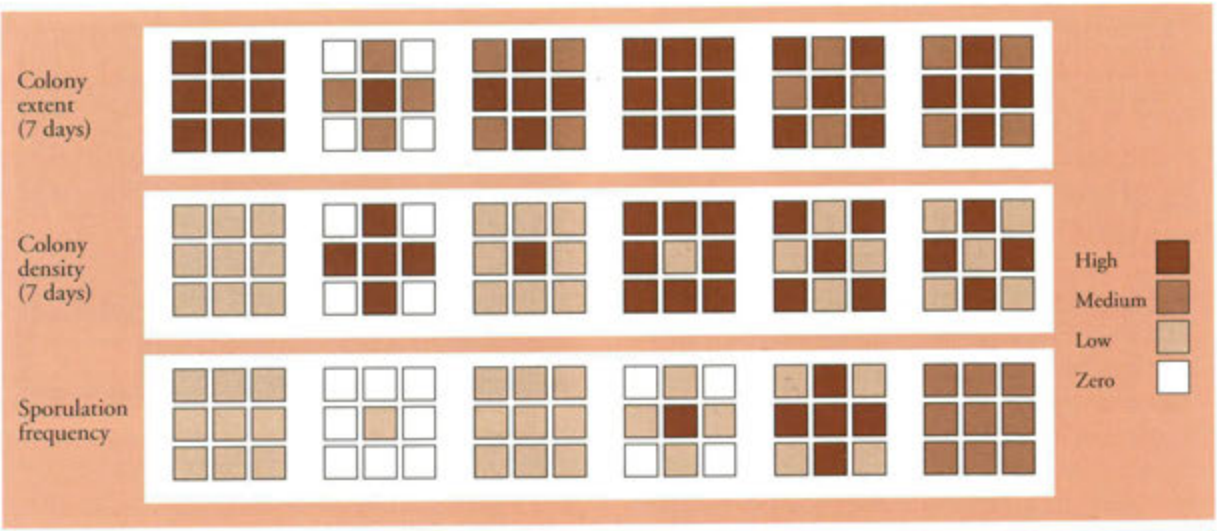


Figure 5 Growth responses of *Trichoderma viride* to heterogeneously distributed agar tiles arranged in the patterns shown in Fig. 4.

Microcosms have been designed where fungi can be grown in conditions of controlled heterogeneity. These consist of 9 tiles of agar media arranged in various patterns of high and low nutrient status (Fig. 4). Nutrients are prevented from diffusing from one tile to another by an air gap between adjacent tiles. A single fungal colony is inoculated onto the central tile and the developing mycelium readily grows across the air gaps, thus colonising peripheral tiles. Different species respond to this system in different ways, for example, *Trichoderma viride*, a potential biocontrol agent, extends on low nutrient tiles faster than on tiles of higher nutritional status, but forms considerably sparser colonies (Fig. 5). Sporulation of this species tends to occur on low-nutrient domains, implying translocation of resources from the nutrient-containing tiles. However, in other species such as *Alternaria alternata*, sporulation is restricted to high-nutrient tiles. In these systems, the arrangement of tiles is heterogeneous but symmetrical; growth responses of *T. viride* and *A. alternata* are concomitantly symmetric. The development of *Rhizoctonia solani*, a root pathogen, is strongly influenced by heterogeneity in the tiles. This species typically shows asymmetric growth where the tesselations include low-nutrient tiles, which may be partly due to slow growth across such domains. The formation of sclerotia, which are heavily pigmented structures constituting a resistant phase of the fungus, shows remarkable sensitivity to heterogeneity in the microcosms. Sclerotia do not form where tiles are all the same, but the substitution of merely the central tile with one of contrasting nutritional status induces sclerotial formation (Fig. 6), as does the more complex mixed patterns. Under these circumstances, sclerotia nearly always form in low-nutrient domains, which must involve translocation of resources from nutrient-containing tiles. Sclerotial formation is also strongly asymmetric, with certain tiles dominating.

The experiments described above rely on qualitative observation of colonisation patterns of agar tiles. At the microscopic level, a fungal mycelium appears complex with many hyphae, often branching profusely in a seemingly random arrangement (Fig. 2). We have demonstrated, however, that the distribution of hyphae in space is not random, rather it is spatially correlated and fractal, i.e. the distribution of hyphae in space in one part of the colony is dependent on the distribution in more distant parts. Furthermore, the fractal dimension, d, which relates to the space-filling effi-

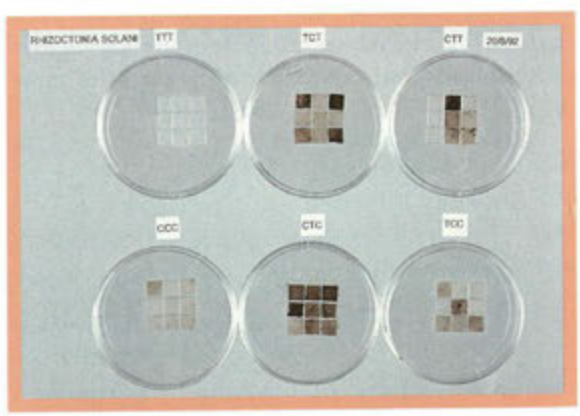


Figure 6 Formation of sclerotia of *Rhizoctonia solani* amongst heterogeneously distributed agar tiles, arranged in the patterns shown in Figure 4. Sclerotia are visible as pigmented aggregates within the tiles

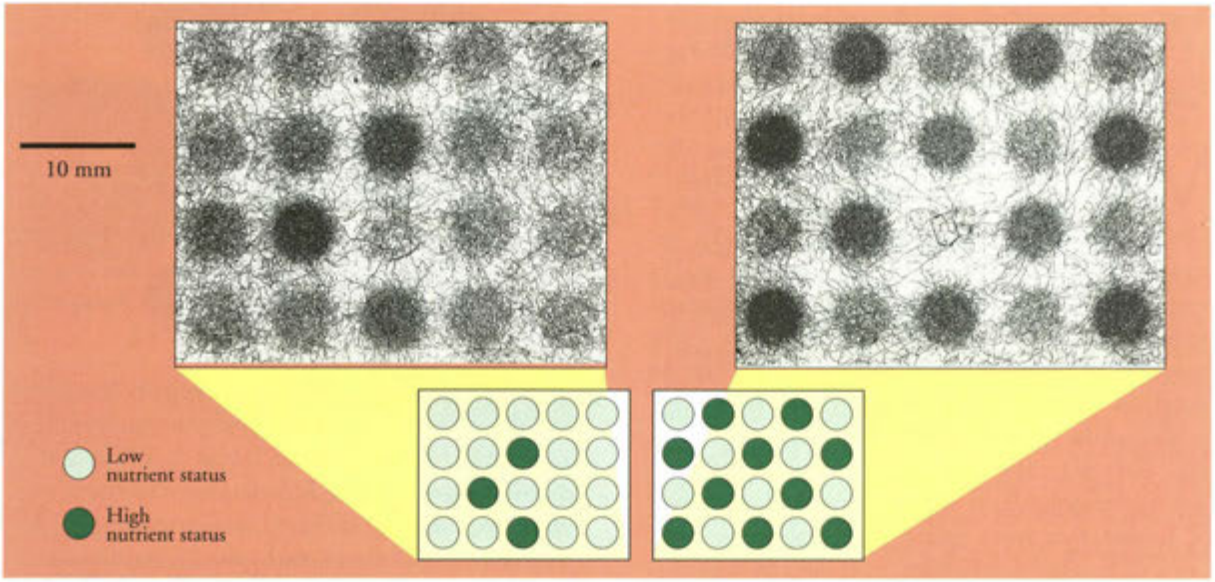


Figure 7 Single colonies of *Trichoderma viride* growing on membrane overlaying circular patches of nutrient resources arranged in heterogeneous patterns.

ciency of the mycelium, has been shown to be strongly related to the nutrient status of the substratum on which the fungus is growing. Thus, where nutrients are low, mycelia develop with low d, and are predominantly in an exploratory growth mode. If nutrients are readily available then branching is prolific, the growth is exploitative of space, and a high d is attained. If a fungus is grown in circumstances where the distribution of nutrients is heterogeneous then localised responses ensue with a variety of explorative or exploitative growth modes being expressed within a single mycelium. We have recently devised microcosms where mycelia can be grown under such circumstances and the detailed distribution of hyphae observed. By a special arrangement of membranes overlaying gel cylinders, it is possible to set up tesselations of relatively high and low nutrient status domains which remain discrete, i.e. there is no physical diffusion between adjacent sites. The responses of *T. viride* colonies to such circumstances support the earlier work using agar tiles, and clearly demonstrate the contrasting explorative and exploitative growth modes alluded to above (Fig. 7). These experiments also demonstrate the efficiency with which *T. viride* can grow given extremely low amounts of nutrient, especially carbon, which is normally limiting in mineral soils. As with agar tile experiments, there is also clear evidence for translocation within the mycelium from zones of higher nutritional status to zones of lower nutrition. Growth on the low nutrient domains where nutrient-containing domains are also present far exceeds that attained on low-nutrient domains alone. These experiments also show the extreme spatial delimitation of the response of *T. viride* about the sites conducive to growth where the cut-off distance around the gel domains is extremely short.

Such experiments are providing new insights into the way mycelia develop and suggest the possible consequences of such responses for soil processes. All species studied so far exhibit translocation of resources from regions of relatively high nutrient status. Different species will influence transport, specifically nutrient dispersal, in different ways. For instance, species such as *T. viride* may tend to disperse nutrients relatively uniformly throughout the soil volume, whilst *R. solani* may tend to (re)concentrate nutrients in localised zones. Sites of sporulation or reproduction relative to resources will also vary, with consequences for the location of inocula which will ultimately give rise to new generations of mycelia. This is important in relation to pathogenic species, since spatial location of inocula will influence the epidemiology of a pathogen. Hyphal proliferation will undoubtedly affect soil structure and aggregate stability. *T. viride* may have a relatively local effect on structure due to the sharp restriction of its proliferation zones with respect to nutrient resources, whilst *R. solani* may influence structure across greater size scales since it shows less delineation. Testing these hypotheses in soil systems presents a major challenge for future research.

Vegetation dynamics

G.R. Squire

Vegetation is in a state of continuous flux, in which the resources of solar radiation, carbon dioxide, water, and mineral nutrients, are taken up, used and released. The result of this flux might appear stable in certain types of natural vegetation, such as tropical forest or temperate moorland. More generally, the result is very unstable: the mass of the stand, the number and type of species in it, and the quality of the products it produces, change rapidly in time and space. Changes sometimes happen suddenly and catastrophically, as when an ecosystem is burned or flooded, then grows back in a different form. They can also happen more slowly over decades and centuries, or repeatedly and systematically during the annual sequence of agriculture. The fluxes in most of the world's vegetation are influenced by human activity, whether intentionally by farmers and pastoralists, or by unwitting or vandalistic behaviour.

Whatever causes the changes, they are brought about through effects on structures and physiological processes in plants. The physiology sets the way the resources are channelled through individual plants, so as to determine their survival in the face of environmental stress, or their success in competition for the resources with other individuals. The decisions that all land managers must make about the configuration of a stand, the timing of operations, and the type and mixture of species all have consequences for survival, competition or both.

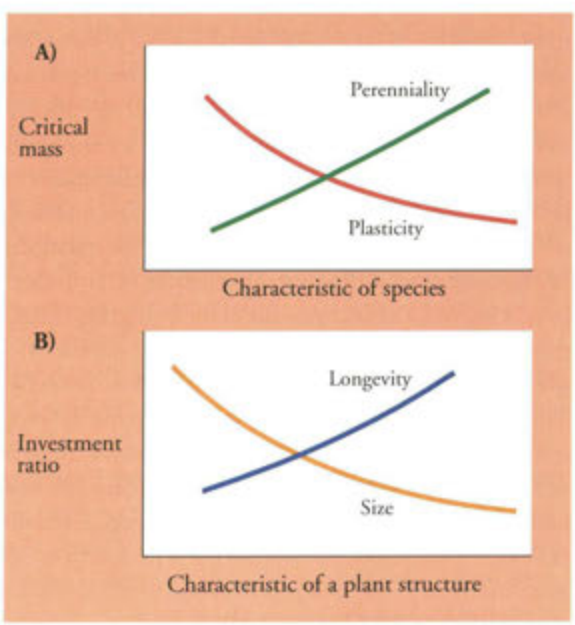


Figure 1 Representation of the effects of general physiological traits on (A) the critical plant mass (or nutrient content) required for reproduction and (B) the ratio of energy (or mass) invested in tissue and the energy-equivalent of resources captured.

The fluxes of light, water and nutrients have been measured through complete stands but rarely through each or any of the different species in a mixed crop, sward or forest. It is technically very difficult to measure fluxes through different species growing together, unless these are separated spatially, in the way, for example, that the foliage of tall cereals is distinct from that of short spreading legumes in tropical intercrops or that of a pine canopy from a bracken understorey. In more complicated vegetation, including natural and semi-natural ecosystems, species tend to be categorised mostly in qualitative terms, or as a functional type (e.g. a rapid spreader, able to colonise). Most mathematical models of spatial and temporal dynamics in complex vegetation similarly define species by qualitative traits, such as degree of invasiveness.

To make progress with vegetation dynamics, it will be necessary to take the quantitative, physical approaches used for monocultures and adapt them for mixtures. Inevitably, research will have to concentrate on a few, crucial traits. One such trait is the critical mass (or energy or nutrient content) that an individual must accrue before it produces structures that enable it to survive or reproduce. An example of this critical mass is that of the vegetative tissue needed before grains can be produced in a determinate cereal. For reproduction in crops, the critical mass of vegetative tissue ranges widely from around 100 kg per individual in some large fruit trees and palms, to about 1 g for the most drought tolerant tropical cereals. For some grasses and many annual weeds, it is only a fraction of a gram. It tends to increase with the degree of perenniality of a species, but decrease with the degree of plasticity (Fig. 1A).

Another characteristic - termed here the investment ratio - combines various traits of the architecture and lightness of leaves and roots with others governing their ability to extract and convert resources. When expressed for example, as an amount of radiation cap-

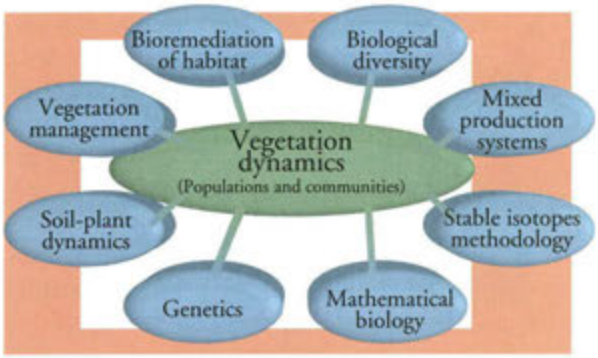


Figure 2 Disciplines at SCRI relating to vegetation dynamics, and some practical applications of the new programme.

tured per unit mass of vegetative tissue (Fig. 1B), the ratio increases with longevity of the leaf canopy, but decreases as the canopy gets larger. The ratio can also be expressed as mass of new plant material returned per unit mass invested, and if, in this form, the ratio is 1, then the plant expands just to stay alive, and has nothing left for reproduction. If the ratio is only slightly above 1, then only those plants with a very small critical mass will be able to survive or reproduce. The critical mass and investment ratio are therefore likely to be intimately related in controlling the outcome of dynamics. The interactions are complicated because traits such as longevity and perenniality tend to influence critical mass and investment ratio in the same direction!

Although these traits condense many physiological attributes, they are nevertheless quantitative and directly related to fluxes of physical resources. The challenge of the new programme in vegetation dynamics at SCRI will be measuring them (and their component traits) in mixed plant communities, and using them as a basis for mathematical modelling of spatial and temporal changes in vegetation. The new work will be based mainly in the field and consist of studies at three scales: the single plant growing in a mixed stand; stands of several interacting populations; and populations (dimensions in kilometres) differentiating and spreading over the countryside. Much use will be made of simple model systems (herb/herb and shrub/herb) maintained at different levels of complexity, for example, and having different numbers of species, having different degrees of perenniality or plasticity.

The new programme will work closely with existing research at SCRI on soil-plant dynamics, mathematics, genetics and stable isotopes (Fig. 2). A further challenge will be applying the approach to provide a sounder scientific basis for management of agricultural and other vegetation, and particularly the remediation of polluted land and damaged ecosystems. In this applied research, the programme will need to collaborate widely with other research groups and with industry.

Soil seedbanks as monitors of the effects of changes in land management

H.M. Lawson & G. McN. Wright

Arable top-soils may contain between 50 and 500 million viable weed seeds per ha, comprising up to 30 different species all with their own characteristics in relation to persistence, dormancy and periodicity. The seeds present may have been blown, shed or carried into the field during the current year or may have remained dormant in the soil from previous years. The weed seed content of the soil is therefore an 'evolutionary memory', reflecting the past history of the land and foreshadowing future weed problems. In the absence of seed return, the seedbank population decreases by between 20 and 50% per year, but one year's uncontrolled seeding can increase populations several-fold. Given favourable conditions for germination, only 2-5% of the seedbank is likely to produce seedlings in any one year. Seedling numbers and species give a useful instant snapshot of the response of the weed flora to a particular set of crop, soil and weed management conditions, i.e. that part of the seedbank capable of exploiting a particular seasonal or rotational niche (the actual flora). Such data are useful for making year-by-year weed control decisions, but they give little indication of the likely response to changes in management over the longer term. For

Figure 1. Seed identification.

this it is necessary to have data on the size, species composition and community structure of the soil seedbank itself (the potential flora).

In arable soils, seedbanks are estimated by means of soil cores, usually to plough depth, taken systematically or at random across plots or fields. Where it is intended to monitor changes over the rotation, it may be preferable to take samples from the same fixed points at regular intervals. Seeds are extracted from soil samples by mechanical sieving and flotation; numbers of viable seeds are counted and species identified later (Fig. 1). The Weed Ecology Group at

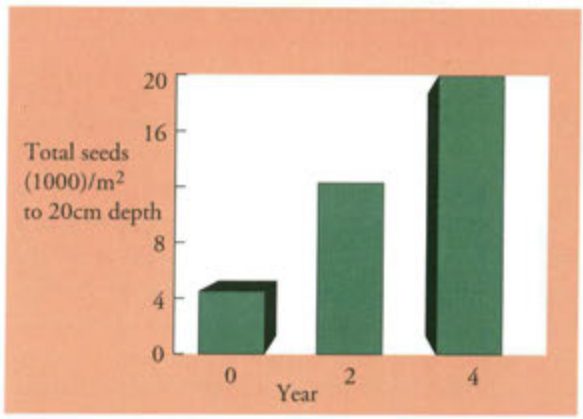


Figure 2 Seedbank increase over four years under cereal crops given no herbicide treatment (mean of four sites).

SCRI is using its seedbank expertise to cooperate with scientists in IACR, ADAS, SAC and Queens University, Belfast in a programme of research into the long-term effects of changes in agricultural practices on populations of weeds, volunteer crops and other wild plants. Results from several of these projects are used to illustrate how seedbank data can identify potential long-term problems or benefits of changes in management practices.

Effect of reducing herbicide inputs Economic and environmental pressures are forcing farmers to consider methods of reducing the amounts of herbicide they apply to their crops. How best can this be accomplished without taking unnecessary risks? The potential cost of not controlling weeds may be very much greater than any short-term savings achieved. In cereal rotations one possibility would be to capitalise on high levels of weed control maintained in previous years by not applying any further treatment for several years. To test the effects of this practice, seedbank populations were monitored in four hitherto intensively-managed arable fields in eastern Scotland on plots where four successive cereal crops were given no weed control treatment. Total weed seed numbers averaged over the four sites trebled over the first two years and increased almost five-fold over a four year period (Fig. 2). Significant yield losses due to weeds did not occur until the third crop at one site and the fourth crop at the other three sites. However, cost-savings in the first few crops were made at the expense of a rapid increase in seedbank populations which could take many years of very effective weed control to reduce to levels unlikely to produce a competitive weed flora. This approach could result over the longer term in greater cost and more herbicide usage rather than any real saving in either respect.

Other less drastic methods of reducing herbicide inputs were included in these experiments. Broad-spectrum herbicides were applied in each crop at full or half recommended rates, either as insurance treatments sprayed irrespective of weed numbers, or only when triggered by arbitrary weed thresholds based on weed density and species composition. Thresholds were exceeded on six out of a possible 12 occasions in the first three crops, but on every occasion in the next two crops, due to a progressive rise in seedling numbers. Total weed seed numbers recorded after four years of these regimes, averaged across the four sites (Fig. 3), showed that halving the dose of the insurance treatment applied every year (and thereby saving 50%

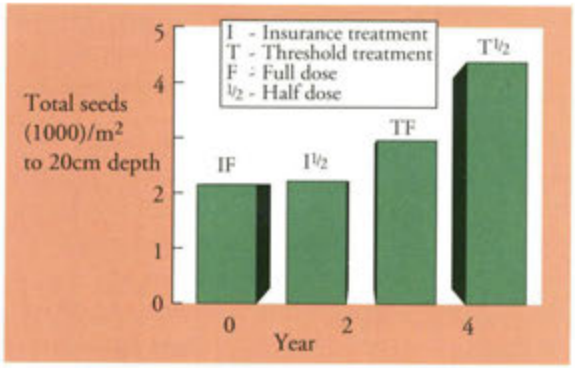


Figure 3 Seedbank populations after four years of different herbicide regimes (mean of four sites).

of herbicide costs) had not incurred any penalty in terms of rising seedbank populations. Application of the full dose when triggered by thresholds saved considerably less, but total seed numbers were nearly 40% higher than on annually treated plots. Half dose threshold treatments gave the greatest reduction in herbicide inputs, but at the expense of doubling the seedbank population. These results indicated a lack of sustainability in the threshold approach, particularly if the herbicides were applied at reduced doses. None of the treatment regimes resulted in a reduction in crop yields over the first four years, but the seedbank data taken in conjunction with other agronomic results led to the conclusion that the application of a reduced rate (not necessarily 50%) of a broad-spectrum herbicide every year was the preferred strategy for reducing herbicide inputs with minimal long-term risk.

Effect of set-aside The introduction of set-aside has brought about a major change in farm rotations in recent years. How can set-aside fields be managed so as to avoid creating future weed problems if and when the field is returned to arable cropping? Alternatively, how can it be managed so as to increase species diversity in fields put permanently into set-aside? The effects of various set-aside management strategies on seedbank populations at four ADAS research centres were examined after three years of treatment. Where set-aside plots had been allowed to develop a natural ground cover, seedbank populations were much larger than on those which had continued under arable cropping (Fig. 4). Seedbank expansion was much curtailed if a grass or grass/clover cover had been sown at the start of the set-aside period. The type of cover had less influence on seedbanks of other plant species than its presence or absence, although the cover species themselves added substantially to total seedbank numbers on sown plots. A number of common arable weed species had been able to exploit the niche created by unsown set-aside, particularly meadow-grasses, black-grass, barren brome and annual sow-thistles. Seeds of several perennial broad-leaved species, such as spear thistle, were found mainly on these plots, but numbers were very small and this group was poorly represented in the seedbank, despite substantial incursion into the sward.

Frequency of mowing set-aside also influenced seedbank numbers and composition over the three year period (Fig. 5). Total seed numbers were lower on plots cut twice or more than on those cut once only in late summer each year. Annual sow-thistles, perennial broad-leaved weeds, barren brome and black-grass were favoured by the single mowing treatment. Seed populations of perennial ryegrass were highest on plots cut once, but white clover showed the opposite effect.

Different management techniques can therefore be used to regulate the size and diversity of seedbank populations in set-aside fields, depending on the objective. Where the field is to be returned to arable

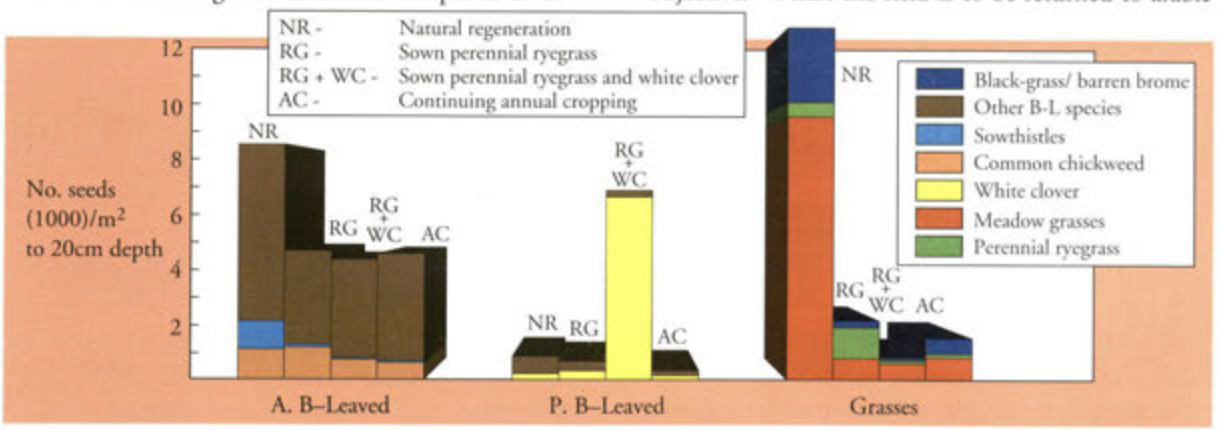


Figure 4 Seedbank changes after three years of set-aside management (mean of four sites)

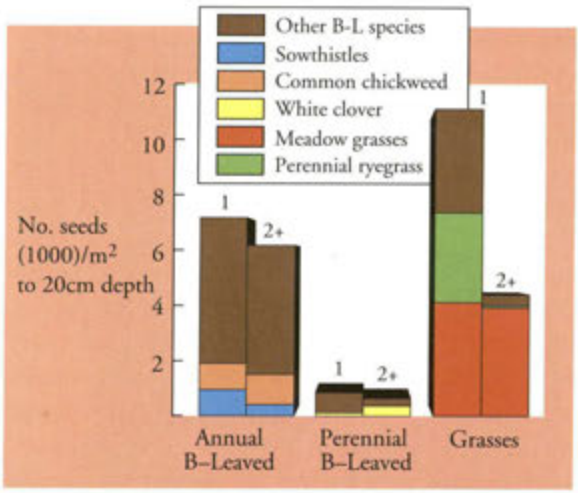


Figure 5 Seedbank changes after three years of cutting once (1) or at least twice (2+) per year on natural regeneration and sown ryegrass plots (pooled) (mean of four sites).

cropping, the use of a sown cover and regular mowing should ensure that the seedbank will not cause major problems to subsequent crops. Alternatively, greater diversity can be encouraged by not using a cover crop and by infrequent mowing. However, it should be noted that increased diversity at these previously intensively cropped sites simply meant an increase in seedbank populations of weed species commonly found in arable land, field margins and grassland. No rare or unusual species were recorded in the seedbank when the trials started or after three years. The introduction of desirable species may be necessary in these situations, but steps would have to be taken to encourage their establishment in the face of competition from weed species also exploiting the set-aside environment.

Species	Years to 99% decline
Common chickweed	11.1 ± 2.44
Field pansy	10.2 ± 1.32
Common poppy	8.7 ± 2.13
Shepherd's purse	6.5 ± 1.70
Forget-me-not	6.1 ± 1.12
Field speedwell	6.1 ± 1.37
Red dead-nettle	5.4 ± 0.68
Ivy-leaved speedwell	5.4 ± 1.06
Annual meadow-grass	4.3 ± 0.88
Cleavers	3.6 ± 0.47

Table 1 Rate of decline of seedbanks, based on fitted exponential curves.

Seed survival Finally, the long-term consequences of allowing weed seedbank populations to increase under various management regimes depend to a large extent on how long seeds of individual species survive in the soil. Joint investigations with IACR Long Ashton and Rothamsted have examined the persistence of seedbanks from a single year's uncontrolled seeding in continuous winter cropping rotations where the plots were ploughed annually and no further seed return was permitted. Rates of decline of seedbanks of individual species were calculated, using fitted exponential curves. Table 1 illustrates the differences recorded across a range of arable species in two experiments. Species such as cleavers and annual meadow-grass were estimated to take only 3-4 years to decline by 99% in numbers, while common chickweed and field pansy could survive for 10-11 years in similar circumstances. Most other species were intermediate in persistence. This type of information will play a crucial part in devising long-term strategies for weed management in crop rotations.

Flexible modelling accessing knowledge and uncertainty

B. Marshall, J.W. Crawford & J.W. McNicol

Our quantitative understanding of biological systems is often expressed as mathematical models which are often inflexible and presented in an unacceptable form. The model is a black-box where potentially valuable knowledge within is inaccessible to the user. The explanation of how an answer is reached is often as important as the answer itself. Uncertainty, a key element in evaluating risk, is rarely considered and crudely handled by such models. Uncertainty derives from many sources, e.g. weather,

inherent variability in the behaviour of systems, market fluctuations and imprecisions in our knowledge (such as soil fertility).

Our current understanding of factors influencing the yield of potato crops and the size distribution of the tubers was used as a test case to develop and evaluate new approaches to encapsulate and access this knowledge. The size of a tuber is one of several important quality characteristics which determines its suitability for selected markets (pre-pack, bakers, crisps, chips). A mathematical model (seed-rate package) was developed in collaboration with ADAS using traditional programming techniques (FORTRAN, PASCAL, BASIC) and is currently in use. Its objective is to find the planting density for a given seed lot of known size and cultivar which maximises the marginal price of market value over seed cost. Its limitation is that this is essentially the only problem it can solve.

A large proportion of the knowledge contained within such models is inaccessible to the user. In practice there is variation, e.g. from one year to the next or one field to another, in how potato tubers perform once planted. Later in the season the grower may have new, more certain information available, e.g. the actual number of stems or the number of daughter tubers produced. At the end of the season, the grower may wish to review the performance of the crop in order to make possible adjustments for future seasons. None of this information can be fed into, or consequences examined by, traditional software packages without reprogramming.

Research in Artificial Intelligence (AI) techniques is developing rapidly. Computing power, a limitation only 5 years ago, has undergone major and significant advances in both hardware and software. The prospect of decision support systems of sufficient complexity and practicality is with us. There are two, complementary approaches to meeting the challenge, *rule-based* and *causal* (probability-based) *models*. We have researched and evaluated both approaches at SCRI.

Rule-based models Rule-based models can encapsulate precisely the same knowledge as that contained in mathematical models. Mathematical equations are replaced by rules of the form

IF (planting density has the value d, seed size has the value s; stem density takes the value f[d,s])

f[d,s] is simply the original mathematical expression quantifying the effect of plant density and seed size on the number of stems produced. At first sight it would appear to be simply a more cumbersome way of implementing the original mathematical model. There are three advantages: model construction is automated, model definition is separated from query definition and qualitative and quantitative information can be combined. A set of rules, each describing one of the many relations having a bearing on tuber size distribution and market value etc., are entered. From this library of rules the complete model is built automatically by linking corresponding inputs and outputs (complete model, Fig. 1). All input and output values are accessible to the user. How they are accessed is determined by the query types available.

A query is no longer a single specific question, "What is the number of daughter tubers produced if the stem density is 30 m^{-2}?". It is the more general case, "What is the value of variable 1 if the value of variable 2 is x?". While still being able to answer the specific question given here, it applies equally to any pair of variables, e.g. "What is the yield of bakers if the total yield is 65 t ha^{-1}?". This one query is also able to answer the reverse, equally important question, "What is the

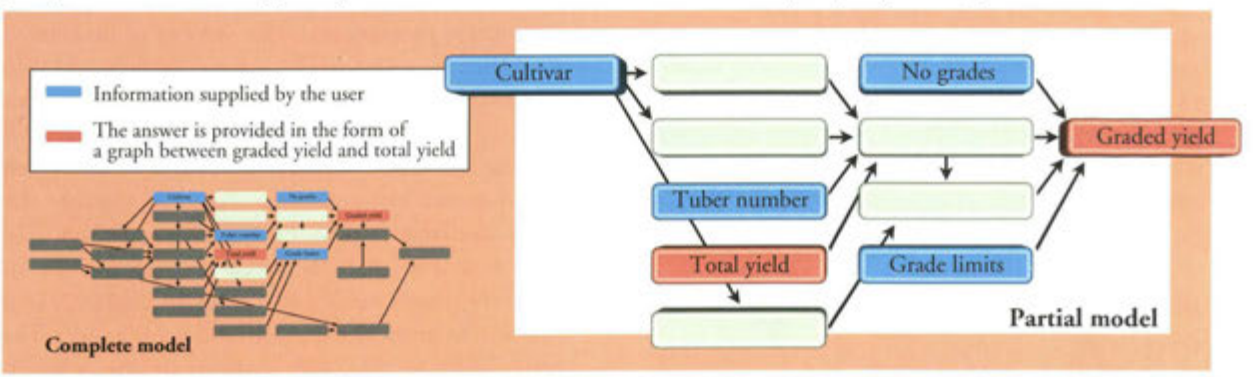


Figure 1 A rule based model of tuber size distribution in potato showing that part which is accessed when a particular conversion (see text) is made.

total yield if the yield of bakers is 30 t ha^{-1}?", with the same ease. Here the rules are being exercised in the reverse direction - what input is consistent with a given output. Another example of a general query is "What value of this variable maximises the value of that variable?". This would answer the specific question of the original seed-rate package, but can answer many more in addition. A further advantage is having defined a set of queries for application to one rule-based model, the set equally applies to any other rule-based model that has been or may be constructed; no further programme development being required. This approach has considerable power and flexibility in accessing and using knowledge effectively.

The result of a 'conversation' with the rule-based model is shown in Figure 1 (partial model). A potato crop is part way through the growing season. The grower has first told the system the cultivar and the market (number of grades and their weight limits) and then asks "How does graded yield vary with total yield?". The system responds first by recognising it has insufficient information to answer the question and asks the user to enter the number of daughter tubers present in the crop. It then answers the main question, producing a graph of graded yield against total yield. Only information essential to answering the question has been requested by the system. Only that part of the model relevant to answering the question has been accessed. The system is ready to continue the conversation answering any further questions the grower wishes to ask in this particular context.

A further problem arises in practice, that is the quality of the information. The market price that the crop may be ultimately sold at can only be a rough estimate when decisions are being made earlier in the season. It is subject to considerable uncertainty and is better represented by a qualitative variable taking the values e.g. 'very low', 'low', 'average', 'high', 'very high'. A mapping is defined between the quantitative variable and its qualitative counterpart, e.g. less than £30/t is 'very low' etc. Having defined appropriate mappings for all variables, the model is then exhaustively exercised to define a full set of qualitative rules. The single quantitative rule shown earlier produces a set of qualitative rules of the form.

IF (planting density is 'low' and seed size is 'small'; stem density is 'low')

The process of abstraction (producing qualitative rules from quantitative rules) makes it feasible to integrate knowledge of a qualitative nature within the same system.

Causal models The full potential of abstraction is only realised when uncertainty can be attached to each qualitative variable. Causal models can attach such uncertainties. Furthermore, they can propagate the consequences of changes in uncertainty using the Bayesian probability theory. The building blocks are the nodes, which are variables such as seed price, seed rate etc. as in the previous model, to which are attached probabilities or uncertainties. Thus the probability that market price is 'very low' may be 2%, 'low' 25% and so forth, with the total probability for each node adding to 100%. The main difference from the rule-based model is the causal link, the connection between the nodes. Rather than a mathematical relation or a set of rules, the link is a set of conditional probabilities. A causal model describing the influence of weather and management on the yield

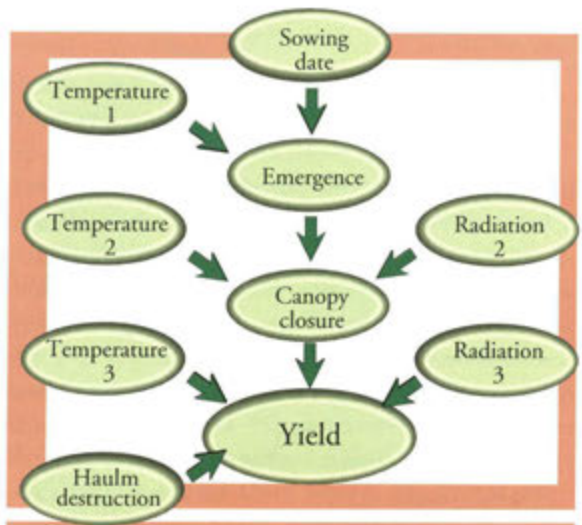


Figure 2 A causal model (directed graph) of the potential yield of potato.

of potatoes is shown in Figure 2. In consultation with experts, the key nodes and causal links are defined. The probability structures are created automatically by a computer programme. The number of probability structures can grow rapidly if more detail is added to the model. As hardware and software continue to advance, so the practical level of complexity will increase. The skill is in constructing a model which incorporates just sufficient detail to capture the important behaviour of the system in question. The next stage is to enter the actual probabilities. With relatively simple models like those in Figure 2, it is possible to enter these probabilities by hand. The nodes 'planting date' and 'temperature' are common parents to the node emergence date and therefore form what is referred to as a 'clique'. However, with

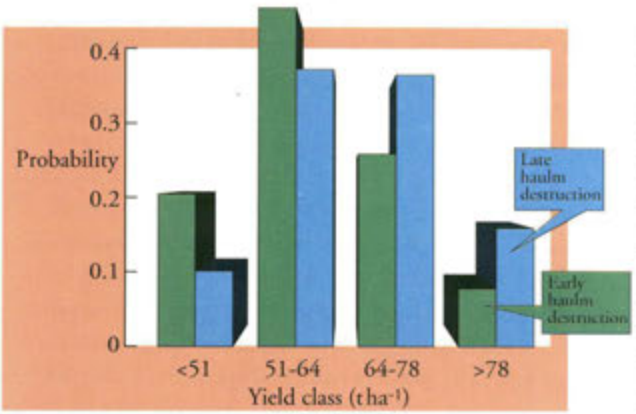


Figure 3 The effect of changing the timing of haulm destruction on the probability distribution of final yield.

more complex models the number of probabilities not only prohibits manual entry but it is also unlikely that there is sufficient data upon which to calculate them in the first place. A practical alternative is to use mathematical or rule-based models to provide simplified representations of the real world. An exhaustive simulation of a mathematical model of water constrained yield of potato, developed at SCRI, was carried out using all combinations of a 30-year run of current weather, planting and harvest dates (approximately 50,000 simulations). The data provided sufficient information on the variability of the system with which to calculate the necessary probabilities and was done automatically with the aid of a computer programme which 'trains' the causal model.

An example of a simple query made to the causal model produced is shown in Figure 3. The grower wishes to assess the effect on yield of changing from an early to a late haulm destruction date. In the first case the grower believes with 100% certainty that the haulm will be destroyed by 4 September. In the second case the belief is changed to 100% certainty that it will be destroyed the third week in September. The consequences are clear, the chance of a low yield has been reduced from over 20% to less than 10%. In contrast to the single value, produced by a mathematical model, there are a range of possible outcomes (due to uncertainties in the weather, planting dates and so forth) with probabilities attached.

As with rule-based models, queries can also be addressed in the reverse direction. This approach was used in a recent study of the impact of climate change on Scottish agriculture. The predictions of climate change are uncertain, on a crude scale relative to the size of Scotland, and undergo frequent revision as understanding improves. The use of crop simulation models is extremely cumbersome and costly in time, requiring a complete repeat of previous exhaustive simulations whenever a new climate scenario is suggested. With the causal model already trained with a range of plausible future climates, it is a simple matter to enter the new beliefs without recourse to exhaustive simulation. Even more effective is to ask how much does the climate have to change for say the probability of high yield to double? One then simply has to keep an eye on the latest climate predictions to check the likelihood of this critical climate scenario being achieved.

Causal models have to allow the uncertainties to be attached and consequences propagated. The use of a restricted but powerful Natural Language Interface has further enhanced the flexible querying of rule-based models. The challenge now is to combine these two approaches so that the explanatory powers of the rule-based system are coupled with the ability of causal models to handle uncertainty. Both features are critical in decision making and risk assessment.

Chemistry

W.W. Christie, B.A. Goodman & I.M. Morrison

This section is an overview of the progress which has been achieved through the chemical expertise of the Institute. It encompasses the investigations of the Chemistry Department, the Spectroscopy Group and the Fibres Group. The topics presented all have the theme of being chemistry-derived; some are covered directly by the Institute's commissioned remit while others are either support work for this remit through other disciplines or are supported financially by outside agencies.

The role of the Chemistry Department within the Institute is to develop and apply novel analytical and synthetic procedures for investigating factors that affect crop production and the quality of plant products. In addition, the Department has the task of ensuring that 'Good Laboratory Practice' is followed throughout the Institute. A 'Quality Assurance Officer' has been appointed to coordinate the process of laboratory accreditation.

A new research programme dealing with the relationships between lipid structure and function in plants and plant products is underway. For example, cyclic fatty acids are produced in vegetable oils when food is fried, and there is concern about possible toxic effects. Nutritional and toxicological studies have been hampered by a lack of knowledge of the detailed structures of these compounds. Accordingly, the nature of the cyclic monoenoic fatty acids formed in heated sunflower oil have been determined by chromatography and mass spectrometry. Fatty acids with cyclopentene, cyclopentane and cyclohexane rings and double bonds in the aliphatic chain were identified. Much of this work is financed by an EEC-AIR grant.

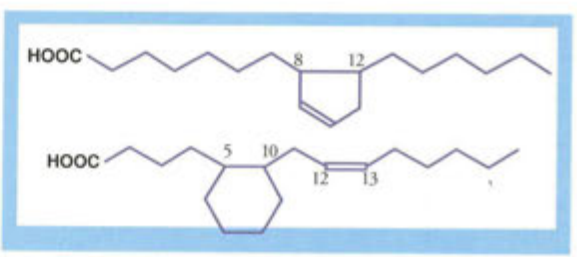
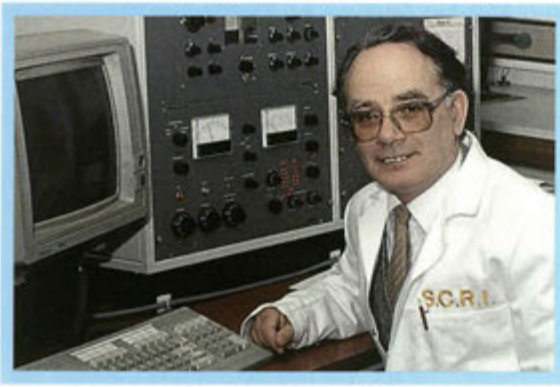


Figure 1 Cyclic monoenoic acids formed in frying oils.

Stable isotope analysis, using continuous flow-isotope ratio mass spectrometry (CF-IRMS), has been applied to a wide range of projects. The methods for measuring natural variation of ^{13}C and ^{15}N in plant and soil material, described previously (*Ann. Rep.1991, 59*) are now carried out routinely with over 7500 samples per annum being processed. A significant proportion originate from outside sources, either from collaborative projects or as analytical services. The methodology is suitable for experiments using very low levels (<0.05%) of stable isotope tracer, exploiting the high analytical precision of the CF-IRMS equipment and giving unambiguous knowledge of the sources being traced as well as greatly reduced tracer costs.

Rapid and simple isotope analysis of plant and soil water has been developed. Existing methods required tedious azeotropic distillation to extract water before analysis. Direct equilibration of plant and soil samples with CO_2 in disposable sample tubes followed by automated CF-IRMS has been validated against azeotropic distillation and is of comparable accuracy and precision at natural isotopic abundance. Batches of over 100 samples can be processed on a daily basis, and samples containing as little as 100 µl water are satisfactory. Similar equilibration of water with hydrogen gas for ^2H analysis is also possible. The method is ideal for low level tracer studies. These techniques are being used to study plant water sources and the ability of root systems to capture water.



Dr. W.W. Christie, Head of the Chemistry Department.

CF-IRMS has proved a useful tool for looking at plant-insect interactions and small insects such as raspberry beetles are particularly suitable. Individual insects can be analyzed for ^{13}C and ^{15}N simultaneously, allowing information to be rapidly collected about large populations.

Phenolic compounds are a diverse group of secondary plant products that have been implicated as defence compounds in a wide range of plants. The potential of capillary electrophoretic techniques for the quantification of such compounds is currently being evaluated. Preliminary studies comparing capillary zone with micellar electrokinetic capillary chromatography suggest that methods based on capillary zone technology may be the more appropriate for the complex mixtures of phenolics present in plant extracts. By this means, an analytical method has been developed for the separation and quantification of a synthetic mixture of some 15 compounds consisting of flavonoids, and cinnamyl and phenol carboxylic acids, that are present in crude methanolic extracts prepared from blackcurrant (*Ribes nigrum* L.) buds.

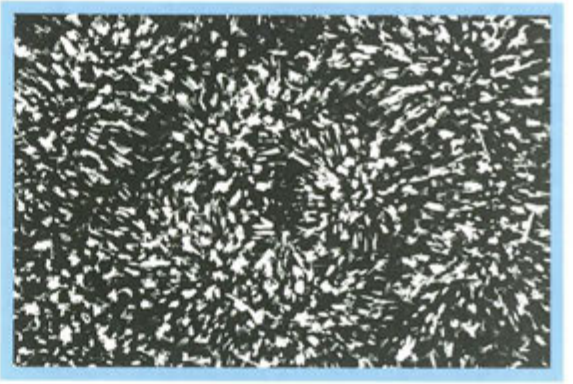


Figure 2 Electron micrograph of waxes on leaf surface.

The surfaces of plants are covered by a thin layer of wax, which serves as a first line of defence against environmental factors, and influences the rate and manner of release of plant defence compounds. Complex mixtures of more than 50 different intact esters in epicuticular wax of *Brassica* spp. have been separated and identified by using combined gas chromatography-mass spectrometry. This methodology is now being used to investigate the effects of species variation and environmental conditions on the qualitative and quantitative composition of epicuticular wax of *Brassica* spp., and for structural analysis of raspberry epicuticular wax.

Automated thermal desorption techniques have been applied to the identification of the major volatile fermentation products produced by pure cultures of both bacterial and fungal micro-organisms from soil. This work, carried out in conjunction with the Department of Cellular and Environmental Physiology, is currently being developed to evaluate the role of volatiles derived from microorganisms in root and soil physiology.

The volatile emissions from a commercial raspberry cultivar, Glen Prosen, have been sampled at six stages of inflorescence development, from green bud through flowering to ripe fruits. Several new compounds were detected in the ripe fruit, in addition to the 'character compounds' ∝- and ß-ionone. This work is part of a collaborative study with the Zoology Department on the raspberry beetle, *Byturus tomentosus*, and should provide a useful picture of the chemical environment experienced by insects in close proximity to the growing plant. Similar methodology is being used to study the volatiles given off by leaves of plants that are either resistant or susceptible to aphid infestation.

A reliable method was developed to allow the routine synthesis of Multiple Antigenic Peptides (MAPs) using the solid phase synthesis equipment available in the Department. Several of the MAPs produced are currently in use by Dr G. Lyon (M & B) and preliminary studies indicate that the antibodies raised are successfully cross reacting with the required native proteins.

Figure 3 DNA sequencer.

The DNA synthesis facility continued operation, meeting increasing demand for oligonucleotides for PCR, RAPD and sequencing applications. Several sequences were made incorporating 5'-OH modifications, including conversion to a primary amine and both biotin and fluorescein tagging.

A series of genomic and cDNA clones isolated from potato have been partially sequenced (with the CMG Department). Synthetic oligonucleotides were made from these sequences and are now being used in mapping studies.

The electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) facilities have continued to make a major impact on research activities and have been used extensively on a variety of problems in which free radicals were implicated. In addition, work has been commenced on the use of these techniques to characterize the chemical environments of metals in paramagnetic metallo-enzymes.

The project on the development of methods for the detection of irradiated food and the investigation of plant senescence processes has advanced on a number of fronts. Optimum conditions for the detection of the cellulose-derived radical have been determined. These experimental criteria have been incorporated into revised methodology for the identification of irradiated foodstuffs that contain cellulose and used to examine the stability of the radiation-induced free radical. The signal intensity falls to about 10% of its original value in 4 months, which thus represents a realistic practical limit on the post-irradiation time of samples that can be investigated.

Measurements have been performed on the roles of free radicals in senescence processes, both normal and pathogen-induced, in selected plant tissues in collaboration with the Mycology Department. The emphasis has been on investigating the interaction between *Erwinia carotovora* and potato tubers, but measurements aimed at investigating the link between free radical generation and phytoalexin biosynthesis have also commenced.

Studies of the roles of free radicals in abiotic processes in plant tissues have been conducted in collaboration with Dundee Institute of Technology. These have involved the measurement of changes in free radical profiles during callogenesis of responsive and recalcitrant potato genotypes and the effects of seed storage conditions on free radical generation in *Carica papaya*, a project which also involved the Royal Botanic Gardens, Kew.

