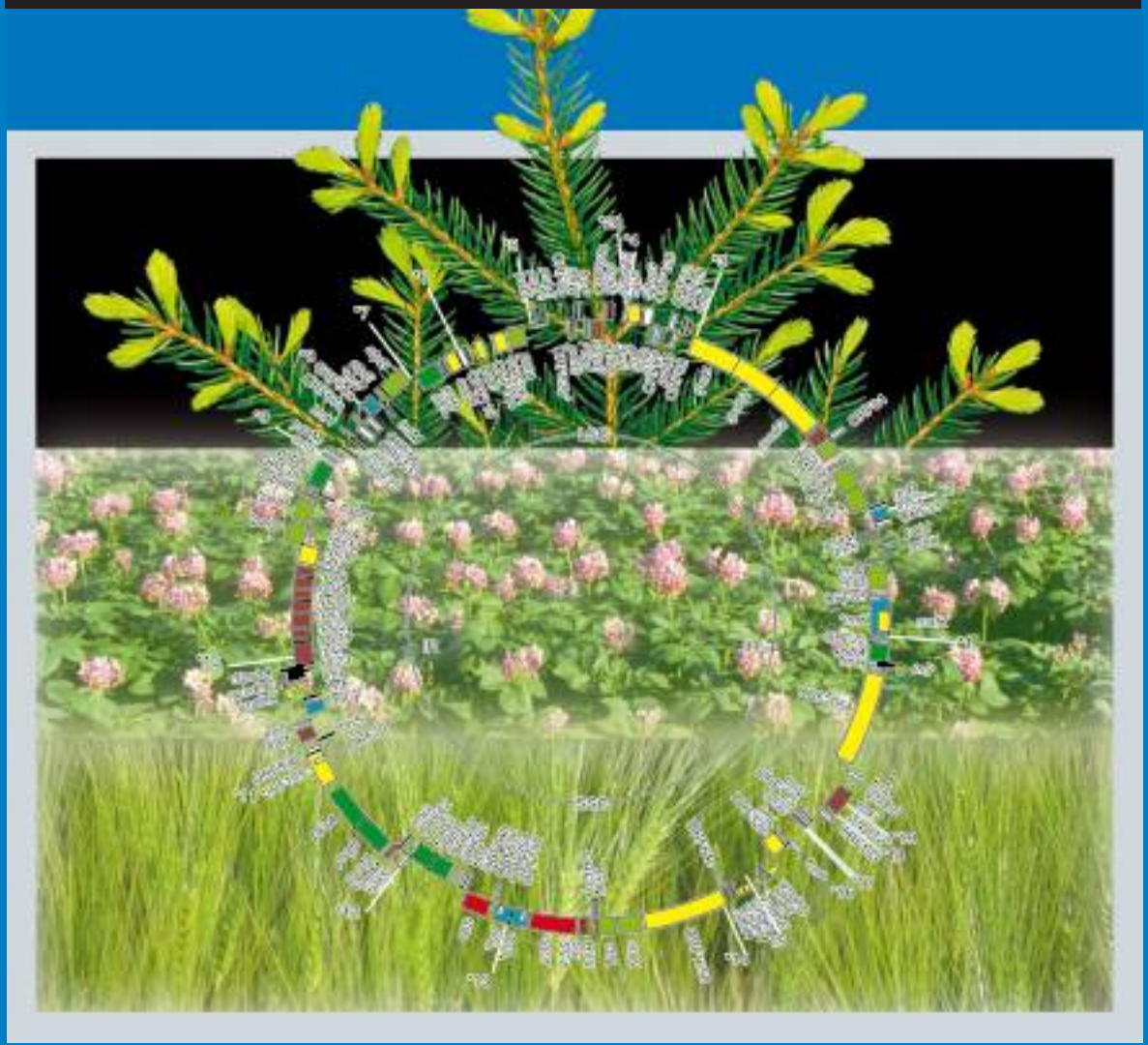
Scottish Crop Research Institute

Annual Report 1994

Contents on page 4



Governing Body

Chairman
J.L.Millar, C.B.E., C.A.

Professor Heather M. Dick, M.D., F.R.C.P. Glas., F.R.C.Path., F.I.Biol., F.R.S.E.
J.B. Forrest, F.R.Ag.S.
J.E. Godfrey
Professor J.D. Hayes, B.Sc., M.S., Ph.D., F.I.Biol.
J.A. Inverarity, O.B.E.
A.M. Jacobsen
Professor D.L. Lee, B.Sc., Ph.D., C.Biol., F.I.Biol., F.R.S.A.
A. Logan
A.N. MacCallum, B.Sc.. (w.e.f. 1/4/95)
Professor T.A. Mansfield, Ph.D., F.I.Biol., F.R.S.
Professor J.W. Parsons, B.Sc., Ph.D., F.I.Biol.
Professor J.A. Raven, M.A., Ph.D., F.R.S.E., F.R.S.
G. Rennie
Professor A.R. Slabas, B.Sc., D.Phil. (w.e.f. 1/4/95)
L.M. Thomson
T.P.M. Thomson

Accountants : KPMG, Royal Exchange, Dundee.

Solicitors : Dundas and Wilson C.S., Saltire Court, 20 Castle Terrace, Edinburgh.

Banking : Bank of Scotland, 11-19 Reform Street, Dundee.

Patent Agents : Murgitroyd & Co, 373 Scotland Street, Glasgow.

Supervisory Medical Officers : Dr Ann Simpson & Dr S Mitchell, Occupational Health Service, Panmure House, 4 Dudhope Terrace, Dundee.

Occupational Health and Safety Agency : Mr J.R. Brownlie, 18-20 Hill Street, Edinburgh.

Scottish Crop Research Institute
Invergowrie, Dundee DD2 5DA, Scotland, UK.

A charitable company (No. SC006662) limited by guarantee No. 29367 (Scotland) and registered at the above address.

Telephone : 01382 562731
Fax : 01382 562426
Telex : 93121 33325 SC G
CGNET : 10087 NQQ003
Electronic Mail
JANET : mail@scri.sari.ac.uk

Editors : D.A. Perry, T.D. Heilbronn
Graphics and Design : I.R. Pitkethly, G. Menzies
Photography : T.G. Geoghegan, S.F. Malecki, G. Menzies

ISSN 0263 7200
ISBN 0 9058 7508 7

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

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

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

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

Contents

Click to go to relevent page

Contents

Report of the Director

John R. Hillman

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

Contrasting with the stable relationships between major powers throughout 1994, conflicts in various parts of the world demonstrated the impacts of religious, national and ethnic identities, and the limited ability of those powers collectively to restore peace. Disintegration of the former Yugoslavia; ethnic strife in Rwanda, the Caucasus and Central Asia; and troubles in Afghanistan, Algeria, Iraq, Liberia, Mexico, Somalia, and Yemen, diverted attention from the emergence of a free South Africa ruled by the majority, an armistice in Northern Ireland after more than 20 years of acute terrorism, re-establishment of an elected authority in Haiti, and enormous gains in the Arab-Israeli peace process. Palau was admitted to the General Assembly of the United Nations (UN) as the 185th member.

Economics

The year marked the 50th anniversary of the UN Monetary and Financial Conference (Bretton Woods Conference). Partly inspired by John Maynard Keynes, representatives of 44 countries met in July

1944 to establish arrangements to ensure an open but orderly world trading system in which capital and payments flowed freely. It was recognised that capital and foreign currency would be necessary to aid reconstruction and assist certain countries meet balance-of-payments needs. The US dollar was agreed as the world's reserve currency and two institutions were established, *viz*. the International Monetary Fund (IMF) and the International Bank for Reconstruction and Development (World Bank). Then, as now, the private sector was seen as the main economic dynamo. Since then, the wisdom of the initiative is manifest in an increasingly open and integrated world economy in which living standards, income, life expectancy, literacy and health standards have risen at a speed and on a scale unmatched by any other period of history. In 1993, over $175 billion of private capital was invested in the less-developed countries (LDCs). The World Bank maintains a major role in funding agricultural research and development.

World economic output and growth recovered strongly during 1994, largely attributable to faster growth in the US and China, a well-established recovery in the UK, an upturn in continental Europe, and a bottoming out of the recession in Japan. IMF estimates indicated a global economic growth averaging 3.1% in 1994 compared with 2.3% in 1993. For the third year in succession, the economies of the LDCs grew faster than those of the more-developed countries (MDCs), at 5.6% compared with 2.7% respectively, and exceeded population growth leading in turn to a slight rise in personal living standards. In the case of the G-7 economies (Canada, France, Germany, Italy, Japan, UK, and the USA) the economic cycle remained desynchronised, whereas the economies of most Asian countries continued to surge.

Inflation rates in most countries continued to fall in 1994, declining to around 2.5% in countries of the Organisation for Economic Co-operation and Development (OECD). Nonetheless, the long downward trend in interest rates essentially ended in 1994, and in some countries the trend was reversed such that real interest rates were regarded as historically high.

Despite the economic recovery, unemployment in the MDCs remained at high levels. Average unemployment in the European Union (EU) rose from 10.6% in 1993 to 11.5% in 1994. A rise in unemployment to 8.5% by the end of 1994 was forecast in the OECD countries. Both the private and public sectors continually restructured and streamlined operations in a quest for efficiency, thereby generating job insecurity. Governments concentrated on reducing their budget deficits, a legacy of the recession and high social-security spending.

In the international stock exchanges, 1994 was regarded generally as a year of decline and volatility, reversing the gains of 1993. There were also large swings in the foreign exchange markets.

Although the IMF expected the external debt of the LDCs to rise by around 8%, similar to 1993, as a proportion of exports of goods and services, it was expected to be significantly lower than in 1993.

Revised estimates from the IMF suggested that world trade grew by 4% in 1993, chiefly as a result of increased trade between the MDCs. In 1994, the volume of international trade grew by 7%, substantially in excess of the long-term growth rate of 5%.

In April at Marrakech, Morocco, the seventh series of international trade negotiations under the Uruguay round of the General Agreement on Tariffs and Trade (GATT), that began in Punta del Este in 1986 and led to a 22,000 page final document, eventually came to fruition. GATT was replaced by the World Trade Organization (WTO), which would oversee compliance with new regulations designed to liberalise international trade and improve the economies of all countries. The pact was scheduled to take effect in January 1995 even though it had not been formally ratified by the 145 signatory nations. In the closing stages of negotiations, the USA and certain other countries proposed confining GATT advantages to those countries observing fair labour standards, but the proposal was opposed by LDCs as a contrivance of MDC protectionism.

In October, the Geneva-based World Intellectual Property Organization opened its new Arbitration Centre (International Centre for the Resolution of Intellectual Disputes). In developing a judicial aspect to its activities, which for more than 100 years have sought to harmonise international laws relating to copyright, patents and trademarks, it will interdigitate with the commercial arbitration services provided by the International Chamber of Commerce in Paris.

All 18 economic powers comprising the Asia-Pacific Economic Cooperation (APEC) agreed in November to liberalise trade through the elimination of trade barriers by the year 2020. Representing 38% of the world's gross national product, APEC represents a

formidable trading bloc. Likewise, the creation of the Free Trade Area of the Americas (FTAA) by 2005 would itself constitute the largest trade organisation in the world. Currently, its members have a combined purchasing power of $13 trillion, and FTAA is envisaged to encompass existing regional trade agreements. Of independent countries in the Americas, only Cuba was not invited to attend the inaugural meeting of 34 western hemisphere nations. In the EU (formally known as the European Community - EC), increased nationalism was noted in the major member nations. Even so, the accession of Austria, Finland, Sweden and Norway was successfully negotiated, although the vote of the popular referendum in Norway led to that country withdrawing from accession. Ratification of the three remaining new members by the existing members was impeded by Spain which made its consent conditional on accession rights for its fishing fleet to UK waters. A compromise was reached at the end of the year clearing entry for the three new members at the beginning of 1995.

EU member states began setting out their stances for the forthcoming Intergovernmental Conference scheduled in 1996 to review the Maastricht Treaty and the EU institutions. European Parliamentary elections took place, with a low turnout and with growing opposition to integration and the aims of the Maastricht Agreement on political, economic and monetary unification, albeit operating with the principle of subsidiarity. There would appear to be deep-seated suspicion or animosity towards centralised government and oppressive bureaucracy. The Prime Minister of Luxembourg, Mr Jacques Santer, was appointed to succeed Mr Jacques Delors as President of the European Commission. France and the UK were physically inter-connected by the opening of the Channel Tunnel (Eurotunnel), one of the greatest engineering feats this century.

During 1994, the UK economy experienced an investment- and export-led acceleration in recovery, and grew by 4%. A favourable combination of rapid expansion, quelled inflation, relatively stable interest rates, booming exports, and falling unemployment was not, however, translated into a new political parameter, the 'feel-good' factor.

Populations

Estimates by the Population Reference Bureau pointed to a world population of 5,607 million in mid-1994, about 90 million greater than the previous year. Overall, the annual rate of increase slightly slowed to 1.6% in 1994 from 1.64% in 1993, reflecting declines in birth rates in both LDCs and MDCs. Over 80% of the population growth in the MDCs took place in the USA. LDCs accounted for an even larger share of world population growth, with 97% of this share occurring in the world's poorest nations. Around 33% of the world's population was below 15 years of age, but the figure was 39% in LDCs. Only 4% of the population in LDCs was over 65, compared with 13% in MDCs, and 65% of the LDC population was rural compared with 26% in MDCs. Birth rates were showing signs of declining in Sub-Saharan Africa for the first time in recorded history. Throughout the world, the total fertility rate (average number of children a woman would bear in a lifetime) was seen to fall. By 1994, with an annual growth rate of only 0.1%, Europe had virtually reached zero population growth, although urbanisation pressures were marked in certain EU countries. Coinciding with migration pressures induced by wars, economic dislocation and social unrest, the MDCs tightened their immigration and refugee laws and procedures.

At the third UN-sponsored International Conference on Population and Development held in Cairo in September, 175 countries debated a 20-year Program of Action, and dealt with interrelated issues such as birthrate control, family planning, abortion and the status of women.

The annual report 'State of World Population 1994', released by the UN Population Fund (UNFPA) flagged a long list of economic, social and environmental concerns that flow from its range of projections, covering the diverging fortunes of different regions, the effects of birth rates and increasing life expectancy. This report discussed fears of an overall global shortage of food of the kind voiced by the 'Club of Rome' school of forecasters in the 1970s, pointing out that during the past 10 years, world food production has increased by 24%, outpacing the rate of population growth. Again, there was an assumption of improving efficiency and yield, which will have to be research-based, taking place on non-deteriorating land. Water as much as food, may prove an increasing cause of friction between peoples, as will the impacts of urbanisation and pressure for immigration.

Environment

The June 1994 deadline for instituting the desertification treaty and action plan agreed in Agenda 21 of the 1992 UN Conference on Environment and Development (Earth Summit) was postponed until

October. Although the UN General Assembly had stated that priority should be given to Africa, various Asian and Latin American countries refused to accept the decision, and the nations most likely to provide the bulk of the funding were uneasy about the potential costs of the provisional plans.

Arising from Agenda 21, an International Conference on Chemical Safety was held in Stockholm under UN auspices. From this gathering, the delegates of the 130 participating countries agreed to establish an International Forum on Chemical Safety to help integrate efforts to promote safety in the trade, use and disposal of toxic substances.

A study commissioned by the countries participating in the Global Environment Facility (GEF) concluded that an independent Secretariat be appointed, removing control from the World Bank, following the lack of agreement between LDCs and MDCs. In March, GEF funds were boosted by $2 billion.

Environmental issues in 1994 included air pollution, depletion of stratospheric ozone, emission targets for "greenhouse" gasses, contamination of fresh water, marine pollution, toxic wastes, radioactive contamination and the effects of power lines. The first meeting of the signatories to the Convention on Biological Diversity, the Rio Treaty, was held in December. In November, the 1982 UN Convention on the Law of the Sea came into force, and included the setting up of the International Seabed Authority.

Plant conservation efforts continued with the trend of international networking. In May, the European Network for Botanical Gardens was inaugurated and in October, following a meeting at the Royal Botanic Garden, Edinburgh, a parallel network was launched. Botanic Gardens Conservation International opened a regional office in Utrecht. The year was marked by new training courses, integrated conservation strategies for various species (e.g. *Sophora toromiro*, *Alsinidendron trinerve*, *Tecophilaea cyanocrocus*, *Silene tomentosa* and *Caesalpina echinata*), and the discovery of two supposedly extinct species - the Wollemi pine in Australia and *Ephemerum capensi* in Lesotho.

Internationally networked plant genebanks and germplasm collections of agricultural significance, such as those held at SCRI, are coordinated through the International Plant Genetic Resource Institute in Italy, and several of the other International Agricultural Research Centers (e.g. CIP, ICRISAT, ICARDA) supported by the Consultative Group on International Agricultural Research. Particularly at the national level, funding authorities have sought evidence of utility of the collections, and tried to displace on to others the burden of maintenance. Categories of goods and services which cannot be classified in the conventional market system give rise to serious problems of valuation, hence the growing appreciation for economic terms such as externalities, costs or benefits, to analyse how certain activities of one kind can affect even apparently unrelated activities, often wealth-creating, elsewhere.

In the areas of environment-economic performance, and the development of environmental satellite accounts, attempts are being made to construct indicators of performance and valuation. Sustainability was a much-banded-about phrase in 1994, but needs to be viewed in its relationship to economics in the widest sense, socio-cultural changes, politics, environmental impacts, moral dimensions and intergenerational equity. Agenda 21 sets out principles for sustainable development, tending to advocate the so-called strong, precautionary position, in which environmental degradation and loss of natural resources are seen to be creating uncompensated losses for the future. Decoupling of economic activity and adverse environmental impact can only be mediated by technical and scientific progress and innovation, but total decoupling is not possible. Nonetheless, in this lies much of the rationale for investment in agricultural, biological and environmental sciences.

Humans are the most important geomorphic agent shaping the surface of the Earth, shifting around 30 Gt (billion tons) per year, not including the 10 Gt per year of river sediment due to agriculture. Wave action, wind, slope processes and glaciers only account for about 6.8 Gt per year, and rivers move about 24 Gt of sediment per year, including the agriculturally derived portion.

Central to the UN Environment Program is the Global Biodiversity Assessment, which arose out of the 1992 GEF Scientific and Technical Advisory Panel on Biodiversity and on Conventions and Research. Draft texts of sections for a forthcoming volume were prepared, and involved an extensive range of authors and co-ordinators. In human history, while around 7,000 species of plants have been exploited, now there are only 103 species supplying 93% of our food supply; of these only 20 supply 90% of world food, and only 3 (wheat, maize and rice) supply 50%. In commercial terms, only 15 species are of

major importance, but there are possibly thousands of other species used as medicines, herbs, spices, flavourings, fumatories and masticatories, and for ornamental, constructional, and lifestyle purposes. Even so, the genetic base is far too narrow.

Agriculture

The world's total agricultural production increased by slightly more than 2% in 1994, according to preliminary estimates of the UN Food and Agricultural Organization (FAO), with much of the recovery attributable to a recovery of output in the MDCs. Output in the 'countries in economic transition' in Eastern Europe and the former Soviet Union may have fallen by as much as 5%. *Per capita* food production rose in both LDCs and MDCs.

International agricultural trade was beginning to be influenced by the agreement reached in the GATT multilateral trade negotiations concluded in December 1993. Although the agreement is designed to reduce progressively the level of specified agricultural subsidies, the subsidies were not eliminated. The European Parliament approved a $98 billion agricultural budget providing price supports and other subsidies under the Common Agricultural Policy (CAP). By December, however, the USA sought compensation for US exports likely to be lost because of tariffs to be raised in 1995 in connection with enlargement of the EU from 12 to 15 members.

World cereal production was forecast by the US Foreign Agricultural Service to increase from 1696 million metric tons (mmt or million tonnes) in 1993 to 1746 mmt in 1994, but increased utilization of cereals from 1749 mmt in 1993 to 1759 mmt in 1994 was forecast to lead to a depletion in ending stocks to just 303 mmt. Wheat production was expected to decline, and even with a reduction in wheat consumption, world wheat stocks as a proportion of wheat use were likely to fall to the lowest levels since the years leading up to the world food crises in the 1970s. Except for the Food Security Wheat Reserve of 4 mmt, the USA had virtually eliminated government-held wheat stocks. Similarly, intervention stocks in the EU had been largely directed as feed towards the domestic livestock market. World production of coarse grain was expected to increase by around 10%, as a result of excellent crops in the USA, India, Eastern Europe and China, but elsewhere aggregate output was reduced.

Total oilseed production was also forecast to increase by more than 10% in 1994-1995, with increases in soy(a)beans, cottonseed, peanuts, sunflower and rapeseed. Oilseeds ending stocks were expected to rise from 20 mmt in 1993 to 30 mmt in 1994, edible vegetable oils from 61 mmt to 64 mmt, and high-protein meals from 128 mmt to 138 mmt. Global demand for vegetable oils outpaced that for protein meal destined for animal feed. Prices for vegetable oils were strong in 1994, with the lowest oilstocks-to-use ratio in 20 years. Palm oil production although substantial was disappointing given the massive increase in plantation area.

Root crops (c. 580 mmt), potatoes (c. 270 mmt), pulses (57 mmt), fruits (370 mmt), nuts (5 mmt) and natural rubber (5 mmt) all sustained production figures similar to 1993.

Global centrifugal sugar consumption of 114 mmt was expected to exceed output for the third year in a row, such that sugar supplies were down to their lowest level in six years, and exports were forecast to decline from 30 mmt in 1993 to 28 mmt in 1994.

An export-retention scheme instigated by the new World Association of Coffee Producing Nations was suspended shortly after coffee prices accelerated in the wake of severe frosts in Brazil. Total production was still expected to grow modestly to over 94 million 60 Kg bags but beginning stocks in exporting countries were forecast to decline from 42 million 60 Kg bags in 1993/94 to 35 million 60 Kg bags in 1994/95. Tea production was relatively constant at about 2 mmt.

World cocoa bean production, set to rise from 2.48 mmt in 1993/94 to 2.54 mmt in 1994/95, has benefited from stronger demand. A new five-year International Cocoa Agreement, structured by the International Cocoa Organization, started to operate in 1994 by attempting to influence prices by production controls.

A new EU quota and licensing system continued to favour importation of bananas from former European colonies in Africa and the Caribbean, many dependent on banana exports. The new regime was ruled by two GATT panels to discriminate unfairly against cheaper, higher-quality Latin-American bananas, and a new 'framework agreement' was reached to address the problem. World cotton production and consumption were expected to be more or less in balance following two years of substantial drawdown in global stocks. Cotton crops in India, Pakistan and China suffered major losses through disease, whereas production leapt in Brazil. In 1994 it was calculated that

Scottish Crop Research Institute, Invergowrie, Dundee and the Sidlaw Hills.

world consumption of all types of fibre for textiles was approximately 39.8 mmt, of which 19.1 mmt (48% of word fibre market) was cotton. Needs for this crop include resistance to pests, diseases and abiotic stress, naturally pigmented fibres, and greatly improved yield efficiency.

Food aid

Food aid and emergencies were much in evidence during the year, not only in parts of the former Yugoslavia. There were special problems for refugees in Rwanda and surrounding countries. Famine conditions were experienced in the Horn of Africa, and major assistance was sought for Ethiopia, Eritrea, Somalia and the Sudan. In West Africa there was an overall improvement but the prolonged civil war in Liberia increased reliance on food aid. In Southern Africa, food supplies were short in Angola whereas a good grain harvest was recorded for Mozambique. Swaziland, Kyrgyzstan and Yemen were added to the FAO list of countries requiring either exceptional or emergency food aid. Afghanistan, Armenia, Azerbaijan, Georgia and Tajikistan also faced difficulties in food supplies, as did Cambodia, El Salvador, Honduras, Mongolia and Nicaragua.

An extension of the Food Aid Convention (FAC), due to expire in June 1995, was granted in December by the Food Aid Committee of the International Wheat Agreement. The FAC is the primary international mechanism for guaranteeing minimum availability of food aid. FAC members are apparently pledged to supply a minimum of 7,320,000 tons of wheat equivalent grain per annum, a slight reduction of 200,000 tons from the previous agreement, and to extend coverage from the LDCs to the poorer countries of the former Soviet Union and Eastern Europe. FAO reported that the equivalent of 13,340,000 tons of food aid in cereals was provided world-wide in 1993-1994, with the former Soviet bloc receiving 36% of

aid, Africa 31%, Asia 21% and Latin America 12%. Cutbacks in grain stocks and restricted aid commitments generally will mean that in 1994-1995, food aid availability will be reduced.

International initiatives relating to food production and aid included (i) the announcement of a World Food Summit in March 1996 to coincide with FAO's 50th Anniversary, (ii) the launch in October of a convention to combat desertification and land degradation, and (iii) the London Guidelines on International Trade sponsored by the UN Environmental Programme and FAO Council proposals to give LDCs a means to protect their population from the adverse effects of chemical usage.

Acquired Immune Deficiency Syndrome (AIDS) was increasingly regarded as a serious obstacle to agriculturally based LDCs, where the disease disproportionately afflicts the most productive age group (15-45 years), and also infects women more than men in societies where women contribute the bulk of the workforce. Mechanisation and improved cultivars could have a special role in AIDS-damaged communities.

Biotechnology

Targets for higher plant biotechnology cut across a diverse range of commercial applications. Since 1986 there has been a dramatic rise in transgenic field tests in many countries, principally Belgium, Canada, China, France, UK, and the US. The main species involved were, and continue to be, oilseed rape, potatoes, tomatoes, tobacco, maize and cucurbits. Virus-resistance was a popular biotechnological theme in 1994. Approval by the US Food and Drug Administration (FDA) for sale of the 'Flavr Savr' tomato, engineered by Calgene Inc. to delay the effects of the ripening process, was a landmark for the MDCs in gaining public acceptance of transgenic foods. Great sensitivity over biotechnological patents was revealed by the reaction to Agracetus Inc. gaining exclusive US rights to the production of genetically engineered cotton.

Biotechnologically based procedures now in commerce extend to diagnostics, precision selection systems, sterile mass propagation, and genetic fingerprinting. More sophisticated and rational approaches to transgenics were beginning to permeate regulatory authorities in several of the MDCs; unnecessary lengthy and convoluted statutory procedures were leading to large-scale switching of investments to less bureaucratic countries.

Food industry

Food-related companies continued to reduce overhead costs, disposing of inadequately profitable operations and reducing workforces. Sales of prepackaged non-alcoholic beverages, juices, mineral waters and convenience foods increased, and there was greater emphasis on products for children. Reduced calorie foods, decaffeinated coffee, wine and 'organic' foods declined in most MDCs; the vegetarian approach was most evident in the EU with a decline in the sales of red meat, but sales increased in the USA and Japan. Enhanced sales of red meat and dairy products in the Asian economies would have an enormous effect on the patterns of world agriculture. Established brand-name manufacturers suffered from loss of profits as a result of supermarket and retailer 'own-brand' products and the wide-scale release of 'copy-cat' products. In the UK, trademark protection was extended to encompass both the appearance of a package as well as the logo.

An enquiry into accidental poisoning by herbs and plant extracts used in cooking was initiated by the Australian authorities. In the US, the Environmental Protection Agency began to review over 80 pesticides, for compliance with the 'Delaney Clause' of a federal law prohibiting the use of carcinogenic chemicals that concentrate during food processing. Food poisoning incidents throughout the world remained at historically high recorded levels. Despite widespread efforts at deregulation, food-related laws advanced strongly. The FDA took the most stringent approach to the implementation of the 1990 Nutrition Labeling and Education Act, imposing new standards for health, nutrition and serving-size labels, to provide consumers with a reference point, the 'daily value' or average diet for a healthy adult.

Timber and wood products

Supplies of wood from natural stands and forests were in strong demand in 1994, and there was an increase in the sales of engineered wood. So-called sustainable plantations supplied just 10% of the world's industrial wood, with Argentina, Australia, Brazil, China, Costa Rica and South Africa speedily developing their plantations. Clear-felling of timbers from the northern latitudes was severely criticised by environmental groups, as were those plantations unable to sustain regional biodiversity. The Convention on International Trade in Endangered Species depressingly failed to list mahogany in its appendix of endangered species. FAO calculates that a global population growth of around 100 million people per year would lead to an annual increase of 77 million cubic metres in wood consump-

tion. For much of the world, wood is the only fuel. Paper and board production had risen to 251.6 mmt by 1993, but pulp production fell to 163 mmt. Paper production, however, increased significantly in line with increases in the recycling of waste paper. Preliminary indications are that these trends continued in 1994. As various Southeast Asian countries such as Malaysia, Thailand and Vietnam, were considering expansion of bulk pulping capacity, European and North American producers were focusing on customised, higher-value papers derived from totally chlorine-free pulping and bleaching technologies. Delignification, restriction of lignin development, cellulose engineering and timber biophysics began to attract research investment.

International research

As reported in the 1993 SCRI Annual Report, scientific research of all kinds throughout the world continued to be under great pressure as funds contracted, activities were reviewed and scientists sought to justify their employment. All too few of those responsible for funding - politicians as well as senior administrators and leaders in both private and corporate sectors - are scientifically literate, and few scientists are seemingly able to convey their achievements, role and potential. Career prospects tend to be somewhat bleak unless the delivery of readily comprehensible achievement is exceptional.

United Kingdom perspectives

At this juncture, it appears that there were complex changes taking place during 1994 in the fortunes of the agricultural, horticultural, forestry, environmental and biotechnology sectors, and their various sub-sectors. Profitability remained under pressure, and with the exception of the environmental and biotechnology industries, employment and investment declined. To a large extent agriculture remained prisoner of various perceptions, namely a sunset subsidy-addicted industry having adverse environmental impacts but receiving favoured treatment, whilst being reluctantly led by supermarkets to meet consumer needs. The reality is far different for key agriculturalists in the major industry that both shapes and acts as custodian of the landscape. Just as for nearly all of British industry, there are too few leading practitioners.

My 1993 Report detailed the tranche of policies brought in since the 1960s aimed at improving the efficiency and priority setting of public-sector science, engineering and technology. There is now a high price to pay in terms of lifestyle for accessing the public purse on the grounds of pursuing science. Such pressures will grow.

Two pivotal activities arose from the science White Paper *Realising our Potential: A Strategy for Science, Engineering and Technology* (Cm 2250, May 1993). First, the Technology Foresight Programme (TFP) started, and second there was a Multi-Departmental Scrutiny of Public Sector Research Establishments (PSREs) carried out by the Efficiency Unit of the Cabinet Office. Both activities involved SCRI.

Technology foresight

The UK TFP is a systematic, ongoing process for assessing those scientific and technological developments which could have a strong impact on industrial competitiveness, wealth creation and quality of life in the next 10 to 20 years. The aims of the much-needed Programme are to form networks and recommend priority areas for research and development funding, and for related education and training. This information would be used by Government in determining policy, and by the Research Councils and others responsible for the allocation of research funds in the public and private sectors.

Agriculture, Natural Resources & Environment	John Hillman	Scottish Crop Research Institute
Manufacturing, Production & Business Processes	David Grant	GEC plc
Defence & Aerospace	Roy McNulty	Short Brothers plc
Materials	John Campbell	Cookson Group plc
Chemicals	Alan Calder	Zeneca Specialties
Construction	Herb Nahapiet	John Mowlem & Company plc
Financial Services	Michael Huges	Barclays de Zoete Wedd
Food & Drink	Peter Lillford	Unilever Research
Health & Life Sciences	Mark Ferguson	University of Manchester
Energy	Gerald Clerehugh	British Gas Research Centre
Transport	Tom Black	Smith System Engineering
Communications	Philip Laven	BBC
Leisure & Learning	Peter Wallis	Specialist Research Unit Ltd
IT & Electronics	John Taylor	Hewlett Packard Laboratories
Retail & Distribution	Graham Winfield	Booker-Tate Ltd

Table 1 Sector panels of the Technology Foresight Programme and their Chairmen.

The programme began in 1993, when the Steering Group on Technology Foresight was established in the Office of Science and Technology, under the chairmanship of Professor Sir William D. P. Stewart, the Chief Scientific Adviser to the Government and former member of the SCRI Governing Body. In Spring 1994, the Steering Group established 15 Sector Panels of experts to carry forward detailed technology foresight work on defined sectors covering the whole UK science base (Table 1). I chaired the Panel on Agriculture, Natural Resources and Environment (ANRE) which had substantially completed its Final Report by the end of the year, publication taking place in April 1995.

The ambit of the ANRE Panel embraced land-based agriculture (including food and non-food products); horticulture; forestry; aquaculture; fossil fuel and mineral extraction; and all aspects of environmental protection (terrestrial, freshwater, marine and atmospheric). In addition to the major direct contribution to GDP by the ANRE sector, it also underpins such major sectors as the food industry, the provision of fresh water, the construction and energy industries, and addresses environmental issues ranging from biodiversity to long-term concerns about the impact of global warming and waste disposal and treatment.

The Panel comprised 21 experts drawn from these diverse fields, supplemented by four Sub-Groups where additional expertise was represented (Table 2). Views of the Panel were augmented by a questionnaire survey of some 50 other experts and a Delphi survey of over 1,100 experts from industry, research institutes and academe. Many workshops and seminars were conducted throughout the UK, and written submissions were received from numerous individuals and organisations. All of these inputs contributed to the final recommendations. Moreover, in considering the recommendations, it was recognised that it would not be possible to use conventional measures of wealth creation in comparing issues concerning the environment and quality of life, with direct market opportunities (such as new products and processes). Issues such as the responsible husbandry of finite resources, sustainability, environmental impact, and animal health and welfare dictate that prioritisation involves political dimensions as well.

Pressures for change In all of the areas considered by the Panel, there are major pressures for change e.g.

Agriculture Increasing international competition and impact of the WTO; use of land for non-food crops and for conservation, development and access; growing resistance to productivity gains at the expense of environmental losses (e.g. pollution, loss of habitat, erosion); threat to plant and animal health from removal of trade barriers; public acceptability of systems for animal-based food production; and decreasing financial support from the CAP.

Marine Increased use for leisure; protection from pollution of the sea, and of food derived from it; improved management of fish stocks; and increasing culture of fish, shellfish and algae.

Oil and Gas Improved recovery from mature fields; recovery from marginal fields or more hostile environments (e.g. deeper water); and decommissioning and dealing with post-production pollution.

a) **Membership**

John Hillman (Chairman)	SCRI
Iain Cubitt (Vice-Chairman)	Axis Genetics
John Casey	Unilever Research
Roland Clift	University of Surrey
Malcolm Crabtree	Leckford Estates
Annette Cutler	Technology Initiatives Ltd.
Ben Gill	NFU
Brian Heap (Agriculture)	Babraham Institute
Julie Hill	The Green Alliance
John Lawton	Imperial College
Ian McConnell	Cambridge University
John Marsh	Reading University
John Mather	Royal Holloway & Bedford New College, London
Marik Meyer	Environmental Consultant
Susan Owens	Cambridge University
David Parkes	Shell Research
Jan Pentreath	National Rivers Authority, Dept of Environment
Neil Roberts (Natural Resources)	Mineral Industry Research Organisation
Mark Tinsley	PC Tinsley Ltd.
Kerry Turner (Environmental)	University of East Anglia
David Brooks (Facilitator)	Consultant
David Watson (Secretary)	OST

b) **Steering Group Assessors**

William Stewart	Chief Scientific Adviser and Chairman of the Steering Group
Derek Burke	University of East Anglia and member of the Steering Group

Departmental Assessors

Ann Eggington	DTI
David Fisk	Dept. of the Environment
David Shannon	MAFF

Table 2 Technology Foresight Panel on Agriculture, Natural Resources and Environment -
a) Membership - 20 members plus secretary, and a facilitator who provides advice on foresight methodology,
b) Assessors provide links with the parent Steering Group on Technology Foresight or Government Departments.

Industrial and Construction Materials Pressures for reuse and modernisation rather than new build in the developed world, and enormous growth in demand in the developing world, as a result of increasing population and rural/urban migration.