Investigations of the reaction of plant volatile oils from oregano, summer savory and thyme with the superoxide radical, $O_2^{\cdot-}$, have been conducted in collaboration with the Scottish Agricultural College. The results show that reaction occurs with their carvacrol or thymol components, leading to the formation of free radical oxidation products. The next objective is to elucidate the free radical pathways.

The free radical scavenging properties of coffee have been shown to involve molecules other than caffeine, with theobromine and chlorogenic acids being demonstrated to have very strong superoxide radical scavenging properties.

A major advance this year has been in the use of quantified maximum entropy procedures for the production of quantitative information. This approach is particularly valuable for spectra with poor signal-to-noise ratios and yields meaningful estimates of uncertainties from a single spectrum.

As in previous years, the research based on nuclear magnetic resonance (NMR) has involved collaboration with Dundee University and projects using both spectroscopic and microimaging (microscopic) techniques have been developed. Solution techniques have been used (a) to investigate metabolic processes by following changes involving chemical forms and the distribution of the label in specifically ^{13}C-labelled

molecules and (b) as an aid in the identification of products from the degradation of plant cell walls, whilst solid state (CP/MAS) techniques have been used in the measurement of cellulose crystallinity and the identification of non-cellulosic impurities in plant fibre products.

There have been a number of notable achievements in the NMR microscopic work stemming from the acquisition of a Bruker X32 Workstation which has made it possible to produce complete 3-dimensional images from a single experiment. This will now facilitate developmental studies over extended periods on single specimens.

The use of highly oxygenated species for the delignification/bleaching of native plant fibres, involving peroxymonosulphate (Oxone), has now been extended to flax (*Linum usitatissimum*) fibre and brassica (forage rape) stems. The removal of the small amounts of lignin-like materials from flax fibre, to values of ca. 0.5%, could be achieved even without a subsequent alkaline extraction. The relatively low levels of non-cellulosic polysaccharides present in flax differed with the treatment. The galactose- and mannose-containing polysaccharides were remarkably resistant. It was only the arabinose- and xylose-containing polymers which were removed and that mainly required the alkaline treatment.

Treatments with another highly oxygenated species, sodium perborate ($NaBO_3.4H_2O$), have commenced and are showing promise. Perborate differs from peroxymonosulphate in that aqueous solutions are already at a pH greater than 7 while the peroxymonosulphate is only active at pH values less than 7. The visual loss of colour is seen in Figure 4 and the necessity for subsequent alkaline extractions, as used with peroxymonosulphate, may be removed. A single treatment would have major benefits for an industrial operation.

Figure 4 Loss of colour of barley straw after perborate treatment (l. untreated, r. treated).

Investigations into the structure and architecture of fibre cell walls has concentrated on the cross-linked dimers of phenolic acids. Both the range of isomers of the different types of dimers and the changes which occur during growth of barley have been addressed. Of the photochemical dimers, both truxillic and truxinic forms derived from ferulic (4-hydroxy 3-methoxy cinnamic) acid were found at all stages of growth and in both apical and basal samples, except in etiolated tissue. This is further confirmation that such dimerisation is due to photochemical reactions. Four other dimers were also detected at very low concentrations. From the mass spectral data, these were dimers of 3-(3-hydroxy 4-methoxyphenyl)-propanoic acid. This is the first report of the presence of such dimers, and their function and occurrence is unclear.

The use of pyrolysis mass spectrometry for the characterisation of oat straw and chemically-treated oat straw (in collaboration with Dr M M Mulder, FOM Institute of Atomic and Molecular Physics, Amsterdam) has greatly expanded our understanding of the changes which occur in plant cell walls particularly on delignification. The fragmentation patterns showed, for example, that chlorite and permanganate treatments, which gave identical wet chemical results, had different effects on the crystallinity of the cellulose. The procedure could also show how the more complex structures in lignin are broken down by the different treatments.

The determination of lignin is one of the major problems in plant cell wall analysis. The most widely used semi-micro method is that using acetyl bromide but it does not give the true lignin content when other phenolic constituents are present, especially with the Gramineae. A preliminary extraction procedure using pyrrolidine has solved the problem and allows a far higher accuracy.

In a previous report it was shown how cereal straw could be solubilised in 100% trifluoroacetic acid at 37°C. The work has been extended to other fibrous raw materials, especially flax fibre and forage rape stems. More than 98% of the flax fibre is solubilised but the forage rape stems are more resistant. The regenerated cellulose from the flax fibre is still very crystalline. The soluble fraction has been shown to contain a series of oligosaccharides whose structure and function is being investigated further.

Current views suggest that the final steps in the lignification of plant cell walls is the production of phenoxy radicals generated by the H_2O_2-mediated action of peroxidases. In common with results from woody species, it has been shown that a non-peroxidase mechanism is also possible in tobacco (*Nicotiana tabacum*). Isolated walls from tobacco xylem can oxidise coniferyl alcohol to lignin-like products in the absence of exogenous hydrogen peroxide and in the presence of peroxide scavengers. Oxidase activity, albeit at low levels, was confirmed and the products were shown to be guaiacyl-type lignins.

In a study of cell wall and endosperm components, a monoclonal antibody, specific for the wheat starch granule surface protein friabilin, was used to demonstrate the presence of a similar protein on barley endosperm. Barley cultivars differed consistently from each other in their content of this protein. Polyclonal antibodies to xyloglucan and human fibronectin did not cross-react with components in extracts of barley endosperm.

Initial results suggest that tissue prints on nitrocellulose from cut stems may be a convenient medium for examining the distribution of transferable cell wall components with antibodies. An immunization *in vitro* was carried out using lectin from *Narcissus pseudonarcissus* as a protein model. Three monoclonal antibodies were selected by ELISA for further evaluation (as well as culture fluid from mixed clones). This technique may be suitable for obtaining antibodies to cell wall components.

Stable isotope analysis at the molecular level

C.M. Scrimgeour

The isotopes of an element have nuclei containing different numbers of neutrons but the same number of protons, and so have the same nuclear charge (atomic number) but different atomic mass. Some isotopes are radioactive and break down to give other elements and emit radiation, but many elements exist as mixtures of stable isotopes. Most of the elements in living systems contain stable isotopes, and those of hydrogen, carbon, nitrogen, oxygen and sulfur are of particular interest. The minor isotopes, ^2H,

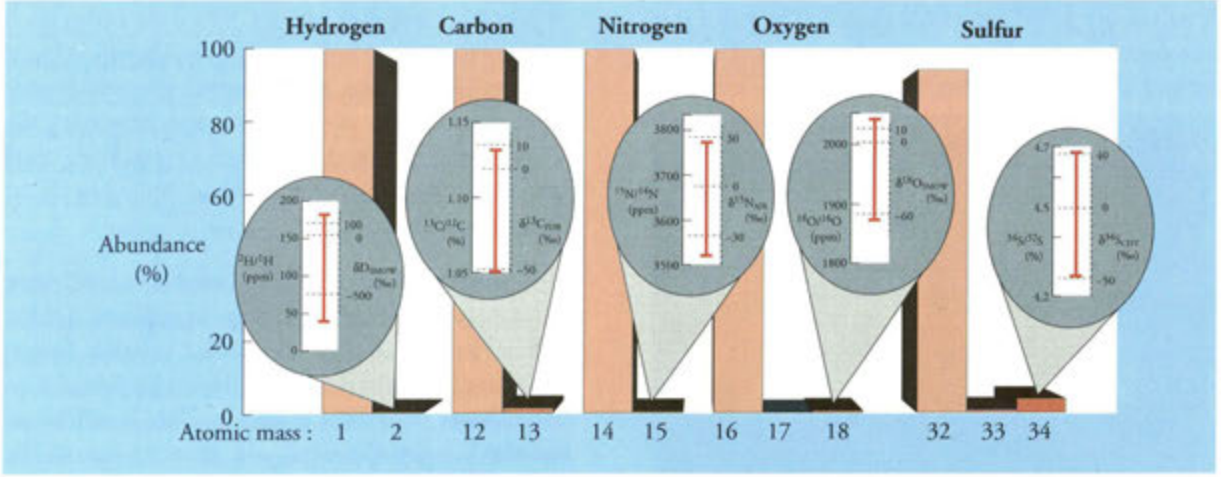


Figure 1 Natural abundance of the stable isotopes of hydrogen, carbon, nitrogen, oxygen and sulfur. The insets show the range of natural variation in isotope ratio along with the corresponding 'δ‰' values relative to the appropriate standards. $\delta = (R_S/R_R - 1) \times 1000$ ‰ where R_S is the isotope ratio for a sample and R_R is the isotope ratio of the standard.

^{13}C, ^{15}N , ^{17}O, ^{18}O and ^{34}S are all rare (0.01 to 4 %) and are all heavier than the major isotopes, ^{1}H, ^{12}C, ^{14}N, ^{16}O and ^{32}S. While the essential chemistry of different isotopes is the same, being determined by the atomic number, the difference in atomic mass may cause a small difference in the rate of reaction or position of equilibrium for reactions involving different isotopes. The resulting natural variation in isotopic composition of these biologically important elements is very small and is usually expressed on a 'δ‰' scale - the difference in parts per thousand from a defined standard - but is more easily visualised if expressed as the ratio of the minor isotope to the major one, conveniently expressed as parts per million (ppm) or percent (%).

Small natural differences in isotopic composition provide valuable information about processes occurring in natural systems *without perturbation* of these systems, and this can be exploited in studies of photosynthesis, plant-water relationships, water and nutrient sources for plants, and food webs (*Ann. Rep. 1991, 59-60*). Differences in isotopic composition can be observed at several different levels, for example, between different plants, between different chemical compounds in the plant parts, and within molecules in which atoms at different positions have markedly different isotopic composition. This within molecule variation gives clues to the biosynthetic pathways leading to particular metabolites, and is also a powerful tool in detecting adulteration or imitation of natural food products.

Measuring the small natural differences in isotope composition poses a considerable challenge in both instrument design and chemical preparation methods. Special isotope ratio mass spectrometers (IRMS) are required which can only operate on a few stable gases such as H_2, CO_2, N_2, N_2O and SO_2 and it requires careful chemistry to convert the sample of interest to one of these gases. Recent developments have increased the sensitivity and speed of analysis by directly coupling sample preparation modules to the mass spectrometer. A continuous stream of helium carries sample gases through the system, and these integrated instruments are known as continuous flow-isotope ratio mass spectrometers (CF-IRMS). The commonest sample preparation system is an elemental analyzer, in which C, N and S in the sample are converted to CO_2, N_2 and SO_2 respectively by high temperature combustion. This is a convenient way of measuring the isotope signature of *all* the C, N, or S in samples such as plant leaves and soils.

Figure 2 Europa Scientific 20-20 and Tracermass CF-IRMS instruments at SCRI.

However, the power of stable isotope analysis is greatly increased if we can measure the isotopic signature of particular compounds and even more so if we can examine specific atomic positions within a molecule. For example, we may wish to compare a plant nutrient in the soil and after entry into the plant, or to follow only a tracer and its metabolites. This presents more of a challenge. Either the component of interest must be separated in a pure form, free from any contaminants containing the same element, or highly selective chemical reactions are required to convert only that compound, or an atom within it, to a suitable gas for IRMS analysis. Sometimes, both techniques must be combined as only a few gas generating reactions have proved suitable for IRMS sample preparation. Some were developed over fifty years ago in the early days of stable isotope work, while others were adapted from equally venerable analytical methods depending on conversion of particular species or functional groups to a measurable volume of gas. Traditionally, this preparative chemistry was carried out using large vacuum lines, often remote from the IRMS. A modern automated gas handling CF-IRMS system speeds up and simplifies many of these analyses and uses cheap disposable sample tubes instead of expensive vacuum glassware. A significant driving force for the development of automated methods was the growing use, during the past ten years, of stable isotope tracers for clinical and metabolic studies in people. For such studies, stable isotopes must be used as radioactive tracers are not acceptable.

Isotopic analysis of amino acids Isotopic analysis of amino acids has many applications in studies of protein metabolism, plant physiology and even the analysis of prehistoric diets, where amino acids from preserved bone collagen are correlated with potential food sources. Before any isotopic measurements are

made, individual amino acids must be separated from the natural mixtures containing about 20 different amino acids. This has been achieved by both preparative liquid chromatography (LC) and preparative gas chromatography (GC). With LC, isotopic fractionation of the amino acids can occur and great care is required to collect the whole of each eluting component. GC has the advantage of reduced isotopic fractionation during the separation process, but the amino acids must be derivatised to increase their volatility. *tert*-butyl-dimethylsilyl (TBDMS) derivatives are particularly good for preparative GC as they are formed in a one step reaction and are easily hydrolysed after the amino acids have been separated. Up to 0.5 mg of each amino acid in a mixture can be isolated in one preparative GC run. For ^{15}N analysis the isolated amino acid can be used directly, as combustion IRMS converts the amino acid N to N_2. Remaining derivatising agents or solvent are not a problem as these are chosen not to contain nitrogen. Similarly, the isotope composition of *all* the carbon can be obtained by combustion, provided care is taken to remove any carbon-containing contaminants. Residues of TBDMS derivatives are volatile and easily removed.

However, it is possible to go one step further and analyse only the carboxyl carbon of amino acids, by using the ninhydrin reaction to convert it selectively to CO_2 (Fig. 3). Here remaining carbon containing contaminants do not matter as the reaction is quite specific for amino acids. The ninhydrin reaction is best known as a colour test for amino acids, but under the right conditions of pH and temperature it results

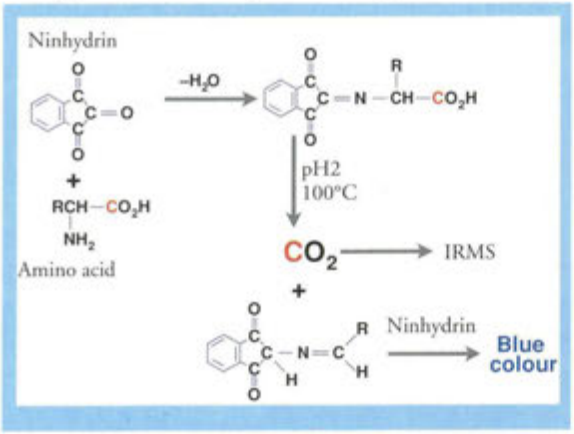


Figure 3 The reaction of an amino acid with ninhydrin. The amino acid carboxyl carbon (C) is released quantitatevely as CO_2 while the remainder reacts with a further molecule of ninyhdrin to produce the characteristic colour.

in quantitative conversion of the amino acid carboxyl carbon to CO_2. These conditions were carefully established by Van Slyke some forty years ago for 'gasometric' analysis of amino acids[1], but can equally well be used for IRMS measurements. Instead of using complex gas measuring apparatus, the reaction takes place in disposable tubes, from which the liberated CO_2 is fed directly to the IRMS through a gas purification system[2]. This is a very powerful tool for studying amino acid transport, metabolism and protein synthesis where amino acids labelled in only one atom can be followed with high precision and sensitivity.

Isotopic analysis of plant and soil water A rather different problem arises when we wish to measure the deuterium (^2H) or ^{18}O content of water when the water is contained in a heterogeneous matrix such as soil or plant tissue. This is necessary, for example, when trying to discriminate between deep ground water and recent rainfall as the plant's water supply. While the source waters are straightforward to analyse, the water contained within plants or soil is less easy. Existing methods have relied mainly on azeotropic distillation to extract water completely from plant or soil samples. Azeotropic distillation involves distilling the water in the material along with a solvent such as toluene. The vapour formed is a mixture of solvent and water, but when this condenses the water does not mix with the solvent. If the solvent is less dense than the water it can return to the boiling mixture while the water remains in a trap, and after a time all the water will be carried over into the trap. Each distillation takes several hours, requires solvent and expensive glassware, and needs samples containing at least 5 ml of water.

Water itself is not suitable for IRMS analysis because of memory effects, and it must first be converted to, or equilibrated with, more suitable gases, CO_2 for ^{18}O and H_2 for ^2H analysis. Equilibration of ^{18}O in water with CO_2 takes 24 h or more at room temperature without agitation. Trial experiments showed that water within intact plant stems and woody twigs equilibrated at a similar rate, and appeared to go to completion. To test this fully, a series of experiments compared direct equilibration in disposable tubes with azeotropic distillation for a range of woody twigs, plant stems and soils. Direct equilibration was found to give comparable accuracy and precision, but sample preparation time is greatly reduced and much smaller samples (containing as little as 100 µl water) can be used. With automated CF-IRMS of the equilibrated CO_2 up to 100 samples per day can be analyzed, mak-

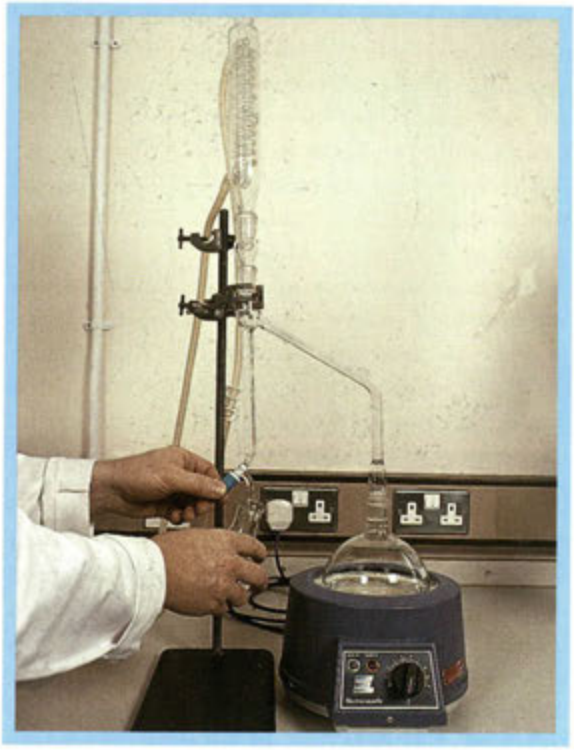


Figure 4 Azeotropic distillation of plant water for isotope analysis.

ing ecological studies of plant water sources a practical proposition.

Water can also be analyzed for deuterium by an equilibration method, using a platinum catalyst to bring about isotopic exchange. Like the ^{18}O analysis, this can be done in disposable tubes, and is suitable for pure water, or biological fluids such as plasma and urine[3]. Direct equilibration with hydrogen was also examined as a way of analysing water in intact plants. However, complete equilibration did not occur unless the sample was first heated to 100°C in the evacuated tube, presumably releasing tightly bound water that does not exchange at a significant rate with other water in the system. This is in contrast to the equilibration with CO_2 where the added CO_2 creates sufficient pH and concentration gradients within the plant to cause equilibration. So far, the method for deuterium analysis is not as accurate or precise as for ^{18}O, and is not suitable for looking at natural variation. However, it is useful for tracer experiments and since D_2O is twenty times cheaper than $H_2{}^{18}O$ it is the preferred water tracer for many experiments.

Water isotope analysis has many other applications, geological, meteorological and clinical, as well as in ecology and plant physiology. Early analytical methods required pure water for analysis, but extensive use of 'doubly labelled' water for measuring energy expenditure in free living people encouraged development of methods where plasma or urine could be used. The direct equilibration of plant material develops this further, and allows measurement of oxygen and hydrogen isotopes derived only from the water in the sample, even in a heterogeneous matrix containing many other compounds with these elements.

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Control of plant fibre quality : Identification and characterization of key enzymes in cell wall synthesis

A. Baty, G.J. McDougall, D. Stewart, J.D.B. Weyers* & I.M. Morrison

Plant fibre is derived from the walls of plant cells and the raw fibre extracted from plant tissues has to be extensively processed for most industrial uses. The main goal of the processing is to remove as much of the non-cellulosic material as possible without disrupting or damaging the cellulose microfibrils of the cell wall. All processing methods incur energy costs, which can be high for the currently used methods, while toxic by-products are produced through the use of delignifying or chlorine-based bleaching agents. Although novel processing methods which avoid environmentally-damaging compounds are being developed (*Ann. Rep. 1992, 61*), there is scope for new approaches to improve plant fibre quality. This could be achieved by targeted breeding programmes in crops whose primary use has not been as a fibre source but

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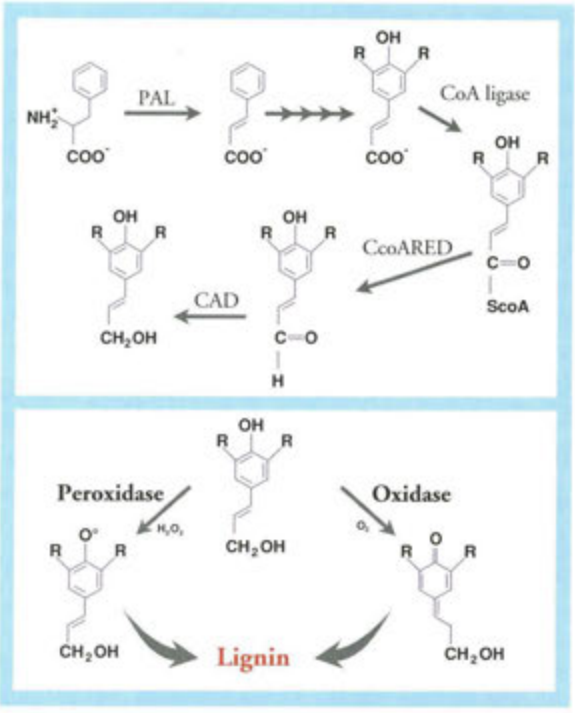

Figure 1 The pathway of lignin biosynthesis. Phenylalanine is converted to cinnamyl alcohol monolignols within the cell. The monolignols are polymerized in the cell wall by the peroxidases and/or oxidases.

not for fibre crops such as flax etc that have been purposely bred for fibre quality.

Another technique to improve fibre quality would be to reduce or modify deleterious or non-cellulosic components in plant cell walls by identifying key enzymes and altering the biosynthetic pathways by genetic means such as antisense technology. This approach could eventually result in plants being manipulated to provide fibres that conform to a set of requirements defined by a particular industry and the consumer. Such 'tailor-made fibres' would require a profound understanding of the biosynthesis of the cell wall components in question. It would be essential that the enzymes selected were absolutely specific for the synthesis of the wall component and that modulation of their activity would directly affect the content of the specific component.

Lignin is a polyphenolic compound which is an integral component of most plant cell walls. Its presence reduces the industrial and agricultural utility of the fibres and its removal is costly in energy and chemical inputs. Therefore, modulation or diminution of lignin content could be economically beneficial. Lignin is synthesized by a unique process by which phenylalanine is converted to cinnamyl alcohol monolignols within the cell by a number of enzymic steps (Fig. 1). The monolignols are extruded into the cell wall space where they are oxidized to phenoxy radicals which polymerize in a free-radical chain reaction to form macromolecular lignin. The polymerization of the monomers is analogous to that of some plastics eg. polystyrene. Although some of the intracellular enzymic steps are specific for lignin synthesis and have been intensely studied, many are common to other cell processes. Therefore, we have concentrated on the enzymes that catalyse the polymerization of monolignols which is the final step in lignin formation. The final enzymic step in a biosynthetic pathway is often important and may be rate-limiting.

Peroxidases and oxidases. Monolignols can be polymerized *in vitro* by the action of peroxidases and hydrogen peroxide or by laccase-type polyphenol oxidases and molecular oxygen. Candidate lignin-specific peroxidase isozymes have been identified in a number of lignifying tissues. They are present in cell walls and their activities and levels increase as cell walls become lignified. They oxidize monolignols more efficiently than other peroxidases. In general, these isozymes tend to have acidic isoelectric points and can become tightly bound to the cell wall during lignification. Results from our recent work have suggested that peroxidases which are extremely difficult to remove from the cell wall may be linked to lignin during its polymerization. Peroxidases linked in this fashion have all the properties expected of lignin-specific enzymes. They are extracted from the cell walls of lignifying tissue, their levels increase as the walls become lignified, and they are acidic isozymes that are particularly able to oxidize monolignols. Therefore, linkage of peroxidases to the wall may be another indication that these enzymes are specific to the polymerization of lignin.

For many years it was thought that only peroxidases were involved in lignin formation in the cell wall but recent work on tree species suggested that laccases, capable of polymerizing monolignols, were also present in lignifying tissue. Oxidase activity can be visualized in a band of actively lignifying cells in tobacco (*Nicotiana tabacum*) xylem by staining with 2,2'-azino-(3-ethylbenz)-thiazoline-6-sulphonic acid (Fig. 2A) and these cells can oxidize a number of chromogenic substrates in the absence of H_2O_2. The oxidase enzyme(s) is/are firmly, probably covalently, bound to cell wall preparations isolated from this tissue and their activity on various substrates including

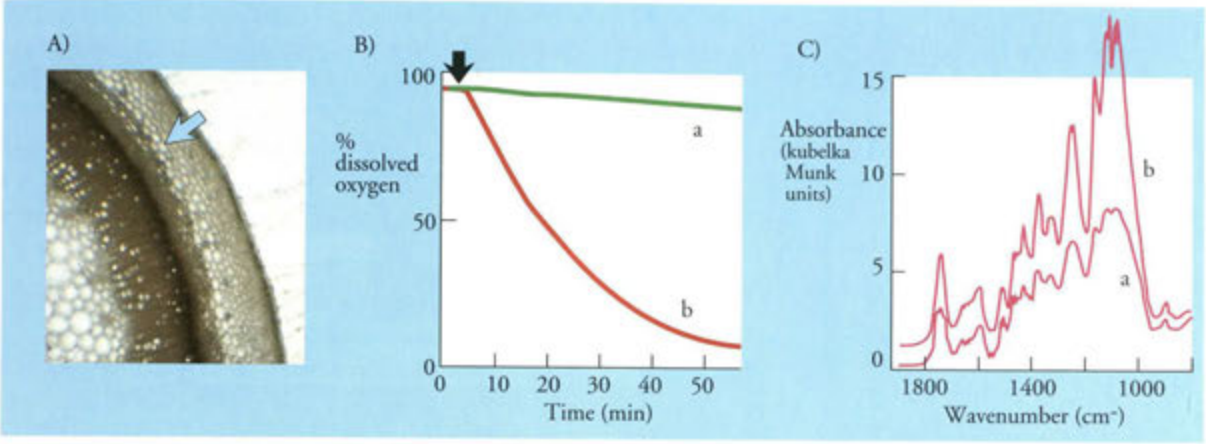

Figure 2 Oxidase activity in lignifying tobacco xylem
A) A ring of xylem cells (arrowed) oxidise ABTS in the absence of added H_2O_2
B) Trace 'a' shows the endogenous rate of O_2 consumption by tobacco xylem cell walls. The rate of O_2 consumption is increased by the addition of 20mM coniferyl alcohol (trace b).
C) Fourier transform infra-red (FT-IR) spectra were obtained from the control xylem walls (a) and the walls after incubation with coniferyl alcohol (b). The enhanced absorbances denoted in spectrum b are characteristic of lignin and confirm that coniferyl alcohol is being polymerised.

the monolignol, coniferyl alcohol, suggests the presence of a laccase-type polyphenol oxidase (EC 1.14.18.1). Oxygen uptake increased when preparations of xylem cell walls were supplied with coniferyl alcohol, confirming the presence of an oxidase (Fig. 2B) and, in addition, they polymerized coniferyl alcohol to lignin-like products which adhered to the cell walls. Fourier transform infra-red (FT-IR) spectroscopic analysis confirmed the polymerization process and spectra from xylem cell walls and walls incubated with coniferyl alcohol are shown in Fig. 2C. The enhanced signals denoted are the result of the deposition of lignin-like material on or in the cell walls. The results demonstrated that laccase-type polyphenol oxidases participated in lignin formation in plant forms other than trees and suggest that they may be widespread in higher plants. These enzymes may be candidate targets for genetic manipulation of lignin content and offer an alternative to previous attempts to manipulate lignin content by ablating lignin-specific peroxidases, which have not been entirely successful. However, as the levels and activities of peroxidases appear to exceed those of laccases in lignifying tissues, the two enzymes may have separate roles in the process. As laccases function without H_2O_2 required by peroxidases, they may carry out lignification reactions around living cells or in the early stages of lignification where the presence of cytotoxic H_2O_2 could cause cellular damage. Peroxidases and oxidase probably cooperate in lignification but further work is required to elucidate the relative roles of the two enzymes.

Other lignification-specific enzymes The presence of H_2O_2 is essential for lignin-forming peroxidases in the plant cell wall and it may be generated by the action of cell-wall-bound malate dehydrogenase. Malate dehydrogenase may oxidize malate, supplied from the protoplast, to form oxaloacetate and simultaneously reduce nicotinamide adenine dinucleotides to NADH in the wall space. NADH is then utilized by cell wall peroxidases in a complex free-radical reaction to oxidize O_2 to H_2O_2. Although this mechanism was proposed some years ago when cell-wall-bound malate dehydrogenase activity was first detected, the enzyme has not been extracted or characterized and attempts to identify and purify it are in progress.

The hydroxycinnamic acids, ferulic and *p*-coumaric acid, are ester-linked to non-cellulosic polysaccharides (NCPs) in the cell wall of some higher plants. These hydroxycinnamate sidechains of the NCPs can also become covalently linked to lignin and, therefore, act as cross-links between their parent polysaccharides and lignin to affect wall architecture. In this way, these hydroxycinnamate groups may act as primers for the initiation of lignification reactions in the wall. Ester-linked hydroxycinnamates appear to be particularly concentrated in the middle lamella between some plant cell walls. This is the same area that lignification of the wall is often first observed. The enzymes that catalyse the transfer of hydroxycinnamates to NCPs in the Golgi apparatus are other candidate enzymes which are being targeted for the manipulation of lignin as the diminution of their activity would alter

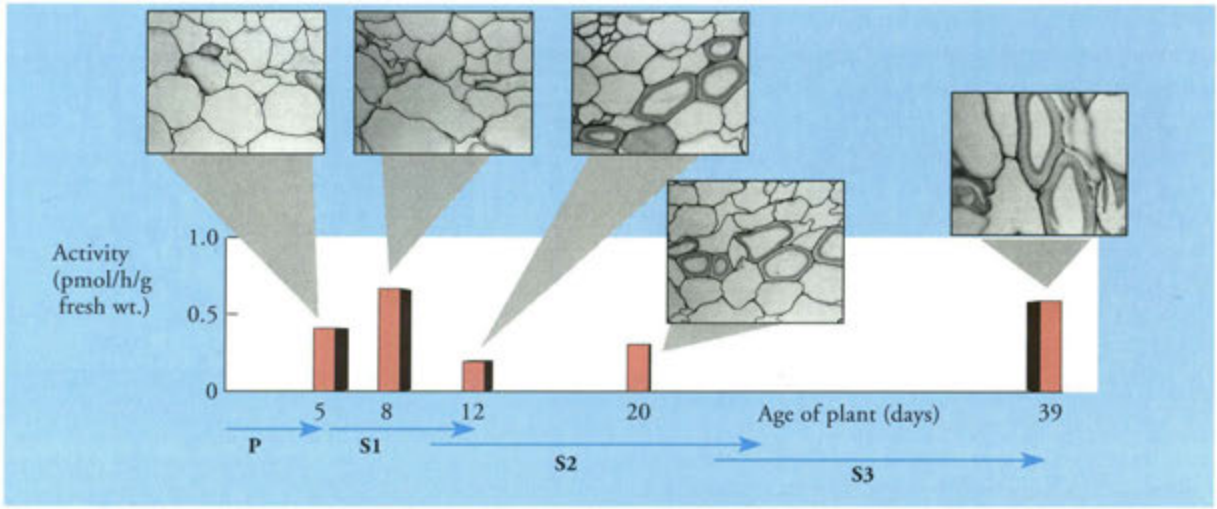

Figure 3 Glucomannan synthesis and the deposition of the flax fibre secondary wall. Glucomannan synthase activity was measured during the development of flax fibres. The activity is high during the deposition of the first layers of the secondary wall (P = primary wall, S1,S2,S3 = stages of secondary wall development).

the content, structure and, through changes in cross-linking, the extractability of lignin.

The biosynthesis of NCPs NCPs have been considered to act mainly as amorphous cements which surround and embed the cellulose microfibrils of the wall. However, their presence is not essential for the assembly of a highly ordered cellulosic secondary cell wall as cotton fibres contain *c* 95% cellulose and lack NCPs. If the content of NCPs in flax fibre (which is around 25%) could be reduced, there would be a concomitant increase in the total cellulose content.

The role of NCPs in flax fibres is currently under investigation. Glucomannan is a ß, (1-4) linked linear polysaccharide containing both glucose and mannose residues and accounts for *c* 2.5% of the dry weight of mature flax fibre and *c* 10% of the NCPs. As glucose and mannose are epimeric isomers, this polysaccharide is structurally similar to cellulose and should hydrogen-bond effectively with cellulose with which it may be closely associated in the wall. Its exact location and role are not known. Flax fibres develop three defined layers of their secondary cell wall over a four week period that follows the cessation of elongation of the hypocotyl of young flax seedlings and the activity of the enzyme glucomannan synthase has been studied through the development of each layer. It was high around the time that the first layer was deposited, then falls and increased again later (Fig. 3). The second rise in glucomannan synthase activity occurred when the fibre wall was near completion but this coincided with the deposition of the first secondary wall layers of another ring of fibre cells. Therefore, glucomannan synthesis appeared to be particularly associated with the initial deposition of the secondary cell wall. The presence of glucomannan in the secondary wall suggests that its synthesis was closely associated with the deposition of cellulose. Most heteropolysaccharides are synthesized by separate and distinct enzymes that transfer each specific sugar residue to the nascent polysaccharide but in glucomannan synthesis a single enzyme catalyses the transfer of both glucose and mannose residues. The unusual mechanism of this enzyme makes it a good target for the genetic manipulation of glucomannan content. Results from these investigations will allow an evaluation of the importance of NCPs in fibre structure.

Antibodies have been raised against oligosaccharides of glucomannan obtained by using a purified ß,1-4 mannanase and are being used to immunolocate where glucomannan is deposited within the structure of the flax fibres and other tissues.

Hormonal control of fibre development An alternative means to improve fibre quality is to manipulate fibre cell development by the use of phytohormones. Fibre development in flax occurs in two phases. Firstly, fibre initials in the procambium of the young cell elongate as the internode extends by a mixture of coordinated growth with, and intrusive growth between, the other cells of the stem. Sometimes the tip branches to avoid an obstacle. A fibre with such a bifurcate tip is shown in Figure 4. When the stem stops extending, the fibres deposit secondary cell wall

material. Preliminary results have shown that the internode length of flax can be increased using auxin and gibberellin and corresponding changes in the fibre lengths are being assessed. Other workers have demonstrated that auxin/gibberellin treatments alter the number, the physical dimensions and the chemical structure of fibres from *Coleus* stems.

The elongated plant fibre cell, with its massively thickened cell wall, represents a huge drain on the plant's resources. The factors that commit a procambial meristematic cell to differentiate into a fibre cell are, therefore, of great economic concern. Understanding the commitment processes may allow us to control fibre initiation and development and ultimately lead to the culture of fibre cells outwith the plant.

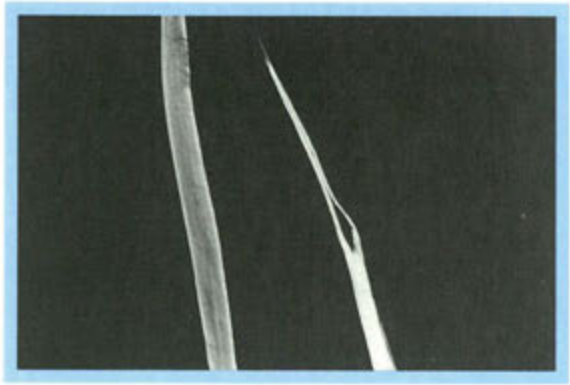

Figure 4 A bifurcate flax fibre cell. Flax fibres branch into two when faced with an obstacle in the stem. This is evidence for intrusive growth of fibre cells through the cells of the stem.

Spin trapping as a tool in free radical research

N.Deighton, S.M.Glidewell & B.A.Goodman

Electron paramagnetic resonance (EPR) spectroscopy allows the selective detection and characterisation of free radicals and paramagnetic metal ions (*Ann Rep 1992, 66*). In favourable cases, the combination of parameters that can be derived from EPR spectra allow for complete characterisation and identification of the free radical or metal centre under investigation. However, due to the inherent instability of many biologically-relevant free radicals, direct detection by EPR is often not possible. In these cases, a technique known as spin trapping has found widespread application.

Spin trapping involves the interception of the unstable free radical with a precursor, usually a nitrone, to produce a relatively stable nitroxide radical which can then be studied by EPR in solution (Fig. 1). EPR spectra generated from free radical adducts of the trap α-(4-pyridyl-1-oxide)-N-*t*-butylnitrone (POBN) generally comprise six lines which arise from interaction of the unpaired electron with one ^{14}N (nuclear spin, I = ±1) and one ^{1}H (I = $\pm^{1}/_{2}$) nucleus. Different conformations of the radical adduct and/or electron-donating/withdrawing properties of the adduct group produce slight differences in the hyperfine couplings.

Much spin trapping work has been performed in recent years and there are now large databases that can be used for identification of the parent free radical from the magnitudes of the hyperfine couplings from the nitrogen and hydrogen nuclei in the adducts. Additional information on the structure of the parent

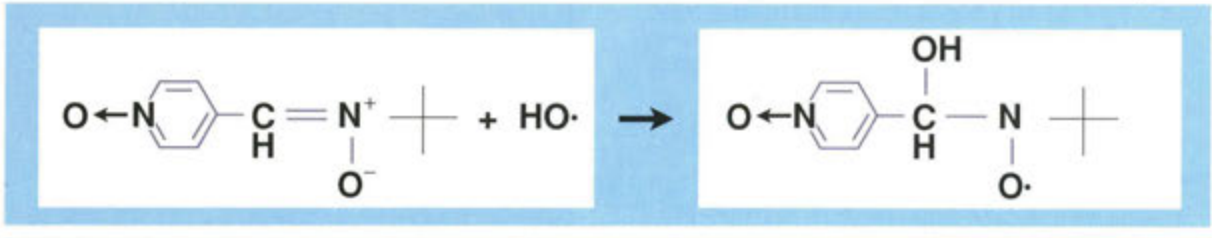

Figure 1 Reaction of the hydroxyl radical with the spin trap POBN.

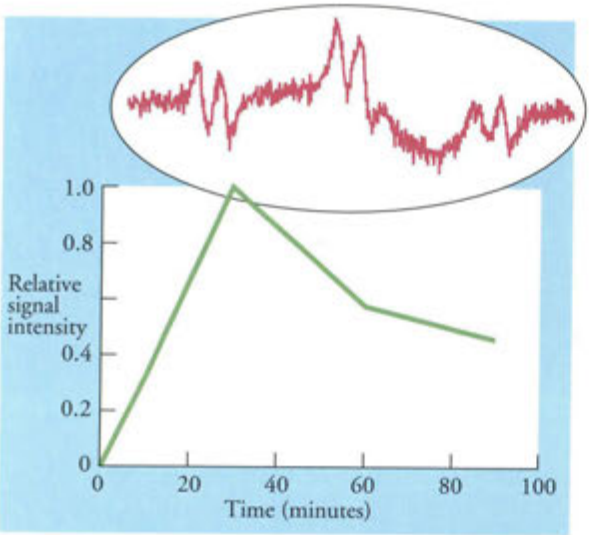

Figure 2 Time course of free radical formation following wounding of soybean cotyledon.

free radical can sometimes be gained through selective isotopic labelling of the free radical precursor which can produce additional spectral splittings (eg. from ^{13}C, ^{17}O). If such characteristic features are not observable in the EPR spectrum, ENDOR spectroscopy may provide the necessary resolution enhancement.

Biological occurrence of free radicals The production of free radicals in biology is strictly controlled in healthy organisms. In the animal world, free radicals have been associated with many medical conditions, particularly traumas, inflammations, Parkinson's disease and cancer. The association with cancer was partly based on the observation that cigarette smoke formed a series of alkyl and alkoxyl radical adducts with the nitrone spin trap α-phenylbutylnitrone (PBN). Wherever membrane disruption has been associated with the development or progression of a disorder, free radicals have been implicated, mostly through spin trapping studies.

Research into free radical mediated events in plants and microorganisms is at a less advanced stage and has been centred mostly on discrete enzymes eg. cyclooxygenases, monooxygenases and of course, lipoxygenase. All three classes of enzyme are currently believed to involve free radical intermediates during substrate turnover and again, the evidence is largely from spin trapping studies. Soybean lipoxygenase has proved a favourite system for study and has been shown to produce alkyl, alkylperoxy and superoxide free radicals from polyunsaturated fatty acid substrates *in vitro*[1].

Spin trapping studies at SCRI In a series of collaborative experiments at SCRI the wound response of soybean cotyledons produced a profile of free radicals that was similar to those from lipoxygenase only minutes after the act of wounding (Fig. 2), whereas, inoculation with *Erwinia carotovora* had no effect on the intensity of the free radical adduct indicating that physical wounding was the event that gave rise to free radical production. Further experimentation is underway to investigate the possible involvement of the free radical burst with cell signalling events that lead to the accumulation of phytoalexins.

A wound response taken to the extreme such as maceration that occurs during chewing food might then also be expected to result in free radical formation. A series of experiments have commenced to investigate these free radical reactions in a range of foodstuffs of plant origin. Following maceration of a small sample of lettuce in the presence of the spin trap POBN two free radical adducts, one of which resembles the HO· adduct (species X, Fig. 3) were detected. The other (species Y) is not yet identified and its large ^{1}H hyperfine coupling of 514μT exceeds that observed for commonly encountered adducts of POBN. Liquid chromatography-mass spectrometry (LC-MS) may reveal the identity of this free radical. Approximately 50% of plant samples investigated have yielded EPR spectra of these two adducts, although samples that

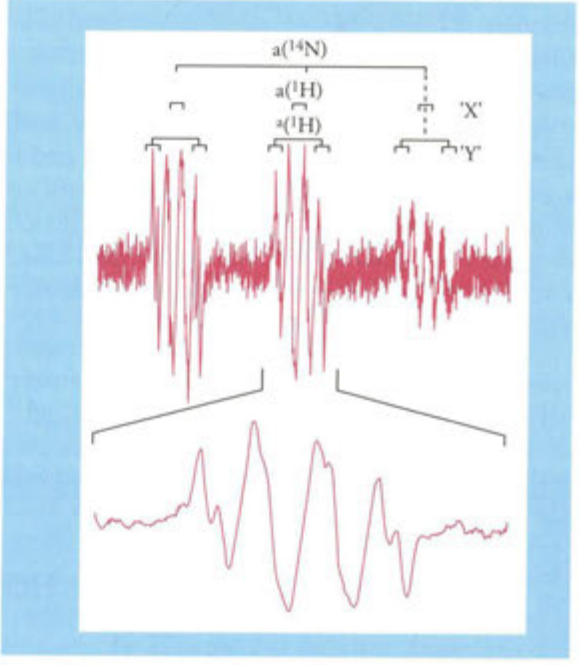

Figure 3 EPR spectrum of POBN adducts following maceration of lettuce.

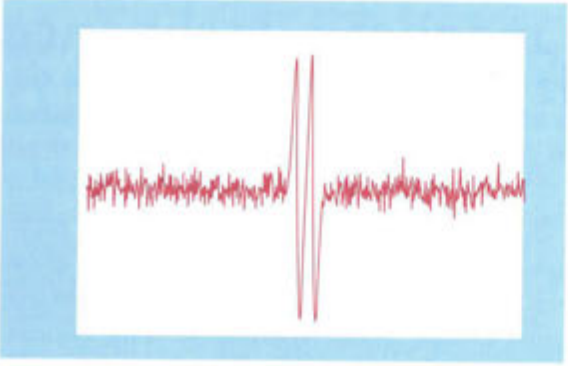

Figure 4 EPR spectrum of ascorbyl free radical from crushed parsley.

are considered rich in ascorbic acid eg. cabbage, parsley and Brussels sprouts produced different spectra which corresponded to the relatively stable ascorbyl radical only (Fig. 4). A summary of the free radicals observed upon maceration is presented in Table 1.

Source	Ascorbyl	X&Y
Broccoli	✖	✔
Cabbage	✔	✖
Celery stalk	✔	✖
Celery leaf	✖	✔
Coriander	✖	✔
Cress	✔	✖
Cucumber	✔	✔
Garlic	✖	✖
Lettuce	✖	✔
Onion	✖	✖
Parsley	✔	✖
Brussels sprouts	✔	✖

Table 1 Free radicals generated upon maceration of foodstuffs.

Free radicals are often produced thermally. Initially concentrating on grape seed oil, a series of experiments have been commenced to assess the role of natural antioxidants in oil stabilisation. A spin trap, (PBN) is dissolved in the oil which is then heated to constant temperature for an extended period, typically 16 h. During this time, EPR spectra are recorded at regular intervals. Adduct formation is observed, and it is believed that the adducts derive from the polyunsaturated components of the oil, eg. linoleic and linolenic acids. The multiple scanning facilities of a

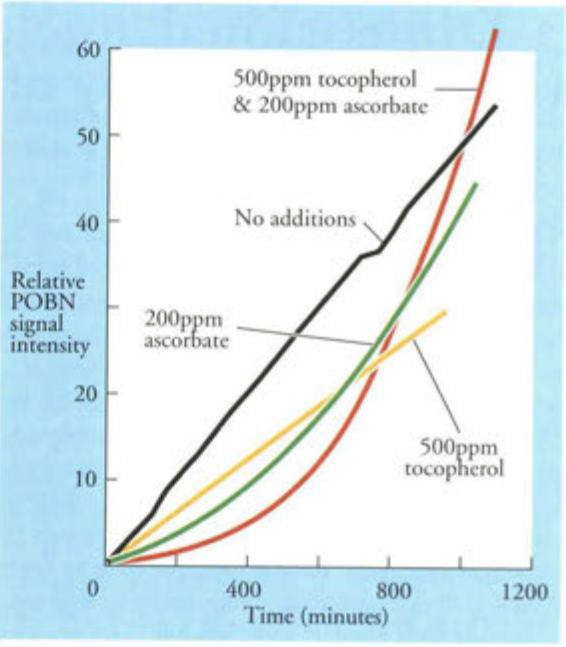

Figure 5 Free radical formation in heated grape seed oil.

fully computer-controlled spectrometer make it possible to perform these measurements with minimal operator involvement.

The effect of α-tocopherol and ascorbic acid added separately or together in lecithin reversed micelles on free radical production in grape seed oil at 60°C are presented in Figure 5. For short periods of heating, addition of α-tocopherol and ascorbate inhibited free radical production and the effect of the two antioxidants in combination was greater than either alone. Over longer periods of heating (>10 h) free radical production was increased in those samples containing ascorbic acid, and samples with combined ascorbate plus tocopherol showed the highest level of free radical production. The generation of free radicals was retarded only in the case where tocopherol was added alone. Stabilisation of vegetable oils by antioxidants is clearly complicated and optimum levels and combinations will probably vary according to the nature of the oil and its planned uses. The EPR methods represent a rapid method for evaluating the efficacy of different formulations at different temperatures.

Reference

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3-Dimensional nuclear magnetic resonance (NMR) microscopy of soft fruit crops

B.A. Goodman, E. Simpson, B. Williamson & R.M. Brennan

The basic principles of NMR spectroscopy and 2-dimensional imaging have been described previously (*Ann. Rep. 1990*, 57) and potential applications illustrated with examples from plant biology and insect physiology. With the recent availability of a powerful dedicated computer workstation in Dundee University, it is now possible to acquire and process 3-dimensional images that consist of $\leq 256^3$ voxels (volume elements), thereby providing a complete non-invasive visualisation of the internal structure of biological specimens with dimensions ranging from several millimetres to a few centimetres. In addition, information on physical and chemical characteristics of the mobile protons can be obtained by adjusting the experimental conditions to study their chemical or physical properties.

All magnetic resonance techniques are based on interactions of magnetic moments with external magnetic fields. In NMR, the sources of such magnetic moments are nuclei with non-zero spins, and resonance between ground and excited state configurations is achieved by absorption of radiation in the radiofrequency (rf) region of the electromagnetic spectrum. The energy required for this transition differs from nucleus to nucleus and hence individual elements can be investigated independently of the rest of the sample.

NMR has inherently low sensitivity and as a consequence it is usually necessary to sum the results of several scans in order to produce spectra with adequate signal-to-noise. A true representation of the specimen is obtained only if the system decays back to its original state before the application of a second and subsequent rf pulses. It does this through a series of interactions with its environment known as relaxation processes, of which the spin-lattice (T_1) and spin-spin (T_2) interactions are the most important (Fig. 1). T_1 and T_2 processes are extremely sensitive to the local environment of the nucleus and it is the variations in their relative magnitudes that form the basis of much of the tissue discrimination that can be seen in NMR images.