Environmental Issues at both global and local levels pervade all of the above activities. The environment provides both an opportunity and a constraint. It can be viewed as a stock of natural capital whose sustainable use is the foundation for wealth creation and an acceptable quality of life. Increased pressure on the environment will result from population growth and redistribution, increased scale of economic activities, demand for potable water, and changes in attitude and lifestyle. There will be increasing pressure on resources (e.g. raw materials, energy stocks) and sinks (waste assimilation capacity of environmental media), leading to possible global climatic changes; loss of biodiversity; pressure on waste management systems; increasing severity of pollution problems; increasing concern over the unsustainability of present human activities; and divergence between expert and public perception of environmental risks.

Likely events in the next 20 years are difficult to predict because of major uncertainties over long-term influences on technology (e.g. CAP, GATT, environmental policies, research funding). Nonetheless, over the next 20 years the UK agricultural sector is likely to be influenced greatly by imports of basic foodstuffs from ever-widening geographical and horticultural sources. Our farmers will need to concentrate on crops and livestock which can be produced most effi-

ciently in this country, probably on an area which continues to decrease. Farm incomes and rural land use, however, will be characterised by increasing diversity (new crops grown for industrial or other non-food purposes) and new recreational, conservation and amenity services for the community. The exploitation of other natural resources will be increasingly influenced by considerations of environmental protection and sustainability. The need to protect and use our environment in a sustainable way has already spawned new industrial sectors, ranging from treatment and monitoring equipment to a wide range of specialist services in activities such as environmental impact assessment, monitoring, environmental audit and pollution control. The market for these 'environmental' goods and services will continue to expand worldwide. The UK must invest in and harness its scientific, engineering and technological capabilities, not only to seize these opportunities, but also to assist agriculture and other natural resource-based industries to respond to widening competition and to the need for environmental protection, while at the same time maintaining their contribution to wealth creation.

Summary of recommendations Investment is required in the following areas:

- Animal, microbial and plant biotechnology and cognate sciences underpinning new products and processes in agriculture, horticulture, forestry, aquaculture, pharmaceuticals, land and water remediation, waste management, fossil-fuel processing, and other industries (e.g. molecular basis of plant and animal breeding, pest and disease detection and control, vaccines, metabolic engineering to provide new uses for terrestrial, freshwater and marine crops and animals). This work is crucial for our understanding of how organisms function and develop, is central to integrative bioscience, and exploits the various genome initiatives in plants and animals.
- Robotics; remote sensor and survey systems; predictive modelling in the presence of uncertainty; artificial intelligence and expert systems. These aspects are essential for agriculture, horticulture, exploration and extraction of fossil fuels, mineral and other natural resources, control of pollution and climate change studies.
- Diet and health; more healthy, attractive and better tasting food products from plants and animals, with improved safety and nutritional value, freshness, convenience, appearance and value.
- Improved technology for utilising forest products, improving wood quality, and finding sustainable substitutes for traditional hardwoods and wood pulps.
- Fin-fish, shell-fish and algae; studies on wild populations and their harvesting, management and utilisation; aquaculture, particularly with regard to biotechnology, breeding, diversification, habitats, containment structures, and environmental impacts.
- Environmental research programmes encompassing monitoring, surveys, further development of data and information systems, process studies, forecasting, prediction of climatic and geological phenomena, hazard warning and impact evaluation studies.
- Integrated ecosystem management, including maintenance, restoration, and utilisation in the context of terrestrial, aquatic, coastal and oceanic systems. Realisation and understanding of the full value of biodiversity and natural and managed ecosystems including sustainable, terrestrial and aquatic farming systems.
- Technologies for site/soil remediation, landfill management, groundwater clean-up, coastal remediation, reduction, recycling, inactivation, biodegradation, incineration, containment and exploitation of domestic and industrial wastes; measures to prevent, reduce or eliminate exposure to toxic substances and their adverse consequences; and techniques to produce, monitor, purify, conserve and distribute potable water, including desalination and other processes.
- Widespread use of life cycle evaluation and management, and eco-design principles and practice studies; evaluation of vulnerability of natural resource production and socio-economic systems to climatic, pollution, and land-use changes; clean, cost-effective sustainable technologies;

building, urban and transport design to reduce pollution and environmental impacts, and to improve energy use.

- Sustainable resourcing of construction materials and other natural resources (including novel materials, reuse of structures, production and use of biodegradable materials).
- Alternative energy sources, including coal bed methane, shale gas, waves, wind, tides, fuel-producing crops (particularly forest products), geothermal, fuel cells, and in the longer term, gas hydrates.
- Structural changes in agriculture, horticulture and waste management will demand greater vertical and horizontal co-ordination, from fundamental research to the primary producer, processor, retailer and consumer, thereby facilitating the speedy uptake of new ideas and technology (e.g. welfare-friendly systems for livestock; utilisation of animal wastes; fishmeal substitutes; new multi-option, pest- and disease-resistant crops; crops as bioreactors; precision agriculture; greater species and cultivar diversity throughout the year; on-farm added value systems; new bioremediation systems).
- Public and political understanding of science and technology, and of the balance between risk and benefit in applying new technologies, and experts' appreciation of the importance of taking proper account of the public's perspective of their work. There is also a need for legislation, training and advice to be soundly based. These aspects are critical to the biotechnology and environmental programmes.

Constraints to achieving these objectives include:

- Supply of educated and trained science, engineering and technology graduates and post-graduates.
- Marketing: better understanding of consumer preferences, and improved co-ordination from researcher to producer, and producer to consumer.
- Financial incentives for investment, particularly for small and medium-sized enterprises and new ventures.
- Experts' appreciation of the importance of taking proper account of the public's perception of their work.
- Underdeveloped technology-transfer systems.

It is essential that these constraints are overcome and that the strong science, engineering and technology base in many areas in the Panel's remit is used fully in policy making in the UK, particularly in regard to environmental regulations.

In recent years, scientific disciplines have become isolated, although many new products and processes require a multi-disciplinary approach. For example, action is needed by funding bodies to ensure that new knowledge from molecular and cell biology is translated into whole organism, population and ecological studies. This is particularly important for topics such as sustainability, biotechnology, bioremediation, plant and animal breeding and pathology, and lifetime studies of resources, and will help to ensure that they contribute to national wealth creation and improvements in the quality of life. Other examples are the integration of microengineering with biotechnology and information technology, to provide small 'smart' robots and sensors; and the linking of artificial intelligence and expert systems with monitoring systems to improve forecasting.

Generic issues Our recommendations were reinforced by similar ones from other Sector Panels (on Chemicals; Communications; Construction; Energy; Food and Drink; Health and Life Sciences; IT and Electronics; Manufacturing, Production and Business Processes; Materials; Retail and Distribution; and Transport). This broad support reflects the extensive range of industry that is underpinned by the activities reviewed by the Panel.

The TFP Steering Group sought to add value to the Sector Panel recommendations by identifying those recommendations which are likely to have the most pervading effects across a number of Sectors, i.e. generic priorities, whilst recognising that in some cases a sectoral recommendation may be of equal or greater importance for future wealth creation prospects. On the basis of wide-ranging criteria that could be brigaded into two others: attractiveness (economic and social benefits and the ability of the UK to capture these benefits) and feasibility (likelihood of scientific or technological breakthrough and the ability of the UK science base to be at or near the leading edge in obtaining tangible results), the generic priorities were prioritised into three broad groups (Table 3). Further development of the TFP was scheduled for 1995-1996.

Key topic areas
Genetics and biomolecular engineering
Bioinformatics
Telepresence, multimedia
Software engineering
Management and business process engineering
Sensors and sensory information processing
Communicating with machines
Security and privacy technology
Environmentally sustainable technology
Health and lifestyle
Optical technology
Intermediate areas
Risk assessment and management
Design and systems integration
Chemical and biological synthesis
Information management
Modelling and simulation
Catalysis
Workplace and home
Biomaterials
Materials
Process engineering and control
Materials processing technology
Emerging areas
Demographic change
Clean-processing technology
Energy technology
Life-cycle analysis
Automation

Table 3 Generic priorities in science and technology - relative assessment of attractiveness and feasibility.

Scrutiny of the PSREs

Foreshadowed in the 1993 Science White Paper, the review was commissioned by the Chancellor of the Duchy of Lancaster and prepared over half a year by an *ad hoc* Team attached to the Efficiency Unit of the Cabinet Office. Their report covered 53 (now 50, following mergers) PSREs in England, Scotland and Wales, but not Northern Ireland. Collectively, in 1992-1993, these organisations employed more than 31,000 staff and spent £1.3 billion, less than one third of the public spend on R&D. Lumped together were bodies that are diverse not only in size (employing 48 to 8000 staff), but also differing in (i) mission (from basic research in a wide range of disciplines to development, testing and statutory work not involving research to any extent), (ii) levels of commercial and academic interfacing, (iii) international roles, and (iv) constitution (e.g. Government Research Establishments, Research Council Institutes, Agencies, and Non-Departmental Public Bodies such as SCRI). Many of these organisations had recently undergone expensive and lengthy major structural and legal changes, or were in the process of merging or changing their legal status.

Patently conceptually similar organisations were not included, such as research groups in Colleges and Universities, Interdisciplinary Research Centres, the Royal Botanic Gardens at Edinburgh and Kew, and a host of other bodies. Their omission must have related to abstruse constitutional reasons (they were deemed to be "private"), rather than the greater extent to which many of them rely on public funding from Government Departments or Research Councils, and to the lesser degree of project and managerial monitoring to which most of them are subject.

No judgements were passed on: (i) national or regional requirements for the research; (ii) the quality of the work carried out; (iii) comparative cost-benefit analyses; (iv) contributions to wealth creation, the UK's competitive position and quality of life; (v) international roles and commitments; (vi) commercial links and contractual obligations; (vii) educational roles; (viii) associated societies; (ix) constitutional legal and accountancy complications; (x) consultations with the range of associated industries; and (xi) existing integrating mechanisms to ensure efficiency. Thus, remarkable as it may seem, scrutiny was not a matter of how well a body does co-ordinated research, or where it does it, or the impact of the research, or crucially, the need for that work.

The Terms of Reference distilled down to the identification and opportunities for early privatisation, rationalisation and changes to current ownership and financing. At the outset, the *status quo* was considered to be unacceptable.

Recommendations were non-specific and included transfer to universities, privatisation; a "Prior Options" process including rationalisation, re-organisation, new Chief Executives to be appointed if organisations were not under Research Council control; and in the absence of a change in organisational structure then Directors of Rationalisation to be appointed to cover all the organisations; competition assessments, rationalisation incentives, open competition to all SOAFD and Research Council funding; and the structural changes to be in place by April 1996 or Directors of Rationalisation to be appointed by April 1995. The consultation period ended on 11 November 1994 (Armistice Day).

The Scottish Crop Research Institute

SCRI is a special centre of international excellence, bridging the public and private sectors, and noted for the high-quality, innovative research encompassing its entire programme. Whilst many PSREs in recent years have been closed, merged or rationalised, SCRI has evolved dynamically, expanding in a highly competitive market. In the last decade the site at Mylnefield has received huge investments in buildings, state-of-the-art scientific facilities, and new scientific staff. Independent Visiting Group reports; performance indicators (e.g. refereed papers per scientist, cost per refereed paper, grant income, EU income etc.); market impact measures (e.g. cultivar area, food processing value etc.); education roles (e.g. university courses presented, research student training etc.); attractiveness for senior visiting scientists, governmental teams and companies; value-for-money; and rate of expansion, are unequalled within the research service. Within the last quinquennium, the establishment of the dramatically successful, award-winning technology transfer company, Mylnefield Research Services Ltd, and the appointment of its outstanding Managing Director, Dr N. W. Kerby, has complemented the efforts in redirecting the science and linking with universities and other institutions at home and abroad.

The mission of the Institute is to undertake an integrated programme of fundamental and strategic multidisciplinary research of the highest quality on agricultural, horticultural and industrial crops, their pests and diseases, and on processes common to all plants; to create and protect wealth, and to improve the quality of life and the environment. It aims to increase knowledge of the basic biological sciences, to improve crop quality and utilisation, to improve biodiversity agricultural sustainability and diversification, and to develop environmentally benign methods of protecting crops from depredation by pests, pathogens and weeds. A broad multidisciplinary approach to address the mission 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 SCRI-based international genetic resources in a region of high phytosanitary conditions, is unique within the UK research service. Synergistic and complementary 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 and MRS Ltd actively seek research contracts from Government Departments and agencies, levy boards, grower organisations, international agencies, commercial companies, local government and Trusts.

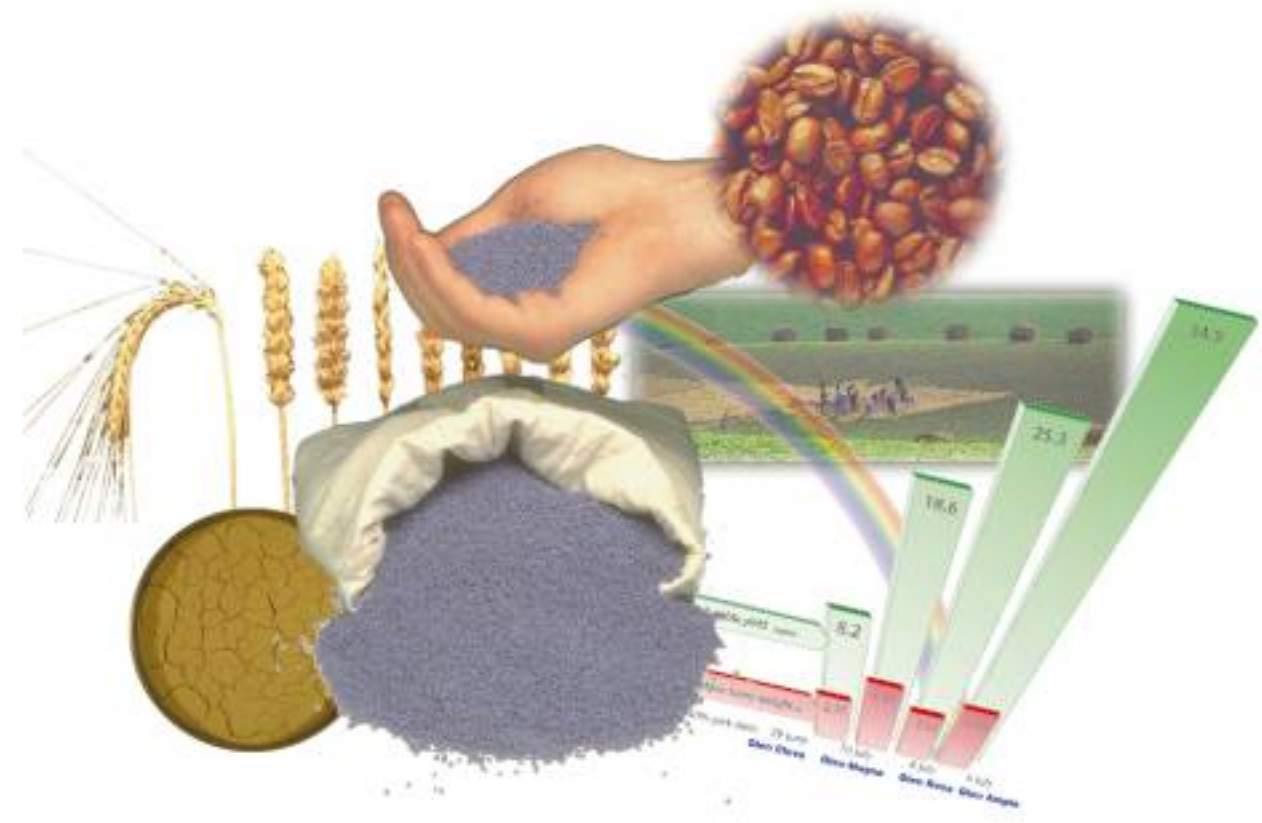
SCRI acts as a parent body for Biomathematics and Statistics Scotland (BioSS), formerly the Scottish Agricultural Statistics Service SASS), which acts as a unit of SCRI under the able leadership of R. A. Kempton. BioSS was set up in 1987 to cover the biomathematical and statistical needs of the five Scottish Agricultural and Biological Research Institutes (SABRIs - SCRI, the Hannah, Macaulay Land Use, Moredun and Rowett Research Institutes) and the Scottish Agricultural College (SAC). High-level consultancy, training and research inputs from BioSS give a major advantage to the SABRI and SAC research programmes. The next Visiting Group to BioSS is scheduled for late 1995.

To the detriment of existing and future generations of scientists there is a danger that all the hard work, invention and discovery that have gone into this Institute over the past 75 years could be dismantled, as a result of the Scrutiny exercise. The next two years will be a challenge.

This 1994 Annual Report details but a small selection of the research achievements of SCRI and MRS Ltd, the commercial successes of MRS Ltd, and the important linking role of the associated Friendly Society, the Scottish Society for Crop Research (SSCR; D. L. Hood, Secretary & Treasurer; A. M. Jacobsen, Chairman). Advances have been made in both fundamental and strategic science, with contributions to the protection and understanding of the environment, and discoveries are reported of direct and indirect benefit to agriculture, horticulture, forestry, land management and biotechnology. It is a reflection of dedicated and talented scientific and support staff in every department and section, that SCRI has attained its global stature and delivered its achievements.

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 commitment and to our development. Regardless of the pressures upon them, they function rigorously,. as always, to the highest professional standards of British public service. Grants, contracts, donations, advice and joint participation in our activities from the SSCR, other governmental departments and their agencies, non-governmental agencies, grower levy boards, local and regional authorities, commercial companies, farmers and other individuals, and learned societies, are also warmly appreciated.

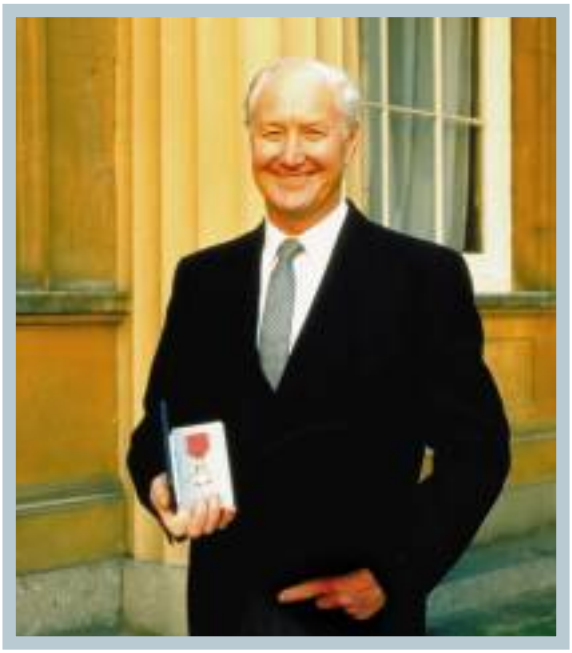
SCRI thrives and justifies its existence in every respect.



People and Events

Retirements Four long-standing, senior colleagues left during 1994.

Professor N L Innes OBE, FRSE retired in June after 10 years at the Institute. He joined SCRI in 1984 as Head of the Plant Breeding Division, and was appointed Deputy Director in 1986. His early career was spent in the Sudan and Uganda, where he became Head of the Uganda Cotton Research Unit in 1972. The following year he took up appointment as Head of Plant Breeding at the National Vegetable Research Station (NVRS), Wellesbourne, where he forged stronger links between public and private sector plant breeders, and was the first public sector Chairman of the British Association of Plant Breeders. He rose to the position of Deputy Director at NVRS in 1977. In 1977 he was awarded the degree of Doctor of Science



Professor N L Innes received the OBE from Her Majesty the Queen on 15 November.

by the University of Birmingham for his research on cotton and was elected Fellow of the Institute of Biology in 1979, Fellow of the Institute of Horticulture in 1986, and Fellow of the Royal Society of Edinburgh in 1989. From 1982-1988 he served on the Board of Trustees of the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India and more recently was elected to the Board of Trustees of the International Potato Center (CIP), becoming its Chairman in 1991. He became President of the Association of Applied Biologists in 1993, and was awarded his OBE for 'services to agricultural science' in the 1994 Queen's Birthday Honours. These honours are tangible evidence of a long, distinguished career in agriculture.

Dr M C M Pérombelon retired in June after a long, distinguished career as a bacteriologist, during which time he gained an international reputation for his expertise and pioneering studies on *Erwinia*, the potato pathogen that causes blackleg of stems in the field, and soft rot in the store. His work led to a revision of the understanding of blackleg, and an explanation of why disease control by traditional measures and seed certification had failed. He has been at the forefront of the development of sensitive tests to assess contamination, and novel methods of control. More recent work has focused on obtaining a better understanding of host-pathogen interaction as a first step towards breeding for resistance. He joined the then SHRI in 1966 and was awarded his PhD by the University of Dundee in 1973, and was an Honorary Research Fellow in the Department of Medical Microbiology at the University from 1989 to 1992. He was awarded an MBE for services to science in the 1995 New Year's Honours List.

Mr H M Lawson retired in August after 28 years as Head of the Weed Science Group at the Institute, and with over 35 years' experience of research on weeds, herbicides, crop management and the integration of weed control into farming systems. He began his working career in Norfolk, where he was responsible for herbicide research and development work in the UK and latterly in Western Europe for Dow Chemical Co. Ltd. In 1966 he left to join the then SHRI, but over the years has retained strong links with agrochemical companies. His research on crop:weed competition and many other aspects of weed and herbicide management is well-known and respected by weed scientists at home and abroad. To local fruit growers, however, he is best known for his work on the control of cane vigour in raspberries. In recent years he established SCRI as a centre of excellence for research on the effects of changes in land management, such as reductions in chemical inputs and set-aside, on weed seed populations in the soil. He was elected a Fellow of the Institute of Horticulture in 1988.

Mr W I A Jack, Head of the Estate Glasshouse and Field Experiments Department, retired in January. He had been associated with the Institute from its earliest beginnings, firstly as Foreman at the West of Scotland Unit at Auchincruive (from 1951), before being transferred to SHRI in 1967 to fill the newly created post of Field Experiments Officer. He became Manager of the Estate in 1973, and in 1975 also took on the responsibility for the glasshouses, which had, until then, been separately managed. He played a central role in field and glasshouse experimentation on which so much of the Institute's, and individual scientists' reputation rests.

Honours Two colleagues received awards in the 1994 Queen's Birthday Honours. Professor Heather Dick, who was appointed a member of the Governing Body in April 1992, was awarded a CBE for her services to medicine and food hygiene. She chairs the Advisory Committee on Microbiological Safety of Food which advises the Government. Professor N L Innes was awarded the OBE for services to agricultural science. This is a fitting tribute to mark a long, distinguished career. Dr M C M Pérombelon received an MBE in the 1995 New Year's Honours List, for services to science, in recognition of his internationally renowned contribution to the understanding of erwinias.

Mr R J McNicol, Head of the Soft Fruit Genetics Department, was presented with the Jones Bateman Cup by the Royal Horticultural Society President, Sir Simon Hornby, on 10 May 1994. The trophy is awarded every three years for significant contributions to research on fruit growing. Previous recipients include the staff of HRI East Malling, Brogdale and HRI Wellesbourne, but this is the first time that the prize has come to Scotland. When the cup is relinquished in 1997, Mr McNicol will automatically receive the Hogg Medal.

Mr R J McNicol was presented with the Jones Bateman Cup by the RHS.

The Peter Massalski Prize, for the most meritorious research by a scientist under the age of 36 at the Institute, was awarded jointly to two members of staff. The winners, Dr Robbie Waugh and Dr Karl Ritz, are both internationally recognised as being at the forefront of their fields.

Visitors 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. Professor T Blundell FRS, Chief Executive of the BBSRC visited the Institute in June, and we were pleased to welcome influential members of the Cambridge University Potato Growers' Association for two days in July. As in previous years there were several groups from The Americas, China and the Commonwealth of Independent States, including a high-ranking trade delegation from Kazakhstan. Distinguished visitors included the Argentinean Ambassador, his excellency Senor Mario Campora, and the Vice-Governor of the Hainan Province of China, Mr Wang Xueping.

Members of CUPGRA visited SCRI in June.

Professor N L Innes, Dr W T B Thomas, Dr A N E Birch and Mr T D Heilbronn, were organisers of the Association of Applied Biologists Presidential Conference on 'The Impact of Genetic Variation on Sustainable Agriculture', held in Dundee in September 1994. Several members of staff contributed to the meeting, and the delegates took the opportunity to visit the Institute as part of the programme.

SCRI: an historical perspective

D.A. Perry

The Scottish Crop Research Institute (SCRI) was formally established recently in 1981 and may be regarded as having a very short history. However, its forebears, the Scottish Plant Breeding Station (SPBS) and the Scottish Horticultural Research Institute (SHRI), from whose amalgamation SCRI arose, both have distinguished histories of achievements which have clearly influenced the directions and strengths of research at the present Institute. Therefore, it is instructive to document some of the major initiatives from their inception and their development to the present day.

SPBS

SPBS was established in 1921, along with several other research institutes, when it became realised by farmers, landowners and the Government that agricultural productivity and efficiency in the UK had fallen well behind that in other countries in Europe and the rest of the world. Furthermore, the UK had recently emerged from the 1914-18 war which had demonstrated its dependence on imports of basic foodstuffs from around the world. Investment in scientific research was seen as one solution to remedy this situation. The initiative to establish a body to improve the yield and quality of varieties of crops grown in Scotland came primarily from the farmers and landowners who were members of the Highland and Agricultural Society of Scotland. A Committee was formed, that later became the Scottish Society for Research in Plant Breeding (SSRPB), to appeal for funds to set up a research station, and the Government of the day pledged to match the amount collected. By 1920 £22,500 had been collected and a total of £45,000 was available. A Government Registration and Seed Testing Station had been established a short time previously at East Craigs, Edinburgh and the new Scottish Plant Breeding Station shared the site, occupying Craigs House on the estate. Montagu Drummond was the first Director of Research, although he moved to the Chair of Botany at Glasgow University within four years. His place at SPBS was taken by W. Robb in 1925 who held the position until retirement in 1950.

The object of the SSRPB was stated to be "the establishment of a thoroughly equipped station for the improvement of agricultural plants. The improvement to be attained partly by selection and partly by the creation of new varieties possessing those qualities which will make them most profitable under Scottish conditions". Thus the objective of SPBS was strictly practical and it was to be guided by the Society rather than the Government. The Society, being composed primarily of interested growers, would ensure that the practical aim of the Station would be met, in so far as it was feasible.

Craigs House, 1921.

The first crops to be grown at Craigs House were oats, barley and potatoes. Collections of varieties were obtained and their performances monitored to determine which were best suited to local conditions. The main tasks were enumerated as:-

1) Collection and classification of suitable living material;

2) Isolation of pedigree strains (pure lines);

3) Comparative trials of varieties, pedigree strains etc;

4) Hybridisation of pedigree strains, varieties and species.

Soon after the establishment of SPBS, improvement of swedes and turnips, and ryegrass, cocksfoot and timothy grasses were included within the remit. Thus the foundations were laid for progress towards the stated aims in all of the crops throughout the 20s and 30s.

The three major figures in this phase of the history of SPBS were W. Robb, the Director who led the work on oats, J.W. Gregor, who conducted the grass programme and W. Black, appointed in 1926, who was in charge of potatoes.

Staff numbers accommodated in Craigs House grew slowly and steadily over the first 25 years of its existence but by 1945 further impetus to the development of

agriculture and the attainment of self sufficiency in basic foodstuffs was given by the emergence from the war years. As a consequence, additional staff were appointed, supported financially by the Department of Agriculture for Scotland, and it soon became apparent that the premises at East Craigs were inadequate for the larger staff numbers and the facilities that they required. In 1947, the University of Edinburgh purchased the Bush Estate south of Edinburgh and negotiations began for SPBS to acquire some of the land on which to build a new research station. The new laboratories and ancillary buildings at Pentlandfield were officially opened by the Secretary of State for Scotland in July 1955. By this time, the Government, through the Scottish Office, was providing most of the financial support with advice from the Agricultural Research Council, although the SSRPB retained a strong interest in the direction of the work of the station, as it had done throughout its history. J.W. Gregor was Director from 1950-1965 during the transfer from East Craigs to Pentlandfield. N.W. Simmonds succeeded J.W. Gregor in 1965 and was replaced by R.C.F. Macer in 1976.

Pollination of brussels sprouts 1964.

Cereals, brassicas and herbage crops

At the time of the establishment of SPBS, oats were a major cereal crop for animal fodder and for human consumption, and the selection and breeding objectives were to produce varieties with early ripening characteristics, resistance to lodging and with low levels of husk and fibre. The earliest varieties produced were Bell and Elder followed by Albyn Express and, much later, in the 50s, Shearer and Pentland Provender. By the late 30s, damage caused by stem eelworm (*Ditylenchus dipsaci*) was recognised and efforts were made to produce resistant varieties. In later years, as barley supplanted oats as the major animal feed crop, work on oats was gradually attenuated and was effectively concluded by 1981.

The programme on grasses was predominantly directed towards selection of land races that were adjudged to be most suitable for growing in Scotland and there was a strong emphasis on improvement of upland grassland through ecological observations of the interactions between genotypes and the local soil and climatic environments. Several strains of cocksfoot, timothy and perennial ryegrass with the prefix Scotia were selected and marketed.

Forage brassicas were, and still remain, an important component of livestock husbandry in Scotland and early efforts at SPBS were directed towards selection and improvement of swedes, turnips and kales. V.M. Davey was appointed in 1926 and developed the concept of pedigree breeding in which repeated selfing increased the homogeneity within the crop without, in some instances, resulting in in-breeding depression. Several varieties which have been released in recent years have their origins in Davey's selection programme. Leafy brassicas, eg kale and rape, were included in the programme from the 1950s and led to substantial research into the genetics and cytology within the genus. Inter-specific crosses were made and differences in ploidy level overcome to create new forms and greater variability. Resistances to clubroot (*Plasmodiophora brassica*) and mildew (*Erysiphe cruciferarum*) were also incorporated by this technique. The culmination of this programme was the production by I.H. MacNaughton of an inter-generic hybrid between *Raphanus sativus* and *B. oleracea* called Raphanobrassica which outyielded all other forms of leafy brassica available. Although the programme on breeding brassicas as forage crops declined throughout the 1980s, current interest in the genus as a source of industrial oils and possibly fibre could lead to a resurgence of research to build on the expertise accumulated in earlier years.

Potatoes

Potatoes received a great deal of attention from the inception of the Station. Collections of genotypes were obtained from various sources but particularly from J. Wilson of St Andrews University, a botanist who had already successfully transferred a resistance gene to late blight (*Phytophthora infestans*) from *Solanum demissum* to *S. tuberosum*. The damaging effects of pests and diseases were particularly recognised in potatoes and much effort directed towards identifying sources of resistance and transferring them into commercially acceptable varieties. Wart disease (*Synchytrium endobioticum*) and late blight were the first diseases to receive attention and produced directly contrasting results. Although wart was extremely damaging, soil borne and long

lived, durable resistance was found early during the research programme and has provided a long term answer to the problem. Resistance was a dominant character and easily transferred into new varieties and the fungus pathogen was relatively immutable. In contrast, late blight proved, and continues to be, a much more intractable problem. Much has been written about the search for a genetic control of blight and further accounts will appear in 1995, the 150th anniversary of the blight epidemic in Ireland and Western Scotland. Sources of resistance were found in wild species, particularly *S. demissum*, and transferred to *S. tuberosum* prior to the foundation of SPBS and latterly extensively by Black and his co-workers. The resistance was controlled by dominant major genes, designated R genes, and they conferred hypersensitivity within the host. However, within a few years of the introduction of an R gene in a new potato variety, the pathogen had adapted and overcame the resistance mechanism causing the new variety to become as susceptible as its predecessors. Successively new genes were identified and introgressed into new varieties only to succumb to further new races. Using a host differential series, Black demonstrated the existence of a total of 11 R genes but by 1944, he was beginning to doubt the probability of success using single major resistance genes. The failure of this approach was illustrated by the fate of Pentland Dell introduced in 1961 which contained three separate R genes, but which had succumbed to outbreaks of foliage blight in England by 1967.

Pentlandfield, 1977.

Black became an international authority on blight and the Station received many isolates for characterisation and distributed many seedlings containing R genes throughout the world. Having become convinced that R genes would never provide a durable control of blight, his attention turned to other mechanisms. During the course of screening work with complex races of the pathogen, it was observed that some genotypes containing the same R genes were less susceptible than others. Attention turned to this field resistance and additional sources were sought within wild species. Although it was polygenically controlled, field resistance was successfully incorporated into new clones. Ironically, the first widely grown field resistant variety was Roslin Eburu exported to Kenya in the early 60s where it gained popularity partly because of its tolerance to blight. Present day varieties such as Brodick, Torridon and Stirling are direct descendants of this programme and show high degrees of field resistance. In modern conditions of European agriculture, large applications of fungicides to control blight remain normal practice and consequently, the demand for durable, genetically determined resistance is not great except from the organic movement. However, should constraints be placed on the usage of fungicides in the future, the products of the SPBS programme and Black's pioneering work will achieve their rightful preeminence.

By 1929, the importance of virus diseases of potatoes had become recognised and an application was made to the Empire Marketing Board for financial assistance towards the cost of buildings and equipment for research on this topic. The unit was established at Craigs House in 1931 and Barton Wright and G. Cockerham were appointed. Barton Wright resigned in 1935 but Cockerham continued and led the group until he retired in 1969. Other members of the group included D.A. Govier and T.M.W. Davidson. It is noteworthy that C.H. Cadman, who later joined the staff of SHRI and became its Director in 1965, was a member of the group from 1939-43. The early work concentrated on characterising the several virus diseases in potatoes, particularly viruses X, Y and S and potato leafroll virus, and distinct strains within each virus were identified. The group were amongst the first in the UK to introduce diagnostic serological tests in 1948. Alongside the work of characterising the viruses, searches for effective resistance to them were underway and commercial varieties were found which contained dominant genes conferring immunity to virus X and some strains of virus Y. In addition, wild strains were screened for exotic forms of resistance to transfer into the breeding programme. Resistance to PLRV was found to be polygenic and present in some cultivated varieties and hybrids with wild species. One successful outcome of the research was Pentland Crown which had a high degree of resistance to PLRV and to virus Y. The emphasis on virus diseases remains to the present with useful sources of resistance available to many of them and now supplemented by transformation tech-

nology using the virus coat protein genes and other genetic constructs.

Resistance to potato cyst nematode (*Globodera rostochiensis* and *G. pallida*) was added to the list of breeding objectives in 1949 when the effects of extensive infestation of ware fields were recognised. By 1951, a gene for resistance to *G. rostochiensis* was demonstrated in *S. andigena* in collaboration with Ellenby at Newcastle. The gene, H_1, was quickly incorporated into a new variety, Pentland Javelin, released in 1968. Javelin did not achieve the preeminence of its competitor, Maris Piper, but the widespread cultivation of varieties with the H_1 gene led to the selection of the second PCN species, *G. pallida*. Resistance to this species has been found in *S. vernei* but, being polygenic, is more difficult to transfer to cultivated varieties. Nevertheless, several recent varieties, e.g. Eden, show good resistance to both *G. rostochiensis* and *G. pallida*.

Throughout the history of SPBS, other diseases have been added to the list under investigation, e.g. common scab, gangrene and blackleg, and there has always been a consciousness of the importance of introducing material that is at best resistant, or at least, not highly susceptible. This emphasis of pest and disease resistance is in contrast to most commercial breeding companies where the objectives of yield, appearance and quality predominate on the assumption that most pests and diseases can be adequately controlled by application of agrochemicals and that introducing resistance is, of necessity, a long term costly exercise. The advantage of a state supported institute such as SPBS was that it did not rely on the profit motive alone and included research on sources of resistance and their transfer to varieties for the public good in the long term.