Principles of NMR imaging The resonance frequency for each type of nucleus in a sample is proportional to the magnitude of its local magnetic field. Thus, when magnetic field gradients are applied across a sample, each nucleus resonates at a frequency that is determined by its position. In general, the volume of interest in a specimen is defined by a linear field gradient and two phase-encoding gradients so that each nuclear spin has a unique resonant frequency and phase which define its position in a set of Cartesian axes (x, y, z).

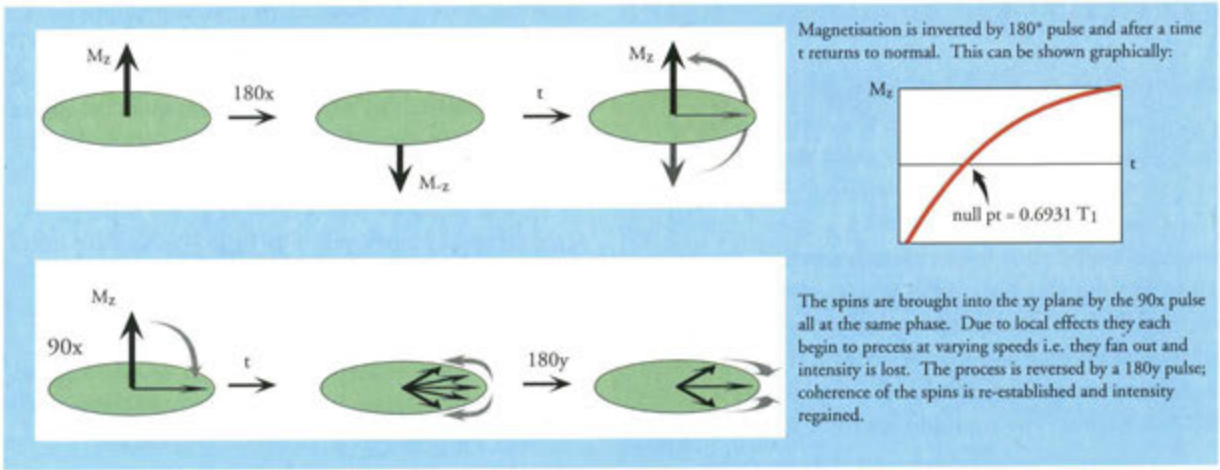

Figure 1 Diagram illustrating a) T_1 and b) T_2 relaxation processes. T_1 represents the rate of recovery from inversion, whereas T_2 represents the rate of loss of coherence of magnetization rf pulse. M_z is the gross magnetization vector (the sum of magnetisation along the z-axis) which varies with time.

In the absence of relaxation effects, the intensity of each voxel is proportional to the number of mobile spins in the corresponding volume of the specimen. However, in most NMR imaging experiments, delays between rf pulses are selected to produce an image in which voxel intensities are weighted according to physical and/or chemical properties of the nuclei. In addition, the power and shape of the rf pulses may be varied in order to produce further discrimination of tissue types. Clearly, the NMR experimental conditions have to be precisely defined in order to produce meaningful images.

Factors influencing image characteristics In biological specimens, several factors can have measureable influences on the magnitudes of either T_1 or T_2. The most significant of these are water concentration, temperature and fluctuations in magnetic field at the nucleus. The predominant causes of local magnetic field variations are (i) unpaired electron density (as found in paramagnetic metal ions or free radicals), (ii) local magnetic susceptibility variations (as caused by for example gas spaces between cells), and (iii) nuclear shielding tensor variations (i.e. changes in the chemical environment of the protons).

Different experimental pulse sequences have been developed in order to probe selectively these different processes. By using a combination of procedures on the same volume of the same specimen it is possible to build up a detailed picture of the physical and chemical characteristics of different types of tissue within a specimen and to measure, in real time, processes such as the velocity of fluid flow within major vascular elements. The complexity of the processes that determine the NMR signal intensity, however, often creates difficulties in the interpretation of the generated image.

Image construction procedures Conventionally, NMR images have been acquired and displayed as a series of 2-dimensional slices. The major weakness of this approach is the difficulty in picturing the complex 3-dimensional nature of most biological structures. In addition, for experiments performed over extended periods of time, difficulties in reproducibly aligning specimens in precisely the same positions within the imaging probe made it essential for the samples to remain untouched throughout the sequence of measurements, thus making it inefficient for investigating slow developmental processes. Recently, computer procedures have become available for complete three-dimensional image construction from NMR data sets. Such techniques are able to make use of multiplanar reformatting, cluster analysis and surface-rendering to produce images that are constructed to display the arrangement of selected tissue types in three dimensions. Three examples are presented below of such use from our current research on soft fruit in collaboration with Dr J.A. Chudek and Prof G. Hunter of Dundee University.

NMR microscopy The major feature of NMR microscopy is its ability to investigate the internal structure of biological materials non-invasively and to observe developmental processes directly on a single live specimen. The high water content of soft fruit makes them ideal subjects for NMR microscopy and internal structural information, such as the arrangement of the vascular system, the presence of gas spaces, surface characteristics, the location of seeds and sites of injury etc., can be revealed as a result of differences in physical and/or chemical environments of protons.

Figure 2 shows a representation of the vascular structure of a fruit receptacle from red raspberry that was produced by a combination of 3-dimensional image reconstruction and surface rendering techniques. The

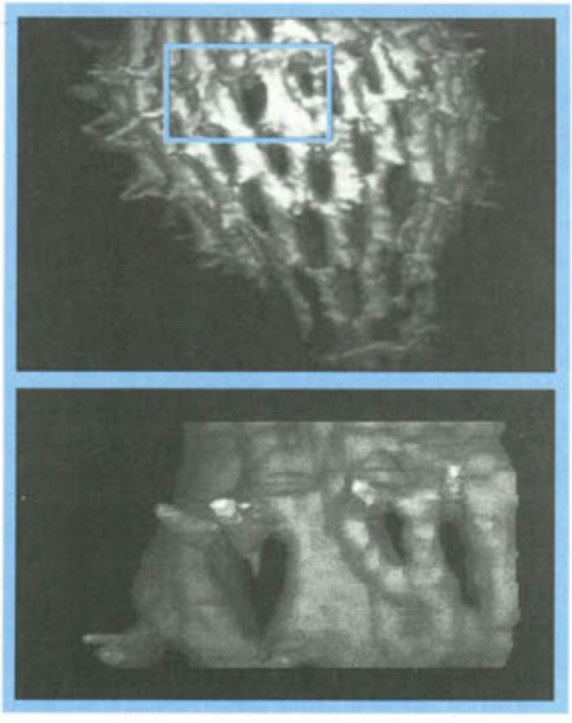

Figure 2 3-dimensional image of vascular architecture in a portion of a fruit receptacle of raspberry, with an enlarged region showing the carpellary traces that previously supplied a drupelet.

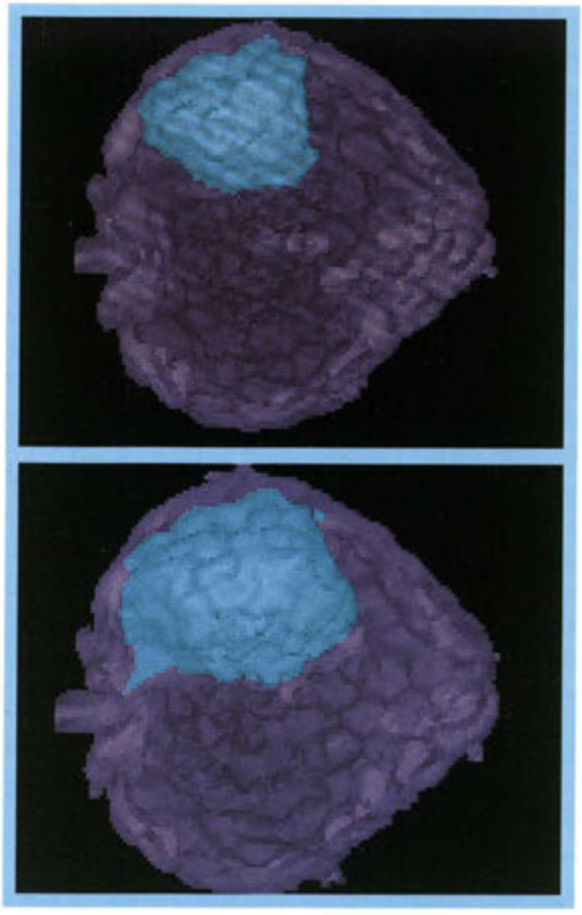

Figure 3 Two surface rendered images that illustrate the spread of a lesion caused by *Botrytis cinerea* in a strawberry fruit examined on successive days.

spiral arrangement of carpellary traces supplying nutrients to the drupelets is clearly displayed and the departures of both the xylem and phloem traces from the stele can be seen in the enlarged portion in the inset. In the future, experiments will be conducted on a super-wide-bore instrument, which is due to be delivered to SCRI in 1994, to study directly the rates of movement of water in such vascular elements in different parts of plants subjected to a variety of external stresses.

An illustration of the progressive development of diseased tissue in strawberry fruit infected by *Botrytis cinerea* is shown in Figure 3. Discrimination between healthy and diseased tissue was achieveable because of the much longer T_2 relaxation times in diseased tissue. Consequently, it is possible to set the experimental parameters to produce image intensity only in the infected region(s) of the specimen. NMR microscopy is thus a powerful method for measuring non-invasively rates of disease development in internal tissue of fruit and other biological material.

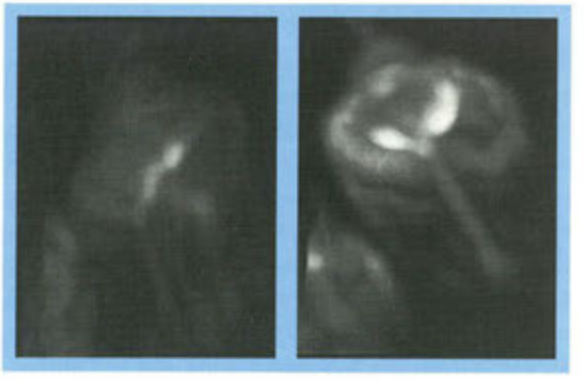

Figure 4 Two images of blackcurrant flowers taken before and after freezing damage
(l - before freezing, r - after freezing).

The effects of freezing injury on flowers of blackcurrant (*Ribes nigrum)* can similarly be visualised using NMR microscopy (Figure 4). The ability to perform this type of measurement is important because examination of freezing stress in plant tissues by conventional means is difficult without introducing artefacts during sample preparation. The main sites of injury, denoted by increased image intensity resulting from water leakage caused by membrane damage, are seen to be in the stylar and ovular regions and future studies will compare frost susceptible and tolerant genotypes under identical conditions in an attempt to gain a better understanding of the physical basis of freezing injury in such woody plant tissues.

Fungal & bacterial diseases

James M. Duncan

The Mycology & Bacteriology Department investigates the genetical, physiological and biochemical bases of pathogenicity in fungal and bacterial pathogens of plants and of resistance to disease in plants. Its work ranges in scope from studies of molecular and pathogenic variation within Phytophthora *and other fungi at taxonomic levels from races to separate species, to mathematical models of disease spread in fields planted with mixtures of genotypes that differ in their resistance to a pathogen. All core work is in some way connected with host resistance. Our strategic goal is to gain a better understanding of the genetical and environmental stability of host resistance, and thus to contribute eventually to environmentally benign strategies for disease control that involve low or reduced agrochemical inputs.*

Blackleg of potato caused by the bacterium, *Erwinia carotovora* subsp. *atroseptica (Eca)*, is important for Scottish seed potato producers. Over the past 30 years much has been learnt through the work of Michel Pérombelon and his team regarding the ecology of the pathogen, epidemiology of the disease and of the closely related bacterial soft rot that occurs in store.

The discovery that *Eca* was ubiquitous in the agricultural environment meant that it could not be excluded for all but the shortest period from production systems and required the development of new strategies for control. Restricting the build-up of inoculum to low levels through early harvesting and a reduced number of propagation cycles through micropropagation are now routine parts of an overall strategy that aims to keep inoculum loads at or below 10^3 live cells per tuber. At this level, the disease is relatively unimportant in subsequent ware crops, almost regardless of environmental factors. In order to minimise the risk of disease, it is important to know the numbers of bacteria present on the seed and this requirement led to the new developments in diagnostic methods. Various systems based on selective media and/or immunology have been devised. The most recent, based on immunofluorescent colony counting (IFC), can detect a few viable cells and provides accurate and rapid

results. As importantly, the statistics and mechanics for sampling from large boxes of tubers are also being developed to a practical level.

New technologies often provide the research worker with fresh insights and the application of IFC to blackleg showed that uneven distributions of *Eca* inocula on infested tubers could affect the disease potential in different seed lots even if the mean level of contamination per tuber was the same among stocks. Knowing the distribution of inoculum within a seed lot may make predictions of subsequent disease levels in ware crop grown from that seed lot more accurate.

Michel Pérombelon has made considerable contributions in studies on pectic enzymes and other systems within the bacterium as pathogenicity determinants and on the nature of resistance in the host. In the latter, his work has highlighted the importance in disease resistance of high levels of pectin methylation in the cell walls of fusion hybrids of the wild species *Solanum brevidens* and the cultivated potato and in subsequent generations derived from them. This has provided new insights into resistance and holds out promise that, in future, new cultivars will have some resistance to the disease, even under the most adverse conditions of anaerobiosis. In the long term, resistance is probably the best strategy for controlling blackleg.

Dr. J.M. Duncan Head of the Mycology and Bacteriology Department.

Research on erwinias at SCRI has made a substantial and internationally recognised contribution towards controlling blackleg and aided the Scottish seed potato industry in particular and potato producers in general. It is fitting that towards the end of his career at SCRI, Michel has highlighted in this Report, those research points past, present and future that he feels are important for integrated control strategies for blackleg and soft rots.

An important environmental component affecting the development of blackleg and bacterial rot in store is free water. Water films exclude air from infected tissue leading to the development of sites of anaerobiosis within which the bacteria can develop unhindered by the active resistance mechanisms of the host which depend on oxidative processes. In the infection of roses and other flowers by *Botrytis cinerea,* it was supposed that free water was necessary for growth and penetration of the host by fungal hyphae. However, studies by Williamson and Harrison working with Louise Harding, from Dundee Institute of Technology, using electron microscopy and cryo-preparation, have shown that spores can germinate and penetrate at high relative humidity without the presence of free water. This is an important finding as grey mould results in unsightly brown blemishes on petals of the flowers and is a serious problem for the cut flower trade. As well as having considerable implications for control strategies for flower producers, the work demonstrates that valuable results can come from short studies employing student labour who, at the same time, receive valuable training in scientific methods.

The work on grey mould clearly recognises the importance of environment in the manifestation of disease. However, its effect on the expression of host resistance can sometimes be overlooked or underestimated. Fluctuations in resistance in response to environment may help to explain inconsistencies in disease rankings that breeders and pathologists often obtain in field experiments screening for resistance. The effect of environment on the expression of resistance is also described by Harrison and Newton in this Report. Surprisingly, for two diseases as different as potato late blight and barley powdery mildew, the conclusions are similar; pathologists must investigate the resistance-environment interactions if they are to understand the variation in expression of resistance in cultivars and selections experienced in the field.

Coffee berry disease, caused by *Colletotrichum kahawae,* is a serious disease affecting coffee. It is cur-

rently controlled by as many as seven applications of fungicides in one season, but losses of up to 50% can still occur. Most commercial varieties are highly susceptible but exploiting resistant germplasm would be a great advance since it would reduce the need for fungicides in coffee-producing countries, many of which cannot afford the expenditure on fungicides. In a seminal study, Nyange, Williamson and McNicol have used the technology of tissue and cell culture to select cell lines with increased resistance to the disease. Cells that survived exposure to the toxin produced by the fungus *in vitro* have been grown on and regenerated into whole plants. Some of these plants will shortly be returned to Tanzania and assessed for their resistance to the disease in the field. Any resistant plants selected in this way will surely prove valuable in future breeding programmes dedicated to finding resistance to this intractable disease.

In the last year, the Department received a LINK award jointly funded by the Horticultural Development Council and the Scottish Office Agriculture and Fisheries Department. The work is on the detection of *Phytophthora* in planting material of horticultural crops. Although aimed at producing practical tests that can detect low levels of contamination of planting material of vegetatively propagated crops by *Phytophthora* spp., the programme is closely linked with the core programme on the molecular biology of *Phytophthora.* DNA suitable for rapid amplification by the polymerase chain reaction and detection and discrimination of up to 10 species has already been identified and is now being developed for first generation tests. Already, the work has shown that a *Phytophthora* species that is very common on raspberry, while closely related to *P.cactorum,* is different enough to be considered as a separate species.

Potato blackleg: Progress and prospects for research

M.C.M. Pérombelon

Introduction The blackleg disease of potatoes caused by soft rot *Erwinia* spp. has created problems for the Scottish seed potato industry. However, after three decades of studies, we now know why past preventive measures have failed and can formulate recommendations for its control. This paper is a personal overview of past and current research which could have a bearing on disease control.

Blackleg terminology Three soft rot erwinias associated with potatoes are *Erwinia carotovora* subsp. *carotovora* (Ecc), *E. carotovora* subsp. *atroseptica* (Eca) and *E. chrysanthemi* (Ech) but only the first two are present on Scottish seed. Blackleg, which is initiated by the pathogen from the rotting mother tuber, is often confused with aerial stem rot which originates in wounds on the aerial stems and is caused by air- or water(irrigation)-borne erwinias. In addition, the range of symptoms expressed varies from a soft rot of the stem to stunting, wilting and desiccation of the plant and is strongly modified by environmental conditions, e.g. a soft rot tends to occur under wet conditions while wilting and desiccation predominates under dry conditions (Fig. 1). Eca predominates under cool moist conditions while Ech occurs in warm (>25°C) conditions which can be dry (Mediterranean) or wet (sub tropics). However, low temperature variants of Ech able to cause blackleg in temperate regions are prevalent on the Continent and have been found in England in crops grown from imported seed. Ecc rarely causes blackleg although it is often present in stems and can take over from Eca in a blackleg lesion. However, it is the most common causal agent of aerial stem rot.

Blackleg epidemiology The belief that the pathogen was transmitted in latently infected progeny tubers from diseased plants led to the development of seed certification schemes based on roguing diseased plants, a procedure which has been used successfully to control many viral diseases. However, these measures have failed to have a significant impact on black-

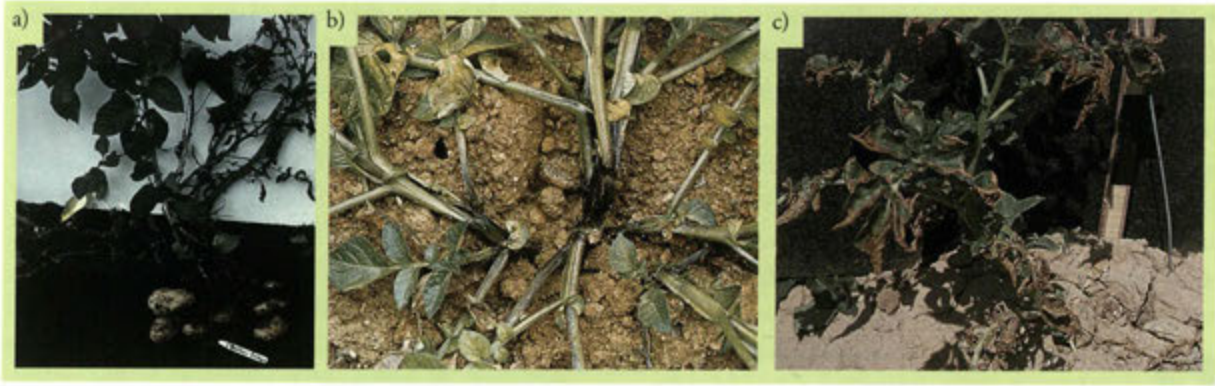

Figure 1 a) Blackleg caused by *E.c. atroseptica* under cool moist conditions. b) Blackleg caused by *E. chrysanthemi* under hot most conditions and c) under hot dry conditions.

leg control and it is now known that seed from blackleg-free crops can produce blackleg plants and *vice versa*. Nevertheless, tubers with stolon end rot from affected plants can be an important source of the bacteria in post-harvest operations and roguing diseased plants is highly desirable.

Surveys done in the sixties in Scotland and elsewhere showed that most commercial seed stocks were extensively contaminated with both Ecc and Eca regardless of the presence or absence of blackleg in the parent crops. When contaminated mother tubers rot, large numbers of the pathogen present are transmitted by soil water to the lenticels of progeny tubers where they can persist throughout the storage period to planting time. These findings led to attempts to produce seed free of erwinias by growing plants from stem cuttings or, more recently, from axenically produced minitubers. However, further surveys in the late seventies showed that a high proportion of stocks derived from stem cuttings were contaminated after the first multiplication cycle and most tubers were highly contaminated after the third cycle. Similar contamination of seed stocks derived from minitubers is probable although the reduction in the bulking time could reduce the risk in most years.

The rapid contamination of initially 'clean' stocks during bulking suggests that, in addition to mother tubers, several sources of erwinias and methods of dispersal and spread within and between crops must be present. The development of sensitive enrichment methods and selective growth media in the seventies confirmed that survival of erwinias in soil was limited and less than the time lapse (3-5 yr) from one potato crop to another in an average crop rotation system. Several potential sources have been identified and their relative importance assessed (Table 1). Crops harvested before mid-August are less likely to be contaminated with air- insect- and leaf-borne erwinias than late harvested crops because widespread leaf contamination and wet conditions favouring rotting of fallen leaves and transmission to progeny tubers tend to occur late. Erwinias from mother tubers can contaminate progeny tubers early in the growing season when wet conditions favour rotting of the mother tubers and spread of the bacteria. Although free water favours rotting and erwinia multiplication in leaf debris and mother tubers, it is not a major source of tuber contamination. Mechanical handling of tubers in the presence of rotting tubers containing large numbers of Eca is an important pathway for the

Source	Dominant erwinia	Contamination of sources*	Tuber contamination	Blackleg	Aerial stem rot
Aerosols	Ecc	+	+	-	+
Insects	Ecc	+	-	-	++
Water	Ecc	++	+	-	+++
Leaves	Ecc	+	+	-	+
Haulm debris	Ecc	+++	++	-	-
Mother tubers	Ecc,Eca	+++	+++	+++	-
Harvesters, graders	Ecc,Eca	+++	+++	+++	-
Blackleg plants					
- haulm	Eca	+++	+	-	+
- tubers	Eca	++	++	++	-

*Relative numbers of erwinias present at the different sources

Table 1 Relative importance of sources of *Erwinia carotovora* subspp. *carotovora* (Ecc) and *atroseptica* (Eca) in relation to seed tuber contamination and blackleg.

spread of contamination, especially if accompanied by severe and extensive wounding of tubers. Contamination of tubers in stocks derived from stem cuttings after the third multiplication cycle is coincidental with more extensive use of mechanical handling.

Cleaner seed production Several cultural measures can be taken to produce cleaner seed based on the knowledge of how contamination occurs. Tuber contamination can be avoided by reducing bulking time of stocks; early harvesting before extensive rotting of mother tubers occurs and bacteria spread to the progeny; and harvesting and grading seed under conditions which avoid excessive wounding and contamination from rotting tubers. Alternatively, methods that reduce seed contamination level can be used, e.g. dry storage in well ventilated stores and thermotherapy using a hot water dipping treatment. However, there is no guarantee that any of the above measures, with the exception of the hot water treatment, will consistently produce healthier seed as they are dependent on environmental and other variables.

Quality control of seed health It is now established that the incidence of blackleg is related to the numbers of the pathogen on seed tubers and, although it is affected by environmental factors, more disease will develop from heavily, than from lightly contaminated seed. The threshold level of seed contamination below which blackleg is unlikely to develop under most conditions is 10^3 Eca cells/tuber and, consequently, the blackleg health status of seed is best assessed by determining the population numbers of the pathogen in tuber peel. Several immunodiagnostic methods have been developed for this purpose which satisfy four main criteria, namely Eca specificity, detection of live cells, sensitivity level down to $<10^3$ cells/ml, and cost and convenience, although they remain to be assessed under field conditions. The methods are viable counts on a selective-diagnostic medium following immunomagnetic separation; immunofluorescence colony staining; and DAS-ELISA applied to erwinia-enriched peel extract dilutions. All of these methods rely on the availability of Eca-specific monoclonal antibodies to differentiate the ubiquitous and serologically closely related Ecc.

The techniques of tuber sampling and interpretation of the assay results remain to be resolved. It is essential that a representative sample of seed is obtained and its optimum size depends on the variation that exists between tubers within the stock. A seed lot containing a proportion of highly contaminated tubers is more likely to give rise to higher blackleg incidence than another with more even contamination throughout, although the average per tuber may be similar. Therefore, it may be necessary to define health status in terms of a disease potential index based on the summation of the weighted number of individually tested tubers in different classes of contamination.

Blackleg resistance In the long term, only resistance to infection will achieve consistent blackleg control, but previous attempts to breed for resistance have failed because this character is absent in conventional potato breeding lines in which only varying degrees of susceptibility are expressed. Moreover, there have been difficulties in devising and applying appropriate screening tests. Knowledge of the epidemiology of blackleg dictates that both tuber and stem resistance are essential for effective blackleg resistance but no cultivar is known that possesses both. Stem resistance tends to confer a greater degree of resistance to blackleg in the field than tuber resistance.

As potato tubers are usually latently infected in the lenticels and wounds and can be stored for several months without rotting, resistance expressed under aerobic conditions must be high. Rotting develops under wet, anaerobic conditions when oxygen-dependent resistance mechanisms, e.g. phytoalexins, phenolics and suberisation are non-operative, suggesting that resistance expressed under anaerobic conditions is essential, although it has been largely neglected in the past. Erwinias produce pectic enzymes that degrade pectin which cements plant cells together. Pectin molecules consist of long chains of rhamnose-galacturonic acid held together by different bonds of which the more important are methyl ester and ionic (calcium) bonds. Recently, pectin methylation was found to be correlated with high resistance of tubers expressed in field conditions in the USA under both aerobic and anaerobic conditions, and in stems of clones derived from protoplast fusion between the non-tuber bearing *Solanum brevidens* and *S. tuberosum* subsp. *tuberosum* (Fig. 2). When the clones were backcrossed to *S.t. tuberosum*, segregation of the resistant character occurred among the progeny, and pectin methylation level of the resistant siblings was as high as 94% which is twice that of the susceptible sibling or *S.t. tuberosum*. The resistance is believed to be derived from *S. brevidens* and probably involves the regulation of enzymes, especially pectin methyl esterase, associated with the degree of pectin methyla-

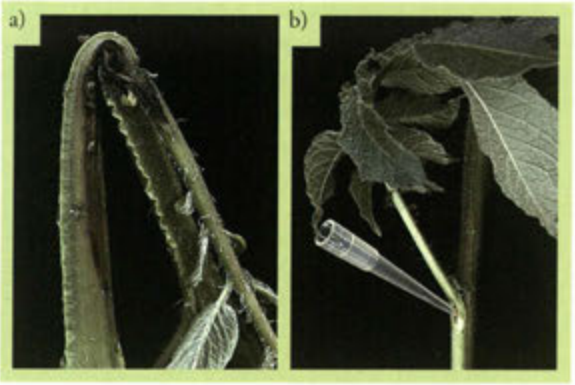

Figure 2. Reaction of protoplast fusion hybrids inoculated with *E.c. atroseptica.* a) susceptible and b) resistant.

tion in cells walls. In addition to *S. brevidens*, other sources of resistance have been found in wild *Solanum* spp. and primitive cultivars from the Andes. The mechanism of resistance could be different from that of *S. brevidens* as it is not apparently related to pectin methylation. These resistance sources could be exploited through a combination of biochemical, molecular biological and genetical analysis to produce more resistant cultivars.

Erwinia pathogenicity Of the pathogenicity determinants that have been identified in the soft rot erwinias, namely iron uptake (siderophores), motility, cell wall structure, and pectic enzymes, the last has been shown to be the most important. The bacteria produce four pectic enzymes (polygalacturonase, pectate lyase, pectin lyase and pectin methyl esterase), usually in several isoforms, with different affinities for different pectic substances which they degrade in different ways. A relatively narrow range of isoenzymes are produced *in vitro* and genes coding for their production and regulation have been analysed. In addition, a regulatory system controlling the synthesis of all extracellular enzymes has been identified which allows the integration of multiple physiological and environmental signals into a cell density-dependent control of the pectic enzymes. Recently, an additional range of isoforms of the four pectic enzymes have been found to be induced *in vitro* by highly methylated pectin and are also produced naturally *in planta* by the soft rot erwinias. The potential to produce such a wide range of isoenzymes suggests that the role of the different pectic enzymes in disease development is more complex than initially thought. If the main preformed useful resistance in potatoes is related to cell wall pectin methylation, further research on the *in planta* production of those enzymes which potentially can break down the host resistance is essential.

Conclusions: an ecological explanation of disease development Temperature and oxygen concentration differentially affect growth and pectic enzyme production of erwinias. Temperature is the main environmental factor affecting the relative virulence of the bacteria, for example, Eca causes more blackleg at low (<25°C) than at high temperatures, whereas the reverse is true for Ecc and Ech. It affects the production of pectic enzymes through the global enzyme regulatory system which down-regulates enzyme synthesis several degrees below the maximal growth temperatures of the pathogens. Bacteria which produce more pectic enzymes at the prevalent temperature will multiply faster and predominate to cause disease. Therefore, competition determines which *Erwinia* sp. or subsp. will predominate in a lesion. Pectic enzymes are also produced by many saprophytic bacteria, and the soft rot erwinias can be regarded as opportunistic pathogens when infecting tubers and even when causing blackleg, which can be construed as an extension of mother tuber rot.

Soft rot erwinias produce an array of pectic enzymes including pectin methyl esterase and pectin lyase that can degrade and demethylate pectin and are followed by other enzymes to macerate potato tissue. Therefore, resistance based on high pectin methylation level derived from *S. brevidens* should be readily broken and, under experimental conditions, the difference between resistant and susceptible inoculated tubers becomes less in long incubation periods. However, in the field, the resistance appears to be durable, possibly because rotting is usually initiated from a much smaller inoculum load than that commonly used to test tubers in the laboratory. In addition, prolonged anaerobiosis is unlikely to occur frequently and growth of initially small numbers of latent bacteria would be lower in tubers with a high pectin methylation level than in a susceptible cultivar when anaerobic conditions prevail, and inhibited on the return of aerobic conditions by the activity of oxygen-dependent resistance mechanisms. It is notable that *S. brevidens*-derived resistance is more clearly expressed when the bacteria are inoculated into stems than into tubers as aerobic conditions are more likely to prevail in the former than in the latter.

Utilisation of non-potato resistance Long term disease control clearly lies in increasing the level of disease resistance expressed in current cultivars. In addition to exploiting the newly found resistance sources from *Solanum* spp., novel approaches targeting weak links in the infection process can be explored

by transferring genes coding for specific inhibitors affecting the pathogen or its pectic enzymes into potatoes. The lysozyme gene from chicken egg white has lytic activity against bacterial and fungal cell walls and has been cloned and transferred to potatoes where it reduced infection by erwinias. In addition, recent research has revealed prospects of incorporating into potato gamma globulins (plantibodies), alone or conjugated with a toxic compound, which are directed against invading bacterial cells or their pectic enzymes. Several inhibitors of different pectic enzymes have been isolated from plants and, as the DNA coding these enzymes from different sources tend to be highly conserved, it should be possible to utilise modified forms active against erwinia pectic enzymes to produce transgenic resistant potatoes. Also promising for potato is the possibility of expressing genes coding for inhibitors or signalling molecule analogues which affect adversely the exoenzyme global regulation and secretion systems of erwinias.

In contrast to the recent past when resistance to erwinias was inconceivable, the problem now is to decide which resistance source and scientific approach should be chosen as the most promising to achieve success in the shortest time.

Interaction of the expression of partial plant disease resistance and the environment

A.C. Newton, J.G. Harrison & R. Lowe

The principle of using partial resistance as a source of potentially durable polygenic resistance to fungal diseases has been proposed for many years but is seldom a high priority in breeding programmes. This is largely because of the difficulties of combining and transferring the characters into agronomically adapted backgrounds while maintaining their expression but not that of other deleterious characters associated with them. Reliability of expression in adapted backgrounds under diverse environmental conditions is also important as the economics of cultivar breeding dictates that new varieties must command ever larger markets to be successful. However, little is known about the expression and potential durability of partial resistance under different environments, especially towards diseases such as mildew (*Erysiphe graminis* f.sp. *hordei*) on barley and late blight *(Phytophthora infestans*) on potato, which have relied upon major gene resistance for control.

Late blight of potato Late blight, caused by *Phytophthora infestans*, is the most serious disease of potatoes and is controlled by frequent applications of fungicides in developed agricultural systems. However, with increasing concern about potentially harmful effects of agrochemicals, the role of genetically-determined resistance in blight control is likely to become more important. *P. infestans* adapts quickly to overcome disease immunity that results from the presence of major resistance genes and resistance to blight that is determined by minor genes is likely to be more durable. Minor gene resistance, also known as polygenic, field, general or horizontal resistance, offers the best approach to controlling blight with a minimum of fungicide use. Although minor gene resistance does not confer immunity to disease, it can reduce substantially the rate of blight progress mainly by slowing down colonisation of leaf tissues. However, the expression of minor gene resistance may depend on the environment. The interaction of genotype with environment on the expression of blight resistance was studied using five potato cultivars in a range of precisely-controlled environments. Resistance was quantified by measuring amounts of *P. infestans* in leaflets with an ELISA system after incubation following a standardised inoculation procedure. The effects of temperature, photoperiod and light intensity are summarised in Table 1.

The results demonstrated that there are at least three forms of minor gene resistance, mediated by temperature, photoperiod and light intensity respectively.

Cultivar	Resistance of leaflets to colonisation affected by Temperature	Photoperiod	Light intensity
Brodick	Yes	Yes	Yes
Shelagh	Yes	Yes	No
Teena	Yes	No	Yes
Bintje	No	No	No
Torridon	No	No	No

Leaflets of cv. Bintje were always readily colonised while those of cv. Torridon were always resistant to colonisation.

Table 1 Interaction of genotype and environment on colonisation of potato leaflets by *P. infestans*.

Temperature and lighting conditions of any geographical location are usually readily reproduced in artificial conditions. Testing the stability of blight resistance of new potato cultivars in different environments that mimic different locations should be adopted as a standard procedure.

Mildew on barley Primitive barley genotypes have been used as donors of partial resistance to develop potentially durable polygenic resistance similar to field resistance to potato blight. Polygenic resistance to mildew has been demonstrated to consist of several measurable components: reduction in infection frequency (IF), increase in latent period (LP) (the time from a spore infecting to producing a macroscopic colony), and reductions in colony size, biomass per colony and sporulation per colony. Fungal biomass in leaves can be measured conveniently by using gas chromatographic analysis of the main cell wall sterol, (3.beta)-ergosta-5,24(28)dienol, or by an ELISA method using a polyclonal antibody raised to mildew spores. Reductions in all these characteristics are shown in genotypes 7204, 7526, 9319 and 9855 compared with Golden Promise in Table 2.

Genotype	Infection frequency * 7°	Infection frequency * 15°	Latent period **	Biomass/colony †	Biomass/(ELISA)
7204	7	22	73%	25	12
7526	-	17	-	-	12
9319	5	-	67%	38	-
9855	6	22	83%	38	21
G. Promise	13	31	84%	40	27
SED	1.2		-	1.1	-

* = Colonies per 3cm leaf segment
** = Colonies visible at date 1 expressed as % of those visible at date 2
† = Arbitrary units

Table 2 Assessment of components of partial resistance to powdery mildew in primitive genotypes of barley.

Some of the resistance components were assessed in F9 (selfed) lines derived from crosses between primitive genotypes with cv Golden Promise. Reduced IF and colony biomass (sterol per colony) was sometimes inherited but was also found in progeny from parental genotypes such as 7163 which did not express these characteristics suggesting transgressive segregation (Table 3). Visual field scores and assessments made by ELISA from random leaf samples in field trials of the primitive parent and the best of the progeny showed good agreement (Table 3).

Genotype	Infection frequency *	Sterol/ colony**	Field score †	Field ELISA**
7163	17	24	10%	2.8
progeny	20-23	11-13	5%	1.6
7204	22	12	9%	2.8
progeny	7-34	18-25	2%	0.7
7526	17	12	6%	2.3
progeny	19-23	17-18	8%	2.3
G. Promise	31	27	20%	4.0
SED	2.5	5.7	2.9	

* = Colonies per 3cm leaf segment
** = Biomass of mildew per unit leaf weight
† = Percentage overall plant infection

Table 3 Expression of partial resistance to mildew in barley breeding lines derived from primitive genotypes.

Some of the variation in resistance expression observed in experiments and field trials may be explained in terms of the environmental lability of partial resistance expression as shown by the variable effects of humidity on resistance assessments (Fig. 1).

Measurements of resistance components are of value to breeders only if they reflect the plant's behaviour throughout its life, particularly in the field. Some measurements are highly correlated positively with each other, while others, such as biomass per colony (BMC) in adult plants, are highly negatively correlated (Table 4). Adult plant infection frequency and mildew biomass in the field are also negatively correlated, indicating that low colony number probably allows larger colonies to form as a result of the lack of competition for nutrients. However, some measurements, such as fungal biomass per colony on seedlings, do not correlate with fungal biomass per colony on adult plants.

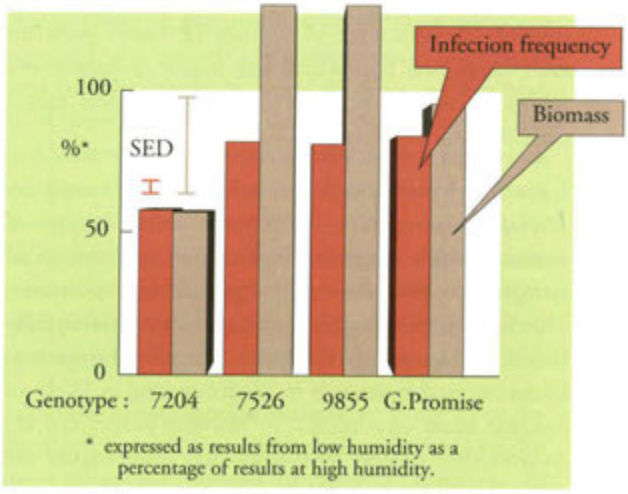

Figure 1 Effects of humidity on expression of partial resistance of barley to powdery mildew.

	B	C	D	E	F
A Seedling IF	-0.451**	0.509***	-0.23	0.058	0.715***
B Seedling BMC		-0.407*	0.136	-0.439**	0.244
C Adult plant IF			-0.569***	0.725***	0.249
D Adult plant BMC				-0.360	-0.451***
E Field score					0.167
F Field biomass					

Table 4 Correlation coefficients between mildew infection assessment methods on barley.

Similarly, seedling infection frequency does not correlate with visual assessments of mildew in the field, and neither fungal biomass per colony on seedlings, adult plant infection frequency or field visual score correlates with fungal biomass in the field. Therefore care must be taken in selecting components of resistance which will be effective in reducing infection in the field. Furthermore, not all field assessments are reflected in yield reductions attributable to disease.

Incorporating partial resistance which is polygenically based into new cultivars is a highly desirable objective from a theoretical point of view. However, the results shown above demonstrate that in practice it can be both difficult to assess, and be subject to interactions with the environment that render it ineffective. Such interactions must be understood before we can reliably predict the durability of disease resistance of new cultivars. These studies are increasing our knowledge of the genetic basis and of the expression of durable resistance so that future breeding programmes can be more directed towards producing cultivars that are disease resistant in a wide range of environments.

Grey mould (*Botrytis cinerea*) of roses

B. Williamson, G.H. Duncan, J.G. Harrison, L.A. Harding* & G. Zimand**

Cut rose blossoms have become an important export commodity in many countries, most notably in Israel and The Netherlands, but substantial losses occur because grey mould, caused by *Botrytis cinerea*, develops during cool storage and transport of the flowers before sale. An additional problem has been the widespread development of strains of the pathogen resistant to many of the fungicides introduced in the last 20 years. Accurate control of the micro-environment and use of microorganisms antagonistic to *B. cinerea* to reduce reliance on fungicides is now an important approach to grey mould control in the glasshouse, but the precise role of moisture, as a liquid or as water vapour around the petals, is not fully understood.

Droplets of water on the surface of plants are known to be important for germination of conidia of *B. cinerea*. They also influence infection because sugars and other nutrients leach from the underlying cells to augment nutrient reserves which become depleted by respiration. Consequently, the duration of surface wetness has been used extensively as a key factor in the prediction of epidemics in several crops attacked by *B. cinerea*.

Accurate control of relative humidity It is extremely difficult to measure and control relative humidity close to the surface of plants. Most methods of measurement are inaccurate and inappropriate for the micro-habitat of fungal propagules in the thin bound-

Figure 1 Lesions caused by *Botrytis cinerea* on rose petals after dry inoculation with conidia and incubation at 100% RH for 24 h.

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ary layers of still air which surround the plant and thickness of the boundary layers varies with air speed and surface topography.

Equipment designed at SCRI for precision control of relative humidity around leaves[1] was used to study the behaviour of *B. cinerea* conidia inoculated on to rose flowers. Dry spores were collected from a 14-day-old culture of the fungus using a sterile camel hair brush and discharged as a spore cloud above the petals. This method of inoculation avoided the loss of spore reserves by leaching and gave the opportunity to test whether the fungus needed liquid water for germination. Newly opened flower heads of rose cv. Scarlet Pimpernel were dry-inoculated with conidia and placed in the incubation chambers with their stalks immersed in water; a single flower which had not been inoculated was included in each chamber as a control. Relative humidities were controlled between 100% to 60% and flowers kept in the chambers for up to 72 h. Samples of roses were examined at intervals after inoculation and the number of fleck-lesions (Fig. 1) appearing at each relative humidity was recorded.

At 94-100% RH most conidia germinated and caused lesions at 15°C within 24 h. In other experiments in which the fungus was applied to petals as a conidial suspension in fine water droplets, the germ tubes arising from conidia were much longer than when applied dry.

Low temperature scanning electron microscopy The behaviour of germ tubes and their penetration of the epidermal cells of the petals was examined by low temperature scanning electron microscopy (LTSEM). This method preserves the surface of specimens in a natural state by avoiding the use of damaging chemical and drying procedures normally used to prepare tissues for the high vacuum inside the microscope. Consequently, the epicuticular waxes, glandular secretions and leachates on leaves that play an important role in the interaction of fungal spores with the plant are preserved *in situ*. After incubating in the humidity chambers, petals were prepared immediately for LTSEM. The specimens were frozen rapidly in nitrogen slush at -210°C, sputter-coated with gold and transferred under vacuum to the 'cold stage' of the electron microscope for viewing while frozen in a fully or partially hydrated state.

Most conidia germinated at 100% RH within 24 h to produce short germ tubes (Fig. 2a) and they penetrated the cuticle without forming appressoria (Fig. 2b). The germ tubes penetrated both the upper and lower epidermal cells of the petals, which had highly ornamented surfaces of differing structure. The upper surface consisted of dome-shaped pentagonal or hexagonal epidermal cells covered in radially arranged ridges (Fig. 2c), whereas on the lower surface the junctions between cells were indistinct because elaborate zig-zag ridges covered the whole area (Fig. 2a). This feature, together with epicuticular waxes, probably contributes to the hydrophobicity of the epidermis and the difficulty of applying fungicide sprays efficiently to flowers.

Frozen petals fractured and examined by LTSEM showed that the hyphae of *B. cinerea* entered the intercellular spaces of the petal within 24 h and were visible passing through cell walls of the mesophyll cells with little evidence of erosion or distortion of the wall (Fig. 2d), suggesting that fungal cell wall-degrading enzymes remain closely associated with the hyphae after secretion.

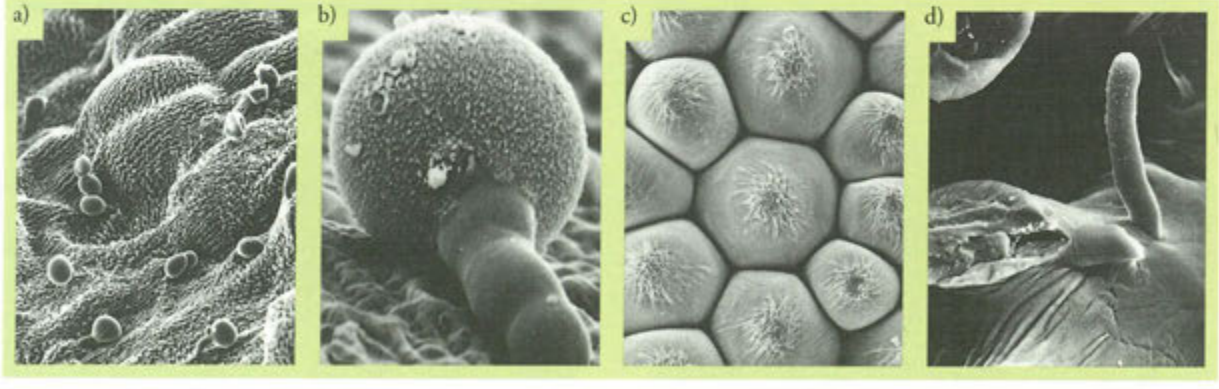

Figure 2 Rose petals examined by LTSEM a. Conidia germinated on underside of petal b. Penetration of lower epidermis without formation of an appressorium c. Structure of upper epidermis of petal d. Hyphae of *B. cinerea* growing from mesophyll cell inside petal.

Conclusions This work has shown unequivocally that *Botrytis cinerea* does not require water droplets to invade rose flowers. The results have important implications for long-distance transport and trade in flowers because it is difficult to keep blossoms in a fresh condition in atmospheres dry enough to reduce the risk of grey mould developing. Droplets of moisture may form in fluctuating temperatures during transport and storage and exacerbate the problem because hyphae spread rapidly in the moisture film. It therefore appears that the best approach to control grey mould may be the introduction of antagonistic microorganisms as biological control agents in conjunction with suitable fungicide sprays. The findings that conidia of *B. cinerea* may germinate in the absence of liquid water on roses has implications for further models developed to predict grey mould epidemics in other crops.

References

[1]Harrison, J.G. & Lowe, R. (1989). *Plant Pathology* **38**, 585-591.

In vitro selection of coffee plants resistant to coffee berry disease

N.E. Nyange*, R.J. McNicol, B. Williamson & G.D. Lyon

Production of coffee on small farms in many African countries is vital to the local economy through export earnings, but is seriously threatened by coffee berry disease caused by the fungus *Colletotrichum kahawae* (Fig. 1). The disease frequently causes losses of more than 50% in yield and quality of coffee beans, but the intensive fungicide spray programmes used for its control are too expensive for most growers to use. In Tanzania, the soaring cost of fungicides on the world market and the low profitability of diseased coffee plants has led the Lyamungu Research Centre to give high priority in coffee breeding to the selection of plants resistant to this disease.

Testing for resistance to the disease can be achieved by inoculating seedlings with the fungus, rather than the berries, but production of disease-resistant coffee trees, through conventional cross-fertilisation and backcrossing of resistant parents with plants showing other important characters, takes more than 20 years. Alternative laboratory-based methods to shorten this breeding cycle are being explored in many countries.

Since it is known that other *Colletotrichum* spp. produce phytotoxins, it should be possible to challenge coffee cells with partially purified toxic fungal products as an aid to the rapid identification of genotypes strongly resistant to the disease.

To develop an *in vitro* screening method for selection of plants resistant or tolerant of the *Colletotrichum* toxin, callus cultures (undifferentiated clusters of cells growing on agar) were initiated from coffee seedlings and the calli transferred to a modified Murashige and Skoog agar medium and subcultured monthly.

The fungus was grown in a shaken liquid medium at 23°C for 21 days and the culture filtrate was centrifuged and freeze-dried. The toxin fraction was extracted with distilled methanol, rotary evaporated until dry, redissolved in sterile distilled water, and stored frozen after filter-sterilisation.

The toxicity of this extract was confirmed by injection into leaves of coffee seedlings (Fig. 2). The extract was then incorporated into the media used to grow calli or suspension cultures of coffee, and after a period of exposure to the toxin, the calli were removed to toxin-free medium and their growth assessed. This was done non-destructively by means of automated image analysis which measured the area of each callus weekly without exposing the cultures to microbial contamination. The viability of coffee cells grown in shaken liquid media after challenge with toxin was assessed microscopically by fluorescein diacetate which

Figure 1. Immature coffee berries infected by *Colletotrichum kahawae* in Tanzania.