Although emphasis was laid on strategic studies of resistance to disease, nevertheless on purely commercial grounds, the products of the genetics programme achieved substantial success as ware varieties. In 1971, Pentland Crown was the leading variety in the UK and, together with Pentland Dell, the two varieties occupied about one-third of the total UK crop. Although the popularity of varieties change, material from the SPBS programme has always maintained a presence in the top varieties grown in the UK. Changes in the administration of publicly-funded research heralded by the reports of Rothschild and Barnes (referred to below) have not hindered the progress of breeding new varieties of potatoes although they have substantially changed the way in which this is achieved. Much closer collaboration and partnership with commercial companies have resulted but they continue to exploit the expertise and improved genetic resources elaborated in the earlier years.

At the heart of the continuing improvement and introduction of new genetic material is the Commonwealth Potato Collection and this is becoming an even more valuable resource as the new technologies of genetic transformation and tissue culture allow combinations of genetic material that were hitherto impossible by conventional crossing techniques.

Barley

By 1962 it became obvious that barley had largely supplanted oats as the dominant cereal for animal feed and trials began in collaboration with Plant Breeding Institute, Cambridge, to trial selections for their suitability for Scottish conditions. A crossing programme was started in 1966 when additional staff were appointed to conduct the trials. By this time, N.W. Simmonds was Director and he criticised the narrow genetic base of the UK cereal crops and advocated a wide crossing programme with exotic germplasm. Early on in the research, selections were made on the basis of high diastase and high amylase characters to improve their acceptability to maltsters. Initially, disease resistance was not considered a priority but by 1970 when the potential of mildew to reduce yields was realised, resistance to this disease and to *Rhynchosporium*, yellow rust and brown rust were included in the programme. Because of the importance of the malting and brewing industry in Scotland, the emphasis on malting quality, its determinants and genetic control remain active research topics today. In addition, climatic conditions prevalent in Scotland may require genotypes that differ from those grown elsewhere.

SHRI

SHRI was founded in 1951, largely as a result of the concerns of the Scottish fruit growers. Raspberries had been introduced as a crop into several locations in Angus and Perthshire around 1900 where they produced excellent crops due to favourable climatic and soil conditions. The crop was grown on smallholdings and most of it was transported by rail to jam manufacturers in England. Initially, large yields were obtained but by the 1920s the vigour and yield of plantations were seriously affected. Strawberries were an important crop in Lanarkshire at around the same time and they suffered a similar decline. A Scottish Horticultural Advisory Committee was established in 1927 to advise the Department of Agriculture and it frequently referred to a need for research to solve the problems of the industry. Investigations into the strawberry prob-

Mylnefield farm, 1951.

lems were centred on the West of Scotland College of Agriculture at Auchincruive and R.D. Reid was appointed in 1930 to investigate possible control measures. He found soil sterilants and fungicides unsuitable and turned to selection of clones that survived on a plot of land that was known to be heavily infested by what was to be identified later as a soil-borne fungus, *Phytophthora fragariae*. He was successful and Auchincruive Climax was introduced in 1947 as the first of a series of strawberry varieties that were resistant to red core. Reid and his co-workers were later transferred to the staff of SHRI although the strawberry breeding unit remained at Auchincruive until 1980.

Professor T. Swarbrick addressing dignitaries at the official opening of the Institute on June 16, 1956.

The problems of the raspberry growers were referred to East Malling Research Station in Kent and during the 1930s infection by viruses was established as the cause of the decline in yield and vigour. R.V. Harris supervised the work from East Malling and C.H. Cadman, who later became Director of SHRI, was appointed as a resident assistant in 1943, transferring to Dundee from SPBS. This then established the Scottish Raspberry Investigation Unit and further staff were appointed including C.A. Wood as pomologist. By 1946 the Horticultural Research Committee recommended the founding of research centres in Scotland for fruit, vegetable and glasshouse crops. Mylnefield Farm near Dundee was identified as a prospective site for the research station and was purchased by the Department of Agriculture for Scotland in 1950 along with the neighbouring farm of Bullionfield which together provided c. 100 ha experimental land. SHRI was formally established in 1951 and took over the staff of the Raspberry Investigation Unit with T. Swarbrick as the first Director.

The emphasis initially was on raspberries and by 1951 a system of introducing virus-free foundation stocks had been developed to provide planting material for new plantations which helped to revive the failing industry. Several aphid-borne virus diseases had been characterised in the crop and Cadman later discovered that soil-borne nematodes transmitted raspberry ring spot. The early concentration on virus diseases resulted in SHRI becoming a major centre for research in this area led initially by Cadman and from 1966 by B.D. Harrison. D.L. Jennings was appointed in 1957 to lead the breeding of new varieties and Glen Clova, the first product of this programme and the forerunner of all the more recent Glen series of varieties, was introduced in 1971. Since then SHRI varieties have dominated the Scottish crop and have been exported worldwide.

Strawberry breeding continued to produce a series of varieties including Talisman and Red Gauntlet at Auchincruive, and blackcurrants and blackberries were also included in the breeding programme at Invergowrie.

Main laboratory and administrative buildings at SHRI, with the new wing completed in 1960.

Alongside the breeding, pathology and agronomy of soft fruit, an active programme on vegetable crops developed, largely to support the increasingly important vegetable processing industry in the east of Scotland. Breeding efforts on cabbage, Brussel sprouts, beans and carrots were initiated, together with agronomic and pathological work.

Aerial view of SHRI, 1965.

The Amalgamation

Throughout the 60s and 70s, the numbers of staff and research topics at both SPBS and SHRI expanded as a result of Government policy to encourage the development of science and technology for the benefit of the UK. The Institutes were administered by the Department of Agriculture for Scotland acting on advice from the Agricultural Research Council who operated on the Haldane principle of minimal interference of research scientists operating within broad terms of reference for the Institutes under its control. This system allowed scientists of the status of Black, Cockerham, Reid, Cadman and others to develop their own lines of expertise and innovation unhampered by excess administration or a high expectation of results. Clearly, the achievements of both organisations in providing advanced technology and improved crop varieties to the benefit of Scottish agriculture and horticulture were evidence of the success of this philosophy. However, a radical change to the administrative system was heralded by the Rothschild report published in 1972 in which it was recommended that a substantial proportion of the funds administered by the ARC in England should be transferred to the Ministry of Agriculture, Fisheries and Food. A Chief Scientist was appointed in the Ministry who would oversee the allocation of funds and the concept of the Ministry as a customer and the ARC as a contractor was established. These moves began the increasing trend towards Government intervention and requirement to demonstrate accountability in the publicly-funded research institutes. The days of the autonomous research institute had passed with the establishment of the Joint Consultative Organisation designed to control and monitor results at each institute. By 1975, the ARC initiated discussions on areas of overlap of research within the Agricultural Research Service and concluded that research on potatoes, forage brassicas and barley should continue at SPBS but that work on grasses and clover should be terminated. Raspberries and blackcurrants would remain at SHRI and culinary vegetables would be transferred to the National Vegetable Research Station.

Further rationalisation was deemed desirable and in 1978 a Working Party was set up by the Secretary of State for Scotland to examine the arrangements for commissioning and organising research at SPBS and SHRI. The Working Party recommended the amalgamation of the two establishments on the site at Invergowrie under the name of the Scottish Crop Research Institute. The combined Institute came into being officially on 1 February 1981 and a substantial capital building programme to accommodate the staff and equipment to be transferred from Pentlandfield was initiated at Invergowrie. In addition, Gourdie Farm was acquired to bring the available land to a total of 200 ha. The transfer to the Invergowrie site was a phased operation and was not completed until 1991. Several factors contributed to the decision to effect the amalgamation and to locate the combined Institute at Invergowrie. Both SPBS and SHRI had become large establishments with clear areas of expertise but also weaknesses and it was perceived that there was considerable complementarity between them that could be exploited by bringing them together. For example, the genetic and breeding expertise on potatoes at SPBS would benefit from the fundamental research on plant physiology and pathology that had developed at SHRI. There would be economies of scale in running one large organisation rather than two separate establishments and there was adequate land for building expansion and for field trials immediately adjacent to the laboratory area at Invergowrie. The remit given to SCRI by a Programme Review Group that reported in 1980 was “to do the research needed to sustain and increase crop production in Scotland and northern Europe ... with emphasis on plant breeding, crop physiology, agronomy and crop protection. It will concentrate on potatoes, spring barley, forage brassicas,

raspberries and blackcurrants." C.E. Taylor, Director of SHRI from 1971 oversaw the negotiations for the amalgamation; J.R. Hillman became Director in 1986.

In 1987, the Macaulay Institute for Soil Research was amalgamated with the Hill Farming Research Organisation and some staff and resources at Aberdeen concerned with plant science, soil microbiology and chemistry were transferred to SCRI. Also in 1987, SCRI became responsible for administering the Scottish Agricultural Statistics Service.

In the years since 1981, further changes have taken place to the structure and remit of SCRI. The Barnes report of 1988 introduced the concept of "near market research" and required the beneficiary of research to pay for it. In agriculture this was interpreted as the farming industry in general and forced researchers to look towards commercial companies and levy boards etc to provide funds to support work which would be of direct practical value to the industry. In particular for SCRI, the designation of the production of new crop varieties as a near market operation created problems, as this had been recognised as a primary function of the Institute at the time of the amalgamation. Nevertheless, there was sufficient adaptability within the system to allow for the formation of a Consortium and to enter into partnerships with commercial plant breeding and seed specialist companies to share the costs and proceeds of introducing new varieties to the market place. The move towards independent income generation from the products of research also led to the formation of Mylnefield Research Services Ltd in 1989 as a commercial trading arm of the parent charitable Institute.

Another major change from the remit given to SCRI in 1981 has been the move from emphasis on research of benefit to northern Britain to a much greater international role. Research on many of the crops investigated at SCRI is relevant to countries growing those crops outside the UK and increasingly the Institute acts as a focus for the introduction of new technologies in crops such as potato and raspberries that are relevant worldwide. Furthermore, research scientists are attracted from many countries to acquire knowledge and techniques developed at the Institute. In addition, the range of crops under investigation has extended to include many that are not grown in Scotland because the facilities available are relevant to tropical and subtropical species. There has also been substantial developments in molecular biology and cell and plant physiology with increases in staff and research projects, many of which have their origin in earlier work at SPBS or SHRI.

The legacy of the early years

Both SPBS and SHRI were founded on the needs of local Scottish growers to solve problems of poor productivity. Many of these problems were pathological in nature and in some cases their resolution lay in attempts to produce new varieties with genetically controlled resistance as demonstrated by the case histories of late blight of potatoes and virus diseases of raspberries outlined above. In the course of these developments, a large body of expertise on crop genetics and breeding was built up and collections of wild species and primitive varieties made to search for and transfer desirable characteristics into crop varieties. Concurrently, it was inevitable that research into the pathogens should be undertaken to characterise resistance-breaking strains and physiological races. Although the increasing sophistication of chemical control methods has to some extent lessened the absolute necessity for durable resistance mechanisms to be incorporated into varieties, increasing public unease about chemical residues in food and the environment and the pathogen's ability to acquire tolerance to chemical controls suggest that intrinsic host resistance will remain a high priority in the future.

The modern technologies of molecular biology rely heavily on the collections and knowledge acquired in the earlier years of the Institute. Furthermore, the selection and establishing the agronomic value of any genetic transformance will depend on the skills developed during conventional procedures of breeding and selection.

SCRI can draw on a long and distinguished history of research and achievement to support and enrich current efforts to improve crop production methods and to understand the basic mechanisms underlying plant growth and development.

SPBS

M. Drummond	1921-25
W. Robb	1925-50
J.W. Gregor	1950-65
N.W. Simmonds	1965-76
R.C.F. Macer	1976-81

SHRI

T. Swarbrick	1951-65
C.H. Cadman	1965-71
C.E. Taylor	1971-81

SCRI

C.E. Taylor	1981-86
J.R. Hillman	1986-present

Table 1 Directors of research.

A quarter century of plant virus research (1966-1991)

B.D. Harrison[1]

The severity of virus diseases in Scottish raspberry crops was a crucial catalyst in the negotiations that led to the establishment of the Scottish Horticultural Research Institute (SHRI) in 1951. Virus research therefore formed part of the SHRI programme from the outset. It prospered under the perceptive leadership of Colin Cadman, and by 1966 SHRI had become the main centre in Europe for work on viruses infecting raspberry and related crops, and on viruses that spread *via* soil. Subsequently, between 1966 and 1991, the research programme broadened, the number of those engaged in plant virus research increased from 14 to 45, and the Scottish Crop Research Institute (incorporating SHRI) became one of the world's leading centres for such work. This article is a brief retrospective view of the main achievements of the Virology Department during this 25-year period.

The programme of research had two main aims. The first was pathological: to identify the causes of virus diseases in crops, to discover how they spread, and to find ways of minimising spread and crop losses. The second was virological: to investigate the bases of viral phenomena, especially mechanisms underlying the biological properties of viruses, by exploiting opportunities offered by the viruses being studied as pathogens. Work on standard laboratory models, such as tobacco mosaic virus, was left to others.

By 1966, work at SHRI had shown that the most serious virus diseases of raspberry in Britain were caused by four different but allied viruses that have isometric particles and soil-inhabiting longidorid nematodes as vectors. These were given the name 'Nepovirus', the first of the currently used generic names of plant viruses to be proposed. Further research on nepoviruses led to several discoveries:

- Bipartite nature of the nepovirus genome, both constituent molecules (RNA-1 and RNA-2) being needed for infection of plants although RNA-1 can replicate alone in protoplasts.
- Genetic determinants for biological properties such as host range, symptom type, seed transmissibility and nematode vector specificity, that are distributed between RNA-1 and RNA-2, as indicated by the properties of pseudo-recombinant isolates in which these RNA species were derived from different virus strains.
- The key role of seed transmission in weeds in the persistence of nepoviruses at a site and their spread from field to field.
- The first satellite RNA that encodes its own non-structural protein.
- The first RNA-linked protein shown to be needed for viral infectivity.
- Evidence that nematode vector specificity is controlled by the nepovirus particle protein.

Research also continued on other viruses infecting raspberry, enabling several previously uncharacterised viruses to be described and procedures for maintaining the UK collection of virus-tested raspberry and *Rubus* stocks to be improved progressively. Notable findings included:

- The best example of virus control by use of genetic resistance to a vector (here the aphid *Amphorophora idaei*), thereby preventing the spread of several aphid-borne viruses at one stroke.
- A novel virus (raspberry bushy dwarf virus) with isometric particles and a bipartite RNA genome that was spread *via* pollen to the plant pollinated and has become the type member of the genus *Idaeovirus*.

Potato viruses were another focus of attention, especially those transmitted directly to tubers by soil-infesting vectors. Noteworthy results included discovery of:

- The key influence of soil type and water content, respectively, on the occurrence and incidence of the nematode-transmitted tobacco rattle tobravirus (TRV), the cause of corky ringspot disease in potato tubers. Disease risk can therefore now be predicted from soil maps.
- Involvement of mitochondria in TRV replication.
- Novel pattern of genome variation in which one segment of the bipartite genome of TRV is strongly conserved and the other is extremely variable.
- The first example of RNA recombination in a plant virus (TRV) in nature.
- The first direct evidence to link an increase in plasmodesmatal permeability with the onset of replication of a virus (TRV).

[1]Head of Virology Department, SCRI from 1966 to 1991; now Professor of Plant Virology, University of Dundee.

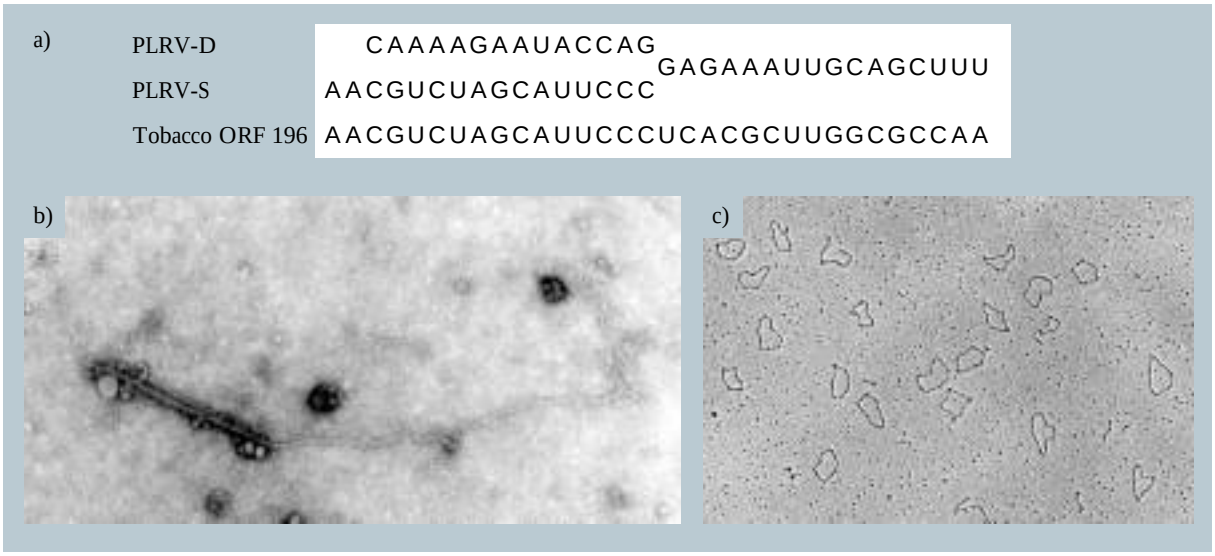


Figure 1 Three noteworthy discoveries. (a) A nucleotide sequence, found at the 5' end of a small proportion of the RNA molecules of a Scottish isolate of potato leafroll luteovirus (PLRV-S), which appears to have been acquired from a tobacco chloroplast RNA (Tobacco ORF 196). Other RNA molecules of PLRV-S, and those of PLRV-D, a Dutch isolate of the virus, have a different 5' terminal sequence (Mayo & Jolly (1991), *J. gen. Virol.* **72,** 2591). (b) Electron micrograph of a filamentous particle of heracleum virus 2 (coated with specific antibody) linked to the longer filamentous particle of the aphid-transmissible heracleum virus 6 (not coated). The association confers aphid-transmissibility on heracleum virus 2, and occurs when both viruses are present in the same source plant (Murant & Duncan (1985), *Ann. Rep. Scottish Crop Res. Inst. for 1984,* 182). (c) Electron micrograph showing that molecules of single-stranded DNA of African cassava mosaic geminivirus are circular (Harrison *et al.* (1977), *Nature* **270,** 760).