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Figure 2. Coffee leaves injected with partially purified culture filtrates from two isolates of *C. kahawae* (red labelled) showing brown lesions, compared to controls injected with water.

is taken up and is cleaved by esterases in living cells to produce a fluorescent product when excited by uv irradiation (Fig. 3). Dead cells do not fluoresce. The viability of suspension-cultured cells was also quantitatively measured by mixing cells with a tetrazolium salt (MTT) and incubating at 30°C for 2 h. The coloured MTT formazan was extracted from cells with acidified isopropanol and measured spectrophotometrically.

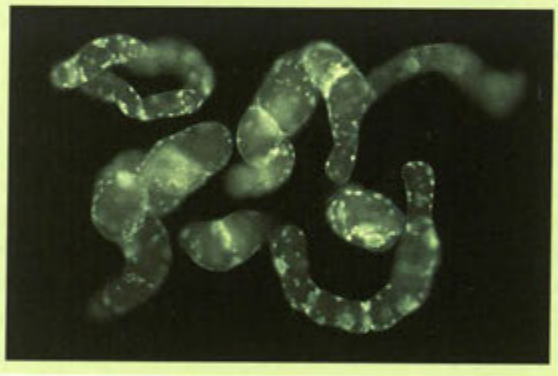


Figure 3. Viable suspension-cultured cells revealed by uptake and cleavage of fluorescein diacetate.

The use of partially purified toxin in callus cultures to identify disease resistant genotypes showed that it was possible to distinguish the coffee cultivar Hybrido de Timor, known to be resistant in the plantation, from selection N39, a known susceptible type. Assessments of the survival of suspended cells in various concentrations of partially purified toxin also differentiated readily between these two cultivars, either by means of the fluorescent staining method, or the MTT colorimetric method (Fig. 4).

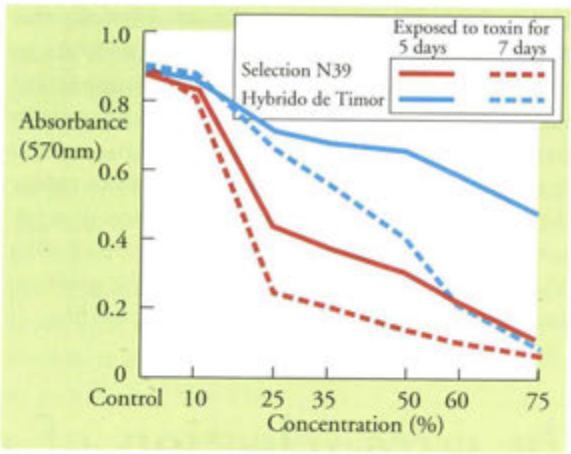


Figure 4 Effect of various concentrations of *C. kahawae* extracts on viability of suspension-cultured cells determined by the MTT colorimetric method.

The next step in the screening process was to transfer surviving callus cultures to media which encouraged the production of plantlets (Fig. 5) so that weaned coffee plants were obtained. These selected plants will then be grown in the glasshouse and later in the field in Tanzania to check that the resistance genes detected in the laboratory are expressed in fruits of full-grown coffee trees.

Figure 5. Somatic embryos of Hybrido de Timor from calli of cell suspension cultures treated for 5 days with 25% partially purified culture filtrates.

The *in vitro* screening methods can be applied to a wide range of coffee germplasm of unknown status for *Colletotrichum* resistance to enable those with strong resistance to be identified and bring new genes into the breeding pool. This approach should reduce the risk of resistance breaking down under heavy disease pressure, reduce farmers' dependence on expensive fungicides and the risk of exposure of coffee drinkers to chemical residues.

Plant viruses

T. Michael A. Wilson

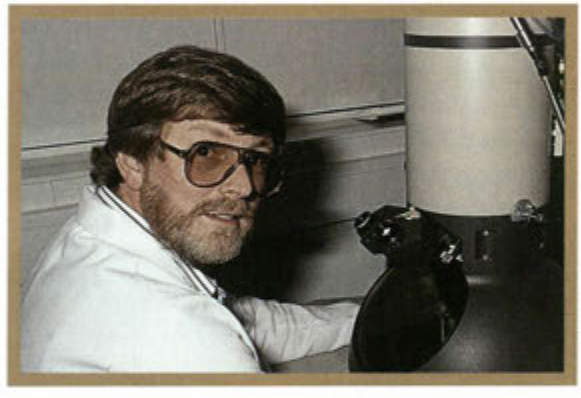
Our completely re-organized, revised 'core programme' of plant virus research was commissioned by SOAFD in 1993. Although more focused, and built around multidisciplinary teams of scientists, our research objectives remain the complete elucidation of selected virus life cycles to permit better detection, diagnosis, characterisation and understanding of these major disease agents in important crops, with special emphasis on potatoes and soft fruit. Fundamental studies in molecular virology and pathology, as well as the molecular genetics of natural or genetically engineered virus resistance in crop plants, present new strategies for future deployment of more durable field resistance against several potentially devastating viruses. In addition to raising crop yield and quality, and insuring against resistance-breaking isolates of viruses, this work aims to reduce the dependence of intensive agriculture and horticulture on chemical sprays to control virus vectors. Our studies have concentrated, therefore, on important viruses or virus-like agents spread by nematodes (nepo- and tobraviruses), fungi (furoviruses), insects (luteo- and potyviruses) or mites. Opportunities also exist to exploit viruses or virus-derived sequences in plant biotechnology and to improve our understanding of plant biochemistry, physiology and anatomy in both the diseased and healthy state.

The ornamental value of variegated abutilon plants is due to infection by the ssDNA-containing geminivirus, abutilon mosaic virus (AbMV). The virus in these plants has lost the ability to be whitefly transmitted and cannot be transferred mechanically. These attractive, AbMV-infected plants are therefore propagated vegetatively causing populations of the virus to become separated in time and space following the original infection event. In an EC-funded project, the level of genetic micro-heterogeneity in several vegetatively isolated clones of AbMV DNA has been measured (p 100).

The agent of blackcurrant reversion disease remains unknown, but dsRNA, detectable in affected plants, is being cloned and sequenced. Transmission of this disease agent is correlated with infestation by the blackcurrant gall mite *(Cecidophyopsis ribis)* and the anatomies of several species of eriophyid mites are described in more detail in this Report (p 116).

The aphid-transmitted potato leafroll luteovirus (PLRV) is one of the most devastating viruses to affect potato crops. We have studied PLRV extensively, sequenced the ssRNA genomes of several isolates and prepared full-

length clones of the virus for reverse genetic experiments to dissect gene functions. Interactions of PLRV with the vasculature of susceptible or resistant potato cultivars have been examined, and PLRV coat protein transgenic potato lines have been created with increased resistance (decreased virus accumulation) following infection by the virus. A form of host gene-mediated resistance to PLRV which acts to decrease virus accumulation has been identified in several cultivars and SCRI breeding clones. Recent evidence indicates that, in at least two breeding clones of *Solanum tuberosum*, resistance to PLRV may be controlled by two complementary, dominant (i.e. epistatic) resistance genes (designated *Rl*). By adding PLRV coat protein gene-mediated resistance to these lines (see p. 103), levels of challenge virus that accumulated in plants grown from infected tubers were as low as 1% of those in susceptible cultivars. Similar, very low levels of virus accumulated in plants of *S. brevidens*, a wild potato species that is a potential source of resistance genes against PLRV. The value of this degree of resistance against PLRV in field-grown crops remains to be tested, but it is likely to be highly effective in diminishing virus spread.



Professor T.M.A. Wilson, Head of the Virology Department

During studies in collaboration with St Andrews University to raise antisera to each of the six proteins encoded by PLRV RNA, each gene was expressed separately from an insect virus vector plasmid grown in insect cells. Unexpectedly, a PLRV coat protein derivative was able to assemble into virus-like particles inside *Spodoptera frugiperda* cells (p. 97). This observation has important immunological and structural implications for future studies on PLRV. Comparative sequencing studies on aphid transmissible and poorly-transmissible isolates of PLRV have suggested that the distal end of the C-terminal coat protein readthrough domain is the determinant of insect transmission.

In collaboration with scientists at IAH, Pirbright and the Molecular Biophysics Laboratory at Oxford University, we have been pursuing the molecular architecture of parsnip yellow fleck picorna-like virus. Improvements in the purification procedure for PYFV eventually provided sufficient virus (>10 mg) to grow crystals for X-ray diffraction studies at the SERC Daresbury facility.

During 1993, competitive SOAFD and EC AIR funding led to major new initiatives in developing improved techniques for post-harvest tuber testing for potato virus Y, as well as recombinant monoclonal antibody engineering and production, using bacteria and plasmid expression or phage display vectors.

A full-length clone of soil-borne wheat mosaic furovirus (SBWMV) RNA2 (3593 nucleotides) has been created and transcribed. However, equivalent clones of SBWMV RNA1 (7099 nts) appeared to be unstable in, or toxic to, bacteria. Serial mechanical transfers of an Oklahoma field isolate of SBWMV in wheat caused rapid deletion of approximately 760 nts from RNA2. The deletion was mapped to the coat protein readthrough domain and sequences flanking the deleted region were determined. Comparison with a stable deletion mutant of Nebraska SBWMV RNA2 sequenced previously (Y. Shirako and T.M.A. Wilson, Rutgers University, NJ) suggested that a structural feature of the RNA rather than a modified readthrough protein function determined the site(s) of spontaneous deletion (see p. 106). In both cases, deletion was associated with increased symptom severity. RNA deletions in the putative coat protein readthrough domain have been noted also in the RNA3 of potato mop-top furovirus (PMTV) (T and R isolates) maintained over many years by mechanical transmission. Recently, another isolate of PMTV has been obtained from soil bait tests at SCRI and found to contain a longer RNA3 component. Virus-free single cystosoral cultures of *Spongospora subterranea* have been established and experiments are in progress to acquire PMTV from the roots of infected source plants and transmit it to virus-free bait plants.

PMTV coat protein transgenic *Nicotiana benthamiana* plants have been found to be highly resistant to challenge by mechanical or graft inoculation with PMTV (see p. 103). A full-length clone of PMTV RNA2 was constructed and transcripts from it directed synthesis of a 51 kDa protein from the first open reading frame despite the presence of a large hairpin loop in the 5' untranslated region of the RNA. Pepscan analysis of

PMTV coat protein revealed six continuous epitopes, one of which was located at the N-terminus of the linear amino acid sequence and exposed along the sides of virus particles.

Strand-specific probes have been used to identify which strand of the groundnut rosette virus (GRV) RNA3 (satellite RNA) molecule predominates in plants infected with satellite-containing GRV isolates. The results defined the positive strand to be that with ORFs I-III described previously (*Ann. Rep. 1992, 85*).

Work on tobacco rattle tobravirus (TRV) coat protein transgenic tobacco plants has shown that they were not resistant to nematode-transmitted virus, *via* either leaves or roots, in contrast to published results from mechanical inoculations. Precise gene exchanges between nematode transmissible TRV and non-transmissible PEBV tobraviruses have revealed that the coat protein alone was insufficient to confer vector transmissibility. Therefore, by analogy with aphid-transmitted caulimoviruses, potyviruses and luteoviruses, an additional virus-coded 'vector transmission protein/helper component' was presumably involved.

Experimental approaches to determining roles for the proteins in the particles of PLRV

M.A. Mayo, C.A. Jolly, G.H. Duncan, J.W. Lamb[1] & R.T.Hay[1]

Potato leafroll virus is an important pathogen of potato crops throughout the world and it causes particular problems in seed crops. PLRV has been studied at SCRI for a number of years both biologically, such as in studies of the epidemiology of PLRV in potato crops and its cytopathology in infected plants, and biochemically, such as in the determination of the composition of PLRV particles. Recent work has led to important discoveries about the genetic structure of PLRV (*Ann. Rep. 1989, 73*) and about different types of resistance to PLRV infection (*Ann. Rep. 1992, 92*).

PLRV, like other viruses in the genus *Luteovirus*, is transmitted by aphids in a persistent, circulative manner. After an aphid has fed on an infected plant, virus particles pass from the aphid gut into the haemolymph where they can circulate around the aphid body and then bind to cells in the accessory salivary glands. The particles then pass into the saliva and thereby enter a new plant when the aphid feeds again (Fig. 1). All luteoviruses behave in this way although the species of vector aphid differ. Luteoviruses do not multiply in the aphid, which suggests that the particles remain intact when passing through the aphid, and that the surface of the virus particle mediates the transport of virus from one part of the aphid body to another. Our recent work has concentrated on the proteins that make up the virus particle.

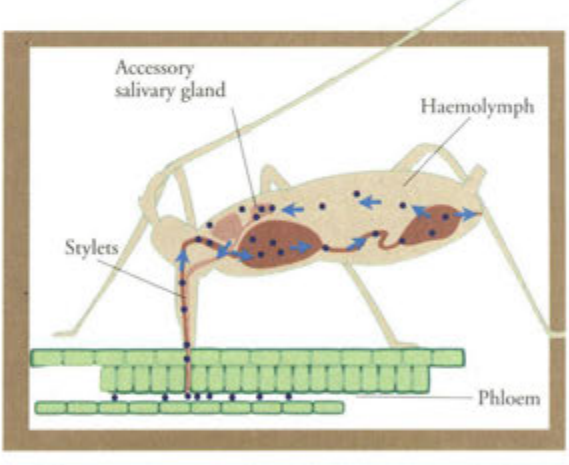


Figure 1 Diagram of the circulation of luteovirus particles in the bodies of their aphid vectors. Virus particles are ingested with phloem contents and cross into the aphid haemolymph at the hind gut region. Once in the haemolymph, particles bind to the accessory salivary gland cells and are then injected into fresh plants when the aphid feeds. Virus particles are thought to remain intact throughout this process.
The diagram is based on work with barley yellow dwarf luteovirus by F. Gildow and colleagues (Pennsylvania State University).

[1]School of Biological and Medical Sciences, St. Andrews University

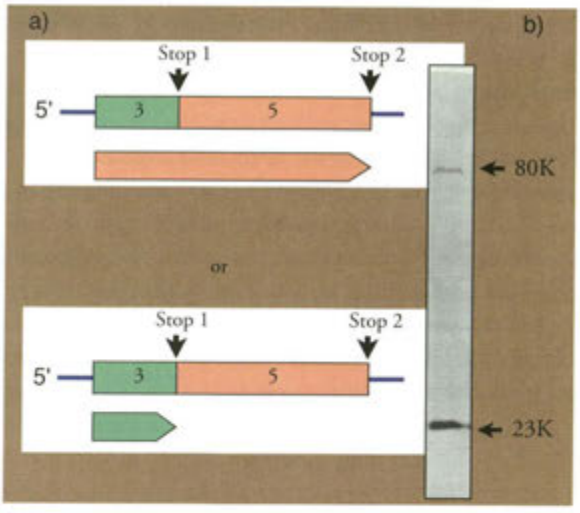


Figure 2 Expression of the genes for the structural proteins of PLRV. (a) Diagram of translation of the sub-genomic mRNA containing genes 3 and 5. Ribosomes translate from the 5' end until they reach a termination codon (STOP 1) to produce P23. A few ribosomes do not stop but continue until STOP 2 to produce P80. P80 therefore contains the P23 sequence.
(b) Immunoblot of protein from a PLRV-infected plant showing the reaction of P23-specific antibodies with P23 and P80.

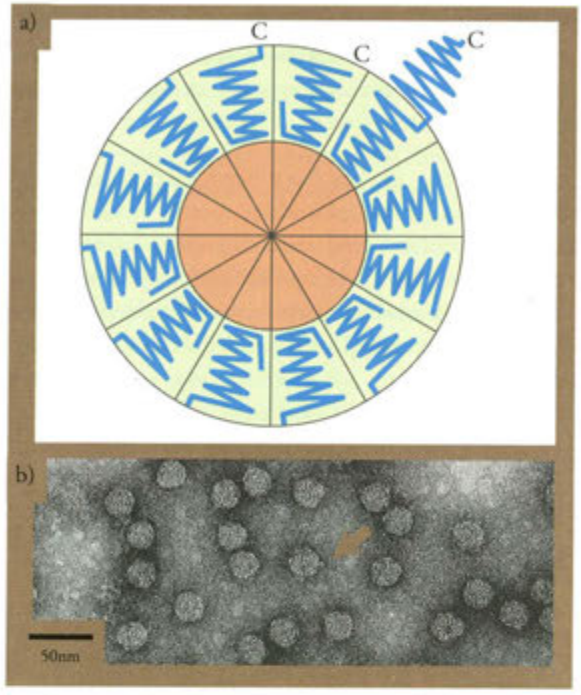


Figure 3 Particle structure of PLRV. (a) Diagram showing the C-terminal extension of P23 to give P80 such that the RT protein is to the outside of the virus particle. C represents the carboxyl terminus of P23 or P80. (b) Electron micrograph of PLRV particles showing some surface features which may be the protruding RT protein.

PLRV particles consist of the genome RNA enclosed in 180 copies of a M_r 23000 protein (P23: coat protein). This protein is made when ribosomes translate a sub-genomic mRNA in infected cells (Fig. 2). However, occasionally these ribosomes do not stop at the normal termination signal in the RNA (STOP 1 in Fig. 2) but continue translation until the next termination signal (STOP 2). This results in the synthesis of a small amount of a M_r 80000 protein (P80) which consists of P23 joined at its carboxyl-end to 507 amino acids of readthrough (RT) protein (Fig. 2). Evidence for this process comes from immunoblots of proteins extracted from PLRV-infected tissues in which both P23 and P80 react with antibodies specific to P23 (Fig. 2). Purified virus particles contain both P23 and P80 in the proportion shown in Figure 2; presumably the part of P80 which is identical to P23 assembles into virus particles such that the RT protein protrudes on the outside. Figure 3 is a diagrammatic representation of the predicted particle structure. In some electron micrographs of PLRV particles it is possible to see protruberances on the particle surface (arrowed in Fig. 3b) which may be the RT protein.

The principal function of P23, like the coat proteins of most plant viruses, is presumably to protect the genome RNA during the transmission of virus particles from one cell or host to another. The role of the RT protein part of P80 is less obvious but clues as to its role come from work with different isolates of PLRV. In surveys of Scottish isolates of PLRV made some years ago, two isolates were obtained (strain V and strain 15) which differed from others in being poorly transmitted by the normally efficient vector, *Myzus persicae*. In an attempt to determine which amino acids are important for transmissibility, we have determined the nucleotide sequences of the P23 and RT protein genes of a number of Scottish isolates including strains V and 15. Comparisons among the deduced amino acid sequences of P23 and RT protein showed that changes in P23 could not account for the differences between isolates. Indeed, at only two positions in the RT protein were there amino acid differences between all efficiently transmissible and all poorly transmissible isolates (Fig. 4). Probably the amino acid changes in the RT protein of poorly transmissible isolates alter the strength with which these PLRV particles bind to receptors in the aphid body and thereby diminish the chances of the virus particles being transmitted. This hypothesis can be tested by reverse genetics in which deliberate mutations are

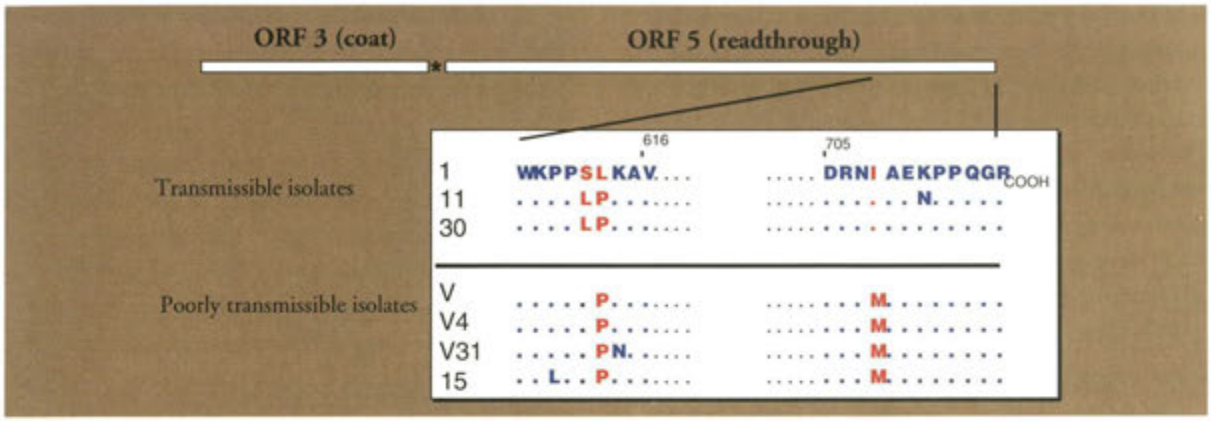


Figure 4 Diagram summarizing the results of sequence comparisons among the structural protein genes of some Scottish isolates of PLRV. Isolates V4 and V31 were obtained by sub-culture from the stock culture of isolate V. Two differences in amino acid sequence were detected between all poorly transmissible isolates and all readily transmissible isolates. The differences (coloured red) were near the C-end of the RT protein.

made in DNA copies of the PLRV genome which is then expressed to make proteins containing the desired changes singly or in combination. To facilitate this, we have used an insect baculovirus gene expression system in which a non-essential baculovirus gene is replaced with a foreign gene. When cells are inoculated with virus containing the recombinant DNA, they become infected with the baculovirus but also produce large amounts of the protein encoded by the foreign gene. Cells infected with recombinant baculovirus containing DNA encoding P23 synthesized relatively large quantities of P23, but it proved difficult to extract the protein from the cells. Immunogold electron microscopy showed that P23 had accumulated in the nuclei of the cells. In an attempt to improve the recovery of P23, an extra nucleotide sequence which encoded a short amino acid sequence including 6 histidine residues, was

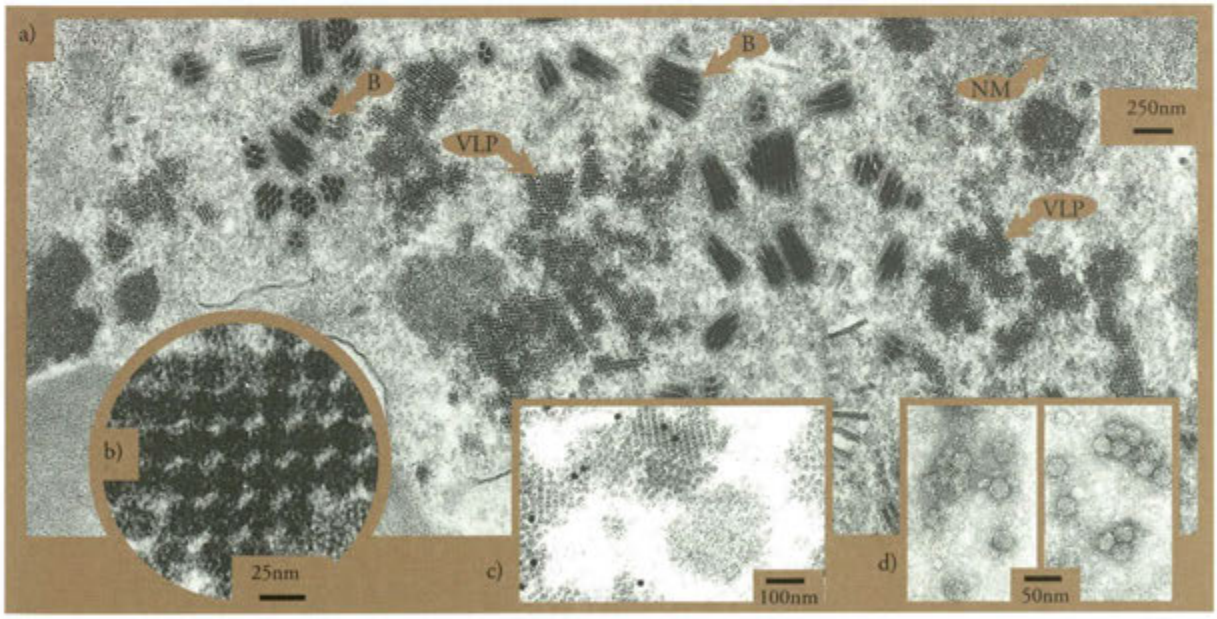


Figure 5 Production of PLRV VLP in insect cells infected with a recombinant baculovirus. (a) A region from an infected *Spodoptera* cell. The nucleus, bounded by the nuclear membrane (NM), contains particles of the baculovirus (B) and crystal-like arrays of VLP. (b) A higher magnification of part of an array of VLP showing the regular arrangement of the VLP. (c) Part of an array of VLP showing a reaction with antibodies to PLRV particles, detected by labelling with gold particles. (d) Comparison of particles trapped by grids coated with antibodies to PLRV from extracts of PLRV-infected plants (left) or *Spodoptera* cells infected with the recombinant baculovirus containing the modified P23 gene.

added to the 5' end of the P23 gene. Insect cells infected with this recombinant baculovirus accumulated the modified P23 also in the cell nuclei (Fig. 5a) but, unlike unmodified P23, it assembled into particles which closely resembled those of PLRV. These virus-like particles (VLP) often appeared to form crystal-like arrays in the nuclei of the cells (Fig. 5b). The VLP were specifically trapped on grids coated with antiserum to PLRV particles (Fig. 5c) and also reacted specifically with gold-labelled antibodies (Fig. 5d).

Our results showed that P23 cannot assemble into particles, at least in this heterologous system, but that the addition of a few amino acids to the amino-terminus of P23 is sufficient for the protein to assemble into VLP. This suggests that the RT protein is not required for assembly of particles but that a minor chemical modification is sufficient. Future work will attempt to determine the minimum modification needed for P23 to self-assemble. However, having a heterologous system for assembling large amounts of VLP from PLRV protein will be of major benefit to work on aphid transmission. VLP can be made that contain a variety of deliberate structural modifications and by feeding these to vector aphids it will be possible to test the ideas developed from the comparative sequencing work and to gain deeper insight into the molecular details of events during PLRV transmission. Results obtained with this system should be applicable to other luteoviruses which include significant pathogens of several important crop plants.

Identification of abutilon mosaic virus variants by DNA fingerprinting

S.M. Macintosh & D.J. Robinson

Abutilon mosaic virus (AbMV) is a geminivirus whose infection in the host plant, *Abutilon pictum 'Thompsonii'*, produces an attractive yellow mosaic pattern on the leaves (Fig.1). As a result, this plant has been vegetatively propagated as a popular ornamental since its first introduction into Europe from Central America in the 19th century. Genetic DNA fingerprinting by restriction enzyme mapping and partial sequencing of polymerase chain reaction (PCR) products from different European AbMV isolates contained within vegetatively propagated host plants has revealed a degree of variation previously unreported.

AbMV has a bipartite, circular, single-stranded DNA genome, typical of whitefly-transmitted geminiviruses[1]. Interestingly, the natural vector of AbMV is the tropical whitefly, *Bemisia tabaci*, which has only recently started to appear in parts of Europe,

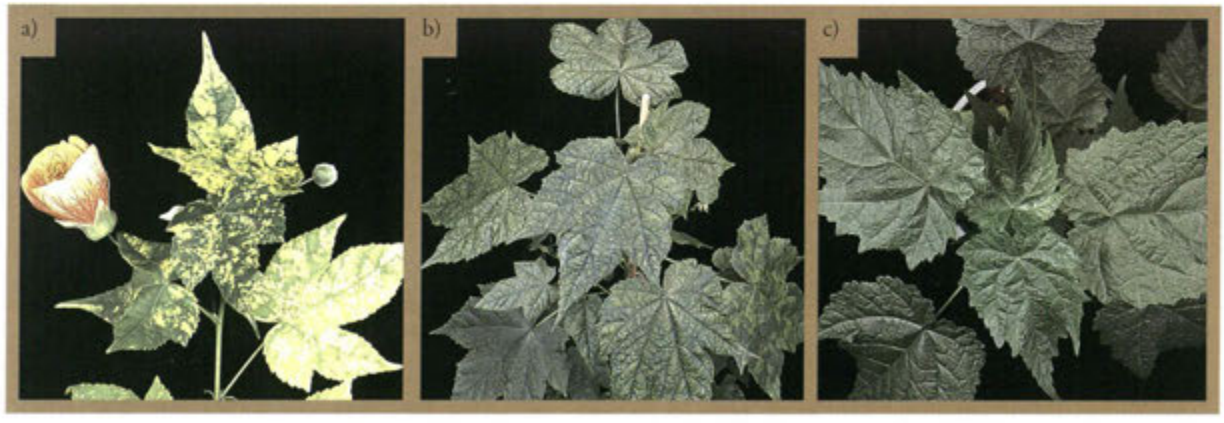


Figure 1 (a) Typical AbMV symptoms in *Abutilon pictum 'Thompsonii'*; (b) A mild variant of AbMV in *A. pictum 'Thompsonii'*; (c) Uninfected *A. x hybridum* 'Ashford Red'.

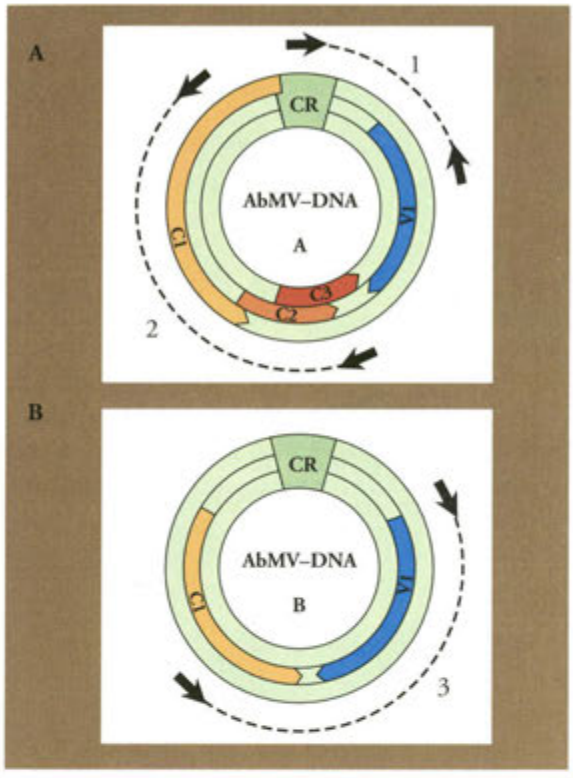


Figure 2 Genome organisation of AbMV showing positions of PCR primers.

and AbMV isolates in vegetatively propagated European *A. pictum 'Thompsonii'* appear to have lost the ability to be transmitted by the vector. They can now be transmitted to healthy abutilons only by grafting, and thus each plant contains in practice an isolated population of the virus. The selection pressures to which these populations have been exposed will have been different from those experienced by the virus in nature. In particular, there has evidently been no selection to maintain vector transmissibility. Variants that cause milder symptoms do occur (Fig. 1b), but they will often have been selected against by propagators, because they are less decorative.

DNA was extracted from leaves of infected *A. pictum 'Thompsonii'* from several sources. Plants D1-6 were derived by vegetative propagation from a single parent obtained from Dundee Botanic Garden approximately 12 years ago. Plant M, a mild variant, came from a private house in Dundee and had been propagated there for at least 20 years. Plants A1-5 were obtained from Hamburg Botanic Garden, but their previous history, and particularly whether they were derived from a single parent, is unknown. Virus-free *Abutilon* x *hybridum* 'Ashford Red' (Fig. 1c) was used as a control.

Selected parts of the AbMV genome were amplified by PCR with three pairs of primers (Fig.2). Primer pair 1 amplified a fragment of 416 bp in DNA-A, extending from within the common region into open reading frame (ORF) AV1, which codes for the particle protein. Primer pair 2 amplified a fragment of 996 bp, overlapping ORFs AC1, AC2 and AC3 in DNA-A, and primer pair 3 amplified a 987 bp fragment in DNA-B, including parts of ORFs BV1 and BC1.

Amplified DNA was then digested with various restriction enzymes chosen to cut the fragments in a predicted way, based on the published AbMV

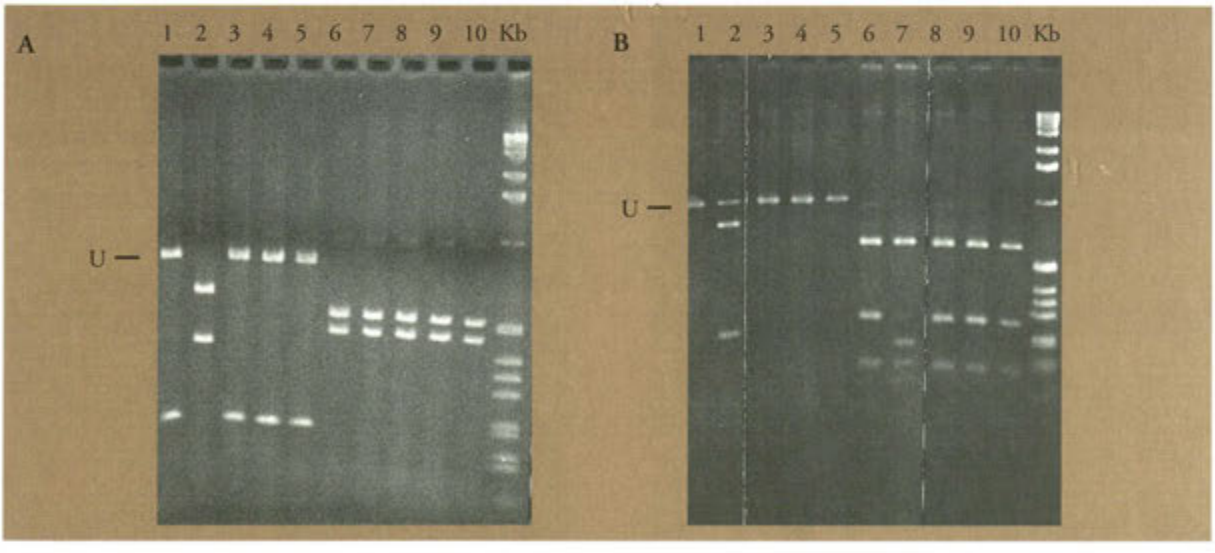


Figure 3 Restriction digests of PCR products of AbMV from plants A1-5 respectively, obtained with primer pair 2. (A) lanes 1-5: *Acc*I; lanes 6-10 *Apa*I ; (B) lanes 1-5: *Eco*RI; lanes 6-10: *Tha*I. Kb= DNA markers (1Kb ladder, Gibco BRL). U= uncut PCR product.

sequence[1]. Figure 3, for example, shows PCR products of AbMV from plants A1-5 obtained with primer pair 2, digested with *Acc*I, *Apa*I, *Eco*RI or *Tha*I. Only with *Apa*I were all five populations cut identically and as predicted (Fig. 3A, lanes 6-10). However, with *Acc*I and *Eco*RI, only A2 cut as predicted (Figs. 3A,B lane 2). Products from A1,3-5 lacked the predicted *Acc*I site, but had a different *Acc*I site instead (Fig. 3A, lanes 1,3-5), and they were not cut by *Eco*RI (Fig. 3B, lanes 1,3-5). When digested with *Tha*I, A1,3-5 had an additional site (Fig. 3B, lanes 6, 8-10), and A2 two additional sites (Fig. 3B, lane 7) compared to the predicted digest which would have given bands of 394 and 602 bp.

The results of the amplification and restriction enzyme digestion experiments with the twelve AbMV isolates can be summarized as restriction maps (e.g. Fig.4) for each of the selected regions of the genome.

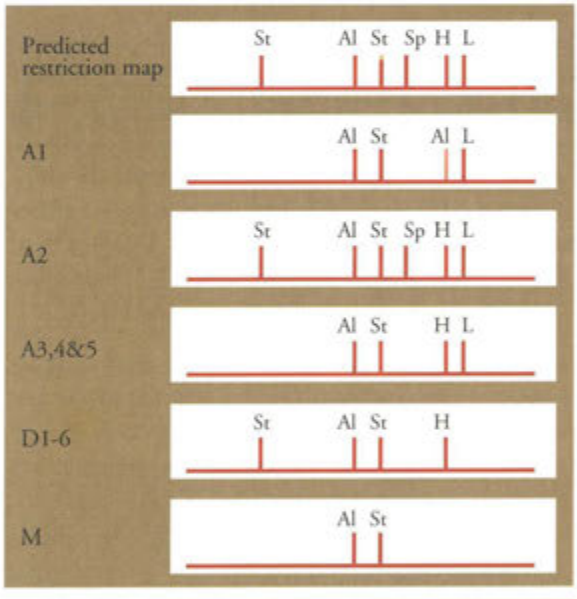


Figure 4 Restriction maps of the region of DNA amplified with primer pair 1 from A1-5, D1-6 and M, compared with the predicted AbMV restriction map[-1]. Enzyme key: Al=*Ala*I, H=*Hind*II, L=*Apa*LI, Sp=*Spe*I, St=*Sty*I. The position of the site indicated by the pale coloured line has not been fully determined.

Overall, in the three areas of the genome studied, none of the isolates was identical to the published AbMV sequence. A2 was the closest, with the restriction map for one of the selected regions matching the predicted map (Fig.4). More diversity was found among the A-isolates than the D-isolates. The mutations detected seemed to be fairly evenly distributed over all the parts of the genome examined. In particular, there were no obvious differences in the frequency of changes between DNA-A and DNA-B, or between coding and non-coding regions.

A portion of each of the DNA-B PCR products of A2 and A4, comprising part of ORF BV1, was cloned and sequenced. Compared with the published nucleotide sequence of this region, the sequence of A2 was 98.75% identical, and that of A4 95.41% identical. There was 94.36% identity between A2 and A4. Fifty percent of the differences in A2, and 31.8% of those in A4 caused amino acid coding changes. Thus, there was little if any bias towards silent mutations. Half of the amino acid changes were conservative, and evenly spread, but some were non-familial, or altered the charge on the protein significantly and were clustered in one area. Coding changes of the latter type could affect the function of the protein.

AbMV DNA from progeny plants derived as cuttings from plants A1-5 was also amplified with the same primers and digested. All gave the same results as their parent with one exception, where an extra restriction site was found in DNA from one progeny A2 plant but not in that from another. Viral DNA amplified from different leaves of the parent A2 plant also showed these two variants. In contrast, no difference was found among samples of AbMV DNA extracted from different leaves of plant M and a sister plant.

Thus, in *A. pictum 'Thompsonii'*, one virus variant usually dominates in each plant. In only one case (A2) was there clear evidence of two variants in the same plant, and even there, one seemed to be dominant in a particular leaf. In experiments where two scions each containing a different AbMV variant were grafted onto uninfected *A.* x *hybridum* 'Ashford Red' plants (Fig.5), one variant appeared to dominate the infection in the stock plant. However, preliminary results indicated that the physiology of the plant was important in determining which variant became dominant.

The level of variation found in these AbMV isolates is comparable with that found in other geminiviruses. However those examples are mainly of insect-transmissible viruses from crop plants, and have usually been associated with differences in symptom appearance in alternative host plants or other biological properties. A much greater amount of variation exists among different virus isolates in crop plants from different locations, but relatively little diversity is usually found among isolates occurring in the same geographical area.

Figure 5 *Abutilon x hybridum* 'Ashford Red' grafted with AbMV-infected *A. pictum* 'Thompsonii'. Symptoms appeared at the tip of the stock plant within 6 weeks of grafting.

It is possible that different wild strains of AbMV were originally introduced into Europe from Central America, and that their diversity continues to be reflected in present day isolates. Alternatively, the isolates may have diverged during 150 years or so of vegetative propagation. Unfortunately, it is impossible to trace back the lineage of European *A. pictum 'Thompsonii'* clones, to determine where and when the viruses in them started to diverge. It is clear that if the mutations have occurred in vegetatively propagated plants, they have accumulated over a long period of time; no changes in the viral DNA were observed in a single cycle of vegetative propagation, and there was little diversity among the virus isolates in plants D1-6, which were derived from a single source relatively recently. It would be interesting to do similar studies on whitefly-transmissible AbMV isolates from Central America to see what level of variation exists among wild populations. The area of the genome that controls insect transmission could also be identified by comparison with European isolates, and the effect of mutations on other biological properties of the virus studied. This would lead to greater understanding of the mechanisms by which the diversity of geminiviruses in crop plants is generated.

Reference

[1]Frischmuth, T., Zimmat, G., & Jeske, H. (1990). *Virology* 178, 461-468.

Exploiting coat protein transgenesis to create improved forms of resistance to two potato viruses.

B. Reavy, H. Barker, K.D. Webster, M. Arif, S. Kashiwazaki, C.A. Jolly & M.A. Mayo

Despite advances in the production of virus-free seed-potato stocks and the control of virus vectors by agrochemicals, virus infection can still cause serious economic losses in potato crops. The development of virus resistant cultivars is a priority in the SCRI potato breeding programme. However, for some viruses there are no suitable sources of natural resistance, and for others resistance is not sufficiently strong to prevent infection when the inoculum pressure is high. The production of new heritable traits using *Agrobacterium*-mediated transformation of potato has been an exciting development in recent years. Of the important virus pathogens of potato which are studied at SCRI, potato leafroll virus (PLRV) and potato mop-top virus (PMTV) provide interesting examples of how genetic modification by transformation is being exploited to create valuable sources of resistance to viruses. We have therefore examined the possibility of developing resistance to PLRV and PMTV by transforming plants with DNA sequences encoding the viral coat proteins, a strategy termed coat protein-mediated resistance (CP-MR).

Figure 1 Effect of inoculation of transgenic and non-transformed (WT) *Nicotiana benthamiana* plants with PMTV. Symptoms of PMTV infection are visible in WT plants (left hand side) but not in transgenic plants (right hand side).

PMTV resistance PMTV causes spraing symptoms (brown arcs and rings) in the tuber flesh of susceptible cultivars, and occurs in Northern Europe, Japan, Israel, South America and Canada. PMTV, a member of the furovirus group of fungus transmitted, rod-shaped viruses, is spread by zoospores of *Spongospora subterranea* which causes the powdery scab disease on tuber surfaces. *S. subterranea* is a common soil-infesting pathogen in many fields in which potato crops have been grown. There are no known natural sources of resistance to PMTV.

The coat protein gene of PMTV was introduced into the test plant *Nicotiana benthamiana* by *Agrobacterium*-mediated transformation. Transformed plants which expressed the coat protein in large amounts were selected and allowed to produce seed. Transgenic and non-transgenic control seedlings were manually inoculated with PMTV. One to three weeks after inoculation, the control plants exhibited disease symptoms and virus accumulation could be detected by ELISA. By contrast, none of the transgenic plants exhibited symptoms (Fig. 1) and no virus could be detected in them by bioassay (i.e. return inoculation of sap to susceptible test plants) (Table 1). Although ELISA indicated that the PMTV coat protein could be detected in a few transgenic plants (Table 1), the mean level of coat protein accumulation in the group of inoculated plants was no higher than in non-inoculated plants of the same transgenic lines and so it was thought to represent expression of the transgene. To test further the durability of CP-MR resistance to PMTV, plants were grafted with scions from infected plants. Notwithstanding the high inoculum pressure thus exerted, no evidence could be found of PMTV multiplication in the stock plants using bioassays. Our results suggested that CP-MR to PMTV was extremely strong in *N. benthamiana* plants, and may represent near-immunity to infection with PMTV. This is the first substantial demonstration of CP-MR to a member of the furovirus group which also contains other economically important viruses such as beet necrotic yellow vein virus and soil borne wheat mosaic virus.

Transgenic line	Virus detection (no. +ve / no. tested) Bioassay	ELISA*
W1	0/6	0/6
W2	0/12	3/12
W7	0/12	1/12
W16	0/6	1/6
W25	0/6	2/6
Non-transformed control	48/48	48/48

* +ve samples are those that give A_{405} values three times greater than those given by non-inoculated plants of the same line.

Table 1 Inoculation of CP transgenic lines of *Nicotinia benthamiana* with PMTV-S and detection of virus replication by bioassay and ELISA.

PLRV resistance PLRV, an aphid-borne virus, occurs world-wide and causes substantial yield loss. A form of host gene-mediated resistance (host-MR) which results in a diminution in both the accumulation of PLRV and the extent of vector-mediated spread between plants has been identified in some potato lines at SCRI. However, introducing this trait into a commercial cultivar by breeding may be time-consuming and difficult.

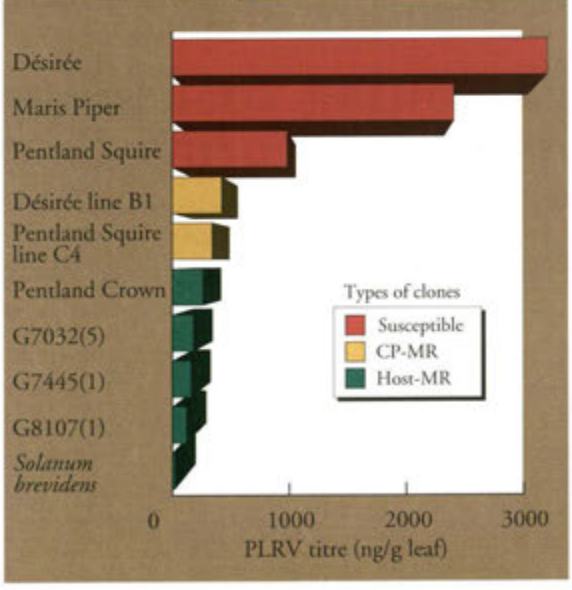


Figure 2 Accumulation of PLRV in potato clones with different levels of resistance to virus multiplication. Estimates are the means of several independent tests.

Both host-MR and CP-MR can cause a significant decrease in PLRV titre in infected plants (*Ann. Rep. 1991, 78*) (Fig. 2). For example, transformation of the susceptible cultivars Désirée and Pentland Squire with the PLRV CP gene resulted in a reduction in virus content of up to 8-fold in infected plants. The amount of virus in the infected transgenic plants was similar to that found in infected plants of the cv. Pentland Crown which has host-MR. Even less PLRV was detected after challenge inoculation of the SCRI breeding clones G7032(5), G7445(1) and G8107(1). While both host-MR and CP-MR to PLRV are effective, the titre of virus in infected plants was not as low as that found in the wild species *Solanum brevidens* infected with PLRV. *S. brevidens* exhibits the most extreme resistance to PLRV so far identified in *Solanum* spp., but it has not been possible to introduce it into cultivated potato by conventional means because of incompatibility barriers.

We have attempted to combine CP-MR with host-MR to further enhance resistance to PLRV multiplication. *Agrobacterium*-mediated transformation was used to introduce copies of the PLRV coat protein gene into cv. Pentland Crown and the three SCRI breeding clones described above. Several transgenic lines were obtained from each clone and propagated in the glasshouse. Progeny tubers were collected from infected transgenic plants following graft-inoculation and resistance to PLRV multiplication was assessed by estimating the accumulation of virus in plants grown from infected tubers (secondary infection). Accumulation of PLRV in transgenic plants which combined host-MR with CP-MR was even less than that in non-transformed plants (Fig. 3). The amount of virus that accumulated in the most resistant transgenic lines containing both host-MR and CP-MR was similar to that found in infected *S. brevidens* and was only 1% of the level of PLRV in infected susceptible cvs such as Maris Piper. We have made tests to assess the spread of PLRV from two CP-MR potato lines described previously (*Ann Rep. 1991, 78*) in a small trial in a screenhouse. PLRV spread from infected plants of two transgenic lines to up to 7% of neighbouring test plants, compared to 22% spread from non-transformed plants. The extremely low titre of PLRV found in transgenic plants containing CP-MR and host-MR should greatly reduce, or even eliminate the spread of PLRV in field conditions because there will be insufficient virus for acquisition by the aphid vectors.

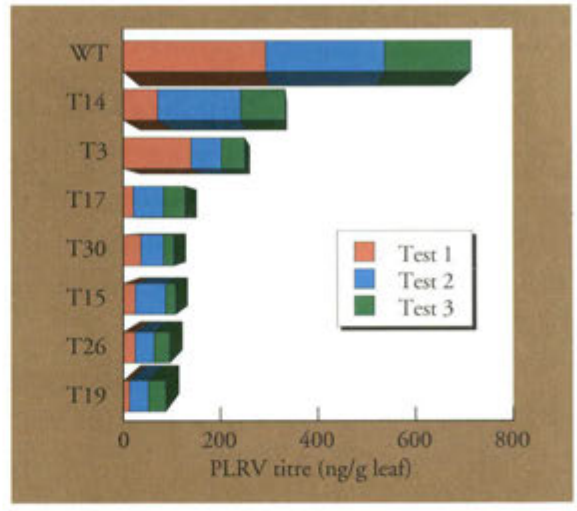


Figure 3 Accumulation of PLRV in plants of CP-transgenic lines of potato clone G7445(1) and in non-transformed control plants (WT). The length of the bars represents the cumulative estimate of PLRV concentration in three tests made at 7-9 day intervals. The virus concentrations in each test are the means estimated from three plants of each line.