• Linkage of the serotype of tobacco rattle virus with nematode vector specificity.

• Transmission of potato mop-top virus by *Spongospora subterranea*, the second example of a plasmodiophorid fungus as a virus vector.

• Tripartite nature of the RNA genome of potato mop-top virus, which therefore differs from previously characterised fungus-transmitted viruses with rod-shaped particles.

In 1979, virologists in Japan devised a much improved method for purifying luteovirus particles, which are confined to phloem tissue and occur in low concentrations. This advance stimulated research at SCRI on potato leafroll luteovirus (PLRV). Several discoveries resulted:

• A protein covalently linked to luteovirus RNA.

• The nucleotide sequence and genome organisation of PLRV RNA.

• The first evidence for recombination between RNA of a virus (PLRV) and that of its host (a chloroplast RNA; Fig. 1a).

• Quantification of the uptake and retention of PLRV by aphids.

• Monoclonal antibodies that distinguish aphid-transmissible and aphid-nontransmissible PLRV isolates.

• A novel type of virus resistance that is expressed as decreased invasion of potato phloem tissue (by PLRV) and is controlled by dominant major genes.

With their experience of growing seed potato crops, some Scottish farmers started to diversify into other vegetatively propagated crops, notably narcissus. Research was therefore put in hand to produce virus-free stocks of the leading cultivars, several previously undescribed viruses were characterised and a comprehensive modern scheme for propagating virus-tested stocks was devised. As a result, Scotland now grows the bulk of the world's highest health status crops of narcissus.

A somewhat fleeting interest in virus diseases of carrot crops paved the way to the discovery of a series of fascinating virus associations in which one member of a pair of viruses depends on the other member to provide the means for the first member to be transmitted by aphids. Four different 'helper systems' were studied and differentiated, novel phenomena discovered and new types of plant virus characterised. The main findings included:

• Evidence that carrot mottle umbravirus lacks a con-

ventional nucleoprotein particle and that its RNA can be packaged in the coat protein of carrot red leaf luteovirus in mixed infections to produce particles that are transmissible by the aphid vector of the luteovirus.

• Changing the helper virus from one luteovirus to another changed the vector specificity of carrot mottle virus to that of its new helper, so emphasising the role of luteovirus particle protein in aphid transmission.

• Dissection of the interaction between the two viruses associated with groundnut rosette disease, one of the three most important plant virus diseases in Africa. The interaction resembles that between carrot mottle and carrot red leaf viruses but with the crucial additional presence of satellite RNA, which causes the disease, depends on the umbravirus for replication and on the luteovirus for aphid transmission and, remarkably, mediates the luteovirus-dependent aphid transmission of the umbravirus. This intricate tripartite system was the first to be found among plant viruses.

• A novel helper system involving two closteroviruses and in which one end of the filamentous particles of the dependent virus becomes attached to one end of the aphid-transmissible helper virus particles, thereby conferring aphid transmissibility on the dependent virus (Fig. 1b).

• A novel dependent plant virus (parsnip yellow fleck virus) with strong evolutionary affinities to picornaviruses such as poliovirus, and its assignment to a new genus, *Sequivirus*.

• The site of retention in the aphid foregut of particles of anthriscus yellows waikavirus, the first such evidence for a virus that is transmitted in the semi-persistent manner.

• Evidence that a near N-terminal tripeptide (DAG) in the coat protein of potyviruses is crucial for their non-persistent transmission by aphids.

Cooperation with cassava pathologists working in Africa led to externally funded research on the whitefly-transmitted geminiviruses which cause Africa's most important plant virus disease, cassava mosaic, and serious diseases in many other crops, mainly in tropical regions. Noteworthy findings were:

• The circularity of geminivirus genomic single-stranded DNA (Fig. 1c).

• A network of serological relationships among whitefly-transmitted geminiviruses such that panels of monoclonal antibodies raised against two of the viruses were used to detect about 30 other related viruses occurring in about 20 crop species in more than 50 (mostly developing) countries.

Figure 2. A.F. Murant (left) instructing a 'mature student', Lord Mansfield, in the art of inoculating plants with viruses, on the occasion of the opening by the latter of the Virology Department glasshouse complex (1982).

• Similarities in the epitope profiles of whitefly-transmitted geminiviruses from the same geographical region, irrespective of their host range, and differences in the epitope profiles of virus isolates causing the same disease in different continents, pointing to a geographical influence in geminivirus evolution.

The mid-1980's were a time when the use of genetic engineering to confer virus resistance was starting to be explored, by transforming plant nuclear DNA with virus-related nucleotide sequences thought likely to have protective effects. The first such system, coat protein-mediated resistance, was reported by others. The second system, satellite-mediated resistance, was discovered soon afterwards as a result of collaborative work with colleagues at Cambridge on cucumber mosaic cucumovirus (CMV). It was established that:

• Plants transformed with a DNA copy of a symptom suppressing satellite RNA of CMV contained satellite RNA transcripts but looked normal.

• When the satellite-transformed plants were inoculated with satellite-free CMV, large amounts of satellite RNA were produced, and both replication of the virus and symptom development were greatly decreased. Activation of the satellite RNA was specific to CMV and the closely related tomato aspermy cucumovirus.

• The satellite-transformed, CMV-infected plants were poor sources of inoculum for vector aphids, but when aphid transmission of the virus succeeded, so also did that of the satellite RNA and few symptoms developed in the newly infected plants.

Electron microscopy provided a key input in many of the advances noted above and numerous techniques, especially those of immuno-electron microscopy, were

adopted and further developed. In addition, new technical aids to research were devised. These included:

• The heat pen, a device for removing wrinkles from ultrathin sections.

• Tungsten-coated glass knives, capable of cutting long ribbons of ultrathin sections of plant tissue and easily produced at a fraction of the cost of diamond knives.

• A freeze-drying block suitable for specimens on electron microscope grids.

Not surprisingly for a research group that has continued to discover plant viruses of novel types, SHRI/SCRI virologists have had a strong and continuous input to the International Committee on Taxonomy of Viruses, coordinating the designation of the first 16 groups (now genera) of plant viruses, and subsequently characterising and naming four other genera. In addition, annual sets of the *AAB Descriptions of Plant Viruses* were commissioned and edited. These now consist of >350 potted summaries of data about individual viruses, and are the most authoritative international reference source. The principles of plant virology were outlined in a text-book written by A.J. Gibbs and B.D. Harrison, and later translated into Russian and Chinese.

Figure 3. B.D. Harrison (left) discussing with Lord Sanderson the production of virus-resistant plants by genetic engineering (1988).

This short account suggests that the Virology Department's work has had a significant influence during a golden period of progress in plant virology generally. A wide variety of discoveries were made by a combination of molecular and traditional approaches, concepts formulated, data sources provided and virus taxonomy advanced. A further influence continues to be expressed through a score of former Ph.D. students, and numerous former visiting workers, who are now engaged in plant virus research in many parts of the world.

Plant genetics

George R. Mackay

Access to cultivars with intrinsic, i.e. genetically based, resistance or tolerance to diseases, pests and abiotic stresses is an essential prerequisite for the development of sustainable agricultural systems. The modification of existing cultivated species and the genetic improvement of others for non-food, industrial uses are equally important in order to maintain biodiversity and provide sources of raw material for renewable energy and manufacture. It was unfortunate that plant breeding was identified in the late 1980's as a 'near market' activity when, clearly, plant breeding and associated research can be as close to, or as far from, the market as the objectives of a breeding programme are set. It is hoped that, as government policy swings towards wealth creation as a legitimate object for public investment in research and development, and the products of biotechnology require evaluation in the field, the value of the skills and expertise of the classical plant breeder will once more be recognised.

Name	Date of release
The Alness	1934
Craigs Defiance	1938
Craigs Royal	1947
Craigs Snow-White	1947
Craigs Alliance	1948
Pentland Ace	1951
Pentland Beauty	1955
Pentland Crown	1958
Pentland Dell	1960
Pentland Envoy	1961
Pentland Falcon	1962
Pentland Glory	1963
Pentland Hawk	1966
Pentland Ivory	1966
Pentland Javelin	1967
Pentland Kappa	1967
Pentland Lustre	1968
Pentland Meteor	1970
Pentland Marble	1970
Pentland Raven	1970
Pentland Squire	1970
Croft	1974
Provost	1981
Baillie	1981
Sheriff	1981
Kirsty	1982
Ailsa	1984
Moira	1984
Morag	1985
Rhona	1985
Shula	1986
Teena	1986
Shelagh	1986
Morna	1986
Glenna	1987
Torridon	1987
Brodick	1990
Stirling	1991
Eden	1991
Glamis	1991
Provan	1991
Cramond	1992
Brodie	1993
Buchan	1993

Table 1 Potato varieties bred by SCRI and released in the UK.

About 75 years ago a group of farsighted farmers and growers recognised the value of the application of the then recently discovered science of genetics to crop improvement. In co-operation with the Department of Agriculture for Scotland, they provided the capital to found the Scottish Society for Research in Plant Breeding at Craigs House, Corstorphine, Edinburgh. By 1994, this early attempt to associate scientists with the real world of agriculture has directly or indirectly led to the release in the UK and worldwide of 56 potato cultivars (Tables 1 and 2). Many of these have made, and still continue to make a significant contribution to the UK economy. Recently released potato cultivars possess combinations of disease and pest resistance, as well as yield and quality characteristics, that are a testimony to advances in the development and application of breeding methods, such as progeny tests for numerous economically important traits. Access to a revitalised Commonwealth Potato Collection and the development of tissue culture based techniques, such as somatic fusion, promise much for the more rapid incorporation of disease resistances from wild species into agronomically superior clones and cultivars. However, these methods take time and, for the foreseeable future, most improved cultivars will owe their origins to the further development of classical breeding and selection methodology based on a sound scientific knowledge of the genetics of the cultivated form, *Solanum tuberosum* ssp. *tuberosum*.

The contribution of SCRI to crop improvement and wealth creation, via improved potatoes, should not, however, be allowed to overshadow the benefits of its research into the genetics and breeding of other species. Historically, varieties of bean (Albyn Tick), grasses (Scotia rye-grass, Scotia cocksfoot), barley (Tweed, Heriot, Tyne), oats (Bell, Albyn Empress, Shearer, Pentland Provender), forage brassicas (Appin and Ballater), horticultural kale (Pentland Brigg), cabbage (Celtic) and swedes (Pentland Harvester, Brora, Airlie, Angus, Melfort) have also provided tangible evidence of the application of science to crop improvement. It is gratifying that, despite withdrawal of core funding of research into brassica genetics, collaboration with private companies such as Nickerson Seeds Ltd and Sharpes International Seeds Ltd have enabled the production and release of several new swede, rape, and kale cultivars in the past few years.

Name	Date of release	Where named
Craigs van Riebeeck	1949	South Africa
Roslin Chania	1960	East Africa
Roslin Eburu	1960	East Africa
Roslin Elementeita	1960	East Africa
Roslin Mount Kenya	1960	East Africa
Roslin Sasumua	1960	East Africa
Roslin Riviera	1961	Scotland
Roslin Castle	1965	Scotland
Roslin Tsangano	1969	Malawi
Roslin Bvumbwe	1969	Malawi
Kenya Akiba	1969	Kenya
Kufri Jyoti	-	India

Table 2 Potato varieties bred by SCRI but released or named elsewhere.

Without public investment in a strategic breeding effort, future production of low cost forage crops such as forage kale or rape is unlikely to attract private funding, but it is pleasing to record continued support from Sharpes for a small-scale swede breeding programme, which will maintain the requisite skills and core expertise that will enable a resuscitation of forage brassica research if, or when, the funding bodies deem it desirable.

Bean breeding has not been pursued for many years, but a small team of researchers in legume genetics have made substantial progress at a basic level. The production of faba bean lines with low levels of the antimetabolites, convicine and vicine, and the application of modern molecular techniques to map these genes and others, offers the opportunity to utilise marker-based selection to increase the efficiency and efficacy of selection. If these new lines are taken up by industry, they could reduce the UK's dependence on imported protein for animal feedstuffs and to diversify agricultural production. The work has also led to valuable collaborative links within the European Union and, if faba bean realises its potential, SCRI is well placed to have the products of its basic research exploited for the economic benefit of the community.

Since the release of barley cultivars Tweed, Heriot and Tyne, there has been a hiatus in barley breeding following the sale of NSDO and adjustments needed to accommodate our association with the consortium of Nickerson and Dalgety. However, a new spring barley line was submitted to National List Trials in 1994. Evidence from trials of advanced lines supported by the SSCR has confirmed that lines well adapted to Scotland may be being discarded on the basis of their performance in Lincolnshire, raising important queries on future breeding policy. In the meantime, research into cross prediction, anther culture and the molecular mapping of quantitative trait loci is providing the knowledge and means to advance the science of barley improvement more efficiently and effectively. Strategic and fundamental research into genetics and breeding methodology at the molecular, cell, and whole plant level will continue to be applied to the benefit of all crop species within the broad remit of SCRI.

Potato breeding at SCRI: from wild species to finished cultivars

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

Selectors, hybridisers and scientific breeders The principal cultivated potato is a tetraploid species (2n = 4x = 48) (*Solanum tuberosum*) which was introduced into Europe, and thence the rest of the world, from the Andes of South America in the late 16th century. By the end of the 18th century it had been adapted to long day conditions through selection by the early cultivators for earlier-tubering, higher-yielding clones derived from seedlings from naturally occurring berries, the consequence of open, largely self-pollination.

Potato breeding in the modern sense began in 1807 in England when Knight made deliberate hybridisations between different varieties by artificial pollination, and flourished during the second half of the 19th century when many new cultivars were produced by farmers and hobby breeders. However, the rediscovery in 1900 of Mendel's published work of 1865 marked the birth of modern genetics, and opened the way to crop improvement by scientific breeding methods based on a sound knowledge of the inheritance of economically important traits. The development of

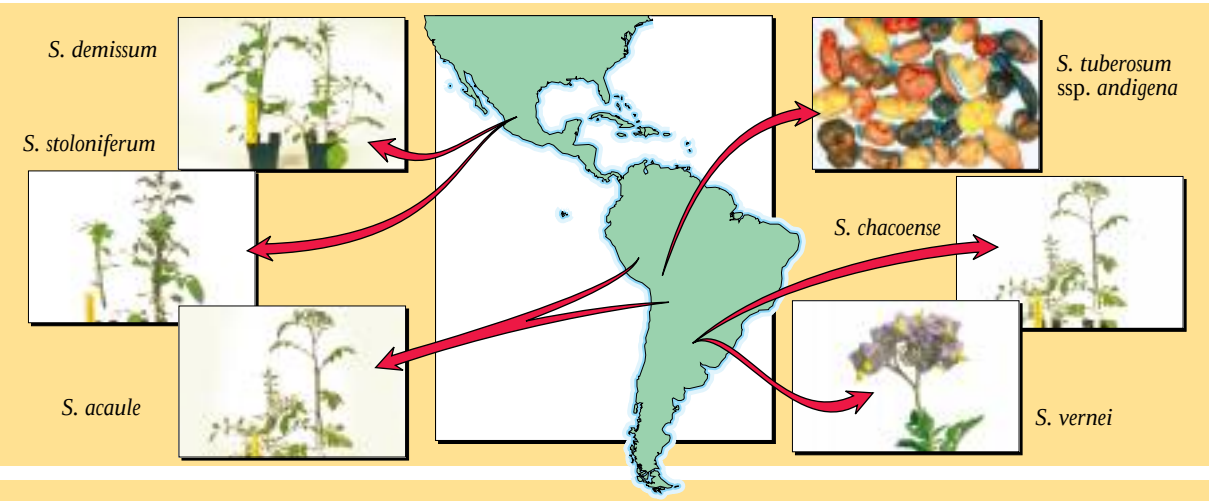


Figure 1 Sources of disease and pest resistance.

such methods for potatoes was one of the challenges which faced the Scottish Plant Breeding Station on its foundation in 1920, and one which still faces potato breeders at SCRI today. Of equal importance has been the need to demonstrate that new methods work in practice, and to release new cultivars so that growers and consumers can benefit from improvements in yield, quality and resistance to pests and diseases. The successes achieved in the latter objective at SPBS and SCRI can be measured by the steady stream of new cultivars from The Alness in 1934, through the widely grown Craigs and Pentland series, to the new crisping cultivar, Brodick, with its resistance to low temperature sweetening, and the most recently released table-use cultivars Buchan and Brodie.

The search for disease and pest resistance Now, as in the past, a successful cultivar has to combine a high marketable yield with good quality, which today means either the table quality demanded by supermarkets or the processing quality demanded by the manufacturers of crisps and French fries. However, potato breeding activity has often been driven by the need for improved disease and pest resistance in a vegetatively propagated crop, where many pathogens are efficiently transmitted from mother to daughter tubers. This has been particularly so at SCRI where objectives are more strategically directed than those of private breeders.

Over the past 75 years, new and durable forms of resistance have been sought for the major pests and diseases, whilst for those considered less important, potential cultivars have simply been screened to avoid extreme susceptibility. Sometimes resistance was found within existing cultivars, early successes being simply inherited immunity to the soil-borne fungus *Synchytrium endobioticum* which causes wart disease, and field immunity to some of the common strains of the mosaic viruses PVX and PVY. In contrast, for resistance to other major pests and diseases it was necessary to explore the wild tuber-bearing and primitive cultivated species of Central and South America. There have been many collecting expeditions to these areas since the pioneering Russian visits in the 1920s, including those of Balls and Hawkes who, in 1939, initiated the Commonwealth Potato Collection (CPC); a valuable germplasm resource now held at SCRI.