Our results with CP-MR to PMTV are particularly encouraging because we have created a form of resistance to PMTV that could be exploited in potato in which there is no natural form of resistance. The work with PLRV is the first report of successfully combining forms of resistance mediated by viral transgenes and by host genes in potato. This represents the integration of genetic engineering with conventional breeding to give resistance that is greater than either approach can provide alone. The success of this work should encourage efforts to improve the resistance of crops to other viruses by using a similar integrated multi-disciplinary approach.

Spontaneous gene deletion events associated with increased symptom severity in a fungus-transmitted virus of cereals

J. Chen, S.A. MacFarlane & T.M.A. Wilson

Introduction Soil-borne wheat mosaic virus is the type member of the *Furo*virus genus which is characterised by having *fu*ngus-transmitted, rigid *ro*d-shaped particles, each containing a single-stranded RNA molecule, two or more species of which comprise the complete genome of each virus. The fungi which transmit these viruses belong to the Plasmodiophoromycetes, which are ubiquitous in soils around the world and persist in the form of resting spores in clusters known as cystosori or spore balls. Resting spores remain viable for many years, or decades, long after the original host plant tissue has rotted. Given adequate moisture and suitable soil temperatures, and possibly even signal compounds released from the root cap or root hair zones, resting spores germinate to produce motile biflagellate zoospores which swim relatively short distances through soil water and enter previously uninfected roots to continue their life cycle. Zoospores of *Polymyxa graminis, P. betae* or *Spongospora subterranea* can transmit specific viruses among the 12-15 known or putative members of the Furovirus genus.

Three Furoviruses are being studied at SCRI. Potato mop-top virus (PMTV) is transmitted by *S. subterranea*, the causative pathogen of powdery scab on potatoes; soil-borne wheat mosaic virus (SBWMV) which infects several members of the Graminae, and Indian peanut clump virus (IPCV) which infects dicotyledonous and monocotyledonous plant species. The latter two viruses are transmitted by *P. graminis* in their respective locations, although the temperature optima and host ranges of their respective vectors suggest genetically different populations. SBWMV causes severe yellowing and stunting of susceptible winter wheat and barley cultivars around the world. Typical crop yield losses range from 10-30% or up to 80% in extreme cases, in fields heavily infested with viruliferous *P. graminis* spores. SBWMV is prevalent in all wheat and barley growing areas of America, Japan, Italy, India, Brazil and mainland Europe. It has not yet been reported in the UK, possibly because the winter wheat and barley cultivars presently grown in the south-east of England have some resistance. Since viruliferous resting spores can be wind-borne with dust and field debris, it is imperative that vigilance for outbreaks of SBWMV is maintained in the UK, especially for those forms of the virus that can overcome the host resistance gene.

Probably the best known and studied Furovirus is beet necrotic yellow vein virus (BNYVV), which causes 'rhizomania' of sugar beet. After being free of this virus in the UK for many years, recent and increasing numbers of outbreaks in East Anglia and southern England are causing concern to the sugarbeet industry. Mostly these have been attributed to viruliferous *P. betae*-contaminated soils being imported on unwashed vegetables or agricultural implements or, less likely, through infected plant tissue. Until recently, plant quarantine regulations have successfully kept BNYVV out of the UK, but increased free trade within the European Union is likely to increase the incidence and distribution of BNYVV, SBWMV and other plant diseases.

The SBWMV genome shows instability In 1984 it was noted that prolonged cultivation of SBWMV field-infected wheat plants in glasshouses at 17°C for 4-8 months, or serial passaging of a field-isolate of SBWMV, from infected to healthy wheat plants, at 2-monthly intervals over 2 years, caused the disease symptoms to become more severe. SBWMV is a bipartite single-stranded RNA virus whose complete genome has been sequenced[1]. SBWMV RNA1 is 7099 nt long and encodes the viral RNA replication machinery as well as the cell-to-cell movement protein of the virus. SBWMV RNA2 is 3593 nt long and encodes the viral coat protein of 19 kDa together with lesser amounts of a coat protein readthrough polypeptide of 84 kDa. Expression of SBWMV coat protein is normally terminated by a UGA stop codon. However, the termination function of this codon can be suppressed (in 10% of translation events) and additional amino acids added to extend the coat protein molecule. At the extreme 3' end of SBWMV RNA2 is a short open reading frame for another protein of 19 kDa which is rich in the amino acid cysteine, but of unknown function. Indirect experiments, involv-

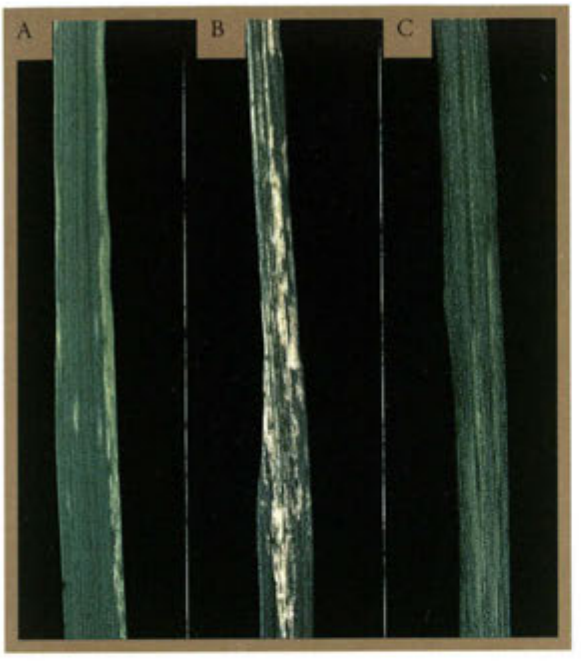


Figure 1 Leaves of *Triticum aestivum* cv. Galahad infected with SBWMV type isolates from Oklahoma (A, first passage Okl-1; B, seventh passage, Okl-7) or Nebraska (C, Lab 1 deletion mutant).[3,5]

ing SBWMV RNA translation in cell-free systems, suggested that the spontaneous deletions occurring in SBWMV RNA2, which gave rise to shorter-than-full-length virus particles, occurred in the coat protein readthrough domain[2].

In addition to the complete nucleotide sequence of RNA1 and RNA2 of wild-type SBWMV from Nebraska[1], the sequence of a stable deletion mutant (Lab 1) of this virus was also determined[3]. No significant differences in the sequences of the longer RNA1 molecule from the two isolates were observed. However, Lab 1 SBWMV RNA2 appeared to have spontaneously deleted two regions of sequence from the portion of the genome encoding the coat protein readthrough domain. The first, of 108 nt, occurred immediately after the UGA stop codon for the coat protein and would simply delete the first 36 amino acids of the readthrough polypeptide. The second, of 1058 nt, occurred in a location which would prevent expression of all the carboxy-terminal 374 amino acids of the readthrough polypeptide. Overall, the 84 kDa coat protein readthrough species would thus be reduced to approx. 38 kDa.

In 1993 we obtained a new isolate of SBWMV from Professor J. Sherwood, Oklahoma State University. This isolate had been obtained from a field in Oklahoma in February 1992 and stored frozen. It was a typical SBWMV isolate from winter wheat or barley crops harvested in late autumn, winter or early spring as it contained only full-length RNA1 and RNA2 molecules, packaged in the corresponding length virions (280-300 nm for the longer RNA1-containing particles; and 140-160 nm for the shorter RNA2-containing particles). This isolate of SBWMV (designated Okl-0) was serially passaged seven times through *Triticum aestivum cv.* Galahad over a period of 32 weeks. After 11 weeks, deleted forms of SBWMV RNA2 appeared in the first population of mechanically inoculated wheat plants and co-existed with the full-length genome component. During subsequent, more rapid passages from plant-to-plant, the proportion of deleted RNA2 molecules increased relative to full-length wild-type RNA2 and, by the fifth passage (20 weeks), no full-length RNA2 remained. Both the stable, deleted Lab 1 isolate of Nebraska SBWMV and the new deleted form of Oklahoma SBWMV (Okl-7) produced more severe symptoms on susceptible winter wheat than the parent virus (Fig. 1).

Multiple harvests of leaf samples from plants infected by SBWMV in each of the seven passages were retained. The leaf tissue provided total RNA populations which could be analysed by northern blotting procedures to discriminate between, and show the

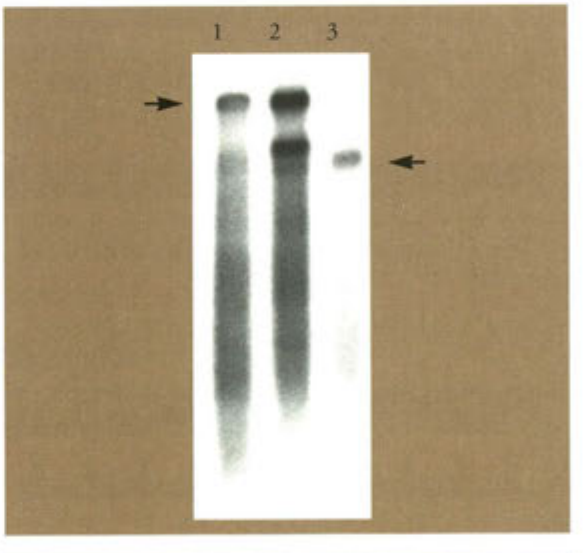


Figure 2 Northern blot of total nucleic acid preparations from wheat leaves infected with SBWMV Oklahoma isolates Okl-1 (lane 1); Okl-3 (lane 2) or Okl-7 (lane 3). A radioactive probe specific for SBWMV RNA2 sequences was used. The sizes of wild-type (3593 nt) and the stable deleted RNA2 molecules (2834 nt) are indicated by arrows to left and right, respectively.

sizes of, SBWMV RNA1 or RNA2 related sequences (Fig. 2). The same total RNA preparations were also used for reverse transcriptase-polymerase chain reaction analyses (RT-PCR) with oligonucleotide primer pairs spanning different regions of SBWMV RNA2 (Fig. 3). This work confirmed that approximately 760 nt had been deleted from all SBWMV RNA2 molecules when the original wild-type field isolate (Okl-0) was compared with virus from the fifth to seventh passages (Okl-5, Okl-7). The original Lab 1 deletion mutant from the Nebraska strain of SBWMV[2,3] was also analysed again and found to be deleted for approximately 1060 nt.

In order to map the deletion sites in Okl-7 and Lab 1 SBWMV RNA2 molecules precisely, the relevant RT-PCR fragments (Fig. 3) were cloned and sequenced (Fig. 4). The results showed that the Oklahoma strain of SBWMV had lost 759 nt between genome co-ordinates 1420-2180 and effectively created a translational fusion between the amino- and carboxy-terminal sub-domains of the coat protein readthrough polypeptide, such that 253 amino acids were lost from the central portion. In contrast, a 1058 nt deletion was confirmed between genome co-ordinates 1468-2527 of the Lab 1 deletion mutant of Nebraska SBWMV which introduced a premature UAG stop codon as predicted from previous work[3]. However, our RT-PCR, restriction enzyme mapping and direct sequencing failed to detect the second 108 nt deletion[3] referred to above in the Lab 1 deletion mutant of SBWMV. Since this material (kindly gifted by Dr Y Shirako, Caltech, Pasadena, CA) was the same as that sequenced previously, we conclude that a cloning artefact or minor sub-population of RNA2 molecules was the source of the original sequence information.

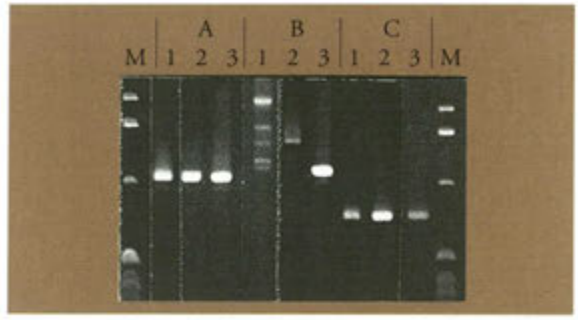


Figure 3 Amplimers created by RT-PCR using RNA templates from wheat infected with SBWMV isolates Okl-1 (lanes A.1, B.1, C.1), Okl-7 (lanes A.2, B.2, C.2) or Nebraska Lab 1 (lanes A.3, B.3, C.3). Flanking tracks contain DNA size markers (in kbp). Lanes designated A and C were amplified with PCR primer pairs specific for the 5'- or 3'-terminal regions of SBWMV RNA2 (approx. 1 kb each). Lanes designated B represent the central (approx. 1 kb) region of SBWMV RNA2 and the different sizes of the bands between isolates are a measure of the extent of deletion in the original RNA molecules.

By analogy with BNYVV[4], we propose that the coat protein readthrough domain encoded by SBWMV RNA2 is involved in virus acquisition and transmission by the fungal vector, and that a strong selection pressure exists on the virus to retain these sequences during natural spread in the field between successive

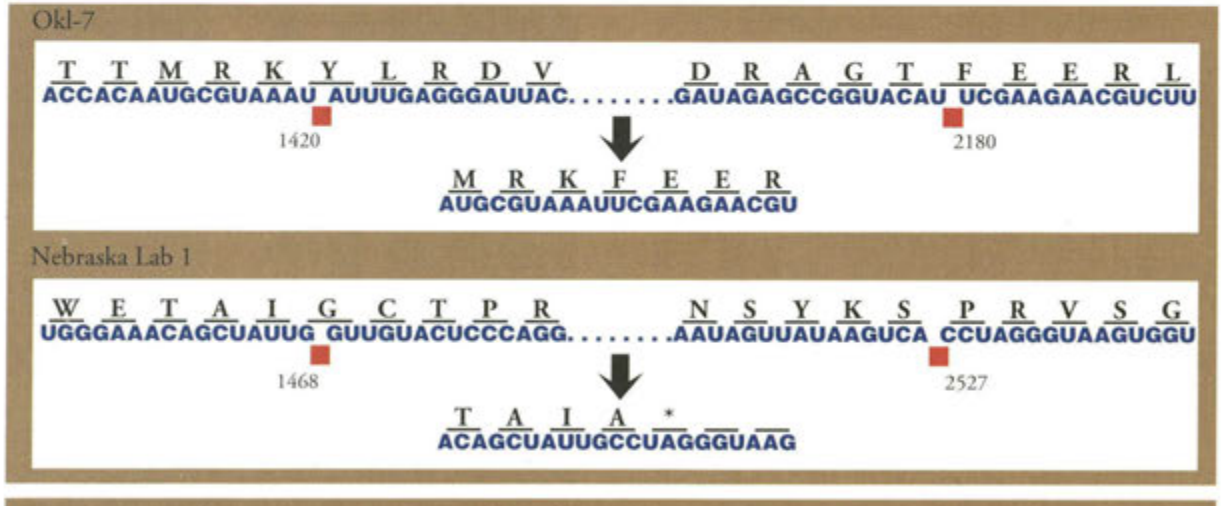


Figure 4 Sequence comparisons of deletions in Okl-7 and Nebraska Lab 1. The effect of each SBWMV RNA2 deletion (mapped between the red squares) on the amino acid sequence of the CP readthrough domain polypeptide is shown above each nucleotide sequence. Numbers are RNA2 genome component co-ordinates based on the complete sequence of the Nebraska isolate of SBWMV.

infection cycles in susceptible host plants. Coding sequences in this region of the RNA were lost rapidly following mechanical transmission to the foliage in contrast to fungus transmission to roots. *P. graminis* transmissions of the deleted forms of SBWMV described above (Lab 1 and Okl-7) are currently being tested by Dr M.J. Adams, IACR, Rothamsted.

It was noticed, during the RT-PCR analysis, that the primary inoculated wheat plants (Okl-1) showed several intermediate-sized RNA2-related bands (Fig. 3, lane B.1), which presumably arose during the process of deletion. These have now been sequenced. A similar phenomenon was noticed in 1984[5] when different patterns of deletion intermediates in SBWMV RNA2 occurred between individual plants brought in from the field and propagated under glasshouse conditions. Computer analysis may soon reveal a sequence or structural basis for the speed and precision of the RNA deletion process.

Conclusions Among the Furoviruses studied to date, some of which contain up to five separate genomic RNA segments, the RNA2 component seems particularly unstable and prone to spontaneous deletion during mechanical transmission. Thus, during cDNA cloning and sequencing of IPCV RNA2, several anomalies, sequence reassortments and potential deletion events were noted [personal communication M.A. Mayo, J. Miller (SCRI) and V. Wesley (ICRISAT, Hyderabad)]. The sequence of West African peanut clump virus RNA2 was published recently[6] and two out of 20 field isolates were found to have deletions of 402 nt, or 968 nt in the region of RNA2 which corresponds indirectly to the coat protein readthrough domain in SBWMV.

A similar phenomenon has been noted in BNYVV RNA2[4] and here deletions in the C-terminal half of the coat protein readthrough domain have been shown to affect transmission by *P. betae*.

In the Oklahoma isolate, deletion occurred more rapidly (11 weeks; Okl5-Okl7) than in the Nebraska Lab 1 deletion mutant and caused more severe symptoms on a susceptible U.K. wheat cultivar (Fig. 1).

In addition to studying the fungus transmissibility of deleted forms of SBWMV, and the molecular events which occur during the spontaneous deletion process *per se*, we are also constructing full-length clones of each of the RNA components of both virus isolates with a view to future recombination and reverse genetic experiments. Ultimately, we hope to understand the structural and genetic interactions between this important virus, its graminaceous hosts and its fungal vector in more detail. In addition, we are examining isolates of the virus from different parts of the world, as well as susceptible and resistant cultivars of winter barley and wheat, with a view to characterising, understanding and exploiting existing (or new) forms of host gene- mediated or transgenic crop resistance.

Molecular analyses of SBWMV, its hosts and its fungal vector are closely connected to studies on PMTV and IPCV, also underway at SCRI. These viruses were chosen not only for their economic and agronomic importance worldwide, as agents of disease, but also because they represent relatively simple molecular genetic access points to study a range of important host plants and fungal vectors

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Nematode and insect pests

D.L. Trudgill

Much of our research is targeted towards relating variation at a molecular level in nematodes and arthropods to variation of biological and agricultural significance. Advances in molecular techniques also offer opportunities for developing novel methods of control, including the development and testing of transgenic plants. These new areas of work have been strengthened by the appointment of an additional molecular nematologist and a molecular entomologist. However, our research is also directed towards making best use of existing methods of control, and both approaches require increasingly detailed knowledge of pest biology, ecology and behaviour. We are now better able to address the gaps in our knowledge because of advances in equipment and techniques. Also, much of our research is collaborative and the multi-disciplinary structure and wide range of facilities continually being developed at SCRI provide a unique environment for integrating many different aspects of crop protection research. In this, visiting workers and Ph.D. students make an important contribution.

We are involved in two collaborative EC contracts; one to determine the number of, and virulence characteristics of the introductions of potato cyst nematode (*Globodera spp.*) into Europe; the second to determine the effectiveness of the bacterium *Pasteuria penetrans* as a bio-control agent for root-knot nematode (*Meloidogyne spp.*). Our part in the latter project, which is co-ordinated from SCRI and involves eleven centres in nine countries, is to use molecular approaches to examine variation in *Meloidogyne spp.* Progress overall includes demonstrating the widespread occurrence of the bacterium in some tropical countries and of a new, extremely virulent species of *Meloidogyne (M. mayaguensis)* in West Africa. Our research, in collaboration with ORSTOM, has produced molecular evidence for distinct groups in *M. arenaria.* In contrast, *M. incognita, M. mayaguensis* and *M. javanica* have each proved remarkably homogeneous.

In related studies, potato cyst nematode populations (*Globodera spp.*) were shown to be much more heterogeneous than *Meloidogyne spp.* and molecular evidence is accumulating to support the view that an extremely

virulent population of *G. pallida* from southern Scotland is a distinct introduction. The use of simple sequence repeat primers has proved particularly useful in these analyses. Other new studies aimed at isolating nematode genes are using changes in mRNA (differential display) to identify genes expressed in response to stimuli from plants, and specific antibodies will be used to isolate and purify nematode gland-cell and surface proteins.

Analysis of ribosomal DNA (rDNA) has proved particularly revealing in entomological studies which seek to identify markers for differences of biological significance between closely related species or biotypes within species. The internal transcribed spacer regions in rDNA have been used to discriminate between related species of mites (*Cecidophyopsis spp.*) and root flies (*Delia spp.*). Polymorphisms in the intergenic spacer regions (IGS) of rDNA have been found in the aphids *Amphorophora idaei* and *Myzus persicae*. This finding has enabled us to show that populations of virulent *A. idaei* ('biotype 2'), which have now been found in many parts of the UK on raspberry cultivars containing the resistance gene A_1, consist of many genotypes, and demonstrates the need to increase the number of resistance genes in breeding programmes for pest resistance. In the development of integrated pest management (IPM) programmes, we are using raspberry beetle and root flies as model systems on which to study the role of plant volatile and surface chemicals in resistance and susceptibility mechanisms. Olfactometer bioassays have identified a complex of flower volatiles as beetle attractants and a synthetic mixture developed in the laboratory is being tested as a chemical lure in the field. In collaboration with colleagues in Switzerland, Denmark, RGB Kew and chemists at SCRI, leaf surface chemicals which stimulate oviposition behaviour in root flies have been characterised and have provided new opportunities for breeding root-fly resistant brassica crops.

Dr.D.L. Trudgill, Head of the Zoology Department.

Collaborative, externally-funded field studies with St Andrews University are examining the effects of various factors on pest and beneficial arthropod populations in raspberry plantations. New IPM strategies which exploit behavioural attractants are being developed, particularly for raspberry beetle, and decreases in pest populations were observed in hedgerow-managed systems compared with stooled management.

Ecological studies on nematodes, partly MAFF funded, are examining the effects of changes in agricultural practices on population densities and species diversity. After three years of set-aside in Scotland, significant increases in numbers of plant parasitic nematodes were observed compared with populations under spring sown crops, but no such differences were observed in winter sown crops in England. The thermal time requirements for nematodes have also been examined and a linear relationship between rate of development and temperature has been demonstrated in several species. Comparisons with *Meloidogyne spp.* show that a high base or threshold temperature for development was associated with a low total thermal time requirement. Collaborative studies, partly funded by the Potato Marketing Board, to model the population dynamics of the white species of potato cyst nematode (*G. pallida*) on partially resistant potato cultivars in the field, and the influence of tolerance differences on yield losses have been completed. The results provided a unique analysis of relationships in field conditions that could form the basis of an expert system to plan rotations and control measures to manage *G. pallida*.

Collaboration with virologists to determine the factors involved in the specificity of nematode transmitted viruses continued and demonstrated that the coat protein gene of tobraviruses was not the only factor determining transmissibility. Electron microscope studies on the mechanism of retention indicated that there might be fine linkages between virus particles and their site of retention in the nematode food canal, supporting earlier NMR studies which revealed mobile arms at the C terminus of coat protein subunits of the virus. The new knowledge gained into the nature of vector specificity is being applied to investigate the epidemiology of spraing disease in potato caused by tobacco rattle virus and is supported by the Potato Marketing Board.

Thermal time and nematode ecology

D.L. Trudgill

It is well known that invertebrate animals are dependent on the temperature of their environment for their vital activities; the warmer the conditions the more rapid the rate of development up to a thermal optimum (T_o). Above T_o the rate of development decreases to a thermal maximum (T_m) as the environment becomes too hot (Fig. 1). Below T_o rates of development decrease with decreasing temperature until all development ceases, at the threshold or base temperature (T_b). Studies with plant related processes such as seed germination have shown that between T_b and T_o the rates of development often increase linearly with temperature. Where this applies, the temperature x time requirement for the process under study is constant (the thermal constant, S, expressed in °C d.). The relationship between temperature and duration of development (D) is expressed by the equation:

$$D = \frac{S}{T_e - T_b} \quad \text{(for temperatures above } T_b\text{)}$$

where T_e is the average environment temperature. The rate of development at any one temperature is the reciprocal of D.

This article presents recent research which explored the relevance of thermal time to nematode and uses as examples two species of root-knot nematodes *(Meloidogyne spp.)*, one of which is tropical *(M. javanica)* and one of which is a temperate species *(M. hapla)*. Durations of development from invasive second stage juveniles (J2) to the first new J2 of the next generation were determined at a range of constant temperatures on tomato which is a good host for both species (Fig. 2). This showed that generation time decreased for both species as T_e increased up to 27°C.

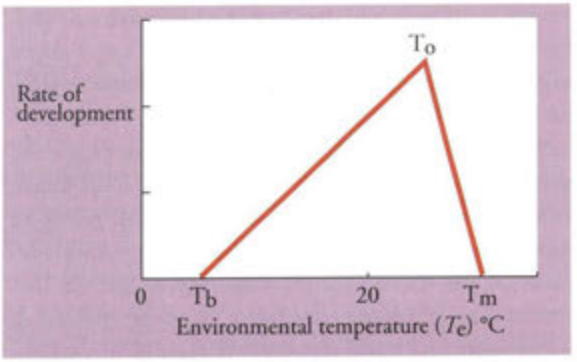

Figure 1 Relation between environment temperature and rate of development for an organism/process which responds linearly.

However, *M. hapla* had a shorter generation time up to 21°C and developed at lower temperatures than *M. javanica*. Above 21°C the duration for development was less for *M. javanica*.

Converting durations into rates of development showed that *M. hapla* had values of T_b and of S of c. 8.3°C and 550°C d. and *M. javanica* had values of c. 13.0°C and 350°C d. (Fig. 3). Hence, the increased T_b of *M. javanica* compared with *M. hapla* is associated with an increase in the slope of the

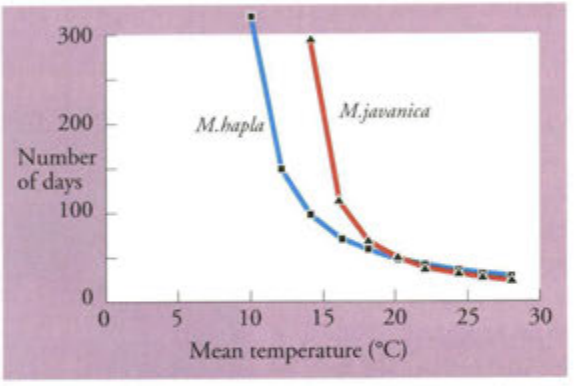

Figure 2 Relation between environment temperature and minimum duration of one generation of *Meloidogyne hapla* and *M. javanica*.

regression between rate of development and temperature. This makes good ecological sense as it enables each species to have a reproductive advantage over the other in the environments to which they are adapted. It also accounts for the observation that tropical

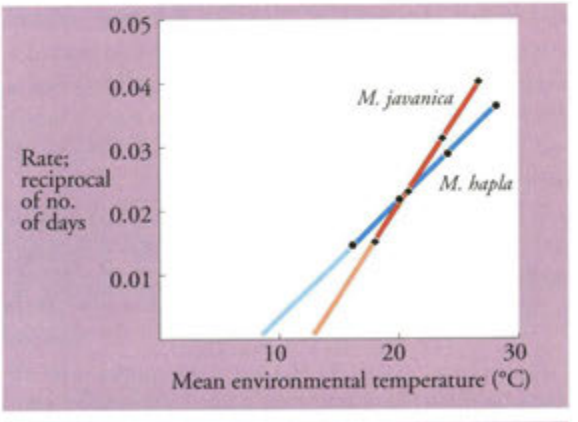

Figure 3 Relation between rates of development and temperature for one generation of *Meloidogyne hapla* and *M. javanica*.

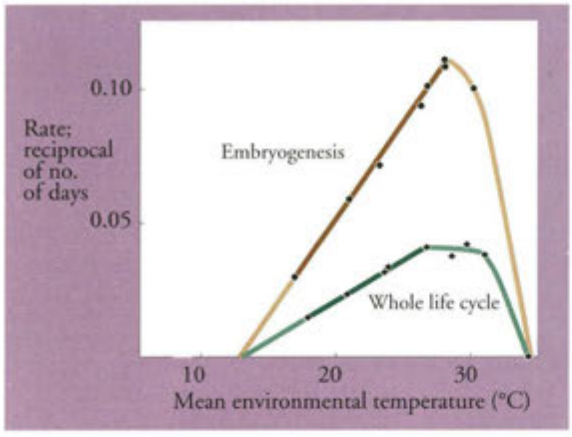

Figure 4 Relation between rates of development and temperature for embryogenesis and whole life cycle.

organisms tend to have relatively high values of T_b while temperate species have low values, and it seems probable that an inverse relationship between T_b and S is widespread in poikilothermic (cold-blooded) organisms.

A complete generation of a *Meloidogyne spp.* involves a sequence of events including growth in size, developmental processes such as embryogenesis, and activities such as finding and invading host roots. Different processes may have different values of T_b but a study of embryogenesis in *M. javanica* (Fig. 4) suggested that the value was not greatly different from that for the whole life cycle. The thermal constant for embryogenesis was 138°C d., about 39% of the total life-cycle requirement.

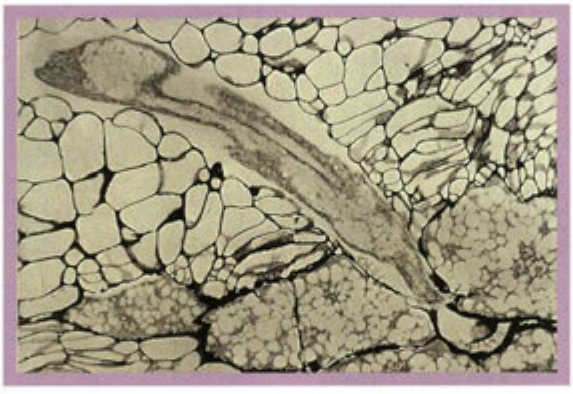

Figure 5 Giant cells associated with a developing *Meloidogyne* female.

The duration of development (D) is a consequence of the amount of development to be done divided by the rate. Comparisons of rates are therefore possible if amounts can be quantified. For example, durations of development of *Meloidogyne spp.* are greatly increased on poor hosts (Table 1) and, as adult size is not greatly decreased, this must be due to much reduced rates of development.

Meloidogyne spp. have high rates of multiplication on good hosts and this greatly contributes to their importance as crop pests. At 25°C they will produce about three generations on one crop, enabling a small population density to increase beyond that which the crop can support without a reduction in yield. This short generation time is the consequence of the rich supply of nutrients provided by the giant cells that they induce in their hosts (Fig. 5) and by aspects of their biology, primarily the adoption of parthenogenetic reproduction and the suppression of functional third and fourth stage juveniles. Even so, their rates of development are slow compared with the bacterial feeding species *Caenorhabditis elegans* which has a thermal constant of c. 43°C d. for one generation, less than one third of that required by *M. javanica* for embryogenesis and c. 12% of that needed for one generation.

A major value of thermal time information is that it forms a basis for understanding nematode ecology. Not surprisingly, most large nematodes have long generation times because the thermal constant is large. Such nematodes thrive best in stable environments. Conversely, species with small adults have short generation times and frequently are able to exploit more transient opportunities. Where species might directly compete it can be used to identify the environments in which each will dominate. The effects of selection pressures, e.g. for low temperature activity, can be predicted if, as is proposed above, S increases as T_b decreases. Also, thermal time information can be used to predict where nematodes may become established. A study with *M. hapla* showed that it could not become established on annual crops in Finland, even through it was being regularly introduced on planting stocks. Similarly, with our present climate it would struggle to survive in Scotland. However, if average summer soil temperatures were increased by 2°C it could become a potential problem.

	% at each stage		
	Tomato	Corn	Sesame
Under-developed juvenile	1	9	63
Developing juvenile	9	33	37
Young female	90	57	0

Table 1 Differences in stage of development of *Meloidogyne incognita* 17 days after inoculation on three crop species of differing host status. Results are from five replicates.

Inter- and intraspecies relationships in root-knot nematodes, *Meloidogyne* spp.

V. C. Blok, M. Fargette, M. S. Phillips & D. L. Trudgill

Root-knot nematodes *(Meloidogyne* spp.) are major pathogens of crop plants that caused yield losses estimated to be worth $77 billion in 1987. In addition, their broad host range exceeding 2000 plant species, world-wide distribution, relatively short life cycles and high reproductive rates make them a challenging agricultural problem.

Root-knot nematodes are obligate sedentary endoparasites which have a sophisticated interaction with their host plants. Infective second-stage juveniles (J2) penetrate the root intercellularly in the region posterior to the root cap and migrate to the developing vascular cylinder where they establish feeding sites. Following injection of oesophageal-gland secretions into the protophloem cells surrounding the head of the nematode, multinucleate 'giant cells' are formed which are used as permanent feeding sites. In addition, cells surrounding the feeding site are stimulated to divide to form a gall (Fig. 1).

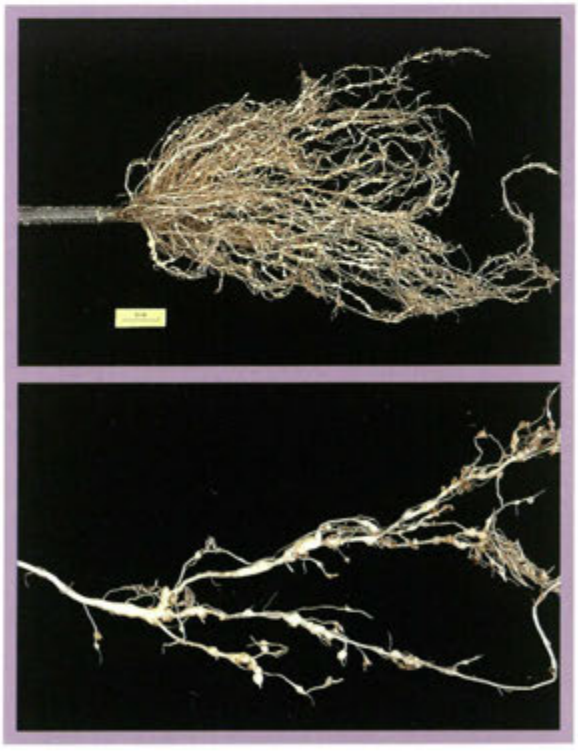

Figure 1 Tomato roots showing root galls produced by *Meloidogyne incognita*

Nematode development is rapid with a second generation starting to feed as soon as 4 weeks after initial invasion and adult females producing up to 2000 eggs. Three generations are possible in one crop cycle and nematode invasion and development impairs nutrient and water uptake by the roots resulting in weakened and low-yielding plants.

Although many *Meloidogyne* spp. reproduce by mitotic parthenogenesis (a form of clonal reproduction), virulent races occur which are able to reproduce on resistant and non-resistant host plants. Several populations able to overcome host resistance in cultivars of tomato, cv. Rossol; sweet potato, cv. CDH; and soybean cv. Forrest were identified from West Africa. Understanding the relationship between virulent and avirulent populations is important for both the use of resistant cultivars in integrated control and for quarantine reasons. However, species and especially races are difficult to separate using classical morphological characters. Consequently biochemical and molecular studies were conducted to investigate the genetic basis of pathogenic variation.

Isozyme studies identified the resistance breaking populations from West Africa as *M. mayaguensis,* a new species previously reported from South America and distinct from the three widespread and economically important tropical root-knot species, *M. incognita, M. javanica* and *M. arenaria.* An analysis of restriction fragment length polymorphisms (RFLPs) of 28 clones from five different *Meloidogyne* spp. using a range of restriction enzymes and probes prepared from a *M. mayaguensis* genomic library confirmed that the virulent West African populations were *M. mayaguensis.* Within species variability was negligible in *M. incognita, M. mayaguensis* and *M. javanica* but within *M. arenaria* there was variation and some populations were similar to *M. javanica.* These results raised questions about the genetic progenitors of both species and the origin of the genetic variation because they reproduce by mitotic parthenogenesis.

Though the RFLP studies were revealing, technically they were limited by the relatively large amounts of high quality DNA required for the analysis and requirement to use radioisotope and thus polymerase

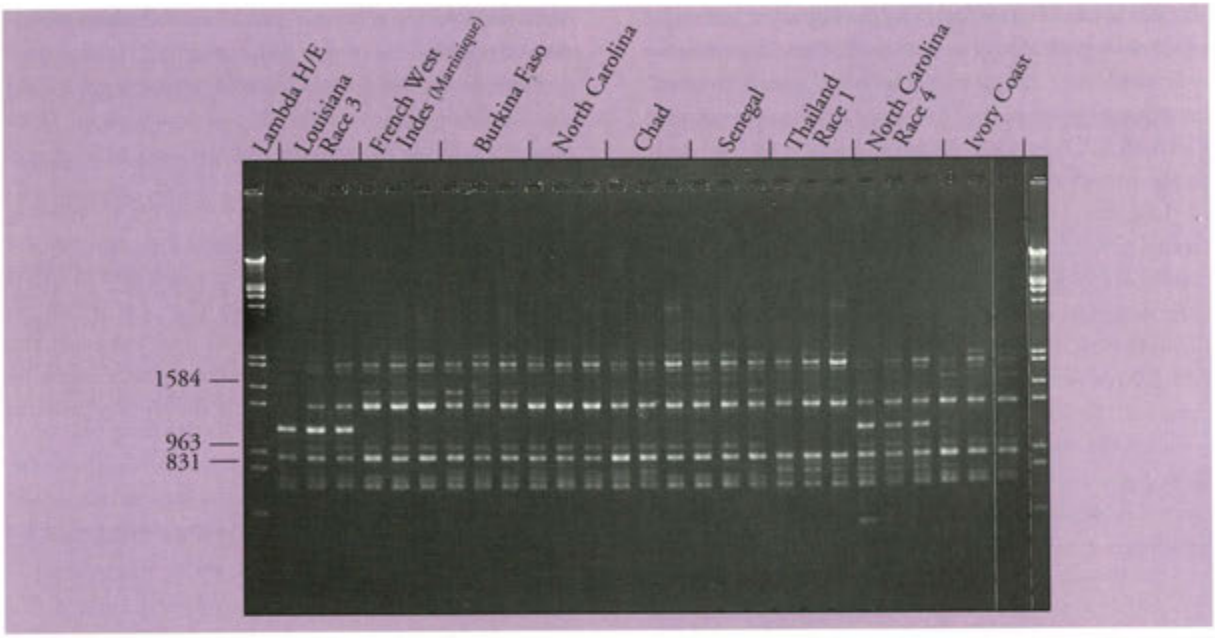

Figure 2 RAPD patterns of nine *M. incognita* populations using primer Sc10-87.

chain reaction (PCR) based techniques were assessed as an alternative. The multicopy ribosomal genes are a classical target for such taxonomic studies. However, following PCR using primers specific for sequences flanking the internal transcribed spacer (ITS) 1 and ITS2 regions, no intra- or interspecies variation was revealed between *M. incognita, M. javanica* or *M. arenaria* indicating a distinct lack of genetic diversity within these species. This is in marked contrast to results obtained with other plant-parasitic nematodes such as *Xiphinema americanum, Globodera pallida* and *Heterodera* spp. where polymorphisms were detected even between populations of one species collected at the same locality.

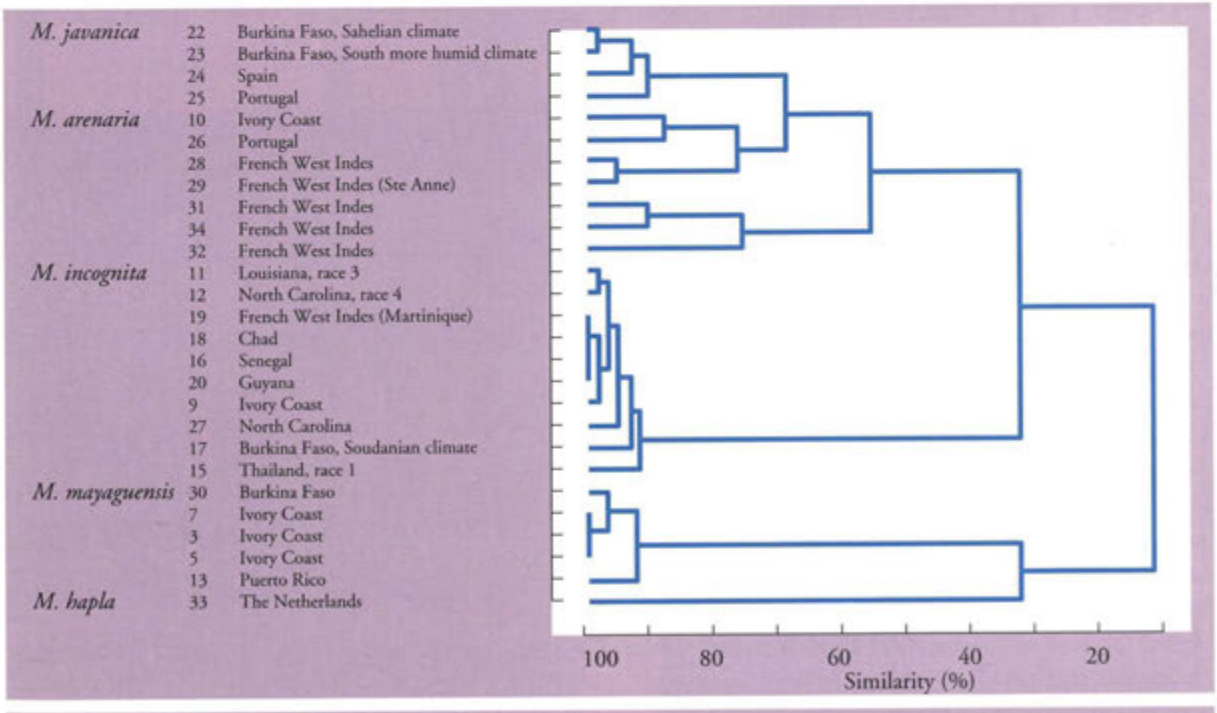

Figure 3 Dendrogram of the similarities between *Meloidogyne* spp.

Greater sensitivity in detecting intraspecific variation in *Meloidogyne* spp. was observed when the genome was randomly sampled using nine small inverted repeat primers in random amplified polymorphic DNA (RAPD) analyses. Figure 2 shows the variation in the patterns of amplified DNA fragments from one of these primers for nine populations of *M. incognita*. Each PCR reaction was repeated three times. These results are typical and showed that there is relatively little variation within the populations of *M. incognita*. Nonetheless differences between the populations which have not been observed by other methods were detected. In contrast, the results from the *M. arenaria* populations showed more differences between lines. In order to assess the diversity between all the populations a similarity matrix (Nei and Lei, 1979) and a dendrogram were generated (Fig. 3) which showed that the RAPD technique produced the same grouping of populations as the RFLP method. It also confirmed the variation within the *M. arenaria* group and the similarity between the *M. javanica* populations and some of the *M. arenaria* populations.

The RAPD analyses proved more sensitive at revealing intraspecies variation in *Meloidogyne* spp. and has the additional benefit of using smaller quantities of DNA than RFLPs. It therefore appears that this technique samples more of the genome and thus enhances the chances of finding molecular markers which might be related to biologically important differences such as virulence.

Reference

1 Nei & Lei (1979) *Proceedings of the National Academy of Sciences* 76:5269.

The ultrastructure and taxonomic evaluation of eriophyid mites of *Ribes*

A. T. Jones, J. W. Amrine*, I. M. Roberts, G. H. Duncan, B. Fenton, G. Malloch, W. J. McGavin & A. N. E. Birch

The eriophyid gall mite, *Cecidophyopsis ribis* (Fig. 1), is the most damaging pest of blackcurrant (*Ribes nigrum*). With the exception of the Americas, where blackcurrant is not cultivated commercially to any large extent, *C. ribis* occurs wherever blackcurrants are grown. It causes the condition known as 'big bud' because the buds enlarge to 2-3 times their normal size due to mite feeding and reproduction which usually results in bud sterility. In addition to causing direct damage as a pest, the mite is the vector of the agent of reversion disease, the most important and damaging virus-like disease of blackcurrant world-wide. Despite research over many years, the causal agent of reversion remains uncharacterised. At SCRI, one of several different approaches being made to identify the disease agent is to study the ultrastructure of infected plants and gall mite vectors feeding on such plants, to identify known or, novel, pathogenic organisms in plant and vector tissues. However, detailed information on the anatomy and

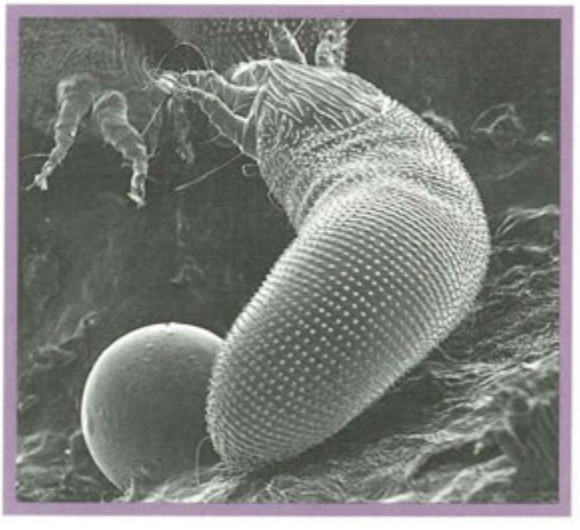

Figure 1 Scanning electron micrograph of the gall mite, *Cecidophyopsis ribis*, in a galled bud of blackcurrant.

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ultrastructure of eriophyid mites is sparse and no information on *C. ribis.* has been published in English. As a first step therefore, it was necessary to determine accurately the internal anatomy of the mite to identify and locate the feeding apparatus and food canal, areas where the reversion disease agent is most likely to be present.

Using electron microscopy of ultrathin serial sections, we have identified the main anatomical features of *C. ribis* and the composite picture they provide is illustrated diagrammatically in Figure 2. Additionally, electron microscopy of serial sections through the anterior portion of suitably orientated specimens allowed a detailed three-dimensional reconstruction to be made of the very complex arrangement of the mouth parts which consist of three separate pairs of stylets, a pair of chelicerae and of pedipalps (Fig. 3). This is the first detailed analysis of the ultrastructure of the mouthparts of this important pest and provides a sound basis for attempts to locate and identify the reversion agent in tissues, and for studies on its biology and feeding behaviour.

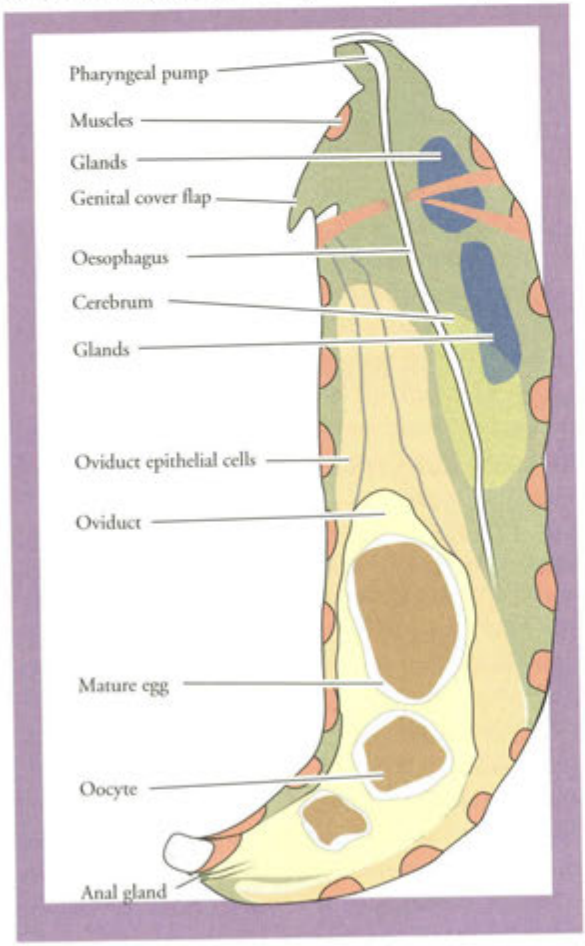

Figure 2 Diagrammatic representation, based on information from electron micrographs of serial sections, of the anatomical structure of *Cecidophyopsis ribis* when viewed laterally.

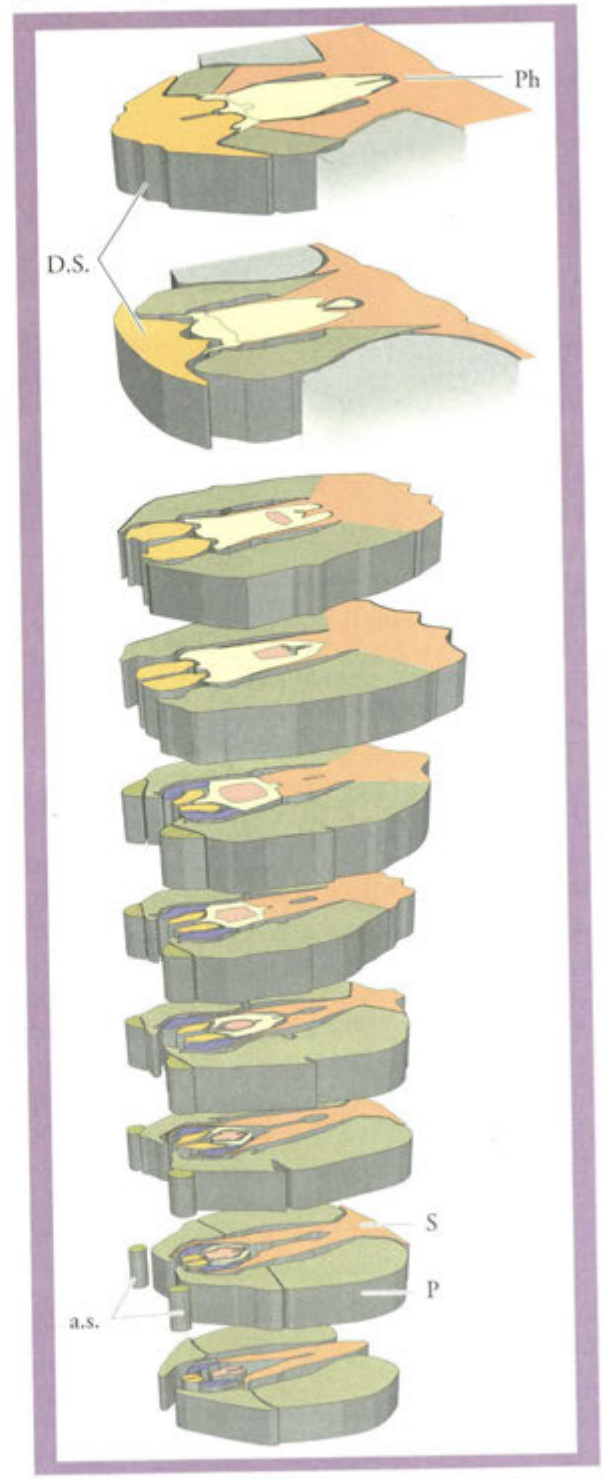

Figure 2 Diagrammatic representation, based on information from electron micrographs of serial sections, of the feeding apparatus of the blackcurrant gall mite.
P= pedipalps, Ph = pharynx, DS = dorsal shield, S = subcapitulum, a.s. = antapical setae.

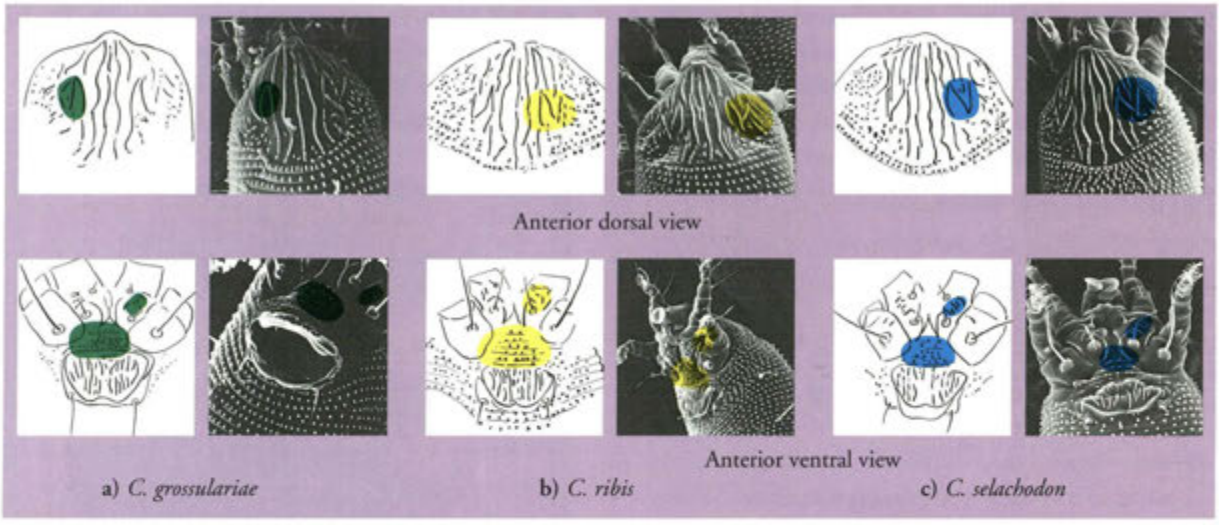

Figure 4 Regions of the dorsal shield (upper) and genital flap (lower) with regions (coloured) that distinguish *Cecidophyopsis* specimens from blackcurrant (a) redcurrant (b) and gooseberry (c).

All commercial blackcurrant cultivars grown in the UK are susceptible to *C. ribis*, and to the reversion agent. Control of the mite, and consequently of reversion disease, is therefore dependent on multiple applications of acaricides that must be timed very accurately to be effective. The organochlorine acaricide that is most effective for control is banned in some countries and under review in others. Because of these difficulties in providing long-term satisfactory chemical control of *C. ribis*, breeding for resistance to the pest is a major priority in blackcurrant breeding programmes. The most widely used source of *C. ribis*-resistance is from gooseberry and is possibly determined by a single dominant gene, *Ce*. However, there are reports of a non-galling eriophyid mite colonising *Ce*-containing blackcurrant genotypes, redcurrant (*R. rubrum* var. *pubescens*) and gooseberry (*R. uva-crispa*)[1]. Furthermore, little is known of the relationships of *C. ribis* to two other *Cecidophyopsis* species reported in the literature to colonise redcurrant (*C. selachodon*) and gooseberry (*C. grossulariae*) or, of the durability of gene *Ce* when expressed in blackcurrant. The situation is made more difficult because the reported morphological distinctions between the three putative *Cecidophyopsis* species on *Ribes* are sometimes inconsistent and often technically difficult to determine without expertise. Not surprisingly therefore, some researchers consider these three putative species to be host-specific biotypes of one species. Clarification of this uncertainty and the development of a more reliable and widely usable means of distinguishing the eriophyid mites on *Ribes* is essential to assess the long-term value of existing mite-resistant germplasm. We also need to gain a greater understanding of the biology and ecology of these mites, especially their *Ribes* host range, in order to assess their relative importance as pests on *Ribes* crops and as vectors of the agent of reversion disease. The need for this information is particularly urgent now that resistance to the blackcurrant gall mite is a major component of blackcurrant breeding programmes world-wide. Attempts to address this lack of knowledge are hindered by the difficulties in studying eriophyid mites of *Ribes* caused by their very small size, apparent host specificity and location within buds or bud scales.

To resolve these problems in mite taxonomy, we have taken two different approaches. Firstly, we have re-examined the detailed morphology of mites from the three *Ribes* species, complementing light microscopy with the higher resolution of surface structure provided by scanning electron microscopy. These studies have shown that mite specimens from the three *Ribes* species were distinguishable by consistent differences in two areas, the dorsal shield and the region anterior to the genital flap. The main distinguishing features in these areas are illustrated in Figure 4. Whilst this approach has clarified morphological characters to distinguish between these mite species, using such characters for identification is very slow, laborious and too expensive for field studies.

Our second approach therefore was to compare the genetic relatedness of mite specimens from different *Ribes* species using molecular techniques to amplify

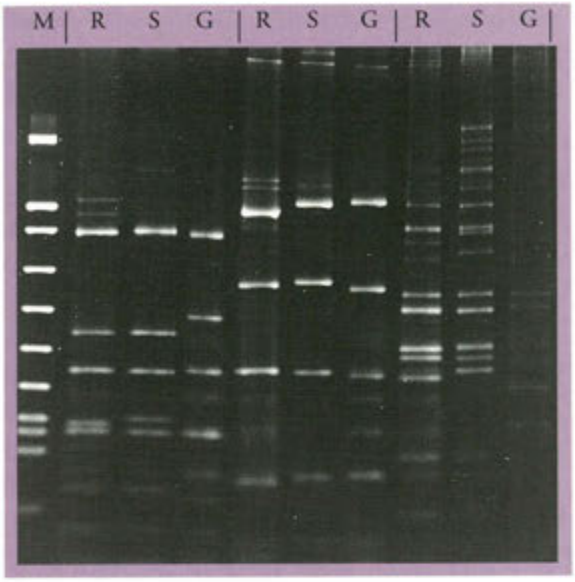

Figure 5 Polyacrylamide gel electrophoresis of PCR-amplified mite DNA following digestion with 3 different restriction enzymes (l to r, *Dde* I, *Mbo* I, *Taq* I). Mite samples were from blackcurrant (R), redcurrant (S) and gooseberry (G). M = DNA size markers.

and analyse mite DNA. Because of the very limited quantities of material available from these tiny arthropods, ribosomal DNA (rDNA), which occurs in more than 1,000 copies per cell, was studied making isolation, PCR amplification and analysis of mite DNA relatively simple. Different regions of rDNA change at different rates depending on the function of the encoded product. The two internal transcribed spacers studied (ITS1 and ITS2) do not encode functional RNA and are therefore not restricted in their accumulation of mutations to the same extent as rRNA coding regions. Consequently, the more rapid accumulation of mutations in these regions causes greater divergence of sequences. Their usefulness as a taxonomic tool is further enhanced by their location between the two highly conserved regions encoding the 18S and 28S rRNAs. This feature, together with the short length of these non-coding regions, facilitates their ready amplification by PCR-related techniques. The PCR products from ITS1/ITS2 were analysed by restriction endonuclease digestion to provide a powerful method for taxonomic studies.

All *Cecidophyopsis* sources gave a primary product of 1.35 kbp by PCR amplification of the ITS region. No variation in the length of this product was detected in agarose gels among any of the mite samples analysed. Using the same technique, DNA from blackcurrant tissue produced a band of 1 kbp which was not present in the PCR products isolated from mites. When the PCR products from mite DNA were cleaved by a panel of restriction enzymes, mite samples from each of the three *Ribes* species gave a distinct fingerprint for each enzyme (Fig. 5). When the same analysis was done on *C. ribis* populations from Sweden they were found to share all the major bands present in Scottish samples suggesting that there is extensive sequence divergence between the three putative mite species (Fig. 5) but minimal divergence within *C. ribis*. These results therefore support our detailed morphometric analysis of mites from the three cultivated *Ribes* species and indicate that they are distinct species. Based on the historical nomenclature they are, *C. ribis* on blackcurrant, *C. selachodon* on redcurrant and *C. grossulariae* on gooseberry.

In addition to complementing traditional taxonomic methods, the molecular studies have provided an experimental basis for rapidly identifying *Cecidophyopsis* species on *Ribes*. This will enable a more critical study of the *Ribes* host range of these different mite species and their possible role(s) as vectors of the agent of reversion disease. Furthermore, the development of molecular techniques to determine and quantify genetic differences, provides the opportunity to study the extent of gene flow between different gall mite populations. Such studies will provide valuable information to assess the likelihood of mite biotype development in response to the possible widespread cultivation of *C. ribis*-resistant blackcurrant cultivars that will be available in the next decade.

Reference

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Scottish Agricultural Statistics Service

R.A. Kempton

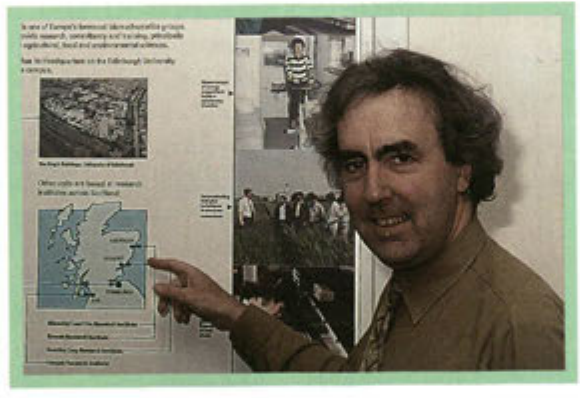
The Scottish Agricultural Statistics Service (SASS) provides statistical and mathematical support to the five SARIs, SAC, Scottish Agricultural Science Agency and, on a contract basis, to other organisations in the agricultural, environmental and food sectors. SASS is administered by SCRI and has staff based at all SARIs and SAC. The Headquarters of SASS is at the King's Buildings science campus of the University of Edinburgh.

One of the strengths of the Scottish System is the opportunity it provides for inter-disciplinary research which crosses organisational boundaries. Over the last two years, several coordinated research programmes have been formally established by SOAFD. In two of these, 'Food poisoning organisms in the food chain' and 'Control of Helminth diseases in livestock', SASS is contributing through the development of mathematical and statistical models which are improving understanding of the biological processes.

Towards the end of the year we took on a more fundamental project to explore the feasibility of applying a systems approach to the Scottish rural economy. In a systems approach, the emphasis is placed on linkages and integration. The linkages between biological processes can be at least as important for understanding and prediction as their detailed individual behaviour, but are often ignored in the commoner reductionist approach to research. Integration is also crucial for effective communication within and across disciplines. A systems approach can thus bring benefits in the form of a greater mutual relevance of research programmes and leads to a greater degree of collaboration instead of inefficient competition. In this project, a small working group from SASS, MLURI, SAC and SCRI has considered a range of representative policy goals and how they might be achieved through a systems approach. This identified some applications where the components of a systems model were already in place, others where the systems approach was feasible in principle, but current methodologies or data were inadequate, and yet others where the approach was not feasible due to the inherent uncertainty and complexity of the systems. A number of areas have been identified where further research is

needed. These include understanding and modelling decision making by farmers, handling uncertainty and 'fuzzy' knowledge in models, and estimating parameters in complex models.

More traditional areas of SASS research activity include the development of methods for field assessment of plant genotypes. Comparison of large numbers of genotypes in a single trial requires efficient design and analysis to control spatial heterogeneity. Research work within the former AFRC Unit of Statistics in Edinburgh led to the development of a family of incomplete (alpha) designs, which combine flexibility with high efficiency. Empirical studies with these designs indicated that, for representative UK cereal trials, the optimal number of plots per block should be approximately the square root of the number of genotypes. Recent work by SASS has shown that further improvement in precision of genotype means can be achieved by arranging experimental plots in a rectangular array, as near square as practicable, and adjusting for both row and column effects. Efficient row and column designs can be constructed using ALPHA+, a computer package recently developed by SASS and statisticians in CSIRO, Australia. An efficient row and column analysis is provided by the REML facilities in the Genstat package. However, ALPHA+ is currently being extended to provide an integrated design and analysis package as part of a collaborative venture with Michigan State University for providing a data management system for the maize breeding programme at CIMMYT.



R.A. Kempton, Director of SASS.

Another interesting design problem arises in plant evaluation trials when there is the possibility of interference from neighbouring plots. This was extensively studied during the year, partly in fulfilment of a MAFF Open Contract held jointly with the National Institute of Agricultural Botany. Interference appears to be frequently associated with plant height and disease susceptibility. Thus in a yield trial with genotypes of differing height, the yield of shorter genotypes can be reduced through shading by taller neighbours; likewise, in a disease screening trial, a highly susceptible genotype may act as a secondary source of infection so that the effectiveness of a resistant genotype in a neighbouring plot is underestimated. To reduce interference, we have assumed that genotypes can be arranged in ordered groups so that interference among genotypes in the same group is negligible, for example, ordering may be by plant height. Based on this grouping, incomplete block designs have been constructed which reduce interference while maintaining the efficiency of alpha designs in controlling spatial heterogeneity. Where prior information on genotypes is not available, adjustment for interference must be made through analysis. A number of models have been developed for this purpose and applied to SCRI potato trials and UK National List trials for a range of crops. The application of these designs and models extends beyond field experiments. For example, similar designs might be used in sequential clinical trials, while the models have been used at SCRI for analysing ELISA results. The research into new designs has benefitted greatly from collaboration with the Biometry Laboratory, INRA-Versailles.

Research in image analysis and spatial processes has continued on a broad front. Work of relevance to SCRI covers microscopy, electrophoresis and photography. In digital microscopy, a method has been developed for measuring the diameters of semi-transparent fibres which are out of focus. Gas diffusion through a Boolean model of a soil aggregate has been simulated on Edinburgh Parallel Computing Centre's CM-200 machine, and related to fractal dimensions. Multimodality microscopy, where images obtained using different optics such as phase and interference contrast are combined digitally with brightfield images, has been investigated as a research tool. In electrophoresis, an algorithm has been developed for correcting the curvature in DNA sequencing-gel

autoradiographs, so that bands are horizontal and in alignment between tracks. This simplifies interpretation and makes more tracks usable in gels which have been subject to warping. Photographs of fungal hyphae and plant roots have been analysed at resolutions greater than those theoretically achievable by a digitizing scanner, by making use of new mathematical results on the sub-pixel restoration of binary images.

A complex rule-based system has been used to model production of the potato crop. Such rule-based systems enable the modeller to query the effect of any variable on any other variable in the model. The addition of a stylised natural language query interpreter has highlighted how powerful this type of model can be as a tool for advisors and researchers. The same crop production system has also been modelled by an expert system, where relationships are quantified by probabilities rather than rules. The potential of the latter approach seems greater but some of the computational problems are still in the process of being solved. An article summarising SASS involvement in artificial intelligence and expert systems projects at SCRI appears elsewhere in this Report.

Finally, SASS has continued to support work at SCRI directed to understanding plant structure at the cellular, genetic and molecular level. This is described in the two articles that follow.

Statistical methods in linkage analysis

Christine Hackett

Knowledge of the organisation of genes controlling economically important traits within the genome can make a considerable contribution to a plant improvement programme. In order to locate a particular gene on a chromosome, it is necessary to have a map of genetic markers linked to one another and spanning all the chromosomes. Until recently, it was not practical to develop such maps because few markers were available. However, recent advances in molecular biology, such as the identification of restriction

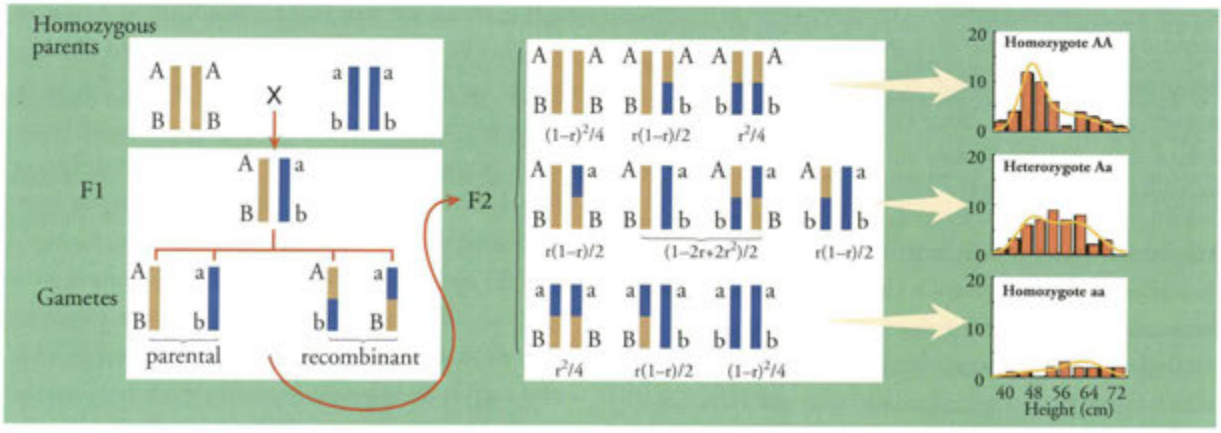


Figure 1 Segregation of two loci, A and B, linked with a recombination fraction r, and observed distributions when B is a QTL affecting height in a barley population derived from a cross between cultivars Gerbel and Heriot.

fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) markers, have remedied this shortage. Large data sets of genetic markers are available at SCRI for barley, field beans and potatoes. There are two stages to the analysis of such data, first the markers need to be assembled into a linkage map and, secondly, genes for the traits of interest need to be located on the map. SASS is involved in developing statistical methods to meet both requirements.

Detection of linkage between genetic markers Genetic mapping is easiest in plants such as barley, maize or tomato where diploid, homozygous parental lines are available. Figure 1 illustrates a cross between two such parents to develop a second generation population (F_2) for mapping. If the parents have genotypes AA and aa respectively at a locus *A*, then approximately one quarter of the F_2 plants will have genotypes AA, half will have genotype Aa and one quarter will have genotype aa. If the parents also have genotypes BB and bb at another locus *B*, then the F_2 plants may be classified according to their genotypes at the two loci. If these loci are on different chromosomes, or widely separated on the same chromosome, they will be inherited independently, with the proportion of, say, AABB plants being 1/4 x 1/4 or 1/16. If loci *A* and *B* are near to each other on the same chromosome, i.e. they are *linked*, then there will be more AABB and aabb plants, and fewer AAbb and aaBB plants, than expected. This is because alleles on the same chromosome tend to be inherited together unless there is a crossover of segments of the chromosome (a recombination event) when gametes are formed. The probability of a recombination between two loci increases with their distance apart on the chromosome and is used as a measure of distance between the loci, called the recombination fraction r. The recombination fraction takes values between 0 (complete linkage) and 0.5 (unlinked).

When a cross has been scored for a large number of marker loci, recombination fractions may be calculated between all pairs of markers and used to construct a map. Figure 2 shows a map of RAPD markers on the long arm of barley chromosome 3HL, which has been constructed using genetic linkage software.

Detection of linkage for a trait Some traits are controlled by a single gene, such as smooth or wrinkled form of the garden pea *Pisum sativum* in Mendel's original experiments. Plants may then be classified by their trait phenotype in the same way as for the genetic markers mentioned above, and the recombination fraction between the trait and each marker may be calculated directly.

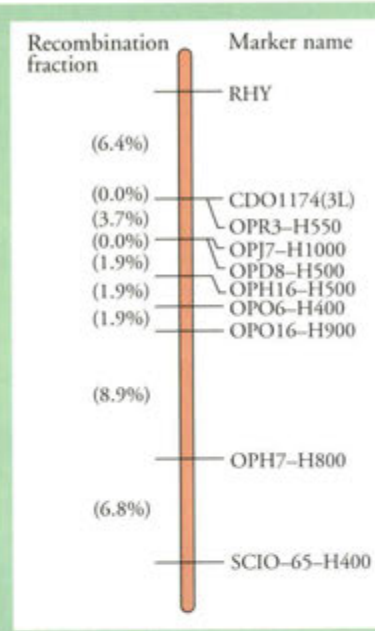


Figure 2 A map of ten markers along the long arm of barley chromosome 3HL. The recombination fraction measures the linkage between adjacent loci.

However, most traits of economic importance in agriculture are affected by the joint actions of several genetic factors (*quantitative trait loci* or QTLs) and modified by the local environment. The phenotypes of such traits, eg yield, height or milling energy, take values on a continuous scale and the individual effects of the underlying QTLs cannot be distinguished. However, the inheritance of the QTLs is the same as for any other gene. Figure 1 may be regarded as representing the inheritance of a QTL, *B*, affecting plant height, which is linked to a marker *A*. Plants with genotype BB will be, on average, shorter than plants with genotype Bb while the tallest plants will have genotype bb. Although the genotype at *B* cannot be observed directly, plant height can be measured and the plants separated into three categories corresponding to genotypes AA, Aa and aa at locus *A*. If neither locus *B* nor any other QTLs affecting height are linked to locus *A* then the mean height will be the same in all categories. However, when a QTL for height is linked to locus *A*, the mean heights will depend on the genotype AA, Aa or aa and this relationship may be detected graphically, or by a one-way analysis of variance. Figure 1 shows histograms of the distributions of height associated with the three geno-

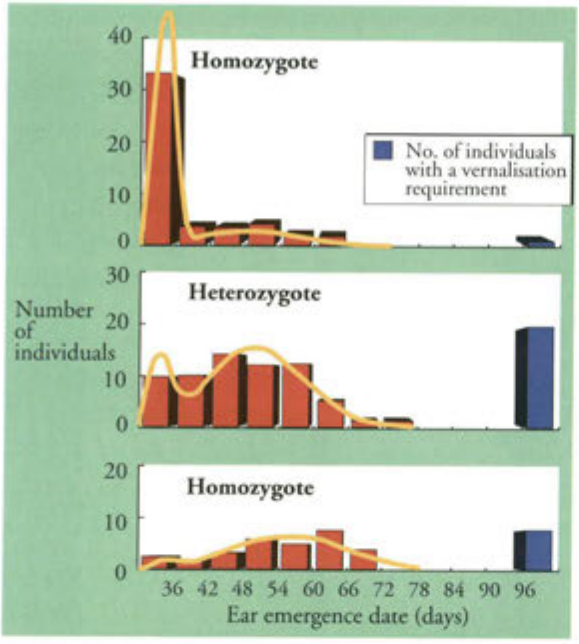


Figure 3 Distribution of ear emergence dates associated with the three genotypes at the β-amylase locus on chromosome 4 of barley. The yellow curves show the fitted distribution of ear emergence dates.

types at the β-amylase marker locus in a cross between a spring barley, cv. Heriot, and a winter barley, cv. Gerbel. Once the presence of a QTL near a marker has been detected, its recombination fraction with that marker may be estimated.

Estimation of the position of a QTL relative to a genetic marker In the cross represented in Figure 1, plants with genotype AA carry a mixture of the three genotypes BB, Bb and bb at locus *B*. Plants with genotype Aa or aa will also carry a mixture of the three genotypes BB, Bb and bb, but in different proportions. The proportions depend on a single parameter r, the recombination fraction between *A* and *B*. For example, if r = 0.25, the proportions among the AA genotypes would be 56% BB, 38% Bb and 6% bb and the proportions among the Aa genotypes would be 19% BB, 62% Bb and 19% bb. If r = 0.10, the proportions among the AA genotypes change to 81% BB, 18% Bb and 1% bb etc. The plants may be assumed to have a normal distribution of heights with different means and standard deviations depending on the genotype BB, Bb or bb. The distribution of heights among the plants with AA genotype is known as a mixture distribution. The distributions of heights among plants with Aa and aa genotypes are different mixture distributions but based on the same means and standard deviations. Such statistical distributions may be fitted by standard statistical techniques such as maximum likelihood. Mixture distributions have been fitted in Figure 1 to model linkage between a QTL for height and β-amylase in barley.

Mixture models may also be adapted for more complicated genetic hypotheses. Figure 3 shows an example for ear emergence date in barley. The distribution of ear emergence date varies with the genotype of β-amylase, indicating that a QTL is linked to this marker. However, some plants with each β-amylase genotype have a vernalization requirement, due to the action of another vernalization gene. The joint effects of the vernalization gene and the QTL may be represented by a more complicated mixture model, which predicts accurately both the number of plants with a vernaliza-

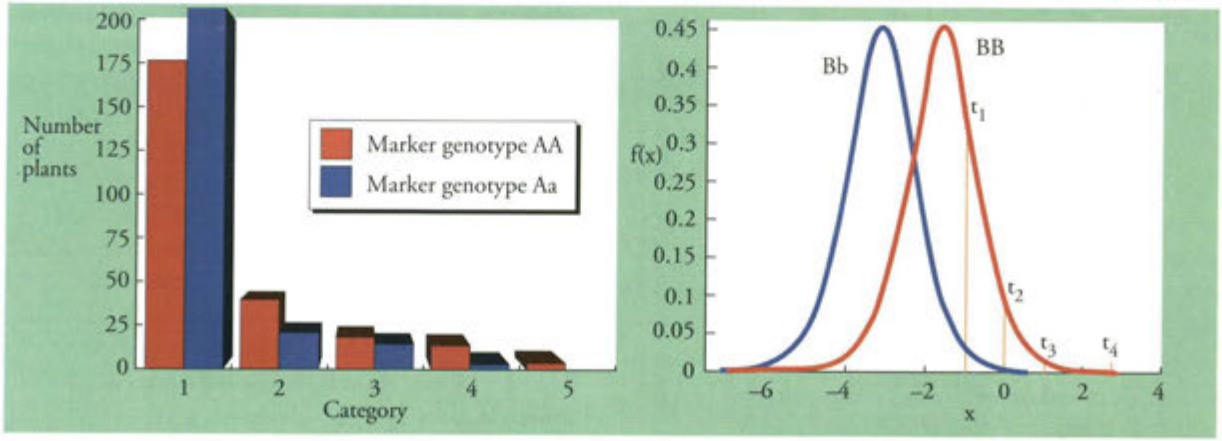


Figure 4 Distribution of ordinal scores (1 to 5 scale) associated with the two genotypes at a locus A in a simulated backcross population, and the underlying continuous distributions and thresholds associated with the QTL genotypes, BB and Bb. The thresholds t_1-t_4 define the categories, eg. the area under the BB curve to the left of t_1 is equal to the probability of a score in category 1 for a plant with QTL genotype BB.

tion requirement and the distribution of ear emergence dates. We are investigating methods for analysing several QTLs for a trait simultaneously, and for combining data from several blocks of an experiment.

Traits with non-normal distributions Most quantitative genetic analyses assume that the trait of interest is normally distributed. However, some important traits, eg disease scores or counts of flowers, do not have normal distributions and this needs to be taken into account in the analysis. Work at CPRO-DLO Wageningen has produced a very general mixture model for QTL analysis. In collaboration with the Volcani Centre, Israel, we have adapted this model for different distributions. Of particular interest are traits that may only be recorded as ordered categories such as low, medium, high, or on a 1 to 9 scale. We can now map QTLs for such traits relative to genetic markers by assuming that the underlying trait is continuous and there exist thresholds defining the categories. Figure 4 shows an example of a trait with five ordered categories, using simulated data. The distribution of scores is associated with the genotype at a linked marker, *A*. The recombination fraction, the underlying continuous distributions and the thresholds are estimated by maximum likelihood. The observed and expected numbers in each category agree well.

The future QTL mapping with populations from homozygous, diploid parents is now well established. However, for some important crops such as potato, rape, raspberries etc., homozygous parental lines do not exist and there is still much research to be done before mapping in these crops can be tackled routinely. The difficulties are apparent even when considering marker loci alone. If the parents in a cross are heterozygous, there may be up to four alleles segregating at each locus in the offspring. Some loci will segregate with 1:2:1 ratios as for an F_2 cross while others will segregate with a 1:1 ratio as for a backcross. If there are four different alleles present in the parents then there will be four genotypes in the offspring in equal proportions. In addition, it may not be known how pairs of alleles are linked, i.e. which offspring genotypes are recombinant types and which are parental. Still more complications occur when an organism is tetraploid or hexaploid, with four or six alleles per parent to be considered for each locus. These areas of research are currently being addressed by SASS in collaboration with the geneticists and molecular biologists at SCRI.

Phylogenetic trees and molecular evolution

F. Wright & R.A. Kempton

In 1983 Joe Felsenstein wrote[1] that "If we were to know the true geneology of all life, draw a diagram of it, and look at it from a sufficient distance, the details of individual matings would blur into single lines for each species and the whole would appear as a great branching tree..... with a geological timescale."

Over the last ten years, so much sequence data have been accumulated that it is now possible to construct an outline of such a 'tree of life', using genes that are common to all species. For long distance relationships like this, ribosomal RNA genes are ideal because they evolve slowly and therefore still contain enough similarity between species to allow evolutionary relationships to be reconstructed. Ribosomal RNA sequence data are available for a range of species, including animals, plants, fungi, protozoa and bacteria. Figure 1 shows a tree of life constructed from a sample of 70 species based on the ribosomal RNA large subunit sequence[2]. Three main clusters are evident: eubacteria, archaebacteria, and the eukaryotes which includes protists, fungi, plants and animals. The root of the tree, where Life is postulated to have begun, is predicted to lie between the archeaebacterial and eubacterial clusters. Evolution is then postulated to have followed a branching process through geological time until the tips of the tree are now reached. The branch lengths represent the estimated genetic distance measured in nucleotide substitutions per position. For example, the distance between man

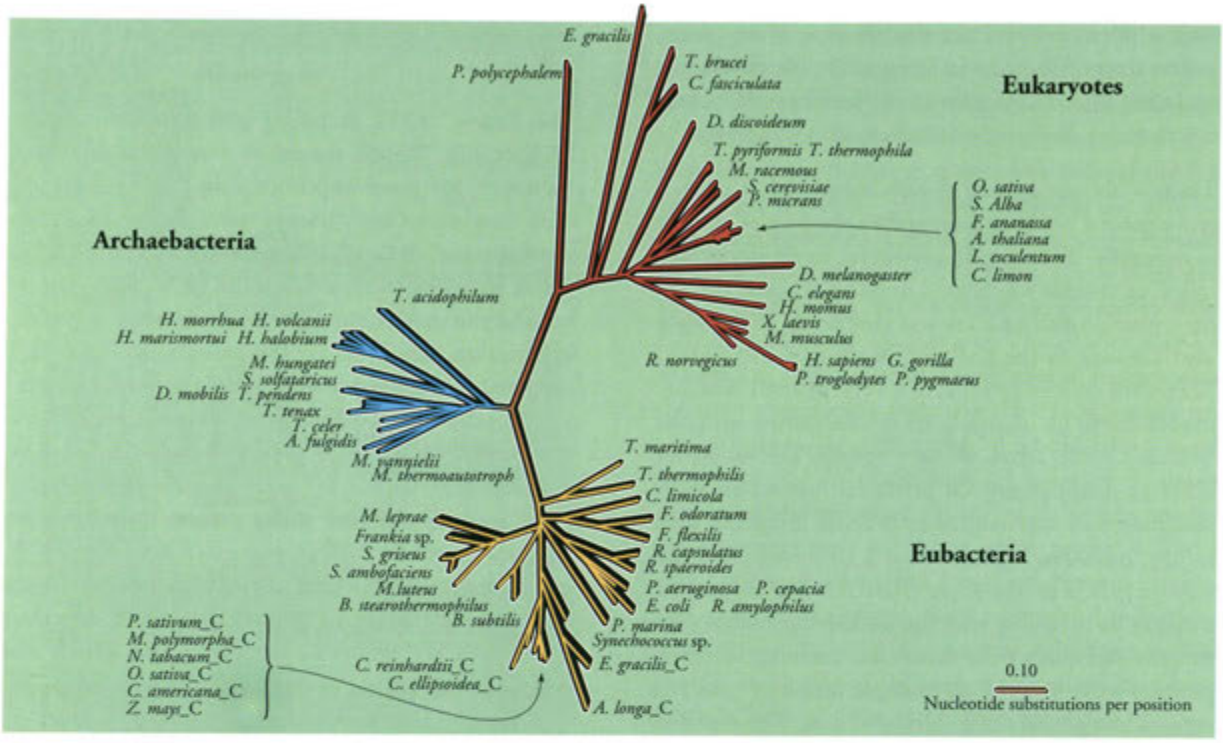


Figure 1 Phylogenetic tree of life showing the evolutionary relationships between 70 current species based on ribosomal RNA large subunit sequences extracted from the RDP database[2]. Labels ending with C denote chloroplast sequences.

(*H.sapiens*) and mouse (*M.musculus*) is 0.175, representing an approximate 17.5% difference at the sequence level.

Molecular sequence data have many advantages over morphological data which have traditionally been used as the basis for phylogenetic trees. In particular, they allow the reconstruction of phylogenetic relationships between taxonomic groups that are very distantly related and for which common morphological characters are not available (as in Fig. 1). Sequence data potentially contain much more information than morphological data and inferences about phylogenetic relationships can thus be made with more statistical power. However, a tree based on a single gene (called a gene tree) needs to be interpreted with caution as the gene may have evolved in a non-branching manner, eg through hybridisation. Even when evolution can be modelled as a standard branching process from a single common ancestor, an observed tree is only one realization of the evolutionary process and will contain statistical noise. Trees based on sequence data from several genes will, however, converge to the species tree.

The construction of phylogenetic trees is not a simple task due to the surprisingly large number of possible trees that can occur (Fig. 2). In most cases we are simply unable to look at all possible trees and assess which best fits the data. Instead, optimisation algorithms have been devised which search a subset of trees. Even then, phylogenetic tree search methods are often very computer-intensive and to produce a tree in a realistic time-scale it is necessary to make simplifying assumptions about how molecular sequences evolve, for example, nearly all methods assume that adjacent positions in a sequence evolve independently. The output from phylogenetic tree methods therefore needs careful interpretation. On

Number of species	Number of unrooted trees
2	1
3	1
4	3
5	15
6	105
7	945
8	10,395
9	135,135
10	2,027,025
20	221,600,000,000,000,000,000
30	8,687,000,000,000,000,000,000,000,000,000,000,000

Figure 2 Too many trees to check them all.

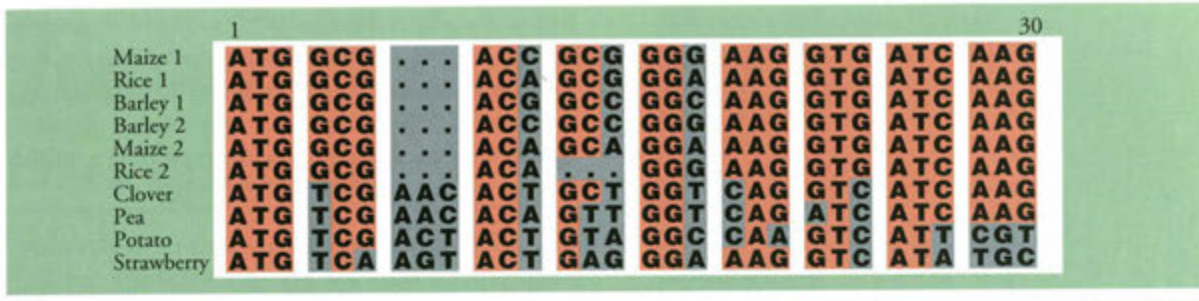


Figure 3 Constructing a multiple alignment of 10 protein-coding DNA sequences.

the more positive side, developments in methodology for tree construction[3] and the availability of enormous amounts of data make this an exciting time for researchers interested in molecular evolution.

Phylogenetic tree construction and testing. The construction of a phylogenetic tree will be illustrated for the study of evolution of a multigene family, the alcohol dehydrogenase (ADH) family in plants[4]. For this purpose, we use 10 angiosperm DNA sequences coming from four dicotyledon species (clover, pea, potato and strawberry) with one available ADH gene, and three monocotyledon species (barley, maize and rice) with two ADH genes. After duplication of the ADH gene in monocotyledons, it is assumed that the two copies evolve independently.

All current methods for phylogenetic tree construction require the sequences to be pre-aligned. This initial multiple alignment step is extremely important and a poor alignment will lead to a distorted tree. If an automatic multiple alignment method is used, the alignment should be checked for any biologically unlikely gaps (e.g. gaps disrupting the reading frame in protein coding DNA). Figure 3 shows the first 30 positions of a multiple alignment of the 10 ADH sequences (the full alignment is 1146 nucleotides long). This was produced using an automatic multiple alignment algorithm, followed by manual editing which included aligning the ATG start codons and placing two gaps at the beginning of the rice 2 sequence rather than a single longer gap inserted by the automatic method.

The aligned sequences were analysed using a Distance Based method of phylogenetic tree construction. This involves calculating a matrix of genetic distances from the nucleotide differences for all sequence pairs, followed by the construction of a tree from these distances. The distance measure used should be corrected for the effect of 'multiple hits', because, as sequences diverge over time, the effect of further nucleotide substitutions at the same position in the sequence will not be apparent. Several methods for correcting for this effect are available, based on different models of nucleotide substitution. A popular method[5] assumes a common substitution rate for all transitions (nucleotide substitutions: A to G, G to A, T to C, C to T) and another rate for transversions (the other eight substitution types).

The simplest method for constructing a tree from a distance matrix is to adapt classical clustering procedures. These start by merging the two closest species to form a cluster; the distance between this cluster and all other species (or clusters) is calculated (the methods differ in how this is done) and the two species, or species and cluster, with smallest distance are merged. This procedure is repeated until a fully linked tree is obtained. A closely related method, the Neighbor-Joining method, allows for different substitution rates in each branch (represented by different branch lengths). Clustering methods have the benefit of being computationally very fast. In contrast, optimisation methods which seek the best fitting tree to the observed distances, while intuitively appealing, may be prohibitively slow for trees involving large numbers of species (Fig. 2).

The Neighbor-Joining tree calculated from the 10 ADH sequences is shown in Figure 4. This suggests that, after separation of dicotyledons and monocotyledons, a duplication event, producing two versions of the ADH gene, occured prior to the divergence of the three monocotyledon species. Thus there are two clusters both containing information about the divergence of maize, barley and rice. (In practice, ADH evolution is more complex and several non-allelic ADH genes have been sequenced for some species.)

It is essential to test the statistical robustness of the derived tree, and this can be done using a 'Bootstrapping' technique. A typical bootstrap analysis involves generating a large number of new samples

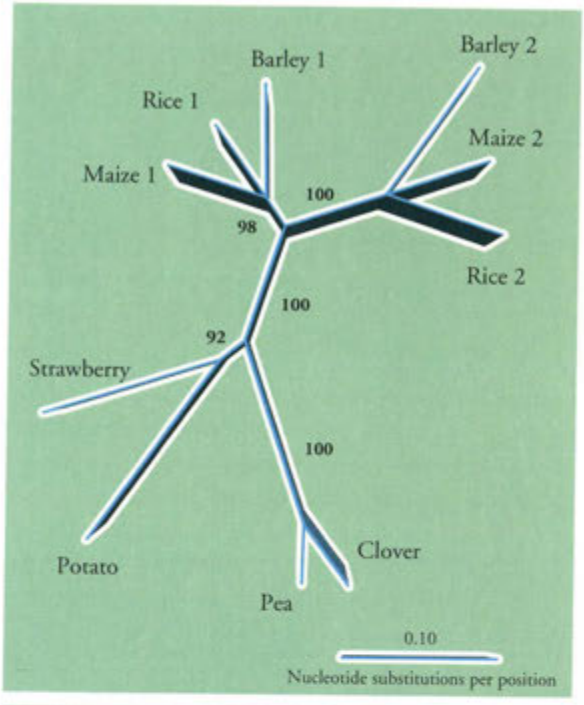


Figure 4 Evolution of the ADH multigene family.

of equal size and observing the variation in trees produced. The new samples are obtained by randomly choosing columns (with replacement) from the sequence dataset, so that some columns are left out while others are repeated. A consensus tree can then be constructed from the individual trees for these secondary samples, together with confidence limits for individual branch lengths. In practice, the method requires a large number of bootstrap samples to be taken (possibly 1000 or more) and hence may only be feasible when a fast tree construction method is used.

The Neighbor-Joining tree in Figure 4 shows the percentage of 2000 bootstrap samples that support each cluster. For example, 100% (or 2000/2000) of the samples of clover and pea formed a separate cluster among the 10 species, while the potato and strawberry cluster was formed in 1842 samples and thus has bootstrap support of 92%. There is also 100% support for the separation of dicotyledons from monocotyledons. In contrast, further separation of the barley, rice and maize cluster has negligible bootstrap support for either gene and is therefore displayed as a trifurcated branching point. With these two exceptions, the high bootstrap values indicate that the overall tree is robust to sampling variation and none of the clusters is likely to have occurred by chance.

Two other commonly used methods for constructing phylogenetic trees are Maximum Likelihood and Maximum Parsimony. Both use a search method and are therefore considerably slower than clustering methods. Maximum Parsimony simply searches for the tree that can be constructed using the smallest number of nucleotide substitutions. It works best when the rate of evolution is slow and varies little between lineages. Maximum Likelihood is based on a model of evolution and searches for the most likely tree under the model assumptions. One advantage of the method is the flexibility over choice of model and the possibility of testing different models and tree topologies. Against this, existing algorithms are very slow to run.

The construction of phylogenetic trees places major demands on computing resources. Although the rapid increase in desk-top computing power and access to parallel computers may temporarily solve this problem, the even more rapid increase in sequence data, while resolving some uncertainties over the structure of current evolutionary trees, will demand yet more computing resources. Clearly, the study of molecular evolution will continue to test the abilities of statisticians, computer scientists and molecular biologists for many years to come.

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Research services

Data Processing

R.J. Clark

The SCRINet local area network was extended to two new Portakabins and three additional SUN workstations were attached. A Novell server was installed to extend the range of Windows and DOS software available. Supported software can be mounted with sufficient licenses to cover demand, with obvious cost benefits to departmental budgets. In addition, Novell netware gives more flexibility to the distribution of printers, e.g. departments can attach their laser printers in an office in their own corridor without the need for extra wiring. Central devices were enhanced by the purchase of a new colour printer and laser printer.

Software available on SCRINet includes statistical analysis packages like Genstat and Minitab, and crop geneticists continue to use CHIP. CHIP is a package for managing genetic databases, generating experiment plans and labels, performing statistical analysis, and selecting progenies. Other UNIX software is used for modelling soil structures and graphics. The Oracle relational database management system is used for safe use of chemicals and for field experiment applications.

SCRINet is connected to the Joint Academic Network (JANET) wide area network for access to external mainframe services such as the Daresbury laboratory for DNA sequencing. Electronic mail facilitates collaboration between scientists at SCRI and co-workers throughout the world via JANET, BT Gold and CGnet.

Scientific Liaison and Information Services

D.A. Perry

The library staff provide information pertinent to the research of the Institute from a wide range of specialist books, reports, leaflets, maps, scientific periodicals, and databases. The library catalogue can be interrogated from any personal computer connected to the Institute network and contains records of the textbooks and recent pamphlets housed in the Library.

Databases in CD-ROM format are available to give references to the periodical literature from 1973 to date in the fields of agriculture, horticulture, pest control, soils and biotechnology. Advice is given on the use of remote databases such as BIDS, university library catalogues and other services available on JANET and the Internet.

Documents not available locally can be supplied through the British Library Document Supply Centre.

The Visual Aids Section provides a comprehensive photographic service using modern techniques with still and video camera equipment. The material produced is used for record, publication, display and publicity purposes. Graphics are produced on an Apple Macintosh system and are used to illustrate scientific results in publications and lectures and to create quality displays in exhibitions such as Scotgrow, Cereals '93 and PMB Open Day. Desk-top published documents ranging from simple leaflets to full colour productions such as this Annual Report are prepared for printing.

The Scientific Liaison Section makes arrangements for and hosts individuals and groups of visitors. It organises exhibitions and assists in arranging conferences such as those sponsored by the Scottish Society for Crop Research and other bodies. The section is responsible for editing the Annual Report and other publications, and for preparing Press Releases. It also maintains contact with European affairs, levy boards and works closely with the commercial concerns of the Institute.

Estate, Glasshouse and Field Experiments Department

W.I.A. Jack

The Department provides a fully equipped and professionally expert service to fulfil the requirements of its clients with regard to the preparation of land, growing medium, sowing, drilling, planting, propagation, plant maintenance, harvest and clearance of residues for the Institute's field and glasshouse research objectives. It may be responsible for an entire package from start to finish or can provide prepared land and/or controlled environment regimes for inputs to be undertaken in varying degrees by scientific clients. Specialist teams equipped with a range of modern machinery and facilities cover brassicas, bulbs, cereals, field beans, fibres, peas, potatoes, blackcurrants, cane fruit, strawberries, novel fruits and trees.

The work undertaken ranges from maintaining genetically engineered plants, virus manipulation and testing; defining data parameters for deriving mathematical models of crops; effects of nutrient, pest, disease, weed environment on crops; and traditional variety trials and maintenance of nuclear stocks.

The Institute has 194 ha of free draining, loamy soil at Mylnefield, Bullion, Gourdie, and Lonsdale. The land rises from 15 m to 122 m, faces south to south-west and is exposed to westerly winds. Windbreaks of both hardwood and conifers are planted at intervals across the prevailing wind track. Each year 60 ha of land is used for experimental crops, and trials are also carried out at the IAPGR farm at Blythbank and other off-station sites. The general crop husbandry is based on a long-term (20+ years) plan of land use and is consistent with good farming practice and sound business management. Unless otherwise specified by experimental requirements, the land is maintained at pH 6.5, high P and K status, not deficient in trace elements, no evidence of previous trial cropping, free from perennial weeds and volunteer crops, and, as far as possible free from soil-borne pests and diseases.

Land is divided into packages of 10-12 ha providing areas for arable crop trials with an 8-year break between crops of the same type and soft fruit trials with a 6-year break. Smaller designated areas of land are provided for specialist requirements. The Department is adequately equipped with a range of up-to-date farm, experimental plot and glasshouse machinery to fulfil the work programme and machinery can be modified as necessary in the farm workshop to suit the requirements of plot work. Water for field irrigation is provided from boreholes through underground ringmains with hydrants every 100 m. There are adequate crop drying, handling and storage facilities.