Utilisation of the Commonwealth Potato Collection Some of the best known sources of disease and pest resistance which have been used in breeding programmes at SPBS and SCRI are shown in Figure 1. The major gene resistances to PVY (Ry genes from *S. stoloniferum*, Ny genes from *S. demissum* and *S. chacoense*) have proved durable and are still useful, as are those to PVX (Rx genes from *S. tuberosum* ssp. *andigena* and *S. acaule*), despite the known occurrence of resistance-breaking strains of the latter in South America. The H_1 gene (from *S. tuberosum* ssp. *andigena*) has also remained effective against the golden potato cyst nematode (*Globodera rostochiensis*) in Britain because Ro1 is still the only pathotype, but its widespread deployment has encouraged the spread of the white potato cyst nematode (*G. pallida*). However, quantitative resistance to *G. pallida* was found in *S. tuberosum* ssp. *andigena* and has been incorporated into cv. Eden and various breeding lines. Furthermore, quantitative resistance to both species of nematode was found in *S. vernei* and incorporated into cultivars Morag and Glenna as well as into vari-

ous breeding lines. These resistances should remain effective for the immediate future. In contrast, the R genes (from *S. demissum*) for resistance to late blight (*Phytophthora infestans*) have been anything but durable, as witnessed by the speed with which Pentland Dell succumbed to new races of the fungus in the 1960s, despite possessing three R genes effective against the prevalent race. It is hoped that the high levels of field resistance achieved in the new SCRI cultivars Stirling and Torridon will prove more durable, despite also being derived primarily from relatively few accessions of *S. demissum*.

In the longer term, new sources of resistance to potato cyst nematode (PCN) and to late blight will be required. Limited recent screenings of the CPC identified new resistances to PCN in the South American species *S. megistacrolobum*, *S. sanctae-rosae* and *S. sparsipilum*, and to late blight in the Mexican species *S. brachycarpum*, *S. papita*, *S. polytrichon*, *S. stoloniferum*, *S. pinnatisectum*, *S. polyadenium* and *S. verrucosum*, and in the Bolivian species *S. circaeifolium*.

The usual first step in the transfer (i.e. introgression) of resistance genes from a wild species is its successful hybridisation with the cultivated potato. The commonest barrier to crosses between tetraploid potato cultivars and closely related wild species is embryo-abortion following endosperm collapse, a phenomenon associated with differences in effective ploidy, called endosperm balance number (EBN), rather than in ploidy *per se*. This has been overcome by bridging crosses, by embryo rescue, and by equalising the parental EBNs through manipulating ploidy levels in various ways. However, the ease of hybridisation can be increased, and its scope widened still further, through somatic hybridisation by protoplast fusion.

Somatic hybridisation by protoplast fusion A programme of protoplast fusion experiments was targeted initially towards introducing PCN resistance. Diploid *S. megistacrolobum*, *S. sanctae-rosae* and *S. sparsipilum* were fused with tetraploid *S. tuberosum* cultivars Brodick and Stirling to produce in excess of 800 putative somatic hybrids (*Ann. Rep. 1992, 14*). Hybrid status of many of these plants, has been confirmed by chromosome number, PCR analysis, PCA analysis and the possession of resistance to PCN attack. Several hexaploid hybrids (2n = 6x = 72) containing resistance to PCN have been identified from the fusions of cv. Brodick with *S. megistacrolobum* and with *S. sanctae-rosae*, and resistant aneuploids (2n = 54 to 71) have been recovered from fusions of cv. Brodick with all three wild species. A total of 240 somatic hybrids will be planted in the field for agronomic assessment in 1995 at our seed site at Blythbank Farm: 107 from *S. megistacrolobum*, 96 from *S. sanctae-rosae* and 37 from *S. sparsipilum*.

The next target is to create fusions between cultivars and breeding lines lacking blight resistance with those wild species recently identified as having resistance, and which have not been used previously in our breeding programme. These include diploid EBN1 species which do not readily cross with the more common diploid EBN2 species, nor with dihaploids (diploids, EBN2) produced from tetraploid EBN4 *S. tuberosum*.

The transfer of resistance genes from hybrids involving wild species to clones with the yield and quality demanded of a modern cultivar has traditionally involved a substantial backcrossing and selection programme over many years. Not surprisingly, plant breeders would like to accelerate the process by limited gene transfer. A number of possible methods of targeted chromosome transfer are being explored at SCRI, including asymmetric protoplast fusion, and early results are promising. In the longer term, it should be possible to monitor the introgression of resistance genes through their linkage to molecular markers, and to speed up the whole process by selecting for markers associated with desirable traits and against undesirable ones. This will be particularly useful for polygenic resistance.

Combining resistances already available with other desirable traits In the immediate future, however, the aim is to combine resistances already available in breeding lines and cultivars of *S. tuberosum* with other desirable traits such as marketable yield and quality. One objective has been to produce clones with three or four copies of major dominant genes which still provide useful resistance; for example, Rx, Ry and H_1 (*Ann. Rep. 1991, 13-16*). When used as parents, all of their offspring are resistant, even when the other parent is susceptible. Their use therefore avoids the need to screen progenies or waste resources on raising susceptible seedlings. A potential cultivar with three copies of the H_1 gene is at present in its second year of National List Trials, thus demonstrating that this approach is feasible.

Another objective is to combine quantitative resistances to late blight and the white potato cyst nema-

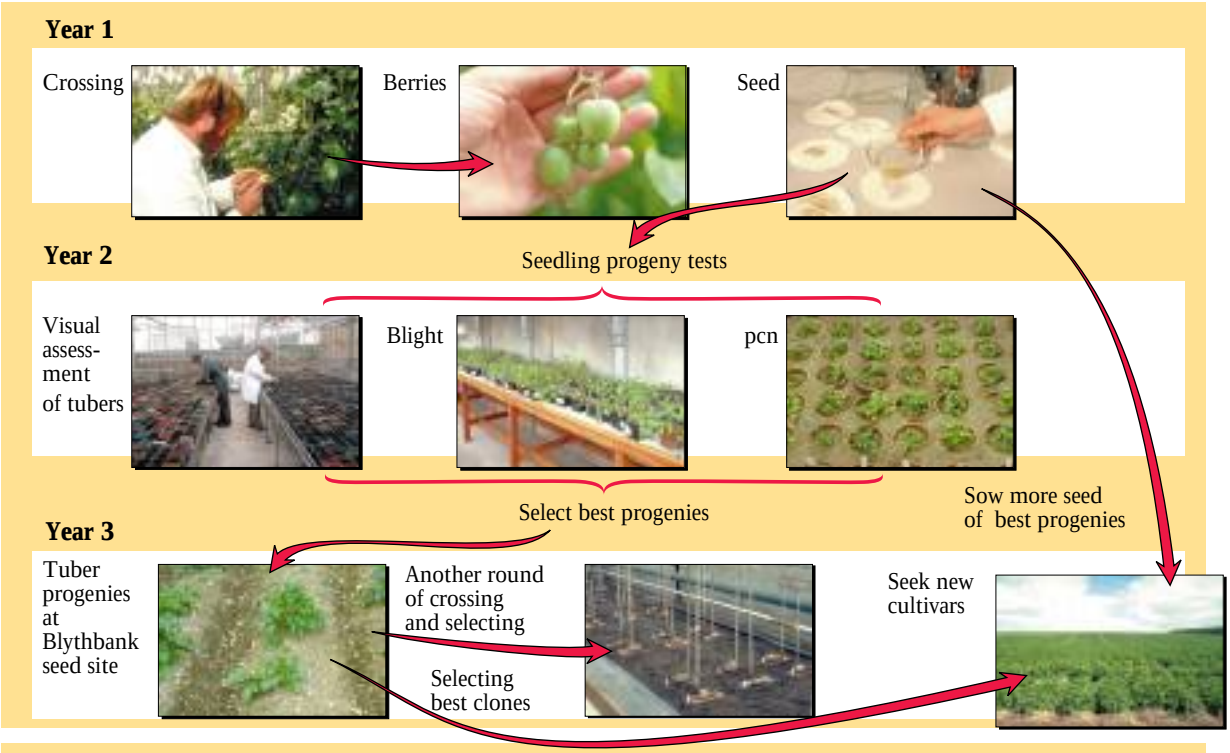


Figure 2 Three year cycle of recurrent selection.

tode with commercially acceptable tuber yields and quality, and to do so as quickly as possible because present day cultivars do not have high levels of resistance to both. Use is being made of seedling progeny tests to assess the resistance of progenies to PCN and to foliage and tuber blight, as resistance in the two tissues is not completely associated (*Ann. Rep. 1991, 13-16*). These can be done within a year of making crosses, and in parallel with a seedling progeny test for visually assessing the commercial potential of tubers. Thus, 168 crosses were secured from 39 suitable parents in 1991 and assessed in seedling progeny tests in 1992. The best 48 progenies were taken to Blythbank seed farm in 1993 as tuber progenies and visually assessed for commercial worth. In future, it should also be possible to add selection for freedom from after-cooking blackening and resistance to low temperature sweetening at this stage. The best three clones from each of the best 36 tuber progenies were selected and intercrossed in 1994 to start the next three year cycle of recurrent selection, because the ultimate goal cannot be achieved in one cycle when many genes are segregating (Fig. 2).

In the meantime, selection based on replicated field trials continues over a number of clonal generations from the clones evaluated as tuber progenies at Blythbank, and from resowings of more true seed of the very best progenies. In due course, those clones with the greatest potential will be evaluated more extensively in collaboration with commercial partners, who will then develop the best as finished cultivars.

Increasing the applicability of tissue culture methods for the improvement of industrial oil crops

S. Millam, A. Craig, E. Moscheni[1] and J.E. Lyon

Introduction Developing and improving industrial markets for crop products has assumed greater importance in recent years due to a number of environmental, political and economic factors, as well as significant breakthroughs in scientific techniques. Food surpluses and consumer demand for 'green' products have raised the potential market for products for non-food use in the energy, chemical and processing industries and they offer opportunities for diversification of the UK agricultural economy.

At SCRI, research and development into crops for industrial oils has employed two approaches; modifying existing crops, and investigating the potential of novel or under-utilised species for specific end products.

Oilseed rape oil is produced at relatively low cost in the UK, and the modification of its oil content to produce higher added value products is a realistic target for improvement. An example of the second approach is an investigation into hitherto under-exploited members of the temperate plant genus *Cuphea*, as potential sources of high value medium-chain fatty acids.

Regeneration of high erucic acid lines of oilseed rape Erucic acid (C22:1) is synthesized by elongation of oleoyl-CoA via eicosenoate and has a number of useful properties and applications. It is very stable at high temperatures with high fire and smoke points and substantial lubricity. Products of erucic acid have a number of current or proposed applications (Fig. 1). Older varieties of oilseed rape contained large amounts of erucic acid. However, because of concern following the findings of feeding experiments on animals in the 1960s low erucic acid varieties for human consumption were developed. Paradoxically, erucic acid is now in short supply as a chemical feedstock; for instance, the supply for usage as a slip agent is only one third of the current demand. Until recently, most of the high erucic acid rapeseed (HEAR) oil was imported from Eastern Europe, as all the rapeseed grown in the west was of the double low type (i.e. low glucosinolates and low erucic acid). However, recent changes to the agriculture in Eastern Europe have restricted the availability of the high erucic acid crop and, consequently, lines have been investigated for use in the UK. There is great interest in improving the currently available HEAR germplasm, including the development of lines with useful added-value by-products.

Methods based on plant tissue culture techniques have been used successfully at SCRI and elsewhere for the improvement of a range of Brassicas and can be used to genetically modify fatty acid profiles[1]. We have used techniques for tissue explant regeneration, and for the isolation, culture and regeneration of protoplasts

Erucamide	- the largest selling derivative of erucic acid and is used as a slip agent in polyolefin film (cling film) with a world market in excess of 171,000 tonnes. Erucamide also has excellent anti-static properties.
Pelargonic acid	- a product of the oxidative cleavage of erucic acid - has uses in plastics, coatings, perfumes, cosmetics and flavours
Brassylic acid	- also a product of the oxidative cleavage of erucic acid - has applications in the manufacture of nylons, polyesters, synthetic lubricants and paints.
Behenic acid	- the hydrogenation product of erucic acid - has a wide range of uses including low irritant quaternary ammonium compounds (common in hair conditioners), mixing and processing aids.
Behenyl fumarate	- is used as a biodegradable polymerised flow improver on North Sea drilling rigs.
Behenyl ketene dimer	- a textile additive used as a waterproofing agent for cotton and as a coating agent for cellulose.

Figure 1 Potential uses for erucic acid and derivatives.

[1] E. Moscheni was a visiting worker from the Dipartimento di Agraria Universita di Pisa, Italy.

	Auxin NAA	Cytokinin BAP	Mean no. shoots per internode (n=40)
SM1	0.0	0.0	0
SM2	5.0µm	0.0	2.60 ± 0.50
SM3	0.0	5.0µm	2.40 ± 0.36
SM4	5.0µm	5.0µm	3.67 ± 0.47

Figure 2 Shoot regeneration studies in HEAR cultivar 'Arthur'.

on three high erucic acid lines of oilseed rape. Of the HEAR lines investigated, plants were regenerated from tissue explants and from mesophyll protoplasts, with the cultivar Arthur proving particularly responsive.

Tissue explant regeneration The high-efficiency stem internode regeneration system developed at SCRI for the transformation of rapid-cycling *Brassica oleracea*[2] was successfully adapted for the regeneration of shoots of three HEAR lines. This method used an 8:1 ratio of cytokinin (BAP) to auxin (NAA) supplement to Murashige and Skoog medium, and produced shoots from internodes at a mean rate of more than four per explant. In a separate experiment using internodes of the cultivar Arthur, shoot regeneration could be obtained using low levels of either a single auxin, a single cytokinin and a combination of both (Fig. 2). This may have significance in the range of subsequent induced fatty acid variation and suggests that the inherent responsiveness of this line would promote its use as a model for further studies.

Protoplast isolation, purification and regeneration Protoplasts were isolated from 12-day old, *in vitro* grown hypocotyl tissue, and *in vitro* mesophyll tissue. The protoplasts were cultured in the dark for 3 days before exposing to light. After 30 days, the cells were overlayed onto a callus-inducing medium and, when calli had developed to a diameter of 1-2 mm, placed onto a high cytokinin media designed for shoot regeneration[3].

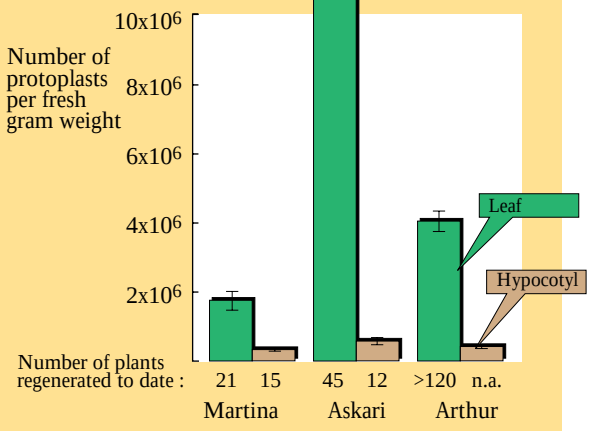


Figure 3 Summary of protoplast isolation and regeneration of HEAR lines.

Populations of HEAR have been generated from both tissue explants and protoplasts (Fig. 3). Preliminary analyses of glasshouse-grown samples have demonstrated interesting changes in fatty acid profiles and they provide a useful germplasm source for studies on the mechanisms of somaclonal variation.

The spring crop of high erucic acid rapeseed particularly suited to Scotland, where both seed and oil yield are among the highest in Europe. Prospects for the more widespread cultivation of this crop are good and it could be grown on set-aside land for non-food use. Our investigations show that the plant is highly amenable to plant tissue culture, thus facilitating potential manipulations and improvements.

Improvement of the genus *Cuphea* by *in vitro* techniques The genus *Cuphea* has been the focus of a number of recent studies as a novel source of medium-chain fatty acids (Fig. 4). Certain members of the genus can also produce unusually high levels of single medium chain fatty acids in their seeds and this desirable characteristic may have implications in modifying other oil crops. Most seed oils contain a mixture of fatty acids, but, for industrial processing, the end-use of a seed oil is targeted at only one of its constituents and plants that produce mainly one component would minimise downstream processing costs. Interestingly, by categorising the species according to their dominant fatty acid an evolutionary trend from C18 in the more primitive species to the shorter chain C8 in the taxonomically more advanced species has been detected.

Figure 4 Examples from the *Cuphea* germplasm collection.

Species	Chromosome numbers	Principal fatty acid
C. viscosissima	6	capric (C10:0)
C. lanceolata	6	capric (C10:0)
C. paucipetala	10	capric (C10:0)
C. leptopoda	10	capric (C10:0)
C. laminuligera	10	lauric (C12:0)
C. tolucana	12	lauric (C12:0)
C. lutea	14	lauric (C12:0)
C. wrightii	22	lauric (C12:0)

Figure 5 *Cuphea* species investigated.

Despite its attractive oil content, Cuphea exhibits a number of phenotypic characteristics that would preclude its unimproved introduction as an agricultural crop. These include indeterminate patterns of development, seed shattering and seed dormancy and are problems common to many undomesticated crops.

Our studies have focused on establishing a range of *Cuphea* species *in vitro*, and developing tissue regeneration protocols that could be adapted for genetic transformation technology. We have also evolved protoplast isolation and fusion methods to establish a basis for an interspecific hybridisation programme aimed at creating material of experimental and commercial interest. Seeds were obtained from Dr L. Angelini, University of Pisa, Italy, where there is a programme of research into the agronomy of *Cuphea* (Fig. 5). *C. laminuligera* and *C. lutea* present the most promising prospects for domestication within the genus[4], whilst other species were selected for oil content or other desirable traits.

Cuphea tissue culture progress Variables controlling plantlet regeneration were investigated using leaves or internodes as a tissue source, and either culture media developed at SCRI or another based on the one previous report of *Cuphea wrightii* regeneration *in vitro*[5].

Figure 6 Regeneration of shoots from a leaf explant of *C. tolucana* after 28 days culture.

Protoplasts were isolated from leaf tissue using the protocol as used for *B. napus* above.

All species produced callus readily from leaf and internode explants. Within 21 days, shoot production occurred on explants of six of the eight species investigated (Fig. 6) and shoot production was markedly higher from leaf explants than from internodes. Protoplasts were isolated for six species, with high rates of recovery after purification, and the protoplasts proved amenable to chemical fusion with *B. napus* protoplasts, though showed a preferential bias towards self-fusion.