The Department maintains virus-free nuclear stocks of *Rubus, Ribes* and *Narcissus.* The *Rubus* and *Ribes* collection, which contains over 100 cultivars, is the basis of commercial production within the UK and a source of healthy plant material for research and commercial organisations world wide. It is continually augmented by new cultivars and seedling lines from the HRI and SCRI breeding programmes.

The 8000 m^2 of heated glasshouses provide fully automated services for all-the-year plant production which now exceeds 500,000 units annually. Glasshouse cubicles range in size from 12 m^2 to 350 m^2 providing specialist support for the varied research packages and for specific purposes including quarantine and isolation. In addition, there are 1000 m^2 of cold glasshouses, polytunnels and net structures.

Facilities for growing plants under controlled temperature, light and humidity regimes range from small 300 litre cabinets to large walk-in rooms. During the year, nine new 600 and 1700 l cabinets were purchased to replace older units.

Engineering and Maintenance Department

S. Petrie

The Engineering and Maintenance Department offers a technical design and maintenance service throughout the Institute. It has the responsibility for ensuring heating, electric, water, telephone and waste services are provided in an effective way and at minimum cost. Preservation of Institute assets is of paramount importance and careful skilled inspections are frequently carried out. Corrective maintenance work takes place to ensure the expected performance and life of equipment, vehicle, plant or building is achieved.

The Department is divided into sections that specialise in a variety of engineering disciplines such as electrical, electronic, refrigeration, heating and mechanical engineering. It provides an engineering design and maintenance service to cover scientific and ancillary equipment and building services including heating, ventilation and air conditioning. There is also a garage section providing maintenance facilities for a substantial fleet of road vehicles, tractors and agricultural machinery. The Department provides a general stores facility and a cleaning and security service. The workshops are generally well equipped to deal with the maintenance tasks assigned to them.

The wide range of equipment and technologies present in the Institute offers a constant challenge to Department staff, nevertheless a very high percentage of repair work is carried out in-house. There are however instances where because of the complexity of product design and restricted access to spares, it has become essential to negotiate a service contract with specialist companies. These contracts are monitored by the Engineering and Maintenance Department.

Major works completed during the year include refurbishment of some of the older glasshouse areas and upgrading of electrical supplies to facilitate future planned expansions in buildings.

Range of new growth cabinets at SCRI.

The controlled environment cabinet area, one of the largest in the country with over 40 cabinets, was the scene of much activity during the year. Nine replacement cabinets were purchased and the facility was completely refurbished in order to house both the new and further planned replacement cabinets in the future.

Mylnefield Research Services Ltd.

N.W. Kerby

Mylnefield Research Services (MRS) Ltd saw the close of a very successful second year of trading and has developed rapidly to facilitate the commercial exploitation of SCRI. The Company now has three full-time members of staff; Dr Nigel Kerby (Managing Director), Ms Anne Cameron who is responsible for costing research proposals and facilitating the management of research programmes and a secretary, Mrs Linda Butler. Two scientists were also appointed on fixed-term contracts (SMART - see below).

Finances The Company's turnover increased by 40% and for the first time was in excess of £1,000,000. Its financial contribution to SCRI included a management fee of £90,000, gift aid of £125,000 and a development contribution towards a new nuclear magnetic resonance (NMR) imaging facility due to be installed during 1994 together with the purchase of various items of scientific equipment. Income is derived from four sectors:- contract research, royalties, scientific services and consultancies (Figure 1). Ninety-five percent of MRS's income was from contract research. Royalty income is expected to increase significantly over the next few years due to the release of a number of new cultivars and the commercialisation of cultivars by the Consortium of Nickerson Seeds Ltd and Dalgety Agriculture Ltd. Income derived from the provision of Scientific Services and Consultancies is very small and the Company hopes to develop and market these areas.

Agreements MRS has specific responsibility for negotiating all external contracts, including those with the European Union.

Blackcurrant	Ben Connan	NSA Plants Ltd
	Ben Loyal	NSA Plants Ltd
Strawberry	Melody	NSA Plants Ltd
	Symphony	Commercial Fruit Plants Ltd.
Raspberry	Glen Lyon	NSA Plants Ltd
	Glen Magna	NSA Plants Ltd
	Glen Ample	NSA Plants Ltd
	Glen Rosa	NSA Plants Ltd
	Glen Shee	NSA Plants Ltd

Figure 2 Licence agreements that were granted during the year for marketing new SCRI fruit cultivars.

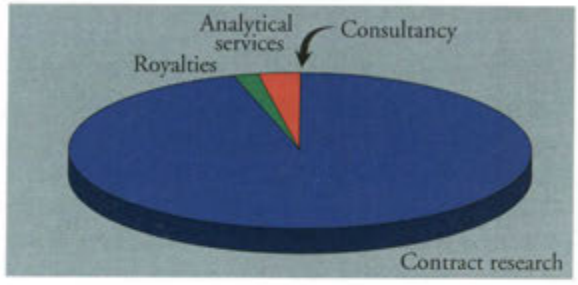

Figure 1 Summary of MRS income for 1992-93.

A new Supplemental Agreement was successfully negotiated with the Consortium (Nickerson Seeds Ltd and Dalgety Agriculture Ltd), clearly defining the period of time that the Consortium has to commercialise advanced SCRI clones that arise from specific publicly funded germplasm enhancement programmes.

Figure 3 Signing one of the soft fruit agreements at Scotgrow 1993 (L-R:- Mr Hugh Darby, Chairman of NSA Plants Ltd; Mr Ronnie McNicol, Head of Soft Fruit Genetics, SCRI; Dr Nigel Kerby, MRS)

Specific and targeted plant breeding, particularly soft fruit and potatoes, are starting to receive substantial support from the private sector and the products emanating from these programmes will increase our royalty income in the future.

MRS received a prestigious Stage 1 Small Firms Merit Award for Research and Technology (SMART) from the Scottish Office Industry Department for the development of soft fruit confectionary products.

MRS gratefully acknowledges the support from SCRI staff and the provision of facilities which were essential for the growth and development of the Company.

CAROS International Limited

D.J. Thomson

CAROS International Ltd is now into its second operational year. Formed as a company limited by guarantee, CAROS' remit is to generate additional income for its members: the Scottish Agricultural and Biological Research Instititutes and the Scottish Agricultural College, by identifying and securing contracts which require the complementary expertise of members of the Consortium.

Inevitably, this means CAROS is looking towards the major international funding agencies such as the European Union, World Bank, World Health Organisation and Asian Development Bank. It takes a pro-active role marketing the wide range of expertise, skills and resources available within the Consortium.

It is a fiercely competitive business. CAROS seeks to establish its credibility in a market place where there are many long-established agricultural consultancy companies with impressive track records of contracts secured and successfully executed. Funding organisations targeted by CAROS may invite only a few consultancies to tender for specific projects and a good track record is an advantage when lobbying to be on their tender lists.

Despite its youth, CAROS has been short-listed four times by the PHARE and TACIS programmes of the European Union since November 1993. These programmes, funded by Directorate General I of the Commission, provide technical assistance to Central and Eastern Europe (PHARE) and the former Soviet Union (TACIS). The focus is on restructuring; helping individual states in the transition from a command economy towards a market economy. Most projects need short-term input from scientists with the highly specialised knowledge only found within research based organisations.

Preparing and tendering a project proposal is time-consuming and costly. The Terms of Reference supplied by the Commission give a broad outline of what is required but a site visit is essential to meet the local agency charged with implementation of the project and identify the true priorities. It is important that this ground work is carried out by an expert who is able to make recommendations on the best course of action and assist with writing the proposal. It is not always possible to find someone from within the consortium who is able to spend a designated week at short notice in one of the remoter parts of the globe, and thus external experts may be used occasionally.

To be selected, it is advisable to tender in association with consultancies from other Member States. Commission contracts must be seen to be spread 'fairly' and the technical assistance offered should be 'balanced'. Thus far, CAROS has entered into association with consultancies from France (1) and Italy (3) and is actively developing relationships with established, reputable companies in other countries.

The biggest challenge CAROS has had to face with projects has been accessing experienced staff available for long-term posts. Each project the company has been shortlisted for so far, in Azerbaijan, Albania, Kyrghyzstan and Samara, has had a requirement for a Team Leader resident for the duration of the project; typically of up to two years. It is frequently difficult for Research Institutions to release key staff members for extended periods without compromising or causing complications with the ROAME commissioning arrangements. CAROS has had to look towards collaboration with various other consultancies.

CAROS is continuing to be shortlisted regularly and approached by other European consortia to join in collaborative bids. The outcome of most bids will not be known until late 1995 at the earliest. The resources are there; within the Consortium is a wealth of expertise backed by a commitment within CAROS to succeed. Dr Thomson now has the support of an assistant, Joan Duffin, and an administrator, Anne Pack, allowing CAROS to direct its efforts beyond Brussels, targeting selected countries and the development banks. CAROS is also focusing attention on the UK Know How Fund with particular reference to Poland and the Baltic States. Research and support staff within all the member institutes have been extremely helpful and their willingness to assist in the identification of suitable CAROS project areas has been invaluable. This has enabled CAROS to target the programmes, countries and, most importantly, funding agencies where the Consortium is most likely to be successful.

Scottish Society for Crop Research

D.L. Hood

The Scottish Society for Crop Research is a registered Friendly Society formed in 1981 by the amalgamation of the Scottish Society for Research in Plant Breeding and the Scottish Horticultural Research Association. It provides a link between SCRI and farmers, processors and other interested bodies by organising meetings for the exchange of information between members and staff of the Institute. It sponsors occasional publications and provides financial assistance to staff for travel and other activities. It is open to membership by any interested person or corporate body on application to the Secretary and it is controlled by a Chairman and Committee of Management. Several crop-orientated sub-committees maintain contact with members on specialised topics relevant to their interests. Membership of the Society was 302 on 31 December 1993.

The Society Lecture was given by Mrs Elizabeth Attridge on 30 March. Mrs Attridge holds the position of Under Secretary at MAFF, with responsibility for the four divisions: Horticulture and Potatoes, Plant Health, Plant Varieties and Seeds, and Agricultural Resources. Her chosen topic was, "Research Needs in a Changing Environment".

The AGM of the Society was held on 6 May when Dr James Duncan, Head of the Mycology and Bacteriology Department at SCRI addressed the members on the complex topic of, "New Plant Passports in the European Community".

The Committee of Management met on two occasions (6 May and 4 November).

Travel grants were awarded to:-

Miss D Kennedy, Mycology and Bacteriology Department, to Skierniewice, Poland.

Dr H V Davies, Cellular and Environmental Physiology Department, to Japan.

Dr G McDougall, Director's Group (Fibres), to Elsinore, Denmark.

Dr Glyn Bengough, Cellular and Environmental Physiology Department, to Stara Lesna, Slovakia.

A scholarship was awarded to Miss H Erard, Virology Department, to help her complete her work on tobacco rattle virus (TRV).

A Soft Fruit Walk was held on Thursday 22 July when topics covered included the latest releases from the raspberry breeding programme, new prototype harvesting machinery, raspberry moth, raspberry root rot, use of NMR imaging, and novel genetic transformation techniques.

A Potato Walk was held on Thursday 19 August where the latest varieties and advance selections were shown. Control of blackleg and host plant resistance to pest damage were illustrated together with nitrogen optimisation leading to a reduction in inputs.

The Society is sponsoring Cereal Trials over a three year period in a programme aimed at breeding spring and winter barley varieties specifically suited to Scotland and Northern Britain in conjunction with Nickerson Seeds Ltd. The first year results have been very encouraging.

A Forage Brassica Open Day was held on Thursday 30 September in conjunction with Sharpes International Seeds Ltd when kale, swede and turnip trials were on view to invited members of the Scottish seed trade.

Officers of the Society

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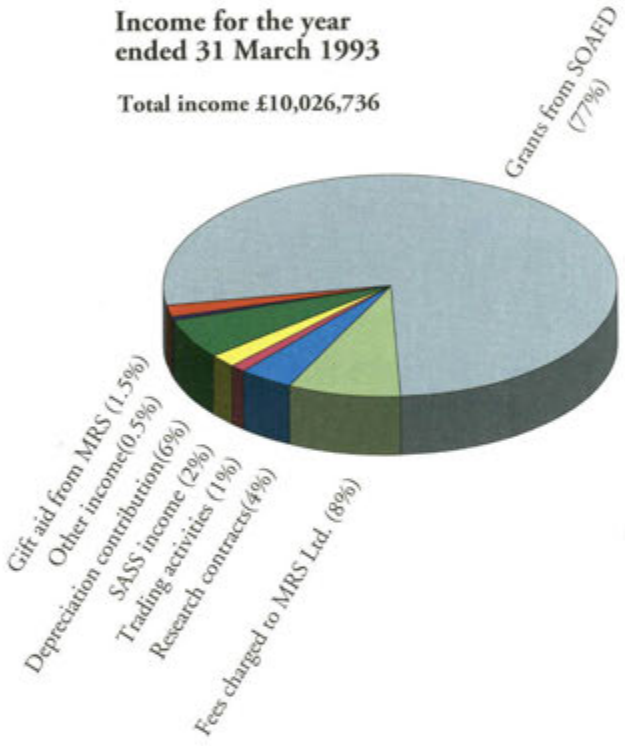
Summary of the Accounts

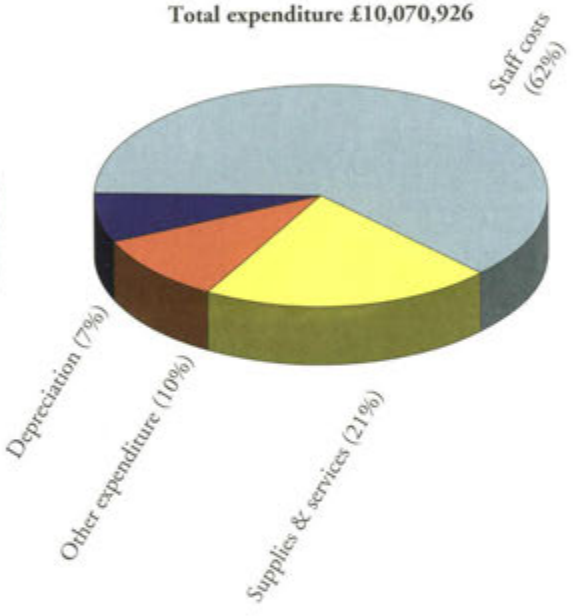
Income for the year ended 31 March 1993

Total income £10,026,736



Expenditure for the year ended 31 March 1993

Total expenditure £10,070,926



Balance sheet at 31 March 1993

Total value £11,456,732

Assets

Fixed assets	94 %
Stocks	1 %
Debtors	5 %

Liabilities

Capital reserve	90 %
Income & expenditure account	3 %
Current liabilities	7 %

Staff list

as at 31 December 1993

Director	Professor J.R. Hillman, B.Sc., Ph.D., F.L.S., C.Biol., F.I.Biol., F.R.S.E.[1,2,3]	UG4
Deputy Director	Professor N.L. Innes, B.Sc., Ph.D., D.Sc., C.Biol., F.I.Biol., F.R.S.E., F.I.Hort.[2]	UG5
Secretary	R.J. Killick, B.Sc., M.B.A., Ph.D., C.Biol., M.I.Biol.	UG7
Assistant to Director	T.J.W. Alphey, B.Sc., Ph.D., C.Biol., M.I.Biol.	UG7

Crop Genetics Department (CG)

Head : G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol.[4,6]	UG6
J.E. Bradshaw, M.A., M.Sc., Ph.D.	UG7
M.F.B. Dale, B.Sc., Ph.D.	UG7
R. Ellis, B.Sc., Ph.D.[6]	UG7
W.H. Macfarlane Smith, B.Sc., Ph.D., C.Biol., M.I.Biol.	UG7
W.T.B. Thomas, B.Sc., Ph.D.	UG7
R.L. Wastie, M.A., Ph.D., F.I.S.P.	UG7
I. Chapman, B.Sc.	SSO
M.J. De,Maine, B.Sc., M.Phil.	SSO
S. Millam, B.Sc., Ph.D.	SSO (Prom. Apr)
G. Ramsay, B.Sc., Ph.D.	SSO
J.S. Swanston, B.Sc., C.Biol., M.I.Biol.	SSO
Ruth M. Solomon-Blackburn, B.A., M.Sc.	HSO
S.A.. Clulow, B.Sc., Ph.D.	HSO
Helen E Stewart, C.Biol., M.I.Biol.	HSO
M.J. Wilkinson, B.Sc., Ph.D.	HSO
A. Young	HSO
Jill Middlefell-Williams, H.N.C.	SO
G.E.L. Swan	SO
R.N. Wilson, N.C.H.	SO
G.R. Young	SO
Eva Bennett	ASO
A. Booth, O.N.C.	ASO
P. Davie, O.N.C.	ASO
Ann Donnelly, H.N.C.	ASO
Norma Dow	ASO
Michelle L.M.H. Fleming, H.N.D., B.Sc.	ASO
Frances Gourlay, H.N.C.	ASO
R. Keith	ASO
Karen I. McIlravey, O.N.C., H.N.C.	ASO
Jane McNicoll, H.N.C.	ASO
A. Wilson	ASO
M.P.L. Campbell	P&GS (E)
Alice Bertie	EWIII/II
J.D. Fuller	EWIII/II
Patricia E. Lawrence	EWIII/II
A. Margaret McInroy	EWIII/II
R. Milligan	EWIII/II (Tr. from EGF Jan)
Moira Myles	EWIII/II
Gail Simpson	EWIII/II (Tr. from M&B Apr)
Joyce I. Young	EWIII/II

Soft Fruit Genetics Department (SFG)

Head : R.J. McNicol, B.Sc. [6]	UG7
R.M. Brennan, B.Sc., Ph.D.	SSO
Julie Graham, B.Sc., Ph.D.	HSO
Sandra L. Gordon, H.N.C.	ASO
Kay Greig, Dip. H.E.	ASO
Amanda J. Thomson, H.N.D.	ASO

Cell & Molecular Genetics Department (CMG)

Head : W. Powell, B.Sc., M.Sc., Ph.D.[4,6]	UG6 (IMP)
J.W.S. Brown, B.Sc., Ph.D.[6]	UG7
B.P. Forster, B.Sc., Ph.D.[6]	UG7
R. Waugh, B.Sc., Ph.D.[6]	UG7 (Prom. Apr)
A. Kumar, B.Sc., Ph.D.	SSO
G.C. Machray, B.Sc., Ph.D.	SSO
C.G. Simpson, B.Sc.	HSO
E. Baird, H.N.C., B.Sc.	SO
Gillian Clark, H.N.C	SO
Jackie Lyon	SO
Nicky Bonar, H.N.C.	ASO
Diane Davidson	ASO
M. Macaulay, H.N.C.	ASO

Cellular & Environmental Physiology Department (CEP)

Head : H.V. Davies, B.Sc., Ph.D.[5]	UG6
K.J. Oparka, B.Sc., Ph.D.[6]	UG6 (IMP) (Prom. Apr)
H.M. Lawson, B.Sc., M.Agr.Sc., Dip. Agric., F.I.Hort.	UG7
D.J. Linehan, B.Sc., Ph.D.	UG7
D.K.L. MacKerron, B.Sc., Ph.D.	UG7
B. Marshall, B.Sc., A.R.C.S., Ph.D.[7]	UG7
D. Robinson, B.Sc., Ph.D.[6]	UG7 (Prom. Apr)
G.R. Squire, B.A., Ph.D.	UG7 (Appt. Oct)
A.G. Bengough, B.Sc., Ph.D.	SSO (Prom. Apr)
J.W. Crawford, B.Sc., Ph.D.[7]	SSO
B.S. Griffiths, B.Sc., Ph.D.	SSO
R.A. Jefferies, B.Sc., Ph.D.	SSO
K. Ritz, B.Sc., Ph.D.	SSO
M. Taylor, B.Sc., Ph.D.	SSO (Prom. Apr)
R.E. Wheatley, B.Sc.	SSO
I. Young, B.Sc., Ph.D.	SSO
G. Goleniewski, B.Sc., Ph.D.	HSO
D.C. Gordon, H.N.C.	HSO
Heather A. Ross, H.N.C., C.Biol., M.I. Biol.	HSO
R. Viola, B.Sc., Ph.D.	HSO
J.S. Wiseman, S.D.H.	HSO
Kathryn M. Wright, M.A., Ph.D.	HSO
Sandra Caul, H.N.C.	SO
Sandra E. Millar, O.N.C., H.N.C.	SO
D.A.M. Prior, H.N.C.	SO
Susan Verrall, H.N.C.	SO
Gladys Wright, H.N.C.	SO
D. Crabb	ASO
G. Dunlop, O.N.C.	ASO
Margaret Garland	ASO
Lesley George	ASO
Diane McRae	ASO

[1] Visiting Professor in the University of Strathclyde
[2] Visiting Professor in the University of Dundee
[3] Visiting Professor in the University of Edinburgh
[4] Honorary Senior Lecturer in the University of St. Andrews
[5] Honorary Senior Lecturer in the University of Dundee
[6] Honorary Lecturer in the University of Dundee
[7] Honorary Research Fellow in the University of Dundee
[8] Honorary Lecturer in the University of Aberdeen
[9] Honorary Fellow in the University of Edinburgh

Chemistry Department (Chem)

Head : W.W. Christie, B.Sc., Ph.D., D.Sc., C.Chem., F.R.S.C.	UG7 (Appt. Jan)
D.W. Griffiths, M.A., Ph.D, C. Chem., M.R.S.C.,	SSO
G.W. Robertson, B.Sc., C.Chem., M.R.S.C.	SSO
H. Bain, H.N.C., L.R.S.C.	HSO
W. Matheson, B.Sc.	HSO
C. Scrimgeour, B.Sc., Ph.D.[6]	HSO (Tr. from CEP Jan)
Winifred M. Stein, H.N.C.	HSO
K. Taylor, H.N.C.	SO
Fiona Falconer, H.N.C.	ASO
Jean Wilkie	EWIII/II
Quality Assurance Officer : T. Shepherd, B.Sc., Ph.D.	HSO

Director's Group (DG)

Fibres	
I.M. Morrison, B.Sc., Ph.D.	UG7
J.M.S. Forrest, B.Sc., Ph.D.	UG7
G. J. McDougall, B.Sc., Ph.D.	SSO
D. Stewart, B.Sc.	HSO
Spectroscopy	
B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C.[6]	UG7
Anne Morrice, S.N.C., H.N.C.	ASO

Mycology and Bacteriology Department (M & B)

Head : J.M. Duncan, B.Sc., Ph.D.	UG6
J.G. Harrison, B.Sc., Ph.D.	UG7
G.D. Lyon, B.Sc., M.Sc., Ph.D., D.I.C.[6]	UG7
M.C.M. Pérombelon, B.Sc., M.Sc., Ph.D.[6]	UG7
B. Williamson, B.Sc., M.Sc., Ph.D.[6]	UG7
A.C. Newton, B.Sc., Ph.D.	SSO
Lizbeth J. Hyman, B.A.	HSO
Diana M. Kennedy, B.Sc.	HSO
R. Lowe	HSO
Shiela Unkles, BSc., Ph.D.	HSO (Appt. Mar)
G. McMillan	SO
Jacqueline Heilbronn, H.N.C.	SO
D.J. Johnston, B.Sc.	SO
Naomi A. Williams, H.N.C.	SO
D.C. Guy, H.N.D.	ASO (Regr. Nov)
Evelyn Warden	EWIII/II

Virology Department (Vir)

Head : T.M.A. Wilson, B.Sc., Ph.D.[2]	UG6
A.T. Jones, B.Sc., Ph.D.	UG6 (IMP)
H. Barker, B.Sc., Ph.D.	UG7
M.A. Mayo, B.Sc., Ph.D., C.Biol., M.I.Biol.	UG7
I.M. Roberts, H.N.C., Dip.R.M.S.	UG7
D.J. Robinson, M.A., Ph.D.[6]	UG7
Lesley Torrance, B.Sc., Ph.D.	UG7
G.H. Duncan, H.N.C.	SSO
B. Reavy, B.Sc., D.Phil.	SSO
S.A. MacFarlane, B.Sc., D.Phil.	HSO
Maud M. Swanson, B.Sc., Ph.D.	HSO
G.H. Cowan, H.N.D.	SO
Sheila M.S. Dawson, H.C.	SO (P/T)
Anne C. Jolly, H.N.C.	SO
Wendy J. McGavin, B.Sc.	SO
Kara D. Webster, H.N.C.	SO
Gillian L. Fraser	ASO
Ann Grant	ASO (P/T)
Wendy Ridley	ASO

Zoology Department (Zoo)

Head : D.L. Trudgill, B.Sc., Ph.D., C.Biol., F.I.Biol.[5,6]	UG6
B. Boag, B.Sc., Ph.D.	UG7
D.J.F. Brown, B.A., Ph.D., C.Biol., M.I. Biol.	UG7
M.S. Phillips, B.Sc.	UG7
W.M. Robertson, N.H.C., F.L.S.	UG7
J.A.T. Woodford, M.A., Ph.D.[6]	UG7
A.N.E. Birch, B.Sc., Ph.D., C.Biol., M.I.Biol.	SSO
S.C. Gordon, H.N.C.	SSO
Vivian Blok, B.Sc., M.Sc., Ph.D.	HSO (Tr. from CMG Oct)
B. Harrower, H.N.D., B.Sc.	SO (Tr. from CMG Oct)
Gaynor Malloch, D.C.R., B.Sc.	SO
R. Neilson, H.N.C., M.Sc.	SO
Ailsa Smith, B.Sc.	SO
Anne M. Holt	ASO
Sheena Lamond	ASO

Data Processing Unit (DP)

Head : R.J. Clark, B.A., M.B.C.S.	SSO
R. Kidger, B.Sc.	HSO
P. Smith, B.Sc.	HSO
I. Black, H.N.C.	SO
S. Clark, H.N.C.	SO

Scientific Liaison & Information Services Department (SLIS)

Head : D.A. Perry, B.Sc., Ph.D.	UG7
T. G. Geoghegan, A.B.I.P.P., A.M.P.A.	Senior Photographic Officer
S.F. Malecki, A.B.I.P.P.	Photographic Officer
G. Menzies	Photographic Officer
I.R. Pitkethly, H.N.D.	Higher Graphics Officer
T.D. Heilbronn, B.Sc., M.Sc.	HSO
Sarah E. Stephens, B.Sc., M.A., A.L.A.	Librarian (Appt. Oct)
Ursula M. McKean, M.A., Dip. Lib.	Assistant Librarian
Kristy L. Grant, B.A.	AO (Tr. from Admin Mar)
Barbara V. Gunn	AA (Tr. from Admin Oct)

Administration Department (Admin)

Secretary : R.J. Killick, B.Sc., M.B.A., Ph.D., C.Biol., M.I.Biol.	UG7
Accountant : S.L. Howie, C.A.	SEO
Assistant Secretary : D.L. Hood, B.Admin., Dip. Ed., L.T.L, A.I.I.M.	HEO
Personnel Officer : I. Paxton, H.N.C., M.I.P.M.	EO
Freida F. Soutar	HEO
Catherine Skelly	EO
Margaret Barnes	AO
Dianne L. Beharrie, Dip. Ed.	AO (P/T)
Maureen E. Campbell	AO
Rhona G. Davidson	AO
Pam Duncan	AO
Wendy A. Patterson, H.N.D.	AO
Sarah-Jane Simms, H.N.D.	AO
Loraine Galloway	SPS
Joyce Davidson	Typist
Jean Findlay	Typist (P/T)
Sheena Forsyth	Typist (P/T)
Elizabeth J. Fyffe	Typist
Maureen Murray	Typist
Myra Purves	Typist
Elizabeth L. Stewart	Typist

Engineering & Maintenance Department (EM)

Institute Engineer : S. Petrie, B.Sc.	SP&TO
D. Gray H.N.C.	HP&TO
A. Low	P&TO
K. Low	P&TO
I.C. McNaughton, H.N.C.	TG1 (Appt. Jan)
G.C. Roberts	TG1
I.M. Scrimgeour	TG1
R. White	TG1
J. Anderson	Craftsman
D. Byrne	Craftsman
K. Henry	Craftsman
F. Howie	Craftsman
E. Lawrence	Craftsman
R.D. McLean	Craftsman
C.G. Milne	Craftsman (Appt. Jan)
R. Pugh	Craftsman
T. Purves	Caretaker
J. Rowe	Caretaker
J. Oldershaw	Boiler/Handyperson
C. Conejo	Handyperson
N. McInroy	EW1
Hazel Duncan	AO (P/T) (Appt. Aug)
J. Flight	Storeman
D.L.K.. Robertson	Storeman

Estate, Glasshouse & Field Experiments Department (EGF)

Head : W.I.A. Jack	SSO
G. Wood, B.Sc., Ph.D., F.E.T.C.	HSO
P.A. Gill, H.N.D.	HSO
J.R.K. Bennett	SO
B.D. Robertson, N.E.B.S.M., H.N.C., Dip. Mgt., M.B.A.	SO
D.S. Petrie	SO
W.D.J. Jack, B.Sc.	P&GS(E
A.W. Mills	P&GS(E)
R. Ogg	P&GS(E)
D.G. Pugh	P&GS(E)
C.R. Dalrymple	EWI
E.A.M. Gardiner	EWI
L.A. McNicoll	EWI
J. Mason	EWI
D.A. Thomson	EW1
J.K. Wilde	EWI
A.J. Adams	EWIII/II
J.T. Bennett	EWIII/II
E.J. Christie	EWIII/II
G. Dow	EWIII/II
B. Fleming	EWIII/II
I. Fleming	EWIII/II
A.C. Fuller	EWIII/II
C. McCreadie	EWIII/II
T.A. Mason	EWIII/II
R. Murray	EWIII/II
Gillian Pugh	EWIII/II
M.J. Soutar	EWIII/II
Angela M. Thain	EWIII/II
C. Walker	EWIII/II
Lorna Doig	AO (P/T)

Scottish Agricultural Statistics Service (SASS)

King's Buildings, University of Edinburgh	
Director : R.A. Kempton, M.A., B.Phil. [9]	UG6
C.A. Glasbey, M.A., Dip. Math. Stats., Ph.D.[9]	UG7
E.A. Hunter, B.Sc., M.Phil.[9]	UG7
Janet M. Dickson, B.Sc.	SSO
G.J. Gibson, B.Sc., Ph.D.	SSO
G.W. Horgan, B.A., M.Sc.	SSO
M. Talbot, F.I.S., M.Phil. [9]	SSO
A.D. Mann, B.Sc.	HSO
I.M. Nevison, M.A.	HSO
G.D. Ruxton, B.Sc., Ph.D.	HSO
F.G. Wright, B.Sc., M.Sc., Ph.D.	HSO
Muriel A.M. Kirkwood, D.A.	ASO
Secretary :Elizabeth M. Heyburn, M.A.	EO
Diane Glancy	AA (P/T)
Karyn Linton	PS (P/T.)
Amy G. Stewart	Typist (P/T)
Ayr Unit	
A. Sword, B.Sc., M.Sc.	HSO
Aberdeen Unit	
Head : M.F. Franklin, B.Sc., M.Sc., Ph.D. [8]	UG7
S.T. Buckland, B.Sc., M.Sc., Ph.D.[8]	UG7
D.A. Elston, B.Sc., M.Sc.	SSO
D.J. Hirst, B.Sc., Ph.D.	SSO
Karen L. Cattanach, M.A., M.Sc.	HSO
Elizabeth I. Duff, B.Sc.	SO
Karen A. Robertson, B.Sc.	SO
Dundee Unit	
Head : J.W. McNicol, B.Sc., M.Sc.	UG7
Christine Hackett, B.A., Dip. Math. Stats., Ph.D.	HSO
T. Connolly, B.Sc., Ph.D.	HSO (Appt. Oct)

Short Term Contracts

SOAFD Flexible Funding

Cell and Molecular Genetics
Jennifer Watters, H.N.D. — ASO

Cellular and Environmental Physiology
Yiqun Gu, B.Sc., Ph.D. — HSO
J. Liu, B.Sc., M.Sc., Ph.D. — HSO (Appt. Apr)
A. Anderson, H.N.D., B.Sc., M.Sc. — SO
N. Ebblewhite, B.Sc. — SO (Tr. from CMG Oct)
Alexandra Holmes, H.N.D., P.G.Dip. Biotech. — EWIII/II

Crop Genetics / Soft Fruit Genetics
R.D. Butcher, B.Sc., Ph.D. — HSO
Aileen Timmons, B.Sc., Ph.D. — HSO
Sharon Dubbels — ASO
Carol Taylor — EWIII/II

Director's Group
N. Deighton, B.Sc., Ph.D. — HSO
Sheila Glidewell, B.Sc., Ph.D. — HSO

Mycology and Bacteriology
D. Cox, B.A., M.Phil. — SO
S. Main — EWIII/II

SASS
D. Hitchcock, B.A., Ph.D. — HSO
Verena M. Trenkel, Dipl. Biol., M.Sc. — HSO (Appt. Nov)

Soft Fruit Genetics
Vasantha Ramanathan, B.Sc., Ph.D. — HSO

Virology
D.A.C. Jones, B.Sc., Ph.D. — HSO (Tr. from M&B Sep)
A. Ziegler, B.Sc., Ph.D. — HSO (Appt. Nov)

Zoology
Annette S. Salowsky-Butcher, Dipl. Biol. — SO

SOAFD Short Term Contract

Cellular and Environmental Physiology
Alison Cooper, H.N.D. — SO
C. Regalado, B.Sc., M.Sc., P.G. Stud. — SO (Appt. Nov)

Crop Genetics
K. Harding, B.Sc., Ph.D. — HSO (Tr. from M&B Nov)

Data Processing Unit
A Mayo, B.Sc. — SO (Appt. Feb)

Director's Group (Fibres)
Julie A. Duncan — EW(III/II) (P/T)(Appt. Aug)

Estate, Glasshouse & Field Experiments Department
A. Grant — P&GS(E) (Appt. Nov)
G.S. Lacey — EWIII/II (Appt. Nov)
M. Torrie — SAWB (P/T) (Appt. Oct)

SASS
S.D. Chasalow, B.A., M.A., Ph.D. — HSO
C. G. Harbron, B.Sc. — HSO (Appt. Nov)
Gayathree Jayasinghe, Grad. Dip. Inst. Stat., M.Sc. — HSO (Appt. Mar)
D. A. McNulty, B.Sc., Ph.D. — HSO (Appt. Oct)

Soft Fruit Genetics
W.T.G. van de Ven, Ir., Ph.D. — HSO
Linzi M. Ross — EWIII/II (Appt. Sept)

Virology
Fiona Carr — ASO (Tr. from DG Dec)

Zoology
B. Fenton, B.Sc., Ph.D. — HSO (Appt. Jan)
J.T. Jones, B.Sc., Ph.D. — HSO (Appt.Nov)

Department of the Environment

Crop Genetics
Eileen T. O'Brien, B.Sc., Ph.D. — HSO (Appt. Apr)
Yvonne M. Charters, B.Sc. — SO (Appt. Apr)

CEC

Cellular and Environmental Physiology
Susan Jarvis, B.Sc., Ph.D. — HSO
Sigrun Holdhus, Cand.mag — EWIII/II

Chemistry
G. Dobson, B.Sc., Ph.D. — HSO (Appt. Jun)

Cell and Molecular Genetics
Mamanu Barua, B.Sc. — HSO (Appt. Nov)
K.J. Chalmers, B.Sc., Ph.D. — HSO
Joanne Russell, B.Sc. — HSO (Tr. from M&B Nov)

Crop Genetics
Lucy Payne, B.Sc., Ph.D. — n/a

Director's Group (Fibres)
Alison Dolan, H.N.C. — SO (P/T) (Appt. Mar)

Virology
Sybil M. Macintosh, B.Sc. — SO
Alison Mackie, B.Sc. — SO (Appt. Jul)

Zoology
M. Armstrong, B.Sc., M.Sc. — SO (Tr. from CMG Nov)
Bridget Carter, B.Sc. — EWIII/II (Appt. Nov)
Paula M. Hebden, B.Sc. — ASO (Appt. Nov)
Jane Roberts, S.N.C. — EWIII/II

CEC / ECSA

Cell and Molecular Genetics
P. Hedley, B.Sc. — SO

Cellular and Environmental Physiology
L.R. Burch, B.Sc., M.Sc., Ph.D. — SSO
Edna Cuthbert, S.N.C., H.N.D. — ASO
Linda Sommerfield, B.Sc — EWIII/II

DTI/Dalgety Spillers

Crop Genetics
D. Matthews, B.Sc., Ph.D. — HSO (Appt. Sep)

Gene Shears

Cell and Molecular Genetics
D.J. Leader, B.Sc. — HSO
J.F. Sanders, B.Sc., Ph.D. — HSO
A. Turnbull-Ross, B.Sc., Ph.D. — HSO

MAFF

Cell and Molecular Genetics
A.L. Craig — SO

Cellular and Environmental Physiology
Sheena J. Rodger O.N.C. — ASO

SASS
Deena C. Mobbs, B.Sc. — HSO

Soft Fruit Genetics
Carol J. Barrett, B.Sc., Ph.D. — HSO (Appt. May)
Emily Cobb, H.N.C. — EWIII/II (Appt. Aug)

McCains PLC

Crop Genetics
D. Todd — SO
Marjorie J. Grant, H.N.D. — EWIII/II (Appt. Aug)

Mylnefield Research Services Ltd

Soft Fruit Genetics
Joao Pontes, B.Sc. — n/a (Appt. Nov)
Ewan Simpson, B.Sc. — n/a (Appt. Oct)

ODA

Cell and Molecular Genetics
I.K. Dawson, B.A., M.Sc. — HSO

Virology
J.S. Millet, B.Sc. — HSO
B. Carter, B.Sc. — EWII/III (Appt. Nov)

ODA/CIP

Beverly Ingram, B.Sc., M.Sc. — HSO (Appt. Jun)
Michele S. Leslie — ASO (Tr. from Vir Jul)

PMB

Cellular and Environmental Physiology
G.J. Lewis, B.Sc., M.Sc. — HSO
M. Young, H.N.D. — SO

Crop Genetics
Lisa F. Palmer, B.Sc. — SO

Mycology and Bacteriology
Alison C. Connelly, B.Sc. — SO

Red Deer Commission

SASS
Susan Ahmadi, B.Sc. — SO (Appt. Jul)

Scotia

Mycology and Bacteriology
A. Reglinski, B.Sc., Ph.D. — HSO

Miscellaneous funding

Cellular and Environmental Physiology
Linda I.D. Marshall, B.A. — ASO
N. Harris — EWV (Appt. Oct)

SASS
Linda J. Williams, B.Sc., M.Sc. — SO (Appt. Oct)

Soft Fruit Genetics
P. Lanham, B.Sc., Ph.D. — HSO
Jane E. Fairlie O.N.C. — EWIII/II (P/T)

Editorial Duties

Name	Position	Journal Title
H. Barker	Editorial Board	*Annals of Applied Biology*
A.G. Bengough	Editor (Joint)	*British Soil Science Society Newsletter*
B. Boag	Editorial Board	*Annals of Applied Biology*
	Editorial Board	*Nematologia Mediterranea*
R.M. Brennan	Associate Editor	*Journal of Horticultural Science*
D.J.F. Brown	Honorary Chief Editor	*Russian Journal of Nematology*
	Editorial Board	*Nematologia Mediterranea*
W.W. Christie	Editorial Board	*Chemistry and Physics of Lipids*
	Managing Editor	The Oily Press
J.M. Duncan	Associate Editor	*Journal of Horticultural Science*
	Associate Editor	*Mycological Research*
C.A. Glasbey	Associate Editor	*Biometrics*
	Editorial Panel	*Applied Statistics*
T.D. Heilbronn	Editor	*SCRI Annual Report*
	Editor	*SSCR Newsletter*
J.R. Hillman	Publication Comittee	*Journal of Horticultural Science*
	Editorial Board	*Agricultural Systems*
	Editorial Board	*Journal of Agricultural Science*
R.A. Kempton	Editorial Board	*Journal of Agricultural Science, Cambridge*
H.M. Lawson	Associate Editor	*Journal of Horticultural Science*
D.K.L. MacKerron	Associate Editor	*Journal of Horticultural Science*
	Editorial Board	*Euphytica*
M.A. Mayo	Editorial Board	*Journal of General Virology*
J. McNicol	Statistical Editor	*Annals of Applied Biology*
I.M. Morrison	Management Committee	*Journal of the Science of Food and Agriculture*
	Series Editor	*Advances in Plant Cell Biochemistry & Biotechnology*
K.J. Oparka	International Advisory Board	*Journal of Experimental Botany*
D.A. Perry	Editor	*SSCR Bulletin*
	Editor	*SCRI Annual Report*
W. Powell	Editor	*Heredity*
	Editor	*Potato Research*
D. Robinson	Associate Editor	*Journal of Horticultural Science*
D.J. Robinson	Editorial Board	*Journal of Virological Methods*
G.R. Squire	Editorial Board	*Experimental Agriculture*
	Editorial Advisory Board	*Crop Physiology Abstracts*
D.L. Trudgill	Editorial Board	*Nematologica*
	Editorial Board	*Fundamental and Applied Nematology*
R.L. Wastie	Editorial Board	*Annals of Applied Biology*
	Editor	*Potato Research*
B. Williamson	Editor	*Annals of Applied Biology*
T.M.A. Wilson	Senior Editor	*Molecular Plant-Microbe Interactions*
I.M. Young	Editor (Joint)	*British Soil Science Society Newsletter*

Awards and Distinctions

Name	Dept.	Degree/Award/Distinction/Appointment
A. Booth	CG	H.N.C. Biological Sciences
J.W. Crawford	CEP	Honorary Research Fellow, Department of Mathematics, University of Dundee
H.V. Davies	CEP	Honorary Senior Lecturer, University of Dundee
Norma Dow	CG	Scotvec National Certificate in Science
J.M. Duncan	M&B	Honorary Lecturer, University of Dundee
		Phytophthora Committee of International Society of Plant Pathology
R.P. Ellis	CG	Honorary Lecturer, University of Dundee
B.P. Forster	CMG	Honorary Lecturer, University of Dundee
S.C. Gordon	Zoo	NEBOSH National General Certificate in Occupational Safety and Health
D.J. Johnston	M&B	Ph.D., University of Dundee
M.A M. Kirkwood	SASS	Higher National Certificate in Computing
F.J. Legorburu	Vir	Ph.D., University of Dundee
B. Marshall	CEP	Honorary Research Fellow, Department of Mathematics, Dundee University
J.S. Miller	Vir	Ph.D., University of St Andrews
Moira Myles	CG	Scotvec National Certificate in Science
R. Neilson	Zoo	M.Sc., University of Dundee
K.J. Oparka	CEP	Honorary Lecturer, University of Dundee
L.G. Pereira	Vir	Ph.D., University of Dundee
W. Powell	CMG	Fulbright Fellowship
		D.Sc., University of Birmingham
D.J. Robinson	Vir	Honorary Lecturer, University of Dundee
C.M. Scrimgeour	Chem	Honorary Lecturer, University of Dundee
C.G. Simpson	CMG	Ph.D., University of Dundee
D. Stewart	DG (Fib)	Ph.D., University of Dundee
G.R. Squire	CEP	Special Lecturer, University of Nottingham
M. Talbot	SASS	Elected to the International Statistical Institute
D. Taylor	CEP	M.Sc., University of Dundee
R.E. Wheatley	CEP	Ph.D., University of Dundee
T.M.A. Wilson	Vir	Honorary Professor, Zhejiang Academy of Agricultural Sciences, Hangzhou, China
G.R. Young	CG	H.N.C. Biological Sciences

Resignations

Name	Dept.	Grade	Month
S.J. Beaney	SASS	SO	June
Linda Bell	CEP	EWII	October
D.L. Borchers	SASS	HSO	November
S.T. Buckland	SASS	UG7	September
Fiona Carr	DG (Fib)	EW(III/II)	November
K.L. Cattanach	SASS	HSO	August
E. Patricia Dashwood	M&B	HSO	March
Hazel Duncan	DG (Fib)	EW(III/II)	April
Janice McDonald	EM	AO	Aug
E.W. Milne	Vir	SO	November
D.C. Mobbs	SASS	HSO	October
Elizabeth Robertson	CEP	ASO	June
A. Salowsky-Butcher	Vir	SO	October
C. Walker	EGF	EWIII/II	September

Staff Retirements

Name	Dept.	Grade	Month
C.C. Carrie	EGF	P&GS(E)	February
C. Conacher	EGF	EWIII/II	July
A.F. Murant	Vir	UG6	April

Redundancies, Voluntary and Flexible Retirements

Name	Dept.	Grade	Month
G.S. Lacey	EGF	EWIII/II	March

Visits Abroad

Name	Country visited	Month of visit	Duration of visit
A.N.E. Birch	Denmark	July	1 week
	Switzerland	November	10 days
Vivian Blok	The Netherlands	January	5 days
	Spain	February	4 days
	Italy	November	1 week
B. Boag	New Zealand	October	6 weeks
D. Borchers	Japan	April-May	2 weeks
	Canada	August	2 weeks
	Tokyo	October	11 days
R.M. Brennan	Poland	July	1 week
D.J.F. Brown	The Netherlands	May	1 week
	Italy	July	2 weeks
	Switzerland	July	1 week
	Portugal	September	2 weeks
	USA.	November	2 weeks
J.W.S. Brown	USA	June	1 week
	Germany	March	2 weeks
S. Buckland	New Caledonia	Jan-Feb	1 month
S.D. Chasalow	USA	August	2 weeks
W.W. Christie	Sweden	January	3 days
	USA	April-May	10 days
	Spain	May	1 week
	France	June	3 days
	France	September	1 week
J.W. Crawford	Japan	May	12 days
	Germany	September	1 week
H.V. Davies	USA	April	2 weeks
	Spain	May	5 days
	USA	July	5 days
	Japan	Aug-Sept	3 weeks
	France	December	3 days
J.M. Duncan	Canada	August	10 days
D.A. Elston	Sweden	August	4 days
J.M.S. Forrest	Germany	November	3 days
B.P. Forster	Slovenia	March	1 week
	Austria	December	1 week
M. F. Franklin	USA	April	9 days
	Germany	May	4 days
C. A. Glasbey	USA	January	1 week
S.M. Glidewell	Italy	June	1 week
B.A. Goodman	France	June	1 week
	Portugal	September	5 days
B.S. Griffiths	The Netherlands	April	1 week
D.W. Griffiths	Denmark	July	5 days
	Switzerland	October	1 week
	The Netherlands	December	4 days
C. Hackett	Israel	May-July	10 weeks
K. Harding	Denmark	September	1 week
J.G. Harrison	France	July	5 days
B. Harrower	The Netherlands	April	1 week
D. J. Hirst	Norway	October	1 week
D. Hitchcock	Norway	May	5 days
G.W. Horgan	Hungary	May	3 days
E.A. Hunter	France	January	3 days
	Italy	May	3 days
	Switzerland	August	4 days
	Italy	September	5 days
N.L. Innes	Bolivia, Peru	April	6 days
	Puerto Rico	May	5 days
	Peru	September	5 days
	USA	October	1 week
	France	November	2 days

Name	Country Visited	Month of visit	Duration of visit
D.J. Johnston	Switzerland	November	1 week
C.A. Jolly	The Netherlands	November	5 days
A.T. Jones	Germany	May	5 days
	Poland	July	10 days
	Canada	August	10 days
R.A. Kempton	Hungary	May	5 days
	France	June	4 days
	Spain	July	5 days
	Uganda	September	8 days
	Spain	September	12 days
	France	November	3 days
D.M. Kennedy	Poland	July	1 week
A. Kumar	USA	July-August	2 weeks
	Germany	October	1 week
H.M. Lawson	USA	February	10 days
G.D. Lyon	Canada	July-August	10 days
S.A. MacFarlane	France	May	3 days
G.C. Machray	Germany	July	3 days
	USA	July	1 week
G.R. Mackay	Canada	July-August	2 weeks
D.K.L. MacKerron	France	July	6 days
	Poland	October	8 days
B. Marshall	France	July	1 week
M.A. Mayo	Peru & Bolivia	February	2 weeks
	India	Aug-Sept	2 weeks
	France	October	3 days
	Italy	November	1 week
G.J. McDougall	Denmark	July	1 week
R.J. McNicol	Poland	July	1 week
S. Millam	Italy	November	2 weeks
J. Miller	Canada	July	1 week
	India	Aug-Sept	2 weeks
A.C. Newton	The Netherlands	November	3 days
	Canada & USA	August	3 weeks
M.C.M. Pérombelon	Belgium	March	5 days
	USA	March	8 days
	Spain	April	8 days
	Belgium	May	2 days
	Canada	July	10 days
	Spain	November	6 days
	France	December	3 days
M.S. Phillips	Spain	February	4 days
	Italy	November	1 week
W. Powell	USA	May-July	10 weeks
	USA	Oct-Dec	9 weeks
G. Ramsay	Germany	June	2 days
	The Netherlands	December	4 days
B. Reavy	Canada	July	1 week
K. Ritz	Germany	September	1 week
	Norway	November	3 weeks
	Germany	December	1 week
W.M. Robertson	The Netherlands	January	2 days
	Italy	November	4 days
D.J. Robinson	Canada	July-August	11 days
H.A. Ross	The Netherlands	October	6 days
G.D. Ruxton	The Netherlands	February	3 days
	Eire	September	3 days
C.M. Scrimgeour	Germany	February	3 days
A. Sword	Greece	June	4 days

Name	Country Visited	Month of visit	Duration of visit
M. Talbot	Hungary	January	4 days
	Germany	March	4 days
	Belgium	June	3 days
	Luxembourg	June	2 days
	Spain	September	6 days
	Luxembourg	October	3 days
	Switzerland	November	2 days
	Greece	December	3 days
M.A. Taylor	USA	July	5 days
	The Netherlands	October	6 days
L. Torrance	India	March	2 weeks
	Austria	January	2 days
	Japan	Sept-Nov	10 weeks
	Belgium	November	2 days
	Belgium	December	2 days
D.L. Trudgill	France	January	1 week
	Spain	February	1 week
	Canada	July	10 days
R.L. Wastie	USA	February	5 days
	France	July	6 days
	The Netherlands	November	3 days
R. Waugh	USA	January	1 week
	Germany	July	3 days
B. Williamson	Germany	September	1 week
T.M.A. Wilson	USA	March	5 days
	USA	April	10 days
	USA	July	5 days
	USA	November	1 week
I.M. Young	Japan	May	12 days
	Australia	Aug-Oct	3 months
M.W. Young	France	July	6 days

Service on External Committees or Organisations

Name	Position	Committee or Organisation
H. Barker	Member	Virology Group Committee, Association of Applied Biologists
A.G. Bengough	Member	Scottish Soils Discussion Group
A.N.E. Birch	Member	Entomology Group Committee - Association of Applied Biologists
	Member	IOBC Working Group 'Breeding for Resistance to Insects and Mites'
R.M. Brennan	Adviser	SmithKline Beecham R&D Committee
D.J.F. Brown	Secretary/Treasurer	European Society of Nematologists
	Member	Society of Nematologists *Ad Hoc* Committee "International Federation of Nematology Societies"
	Member	European Plant Protection Organization *Ad Hoc* Committee "*Xiphinema american*-group nematodes"
	Member	Institute of Biology "IOBS Link Group"
J.W.S. Brown	Co-ordinator	Commission on Plant Gene Nomenclature of the International Society for Plant Molecular Biology (snRNA genes)
J.W. Crawford	Panel Member	AFRC/SERC Biomathematics Panel
	Member	Management Group, Centre for Non-linear Systems in Biology
	Member	Working Group, SOAFD Feasibility Study of a Systems Approach to Studying Agriculture and Land Use in Scotland
R.P. Ellis	Member	BSPB Cereal Crop Group
	Member	SAC Recommended List Consultative Committee
	Technical Secretary	SSCR Cereal Sub-committee
B.P. Forster	Co-ordinator	International Committee on Barley Chromosome (4) Genetic Mapping
K. Harding	Representative	WHO Workshop
T.D. Heilbronn	Publicity Officer	Association for Crop Protection in Northern Europe
J.R. Hillman	Chairman	SCRI/SASA/COSAC Liaison Group
	Chairman	Tayside Biocentre Group
	Deputy Chairman	Board of Directors, Mylnefield Research Services Ltd
	Member	Board of Directors, CAROS International
	Member	AFRC Plants and Environment Research Committee
	Member	SOAFD Joint Management Board
	Member	ECRE Board of Management
	Member	SNSA Adviser to Committee
	Member	Senate, University of Dundee
	Member	University of Strathclyde Sub-Board for the Degree of B.Sc. in Horticulture
	Member	SSPDC Management Committee
	Member	Tayside Economic Forum
	Advisor	International Foundation for Science, Stockholm
E. A. Hunter	Member	FLAIR Concerted Action No 2 Management Committee
N.L. Innes	Chairman	Governing Board & Executive Committee, CIP, Peru
	President	Association of Applied Biologists
	Member	University of Dundee Botanic Garden Committee
	Member	Botanic Gardens Conservation International, Kew
	Member	UK Genetic Resources Committee
	Member	MLURI Promotions Board
	Member	EUCARPIA Tropical Crops Steering Group
A.T. Jones	Director of Studies	International Study Course on 'Modern advances in plant virology: virus transmission, detection and resistance', sponsored by The British Council
	Member	EPPO Panel on Certification of Pathogen-tested Fruit Crops
R.A. Kempton	Council Member	International Biometric Society
	Member	Scientific Review, Biometry Laboratory, INRA-Versailles
H.M. Lawson	Chairman	UK Weed Liaison Group
	Member	BCPC R&D Subcommittee - Weeds
	Chairman	Scottish Weed Group Experimental Committee
G.D. Lyon	Member	AFRC Steering Group to organise workshop on opportunities for the manipulation of plant disease resistance
W.H. Macfarlane Smith	Member	AFRC Joint Committee on Health & Safety
	Member	BSPB Oilseed & Industrial Crop Group
	Member	AFRS Safety Officers Group
	Member	SARI Safety Officers Group
	Member	NPTC Plant Variety Development Panel
G.R. Mackay	Chairman	EUCARPIA Potato Section
	Member	Interdepartmental and Users Committee of Quarantine Users Committee - SASA
	Member	BSPB Potato Crop Group

Name	Position	Committee or Organisation
D.K.L. MacKerron	Secretary	Potato Crop Sub-committee, SSCR
	Secretary	Physiology Section, EAPR
B. Marshall	Member	AFRC Soil Science Steering Group
	Member	NERC, Terrestrial Life Sciences Research Grants and Training Committee
	Coordinator	AFRC Soil-Plant-Microbial Interactions Working Party
	Deputy Head	Management Group of Centre for Non-linear Systems in Biology
M.A. Mayo	Member	Executive Committee of International Committee for Taxonomy of Viruses (ICTV)
	Member	Virus data subcommittee of ICTV
	Chairman	Satellite study group of ICTV
U.M. McKean	Member	Scottish Agricultural Librarians Group
R.J. McNicol	Member	HDC Soft Fruit Trialling Sub-Committee
	Member	SSCR Soft Fruit Committee
	Adviser	SNSA Committee
	Adviser	SSFG Ltd Board
	Adviser	Soft Fruit Committee of Horticulture Research International
I.M. Morrison	Member	LINK-Crops for Industrial Use, FIBSTORE Project Committee
A.C. Newton	Member	UK Cereal Pathogen Virulence Committee
	Membership Secretary	British Society for Plant Pathology
	Newsletter Editor	European and Mediterranean Cereal Rusts Foundation - Diversification in Crops Working Group
K.J. Oparka	Member	International Organising Committee Phloem Transport Conference, Canterbury, UK, 1995
	Member	International Organising Committee Plasmodesmata Conference, Tel Aviv (Israel) 1996
M.C.M. Pérombelon	Member	COST 88 Bacteriology Committee
D.A. Perry	Treasurer	Association for Crop Protection in Northern Britain
	Treasurer	British Society for Plant Pathology
	Secretary	CAROS International Ltd Board
B. Reavy	Member	AFRC Protein Engineering Liaison Committee
K. Ritz	Member	AFRC Soil-Plant-Microbial Interactions Working Party
	Member	SOAFD Soil/Plant/Microbe Group
	Member	Management Group, Centre for Non-linear Systems in Biology
I.M. Roberts	Safety Representative	Royal Microscopical Society
	Chairman	AFRC Electron Microscope Advisory Group
D. Robinson	Member	AAB Plant Physiology Group
D.J. Robinson	Member	Advisory Committee on Releases to the Environment
	Member	Society for General Microbiology, Virus Group Committee
G.R. Squire	Member	SOAFD Working Group on Vegetation Dynamics
S. Stephens	Member	Tayside Chief Librarian Committee
	Member	Scottish Agricultural Librarians Group
M. Talbot	Member	Statistics Group of UK Plant Varieties and Seeds Committee
	Member	Technical Working Party on Automation and Computer Programs of the International Union for the Protection of Plant Varieties
W.T.B. Thomas	Convenor	AAB Plant Breeding & Genetics Group
L. Torrance	Member	Management Committee COST-88
D.L. Trudgill	Co-ordinator	EC/STD project on biocontrol of *Meloidogyne* spp.
M.J. Wilkinson	Member	UK Plant Genetic Resources Group Interdepartmental Post-quarantine Users Committee
T.M.A. Wilson	Group Member	The Church of Scotland Society, Religion and Technology Project, Working Group on Ethical Issues in Genetic Engineering of Non-human Life Forms
	Member	International Advisory Board, VIIth International Conference of Comparative and Applied Virology
J.A.T. Woodford	Regional Hon. Sec	Royal Entomological Society
F.G. Wright	Member	AFRC Protein Engineering Liaison Group
I.M. Young	Member	British Soil Science Society Council
	Member	AFRC Soil Physics Working Party
	Member	SAC Soil Technology Group

Short term workers and visitors

Name	Country of origin	Dept.	Month/yr of arrival	Length of stay
N. Ahmed	Bangladesh	SASS	Jul 93	6 months
S.B. Andersen	Denmark	CMG	May 93	6 months
S. Anwar	Pakistan	Zoo	Apr 92	1 year
S.Ariffin	Malaysia	Chem	Jun 93	2 months
M.I. Arroyo	Spain	CEP	Jul 93	1 month
G. Asmar	UK	CMG	Oct 92	1 year
Estelle Bailey	UK	CG	Apr 93	6 months
R. Barlow	UK	CMG	Oct 92	3 months
C. Barrera	Peru	Vir	Apr 93	1 year
A. Barton	UK	Zoo	Apr 93	11 weeks
M. Binns	Canada	SASS	Jan 93	7 months
J.P. Braselton	USA	CG	Sep 93	9 months
Samantha Budd	UK	CEP	Oct 93	6 months
A. Buser	Switzerland	Zoo	Sep 93	1 week
D. Carter	UK	M&B	Oct 92	3 months
N. Cerovska	Czech Republic	Vir	May 93	1 month
L. Cornette	Belgium	CEP	Feb 93	5 months
M. Davidson	UK	CG	Oct 93	8 months
Prabhjyot Dehal	UK	CMG	Sep 93	6 months
H. Elangwe	Cameroon	CEP	Nov 92	11 months
Janet Elliot	UK	CG	Jan 93	8 months
D. Engelhardt	Austria	Vir	Jun 93	2 months
W. Engst	Germany	Chem	Nov 93	1 month
J.B. Fernandes	Brazil	Chem	Feb 93	1 year
D. Fisher	USA	CEP	Aug 93	1 year
M. Fourmann	France	CMG	Sep 93	2 months
N. Francis	UK	CG	Apr 93	6 months
M. Frost	UK	Zoo	Oct 93	7 months
F. Georget	France	CG	Jul 93	3 months
Anna Gluska	Poland	CEP	Jun 93	3 months
Joanna Gramshaw	UK	CEP	Sep/Dec 93	2 weeks
L. Hakim	Bangladesh	CMG	Mar 92	1 year
A. Hamilton	UK	Zoo	Aug 93	5 months
L.A. Harding	UK	M&B	Apr 93	6 months
C. Hernandez	The Netherlands	Zoo/Vir	Dec 93	2 weeks
J.F.J.M. van den Heuvel	The Netherlands	Vir	Aug 93	1 week
L. Hiller	USA	CEP	Jul 93	1 year
S. Holm	Sweden	SASS	Mar 93	1 week
S. Hughes	UK	Zoo	Aug 93	5 months
S.J. Jagtap	UK	CG	Jul 93	6 months
H. Jansen	The Netherlands	CG	Oct 92	5 months
Karen Johnstone	UK	CG	Jan 93	10 months
P.M. Jones	New Zealand	Zoo	Jul 93	1 week
Gabriella Knapova	Germany	M&B	Jan 93	1 week
R. Koeman	The Netherlands	CG	Jan 93	3 months
H. Koike	Japan	CEP	Feb 93	1 week
P. Kunz	Switzerland	Zoo	Sep 93	1 week
C. Laburn	UK	ESF	Sep 93	3 months
Rachel Legg	UK	Zoo	Aug 92	1 year
M. Liskova	Slovakia	Zoo	Apr 93	3 months
J. Logan	UK	SASS	Aug 93	1 year
S. MacDonald	UK	M&B	Apr 93	4 months
J. Marshall	New Zealand	Zoo	Sep 93	1 week
R. Martinez-Carrasco	Spain	CEP	Jul 93	3 weeks
W. Mazurczyk	Poland	CEP	Sep 93	10 days
S. Mejza	Poland	SASS	Mar 93	1 week
J. McWhirr	UK	Chem	Oct 93	5 months
Emma Moxey	UK	CG/Zoo	Aug 93	10 months
F. Murray	UK	Admin	Mar 93	6 months
R. Naidu	India	Vir	Jun 93	2 months
T.L. Niblack	USA	Zoo	Jun 93	1 week
B. Nikolova-Damyanova	Bulgaria	Chem	Sep 93	3 months
Catherine de Nova	New Zealand	SFG	Oct 92	6 months
C. Oncino	France	Vir	Jun 93	1 month
T. Pagella	UK	CG	Aug 92	8 months
C.G. Palivan	Roumania	DG (Spec)	Jul 93	3 months
H. Palivan	Roumania	DG (Spec)	Aug 93	1 month
E. M. Qannari	France	SASS	Apr 93	1 week
D. Quarm	UK	Zoo	Jan 93	9 months

Name	Country of Origin	Dept.	Month/yr of Arrival	Length of stay
K. Rappoldt	The Netherlands	CEP	Nov 93	1 week
Y. Robert	France	Zoo	Oct 93	3 days
H. Rogasik	Germany	CEP	Dec 93	3 weeks
B-J. Ruissen	The Netherlands	CEP	Jun 93	4 months
S. Schmidt	Slovakia	Chem	Jun 93	1 week
S. Scott	USA	Vir	Jan 93	6 months
S. Sharma	India	CMG	Oct 93	10 months
J. Stewart	Canada	CEP	Jul 93	10 months
D. Taylor	UK	CEP	Apr 93	6 months
P. Tien	China	Vir	Nov 92	6 months
E. Tucker	UK	M&B	Apr 93	5 months
E. van Loon	The Netherlands	CEP	May 93	3 months
T. Vaughan	UK	CMG	Sep 93	6 months
L. Vicente	Portugal	DG (Spec)	Jun 93	2 weeks
M. van Vuuren	The Netherlands	CEP	Nov 92	4 months
R. Wahad	Malaysia	Chem	Jun 93	2 months
J. Wishart	UK	Zoo	Jul 93	3 months
Emma Woodward	UK	CEP	Oct 93	6 months
Y. Yanai	Japan	CEP	Mar 93	10 months
G.W. Yeates	New Zealand	Zoo	Aug 93	1 week

Longer-term visitors and Research Fellows

Name	Country of origin	Dept.	Month/yr of arrival	Length of stay
Anne-Sophie Bournay	France	CMG	Oct 93	2 years
F. Chaubron	France	CEP	Mar 93	18 months
Jill Ellis	UK	CG	Jan 91	3 years
Linda L. Handley	UK/USA	CEP	Jun 91	5 years
Rhonda Meyer	Germany	CMG	Mar 92	2 years
B. Venuat	France	CEP	Sep 92	2 years
P. Whitty	UK	CMG	May 92	3 years

Postgraduate Students

Name	Dept.	Subject
I. Abdalla	SASS	Automatic detection of tissue boundaries in ultrasound scans of pedigree sheep.
J. Allainguillaume	CG	Accelerated gene localisation in potato.
A. Anderson	CEP	Quantification and evolution of qualitative theory of soil structure.
J. Angel-Diaz	Vir	Molecular approaches to the control of raspberry bushy dwarf virus.
M. Arif	Vir	Potato mop-top furovirus transmission.
Siti A. Mad Arif	CMG	Plant genetic transformation and gene expression.
Miray Arli	Vir	Studies on potato mop-top virus multiplication.
G. Asmar	CMG	Characterisation of plant cDNAs encoding putative RNA helicases.
E. Baird*	CMG	Potato molecular biology.
R. Bargota	CEP	Starch synthesis in *Vicia faba.*
S.N.B. Barr	CG	Somatic hybridisation of tetraploid and wild potato.
U. Barua	CMG	RAPD methods of detecting polymorphisms in barley.
Annette Baty	DG-fib	Control of cell wall biosynthesis during differentiation of fibre cells.
M. Biggs	SFG	Factors influencing the development of cold tolerance and dormancy in woody perennials.
Wendy Breese	M&B	Downy mildew of *Rubus* cane fruits.
Karen Brierley	DG-spec	NMR spectroscopy for characterisation of the coat protein of pepper ringspot virus.
Yvonne M. Charters	CG	Investigations into the survivability of oilseed rape in model feral situations.
F. Chaubron	CEP	Cloning of sugar metabolising genes from sugarbeet.
J. Chen	Vir	Molecular biology of fungus-transmitted cereal viruses.
K. Cheung	CMG	Transformation of groundnuts.
F.A. Comerford	CMG	Lamins in the plant nuclear membrane.
G.H. Cowan	Vir	Development of antibodies to non-structural proteins of plant viruses.
D.F. Cox	M&B	Development of non-linear mathematical theory of plant disease epidemiology.
Sarah Cox	Vir	Molecular approach to the study of virus-like diseases of *Ribes.*
Clare Croser	CEP	Aspects of root growth through compacted soil.
J. Curtis	Vir	Narcissus latent virus.
I. Dawson	CMG	Molecular diversity of tropical tree species.
Pauline Douglas	M&B	Control of plant defense responses by reversible protein phosphorylation.
Lisa Duncan	Zoo	Study of the surface molecules of plant parasitic nematodes.
Sarah Fennel	CMG	Biochemical and molecular markers of *Arachis.*
J. Forster	CEP/CMG	Genetic manipulation of nitrate reductase activity in potato.
Valerie Godfrey	M&B	Pectin lyase production in *Erwinia caratovora* ssp. *atroseptica.*
J.I. Hamilton	CMG	Molecular characterisation of RNA binding proteins in pre-mRNA splicing.
Patricia M. Harbour	CG	Biological control of potato storage diseases.
B.E. Harrower*	CMG	Molecular biology of nematodes.
P.E. Hedley	CMG	Genetic manipulation of sugar metabolism in tubers of potato.
R.J. Hopkins	Zoo	Resistance to cabbage and turnip root fly in swedes.
D-J. Hwang	Vir	Expression and assembly of plant viral coat protein into virus-like particles.
Lizbeth Hyman*	M&B	Characterisation of pectolytic bacteria by monoclonal antibodies.
D.J. Johnston*	M&B	Latent infection of flowers by *Botrytis cinerea.*
Anne Jolly*	Vir	Comparison of potato leaf roll virus strains in the P 5 gene.
D.J. Leader	CMG	U5snRNA genes from potato and maize.
G.J. Lewis	CEP	Methods for simulation of water and nitrogen use in potato.
Anne Maddison	CMG	Molecular dissection of invertase gene expression in potato.
I. Manoussopoulos	Vir	Mechanisms of aphid transmission of potyviruses.
Pauline McConway	M&B/CMG	Molecular biology of potato resistance to erwinias derived from *Solanum brevidens.*
Sarah Miller	M&B	Assessment of the potential to control potato diseases by resistance elicitors.
R.J. McNicol*	SFG	Investigations into running off in blackcurrants.
A. Mortazavi-Back	CG	Aspects of breeding potatoes for dry rot resistance.
Elizabeth Murant	CEP	Endocytosis in plant cells.
F. Nabugoomu	SASS	REML estimation in a series of varietal trials.
Martha Namfua	Zoo	Management of nematodes associated with coffee.
N.E. Nyange	SFG	Breeding for resistance to coffee berry disease and coffee rust.
C. Orozco-Castillo	CMG	Molecular diversity and genetic linkage mapping of *Coffea* sp.
H. Pakniyat	CMG	Genetic control of salt tolerance in barley.
Sara Preston	CEP	The role of microorganisms in the genesis and stabilization of soil structure.
D.A.M. Prior*	CEP	Effect of uptake and partition of sucrose and xenobiotics within plant cells.
J. Provan	CMG	Large scale cloning of plant DNA.
G. Rhandawa	CMG	Manipulation of potato genes.
W.Q. Ribeiro	CMG	Genetic variation in *Phaseolus vulgaris.*
Heather A. Ross*	CEP	Investigation of the control of sugar breakdown.
Joanne Russell	CMG	Genetic fingerprinting of cocoa.
Jessica Searle	M&B	Population Genetics of *Rhynchosporium secalis.*
J. Shaw	SASS	Techniques for discrimination of seed types using imaging measurements.
J.S. Swanston *	CG	Malting and brewing properties of novel barley starch combinations.
F.N. Wachira	CMG	Molecular variation in tea.
R.E. Wheatley*	CEP	Nitrogen transformation in cultivated soils.
A. Wilson*	CG	Gene position in a synthetic *Brassica napus* .
Mary Woodhead	CEP/SFG	Regulation of anthocyanin gene expression in blackcurrant.
Stella Xenophontos	CG	The epidemiology of spraing disease (tobacco rattle virus) of potato.
M.W. Young	CEP	Predictive models for the nitrogen requirements of potato crops.

* Permanent members of staff

SCRI Research Programme
1993-1994

SOAFD funded research programme showing: SCRI Project number; SOAFD number; Title (prefixed ROA for ROAMEd core projects, IFS for Increased Flexibility Scheme projects, FF for Flexible Fund projects and LINK for SOAFD-LINK projects); Scientific Project Leader. In addition to this list there are c. 200 research projects undertaken on behalf of various bodies, including other Governmental bodies, commerce and levy boards.

040	SCR/006/91	ROA Genetic architecture of tetraploid potatoes and production of enhanced germplasm	Bradshaw J E
045	SCR/007/91	ROA Develop, improve and use screening methods for resistance to diseases and pests of the potato	Wastie R L
046	SCR/008/91	ROA Develop and use screening tests for biochemical compounds in potatoes	Griffiths D W
047	SCR/017/91	ROA Maintenance, improvement and evaluation of the Commonwealth Potato Collection	Wilkinson M J
109	SCR/064/91	ROA Biology and population dynamics of agricultural pests especially plant-parasitic nematodes	Boag B
114	SCR/061/91	ROA Analysis of the inheritance of resistance to and complementary virulence of potato cyst nematodes	Phillips M S
152	SCR/042/91	ROA Identification and exploitation of genetic markers in crop improvement	Forster B
157	SCR/028/91	ROA Physical and chemical characteristics of fibres from fibre-producing herbs, shrubs and trees	Morrison I M
158	SCR/029/91	ROA Control of differentiation and development in plant fibre cells	Morrison I M
159	SCR/030/91	ROA Novel and conventional processes for the extraction and modification of fibres from plant sources	Morrison I M
180	SCR/062/91	ROA Mechanisms determining specificity and efficiency of transmission of tobraviruses by (Para)Trichodorus nematodes	Brown D J F
181	SCR/063/91	ROA Mechanisms determining specificity and efficiency of nepovirus transmission by longidorid nematodes	Brown D J F
187	SCR/009/91	ROA Investigate the genetics and biochemistry of the low temperature sugar stability characteristics of potato for use in fried food products	Mackay G R
205	SCR/031/91	ROA Evaluate the potential of NMR spectroscopy for the determination of the composition and structure of plant fibre and fibre products	Goodman B A
215	SCR/067/91	ROA Biology and ecology of pests and beneficial arthropods associated with cane and bush fruits	Woodford J A T
261	SCR/007/90	ROA Introduction of exogenous DNA into *Rubus, Ribes, Fragaria* and other soft fruit genera using *Agrobacterium tumefaciens*	McNicol R J
263	SCR/004/90	ROA Incorporation of the Birmingham Potato Collection into the Commonwealth Potato Collection	Wilkinson M J
264	SCR/005/90	ROA Correlating glasshouse and field performance of true (botanic) potato seed populations	Clulow S A

265	SCR/006/90	ROA Development and evaluation of methods for specific applications of high-technology instrumentation for the SCRI research programme	Christie W W
274	SCR/002/90	FF Sensitivity analysis of crop performance with development to aid crop management in an altered climate	Crawford J W
275	SCR/001/90	FF To determine the factors modifying the transcription of genes controlling carbohydrate metabolism	Davies H V
281	SCR/053/91	ROA Investigation of the genetic control of characters determining crop performance in barley	Ellis R P
282	SCR/054/91	ROA Development of improved methods of generating and evaluating variation in barley for a range of important characters	Thomas W T B
283	SCR/055/91	ROA Investigation of the genetical determination of biochemical components that relate to cereal quality with the aim of improving selection procedures in breeding programmes	Swanston J S
284	SCR/056/91	ROA Anther and isolated microspore culture in cereals and legumes	Ramsay G
285	SCR/058/91	ROA Anti-nutritional factors in faba beans	Ramsay G
286	SCR/057/91	ROA Tissue culture and transformation in legumes	Ramsay G
287	SCR/059/91	ROA Biochemical markers in faba beans	Ramsay G
288	SCR/019/91	ROA Devise techniques for modifying the competitive relationship between fruiting and vegetative phases in raspberry	Lawson H M
289	SCR/020/91	ROA The collection, evaluation and conservation of genetic resources of perennial soft fruit genera	McNicol R J
290	SCR/021/91	ROA The development of molecular and biochemical markers in woody perennial fruit crops	Brennan R M
291	SCR/022/91	ROA Investigations of the genetics and mechanisms of pest and disease resistance in *Ribes*, *Rubus* and other soft fruit genera	McNicol R J
292	SCR/023/91	ROA Investigation of mechanisms and genetic control of low temperature tolerance in perennial fruit crop genera	Brennan R M
293	SCR/024/91	ROA The floral biology of perennial soft fruits	McNicol R J
294	SCR/025/91	ROA Gene flow from cultivated to feral populations of soft fruit species and its implications for the release of genetically engineered plants	McNicol R J
296	SCR/027/91	ROA Produce and maintain virus-tested stocks, assess resistance and index British and imported *Ribes* and *Rubus* for virus infections	Jones A T
297	SCR/010/91	ROA Development and exploitation of tissue culture techniques, in particular microspore culture technology in *Solanum tuberosum* L.	Millam S
299	SCR/012/91	ROA Exploitation of protoplast technology in the development of new material and in the introduction of new genes into existing material	De,Maine M J
300	SCR/013/91	ROA The mechanisms of dihaploid formation following pollination of tetraploid potatoes with dihaploid inducer clones	Wilkinson M J
301	SCR/014/91	ROA The production of dihaploids and their use in improving the efficiency of germplasm enhancement and genetical study of *Solanum tuberosum*	De,Maine M J
302	SCR/018/91	ROA The production of hybrids between dihaploids of *Solanum tuberosum* and diploid *Solanum* species as a means of producing novel sources	De,Maine M J

		of material for germplasm enhancement and genetical studies at the diploid level	
303	SCR/015/91	ROA Inheritance of resistance to potato virus diseases and production of resistant enhanced potato germplasm	Solomon-Blackburn R
304	SCR/016/91	ROA The production, maintenance, distribution and associated management of facilities to produce disease-free tubers of genetic stocks of potato clones	Chapman I M
305	SCR/034/91	ROA Genetic studies within the family Brassicaceae, as model systems for the study of cytotaxonomy, polymorphism and gene introgression	Millam S
309	SCR/033/91	ROA To determine the biochemical and molecular mechanism associated with seed dormancy in woody species	Davies H V
311	SCR/069/91	ROA Study changes in the status of agricultural pests, especially plant-parasitic nematodes, due to alterations in agricultural practices and land use	Boag B
312	SCR/070/91	ROA Determine the thermal-time relationships for developmental processes in representative plant parasitic nematodes	Trudgill D L
313	SCR/071/91	ROA Mechanisms of host plant recognition, resistance and susceptibility to insects and mites	Birch A N E
314	SCR/072/91	ROA Mechanisms of resistance to virus vector aphids	Woodford J A T
316	SCR/074/91	ROA A biochemical and molecular study of the introduction of potato cyst nematodes (PCN) into Europe and their spread and virulence characteristics	Phillips M S
318	SCR/076/91	ROA The ecological and nutritional significance of changes in plant biochemistry induced by insect and mite attack	Birch A N E
321	SCR/077/91	ROA Chemical characterisation and properties of the cuticle in plant parasitic nematodes	Robertson W M
322	SCR/081/91	ROA Molecular analysis of species and virulence group relationships in *Meloidogyne* spp.	Trudgill D L
323	SCR/078/91	ROA A molecular and biochemical study of collagen differences associated with speciation in Meloidogyne spp., and host specificity of the nematode bacterial parasite *Pasteuria penetrans*	Robertson W M
324	SCR/079/91	ROA Involvement of carbohydrates in retention and release of virus particles in vectors	Robertson W M
325	SCR/080/91	ROA Mechanisms of nematode damage and tolerance in relation to resistance and better strategies for control without using nematicides	Trudgill D L
326	SCR/050/91	ROA Physical and physiological constraints on the growth and activity of plant root systems	Robinson D
327	SCR/049/91	ROA Relating soil structure to biological function	Young I M
329	SCR/047/91	ROA Strategies of drought tolerance in arable crops	Bengough A G
330	SCR/082/91	ROA Dynamics of microbial populations in relation to environmental factors	Griffiths B S
331	SCR/046/91	ROA Transport of substances through soil: regulating and mediatory role of microbes	Ritz K
332	SCR/045/91	ROA Interactions between environment and microbial transformations in root zone soils	Wheatley R E

333	SCR/044/91	ROA Identification and quantification of root exudates	Shepherd T
334	SCR/032/91	ROA Monitoring and prediction of weed and other wild plant populations in and vegetation management strategies for crops, uncropped areas and rotations	Lawson H M
339	SCR/043/91	ROA Mechanisms of uptake and transport of xenobiotics	Oparka K J
340	SCR/004/91	FF Computation of safe isolation distances for field-grown genetically modified crops	Mackay G R
341	SCR/005/91	FF Binary image restoration at subpixel resolution from multi-level data	Glasbey C
344	SCR/002/91	FF Investigate techniques for identification of foodstuffs of plant origin subjected to ionizing radiation, determination of received radiation dosages and elucidation of the role of free-radicals in senescence related processes	Goodman B A
357	SCR/357/92	ROA To determine the regulation of cell to cell transport and carbohydrate flux in sugar and starch-storing tissues	Davies H V
358	SCR/358/92	ROA Molecular mechanisms involved in tuberisation in potato	Taylor M
359	SCR/359/92	ROA Quantifying the benefits of genetic resistance to late blight and other diseases of the potato in pesticide-free (organic) farming systems	Mackay G R
360	SCR/360/92	ROA Develop techniques of molecular spectroscopy and utilize them in the resolution of problems involving composition, structure and/or biochemical processes in biological species of importance to agric.	Goodman B A
361	SCR/361/92	ROA Genetic control of pathogenicity and host specificity at the molecular level in the fungal pathogens *Phytophthora* and *Rhyncosporium*	Duncan J M
362	SCR/362/92	ROA Physiology and biochemistry underlying resistance of potato to late blight (*Phytophthora infestans*) and bacterial soft rots (*Erwinia*), barley to mildew (*Erisyphe graminis*) and soft fruit to grey mould (*Botrytis cinerea*)	Lyon G D
363	SCR/363/92	ROA Biochemistry of pectic enzymes produced by *Botrytis* and soft-rotting *Erwinia* spp. and related saprophytic bacteria	Williamson B
364	SCR/364/92	ROA Epidemiological and etiological studies of bacterial and fungal pathogens of potatoes, cereal crops and raspberries	Perombelon M
365	SCR/365/92	ROA Quantify the effects of water and nutrient stresses on the physiology of growth in crops (using potato and field bean as examples)	MacKerron D K L
366	SCR/366/92	ROA Quantify the effects of environment on growth and vegetative developmental processes in potato and woody crop species	MacKerron D K L
367	SCR/367/92	ROA Post-transcriptional processes in plant gene expression	Brown J W S
368	SCR/368/92	ROA Molecular genetics of plant-parasitic nematodes: exploitation of the *Caenorhabditis elegans* model	Blok V C
369	SCR/369/92	ROA Genetic manipulation in higher plants: in vitro regeneration, genetic transformation and gene isolation in plants	Kumar A
381	SCR/381/92	ROA Application of non linear mathematics and fractal geometry to topics on spatio-temporal dynamics in heterogeneous media: diffusion and microbial dynamics in structured soil, morphogenisis and epidemiology	Crawford J W
382	SCR/382/92	FF Investigation into oil seed rape as a possible cause of human allergy and the chemical, palynological and mycological factors which may be involved	Macfarlane Smith W H

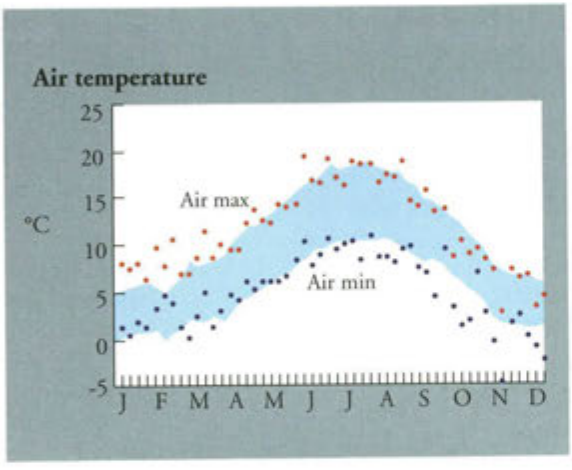
386	SCR/386/92	ROA Biochemical and molecular variation in *Myzus persicae* and associated aphid vectors of potato leafroll virus and the potato virus Y complex	Woodford J A T
387	SCR/387/92	ROA Aphid vectors of potato virus Y complex in Scotland in relation to environmental change	Woodford J A T
388	SCR/388/92	FF Development and evaluation of a quantitative theory of soil structure and its relation to transport processes	Young I
389	SCR/389/92	FF Development of non-linear mathematical theory of plant disease epidemiology using as model systems, scald and powdery mildew in barley, blight in potato and redcore in strawberry	Newton A C
390	SCR/390/92	FF Breeding and selecting raspberry cultivars for suitability to machine harvesting with improved processed quality, and for greater shelf-life for the fresh market	McNicol R J
392	SCR/392/92	FF Transformation of antisense and fusion constructs of spliceosomal genes into potato	Brown J W S
393	SCR/393/92	FF Combined NMR and mathematical study of major metabolic pathways in higher plant cells	Viola R
394	SCR/394/92	LINK A molecular approach for the detection and diagnosis of the agent of reversion disease and of other virus-like agents of black currant [SmithKline Beecham/SOAFD]	Jones A T
395	SCR/395/92	LINK Detection of *Phytophthora* diseases in horticultural planting stocks by the Polymerase Chain Reaction (PCR) [HDC/SOAFD]	Duncan J M
396	SCR/396/93	ROA Synthesis of novel chemical compounds for use in studies of plant biochemistry and physiology	Shepherd T
397	SCR/397/93	ROA Novel methodology for the determination of lipid structure and its application to plant biochemistry and food lipids	Christie W W
398	SCR/398/93	ROA Chemical strategies for the study of natural defence compounds present in plant species, including faba beans, potatoes, brassicas and soft fruit crops	Griffiths D W
399	SCR/399/93	ROA *In situ* identification and localisation of plant cell wall components, particularly from fibre cells, by the production of cell wall specific antibodies	Forrest J M S
400	SCR/400/93	ROA Chemical and immunological methods for the isolation and characterisation of proteins, xyloglucons and other cell wall components from endoderm tissue, using barley as a model species	Forrest J M S
401	SCR/401/93	ROA Molecular ecology of arthropod pests, with particular reference to speciation and host adaptation of aphids in the genus *Amphorophora*, mites of *Ribes*, beetles attacking raspberry and Dipteran pests	Birch A N E
402	SCR/402/93	ROA Properties, variation, detection and control of the agents of virus and virus-like diseases of *Rubus*, *Ribes* and *Fragaria*	Jones A T
403	SCR/403/93	ROA Molecular basis for variation and genome organisation of nepoviruses	Jones A T
404	SCR/404/93	ROA Functions and modes of action of the gene products of luteoviruses, especially potato leafroll virus, with particular reference to virus transmission by aphids	Mayo M A

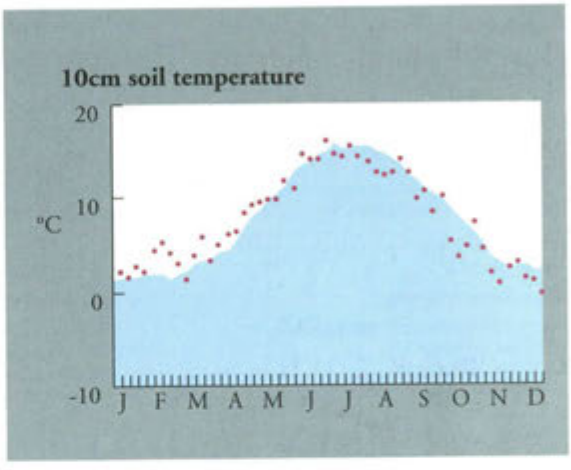
405	SCR/405/93	ROA Structure and function of the genomes of tobraviruses (specifically tobacco rattle and pea early browning viruses), with particular reference to virus variation, transmission and pathogenicity	Robinson D J
406	SCR/406/93	ROA Fungus-transmitted viral pathogens of potato, cereal and peanut: fundamental model studies and comparative analyses of their genomes, gene expression, transmission by fungi and molecular cytopathology	Wilson T M A
407	SCR/407/93	ROA Fundamental studies on the structure and function of nucleoprotein particles of whitefly-transmitted geminiviruses	Robinson D J
408	SCR/408/93	ROA Methods and techniques for electron microscopy and their application to the study of viruses and other plant pathogens	Roberts I M
409	SCR/409/93	ROA Establish methods for cloning antibody-coding sequences to produce recombinant antibodies from bacterial cultures	Torrance L
410	SCR/410/93	ROA Host gene-mediated and transgenic resistance: a study of inheritance, expression and molecular mechanisms to improve crop protection against four important potato viruses	Barker H
411	SCR/411/93	ROA Model studies on the molecular pathology of virus-plant interactions with particular emphasis on turnip mosaic potyvirus in *Arabidopsis thaliana* and tobacco mosaic virus coat protein in chloroplasts	Wilson T M A
412	SCR/412/93	ROA Transformation of *Rubus, Ribes, Fragaria* and *Vaccinium* and evaluation of the biological value of the resultant transgenic plants	McNicol R J
413	SCR/413/93	FF Development of improved diagnostic tests for potato virus Y in a post-harvest tuber testing scheme	Barker H
414	SCR/414/93	FF Carbon partitioning: role of rhizosphere carbon-flow in regulating soil microbial diversity and activity	Griffiths B S
415	SCR/415/93	FF Antibody gene repetoire cloning to produce a diverse array of specific antibodies	Torrance L
416	SCR/416/93	FF Food web analysis of below-ground components of grassland ecosystems using natural abundances of stable isotopes	Handley L L
4001		SEED Identification of mature characters in the juvenile phase of woody species	McNicol R J
4003		SEED Stable isotope research	Scrimgeour C M

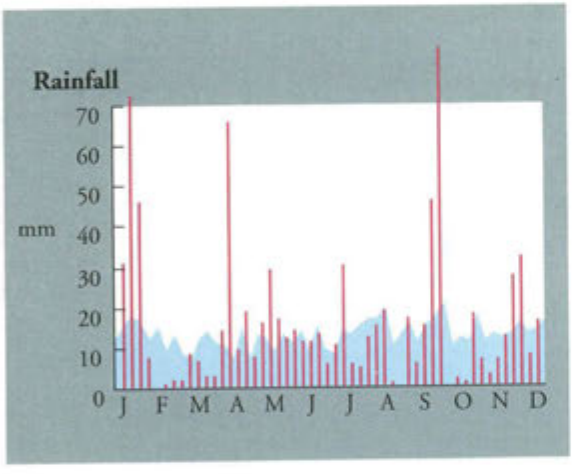
Meteorological Records

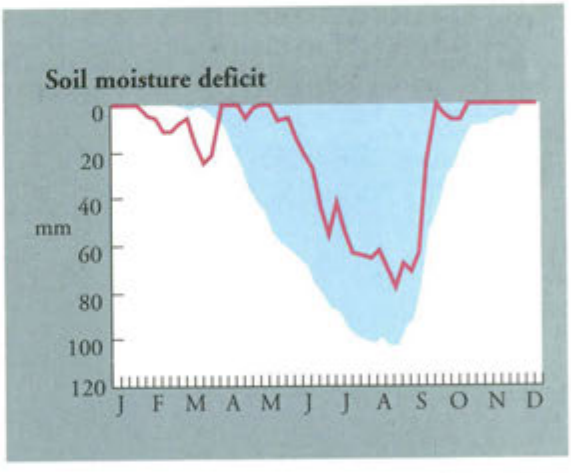
D.K.L. MacKerron

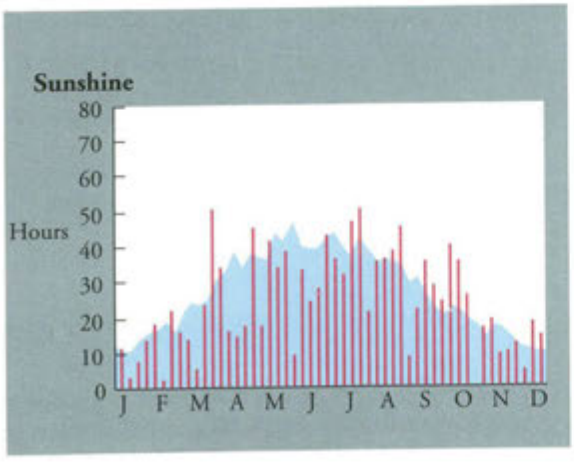
Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 1993 and the long term average for 1961-1990( ).

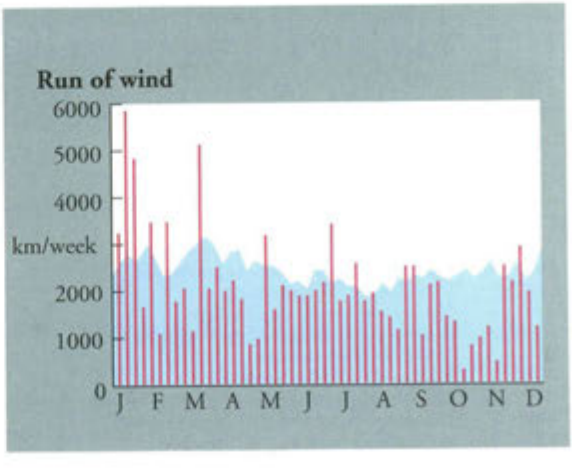












Institutes supported by the Biotechnology and Biological Sciences Research Council

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BBSRC Computing Division	West Common, Harpenden, Herts AL5 2JE	05827-62271
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Laboratory of Molecular Signalling	Dept of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ	0223-336600
Institute for Animal Health	Compton, Near Newbury, Berkshire RG16 0NN	0635-578411
Compton Laboratory	Compton, Near Newbury, Berkshire RG16 0NN	0635-578411
Pirbright Laboratory	Ash Road, Pirbright, Woking, Surrey GU24 0NF	0483-232441
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Broom's Barn Experimental Station	Highham, Bury St. Edmunds, Suffolk IP28 6NP	0284-810363
Institute of Food Research	Earley Gate, Whiteknights Rd, Reading RG6 2EF	0734-357055
Norwich Laboratory	Norwich Research Park, Colney, Norwich NR4 7UA	0603-56122
Reading Laboratory	Earley Gate, Whiteknights Rd, Reading RG6 2EF	0734-357000
Institute of Grassland and Environmental Research	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	0970-828255
Aberystwyth Research Centre	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	0970-828255
North Wyke Research Station	Okehampton, Devon EX20 2SB	0837-82558
Bronydd Mawr Research Station	Trecastle, Brecon, Powys LD3 8RD	0874-636480
Trawsgoed Research Farm	Trawsgoed, Aberystwyth, Dyfed SY23 4LL	09743-615
John Innes Centre	Colney Lane, Norwich NR4 7UH	0603-52571
Roslin Institute	Roslin, Midlothian EH25 9PS	031-440-2726
Silsoe Research Institute	Wrest Park, Silsoe, Bedford MK45 4HS	0525-60000
Horticultural Research International	Wellesbourne, Warwick CV35 9EF	0789-470382
HRI, East Malling	West Malling, Maidstone, Kent ME19 6BJ	0732-843833
HRI, Littlehampton	Worthing Road, Littlehampton, West Sussex BN17 6LP	0903-716123
HRI, Wellesbourne	Wellesbourne, Warwick CV35 9EF	0789-470382

Scottish Agricultural and Biological Research Institutes

Hannah Research Institute	Ayr, Scotland KA6 5HL	0292-76013
Macaulay Land Use Research Institute	Craigiebuckler, Aberdeen AB9 2QJ	0224-318611
Moredun Research Institute	408 Gilmerton Road, Edinburgh EH17 7JH	031-664-3262
Rowett Research Institute	Greenburn Road, Bucksburn, Aberdeen AB2 9SB	0224-712751
Scottish Crop Research Institute	Invergowrie, Dundee DD2 5DA	0382-562731
Scottish Agricultural Statistics Service (Administered by SCRI)	University of Edinburgh, James Clerk Maxwell Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JZ	031-650-4900

List of Abbreviations

AAB	Association of Applied Biologists
ADAS	Agricultural Development and Advisory Service
AFRC	Agricultural and Food Research Council
AFRS	Agricultural and Food Research Service
BCPC	British Crop Protection Council
BSPB	British Society of Plant Breeders
BTG	British Technology Group
CAPS	Cleaved AmplifiedPolymorphic Sequence
CEC	Commission of the European Communities
CIP	International Potato Centre - Peru
COST-88	European Co-operation in the field of Scientific and Technical Research
EAPR	European Association for Potato Research
ECRE	Edinburgh Centre for Rural Economy
ECSA	European Chips and Snacks Association
EHF	Experimental Husbandry Farm
ELISA	Enzyme linked immunosorbent assay
EPPO	European Plant Protection Organisation
ESTs	Expressed Sequence Tagged Sites
FF	Flexible Funding (SOAFD)
FLAIR	Food-Linked Agro-Industrial Research
GIUS	Glasshouse Investigational Unit for Scotland
H-GCA	Home-Grown Cereals Authority
HDC	Horticultural Development Council
HPLC	High Performance Liquid Chromatography
HRI	Hannah Research Institute
IACR	Institute of Arable Crops Research
IOBC	International Organisation for Biological Control
ISHS	International Society for Horticultural Science
ISPP	International Society for Plant Pathology
IVEM	Institute of Virology and Environmental Microbiology
MAFF	Ministry of Agriculture Fisheries and Food
MLURI	Macaulay Land Use Research Institute
MRI	Moredun Research Institute
NERC	National Environmental Research Council
NFT	National Fruit Trials
NIR	Near Infra-Red
NMR	Nuclear Magnetic Resonance
NPTC	National Proficiency Test Council
ODA	Overseas Development Administration
ORSTOM	Organisation for research in science and technology over-seas
PCR	Polymerase Chain Reaction
PMB	Potato Marketing Board
PVRO	Plant Variety Rights Office
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RRI	Rowett Research Institute
SABRI	Scottish Agricultural and Biological Research Institutes
SAC	Scottish Agricultural College
SARI	Scottish Agricultural Research Institutes
SASA	Scottish Agricultural Science Agency
SASS	Scottish Agricultural Statistics Service
SCRI	Scottish Crop Research Institute
SET	Scottish Enterprise Tayside
SNSA	Scottish Nuclear Stocks Association
SOAFD	Scottish Office Agriculture and Fisheries Department
SSCR	Scottish Society for Crop Research
SSPDC	Scottish Seed Potato Development Council
STS	Sequence Tagged Sites
TRIO	Tayside Regional Industrial Office
UNDP	United Nations Development Programme
WHO	World Health Organisation

The Scottish Crop Research Institute

Site plan

Access to Scottish Crop Research Institute

SCRI is on the east coast of Scotland, midway between Edinburgh and Aberdeen.

It is located at Invergowrie on the main A90 road 6km west of the centre of Dundee.

British Rail has direct InterCity services between Dundee and London, Edinburgh and Glasgow and other UK cities.

Flights are available to Dundee Airport from Manchester and Aberdeen, and scheduled services operate from many domestic and international destinations to Edinburgh and Glasgow