	Regeneration Callus incidence	**Rate/explant** Shoot production	**Protoplast yield** 10^6 per g
C. viscosissima	87%	1.0 ±0.2	7.52 ±2.4
C. lanceolata	97%	1.2 ±0.2	4.00 ±1.8
C. paucipetala	100%	None	11.34 ±3.2
C. leptopoda	97%	2.1 ±0.4	n.a.
C. laminuligera	20%	None	11.52 ±2.0
C. tolucana	100%	3.1 ±1.1	15.36 ±4.2
C. lutea	100%	0.7 ±0.2	1.02 ±0.3
C. wrightii	60%	7.2 ±2.3	n.a.

Figure 7 Response of a range of *Cuphea* spp. *in vitro*.

Summary This is the first report of the *in vitro* culture of seven *Cuphea* lines and indicates the applicability and potential for tissue culture manipulations of examples of this genus.

In the long-term the domestication of species such as *Cuphea* may be better than modifying existing crops to avoid reliance on a single species only for the production of a range of oils. The benefits arising from the production of industrial oils from a sustainable agricultural system, and the productive use of set-aside land are manifest. The methods of conventional tissue culture offer a cost-effective and easily transferable technology compared with the methods of gene isolation, cloning and transformation is use in other research programmes.

References

1 Millam, S., Craig, A., Robertson, G., Griffiths, D.W., Christie, W.W. & McNicol, J. (1994). *Abstracts VIII International Congress of Plant Tissue and Cell Culture*, 119.

2 Millam, S., Wen Lanying, Whitty, P., Fryer, S., Burns, A.T.H. & Hocking, T.J. (1994). *Cruciferae Newsletter* 16, 65-66.

3 Barsby, T.L., Yarrow, S.A. & Shepard, J.F. (1986). *Plant Cell Report* 5, 101-103.

4 Moscheni, E., Angelini, L.G. & Macchia, M. (1994). *Industrial Crops and Products* 3, 3-9.

5 Janick, J. & Whipkey, A. (1986). *Horticultural Science* 21, 135-137.

Aspects of environmental risk assessment for genetically modified plants with special reference to oilseed rape

A.M. Timmons, E.T. O'Brien, Y.M. Charters & M.J. Wilkinson

Intensive research over the past 10 years has resulted in the production of genetically modified lines of several important crop species including oilseed rape (*Brassica napus*), potato (*Solanum tuberosum*) and raspberry (*Rubus idaeus*). Many of these lines have increased market potential which has led to commercial pressure for their release as new cultivars although any release must be preceded by a rigorous risk assessment in statutory controlled field trials. However, concern that any release into the environment may allow transfer of the inserted genes to neighbouring commercial fields, feral (escapes from cultivation) and volunteer populations or natural populations of wild relatives has aroused intense debate. Paucity of information on pollen and seed dispersal ranges, the geneflow dynamics of agricultural fields, the distribution of cultivated and feral populations and on the ecological status of potential feral populations has done little to alleviate these concerns and could lead to a polarization of views based on conjecture rather than fact. If the advances in biotechnology are to be applied, then any risks, real or perceived, must be quantified.

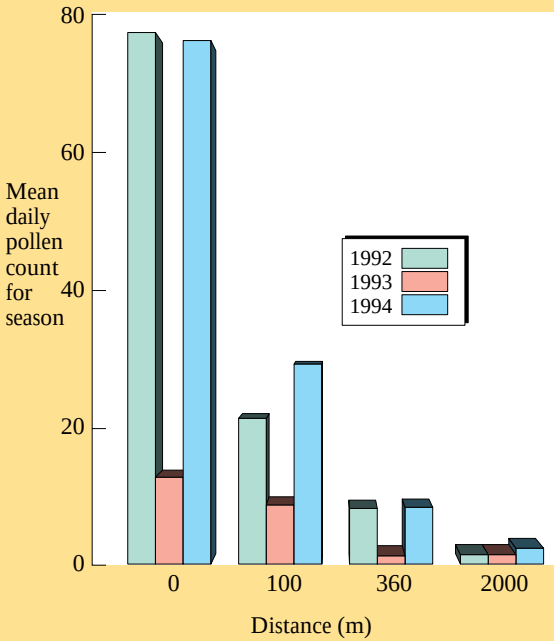


Figure 1 Mean daily pollen counts for the flowering season of oilseed rape in 1992, '93 and '94 (54, 47 and 42 days respectively) recorded at four distances from a field margin (0, 100 and 360 m and 2.5 km in 1992, 2.0 km in 1993 and 1.5 km in 1994).

Large-scale commercial fields of genetically modified crops were not available in the UK and work at SCRI has used fields of non-genetically modified oilseed rape to estimate pollen movement and geneflow by using naturally occurring genetic variation between cultivars of the same species. The results can then be used to assess the likely movement of introduced genes from fields of genetically modified crops.

Pollen movement Detailed pollen profiles were constructed along a linear transect from an oilseed rape field covering a distance of up to 2.5 km. Airborne pollen densities were found to decline with distance although there was considerable variation between years in both pollen concentrations and in the rate of decline (Fig. 1). In all years, the pollen concentrations detected at 360 m were 10-11% of that recorded at the field margin. Low pollen densities were consistently recorded at 1.5-2.5 km from the source which did not differ significantly between years and so probably represents background levels. The most striking feature of these data was the large disparity between the high densities of pollen recorded from large-scale agricultural fields and the lower levels reported by other workers from small-scale trial plots. Oilseed rape pollen seems to be released in large quantities and dispersed further than had been predicted from small-scale trial plots. Caution should be exercised, therefore, in the interpretation of small scale experiments when trying to predict the levels of geneflow likely to occur under standard agricultural conditions.

Long-range geneflow It was not known if the low levels of pollen consistently detected at 2 km were sufficient to effect significant levels of geneflow. Emasculated (and depetalled) oilseed rape plants placed at increasing distances from an oilseed rape field were used as bait plants for airborne pollen.

Figure 2 Aerial view of small section of area surveyed in Tayside region of Scotland (May, 1993).

Some seed set was observed on all of the bait plants. The percentage seed set declined with distance and correlated well with the decline in airborne pollen concentrations. Seeds produced on plants 2 km away from the source gave rise to plants with 38 chromosomes that were phenotypically normal *B. napus*, thereby demonstrating the capacity for long-range geneflow up to this distance.

Distribution of cultivated oilseed rape fields and potential feral populations A ground survey was conducted over two years, covering 70 km^2 in the North East Fife and Tayside regions of Scotland to investigate the distribution of cultivated oilseed rape and non-cultivated, potentially feral populations (Fig. 2). The mean distance between cultivated winter and spring sown oilseed rape fields and feral populations identified within the survey area was 1.0 km and 1.2 km respectively. Approximately 10% of the feral populations occurred within 100 m of cultivated fields.

Characterization of feral oilseed rape populations Feral populations of oilseed rape varied in size from individuals to populations exceeding 1000 plants (Fig. 3a, b). The seed return per plant and germination frequency varied greatly between sites. Many of the sites mapped were subject to weed management practices (cutting, spraying or rotovating) which prevented many plants in a population reaching maturity. However, 24% of the populations surveyed successfully set seed although relatively few populations were located at the same site for more than one year.

Geneflow between agricultural fields The mean distance between cultivated oilseed rape fields was 0.9 km and approximately 10% were situated within 100 m of one another. These distributions suggest that

Figure 3 (a) Population of oilseed rape in field margin (North East Fife, April 1993); (b) Population of oilseed rape occurring on ex-agricultural soil (Tayside, June 1993).

the potential exists for geneflow to occur between agricultural fields under current farming practices. The main problem associated with attempts to deter-

Figure 4 Large-scale screening of seed progeny collected from measured intervals within a commercial field of a winter sown oilseed rape cultivar (Falcon) situated adjacent to a field of spring sown cultivar (Comet). Flowering 'hybrid' seed (produced as a result of inter-cultivar crosses) can easily be identified amongst large numbers of non-flowering intra-cultivar crosses and selfs.

mine the frequency of geneflow occurring between adjacent agricultural fields is detecting potentially very low frequencies of hybrid seed (produced by cross pollination between donor and recipient cultivars) among large numbers of seed produced by intra-cultivar crosses and selfing. To overcome the problem of scale, detection of hybrids was approached using a 2-phase screening system which involved morphological markers to carry out the initial large-scale screening of seed progeny (approx. 350,000) (Fig. 4) followed by a second phase using molecular markers (RAPDs) to confirm the reliability of the initial screening. In oilseed rape there are few morphological marker genes suitable for efficient large-scale screening of commercial cultivars. However, the vernalization requirement of winter oilseed rape varieties was found to be a suitable marker for large-scale screening in the field to test for cross-pollination between cultivated fields of winter and spring varieties. Using this approach, geneflow between fields was detected at a distance of 100 m from the source field margin. A similar approach is being applied to adjacent fields of raspberry and potato and, in the case of raspberry, geneflow has already been detected at 120 m.

Acknowledgements This work is being funded by the Scottish Office Agriculture and Fisheries Department (Contract No. FF340) and the Department of the Environment Research Programme (Contract No. PECD 7/8/237).

Genetic improvement of trees

R.J. McNicol & M. Van de Ven

Improved tree breeding to meet the needs of world energy, building and fibre (paper-making) requirements, in an environmentally sustainable manner, requires a major advance in the understanding of the nature, source and interaction of the genes controlling commercially important traits in trees. A firm basis is being established in genome analysis in legumes and brassicas; however, woody and technically difficult and long-term crops like trees have been largely ignored. Sitka spruce accounts for about 60% of the timber production in the UK and yet the breeding of the crop is at a relatively under-developed stage, despite it being introduced into the UK in the 1920's. This is largely because it has a long juvenile stage which makes each breeding cycle a protracted affair. It takes about 15 years for a germinating seedling tree to start flowering, and perhaps a further 20 years before mature aspects of such a tree can be fully assessed to permit the selection of superior genotypes.

Recent advances in molecular techniques being used at SCRI, eg RFLPs and PCR based techniques such as RAPDs, offer the very real prospect of identifying molecular markers that can be linked to important traits and hence permit the early selection of potentially desirable genotypes prior to field planting. The prospect for greatly increasing the efficiency and speed of the breeding and selection processes in tree genetics programmes is therefore very good.

The programme at SCRI has aimed to combine existing expertise in genetics, breeding, wood chemistry and molecular skills of SCRI, and the germplasm and

Figure 1 Mature trees.

Figure 2 Trees are managed for early seed production by grafting onto root stocks.

knowledge of the Forestry Authority, to greatly improve present breeding efficiency, so that enhanced germplasm can be selected quicker and more effectively for ultimate planting within the UK. The successful completion of these objectives will be translated into higher quality, and increased production of timber and fibre in the UK under a sustainable agroforestry system.

Sitka spruce was introduced into Britain around the end of the last century from North America, predominantly from Queen Charlotte Island, British Columbia and Alaska. Superior or 'plus' trees are selected from plantations around Britain on criteria such as growth rate, height, straightness, branching habit, disease resistance and timber quality (wood density). Cuttings from these trees are then taken for grafting onto rootstocks, and thereafter maintenance in a gene bank, until the outcome of progeny testing of open pollinated seed is determined. If the superior

Figure 3 A seed orchard of sitka spruce with trees well spaced to enhance cross pollination.

trees are shown to have good General Combining Ability (GCA), by out-performing controls by some 10-15%, then they will be used in the breeding programme. This entails vegetatively propagating these superior trees and planting them in isolated seed orchards, where the layout is designed to achieve maximum out-crossing between the genotypes.

Plus-trees of sitka spruce from two orchards were provided by the Forestry Authority. Since little was known of the relatedness or origin of these trees and of the actual extent of out-crossing within and between the seed orchards, we initially set out to select suitable molecular markers to fingerprint them. After developing protocols which permitted the extraction of DNA of sufficiently high quality to carry out PCR amplification procedures, we tested 30 RAPD primers, and from these selected seven which were able to differentiate between the 55 genotypes.

We have now developed our DNA extraction and molecular techniques to allow us to establish the degree of out-crossing in sitka spruce seed orchards. In addition, this technique permits the determination of the pollen donor which provides a potentially invaluable and novel technique for monitoring gene flow in this and other tree species.

It is our intention that with the further development of these protocols, we will be able to minimise the degree of inbreeding and pollen contamination by undesirable genotypes within seed orchards. This is significantly important as a first step in the improvement of commercial tree species within the UK through application of molecular techniques.

New swede cultivar

Invitation (SS16): Invitation successfully completed official trials in 1994 and has been placed on the National List in 1995. It is SCRI's first cultivar with resistance to clubroot as well as to powdery mildew. Clubroot resistance was introduced from stubble turnip (in fact European Clubroot differential 04) through a synthetic *Brassica napus* produced in 1976. Four backcrosses to modern cultivars with selection for clubroot resistance ensued before commencing four generations of selfing with family selection for agronomic characters, as well as single plant selection for clubroot resistance. Invitation is a general purpose swede with high yields of dry matter at a high dry matter content. It has purple skinned, intermediate shaped roots with yellow coloured flesh. It is being marketed by Sharpes International Seeds Limited.

New raspberry cultivar

Glen Ample (7815B8): derived from a complex cross involving Glen Prosen and the Canadian cultivar Meeker. It combines exceptionally high yielding potential with excellent flavour. The canes are spine-free, upright and of good vigour. Internode distances on floricanes are short and fruiting laterals are erect, giving good fruit presentation for easy harvesting. Fruit is bright and larger than Glen Rosa (3.3 g), averaging at 3.9 g. Glen Ample is a high yielding, fresh fruit cultivar that forms fruit of good quality under large scale trialling conditions in England. Under Scottish conditions, the fruit is less cohesive and may only be suitable for processing.

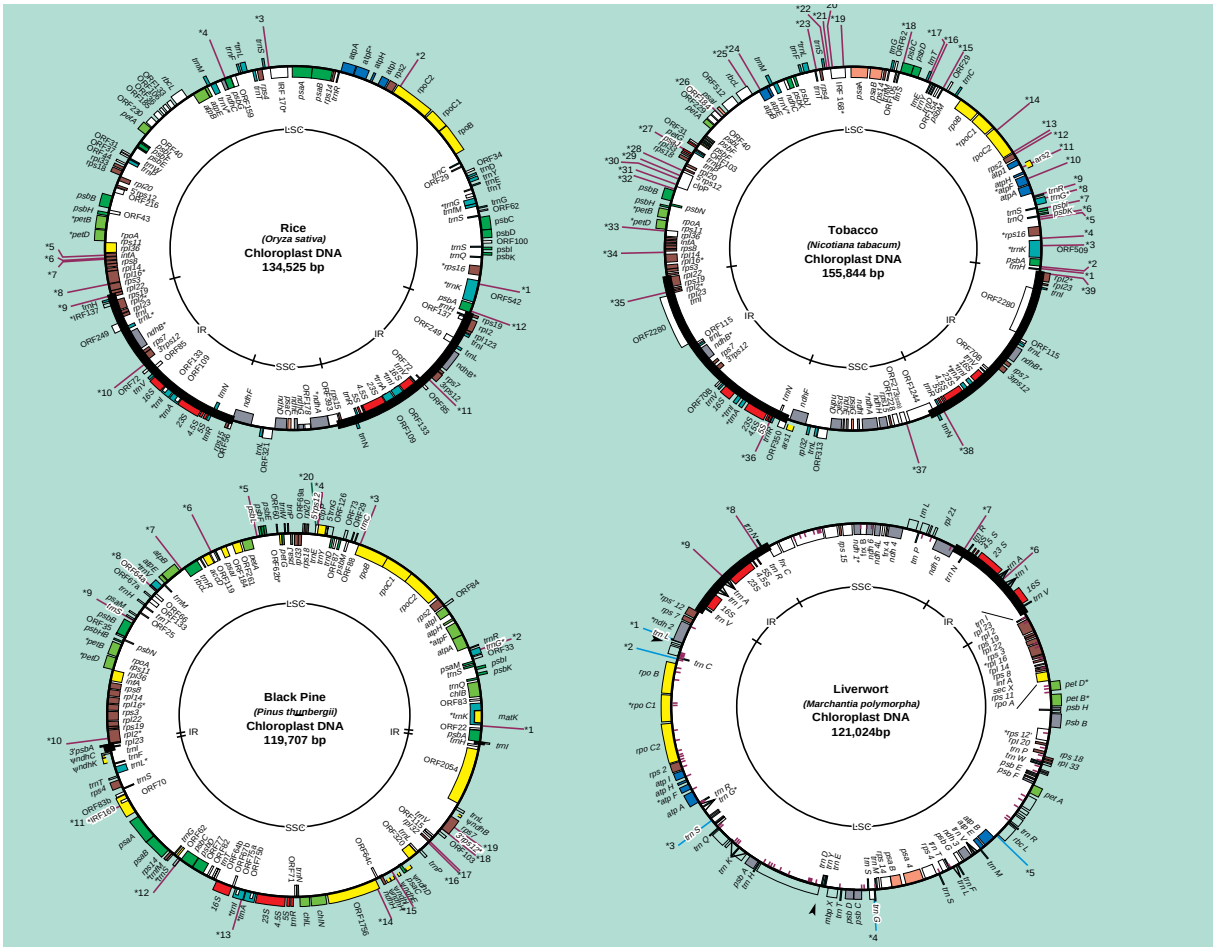
Molecular biology

Wayne Powell

The Cell and Molecular Genetics Department was created a mere 10 years ago to meet the dual challenge of harnessing modern technology and initiating several new important research programmes. Its success and achievements are partly reflected by the manner in which molecular and cellular biology now permeates much of the research activities of the Institute. During the past decade there has been a change in our scientific culture and we are more dependent on accessing external funding from a wider range of sources than previously. In addition, the rapid pace of research today poses new challenges where the need and desirability of national and international co-operation together with scientific exchange are vital. We remain flexible in responding to change in research direction and committed to enhancing interaction between government and commercially-funded research organisations.

The research programme on the analysis of pre-mRNA splicing in plants is pre-eminent and continues to arouse considerable scientific interest. It is the only programme in the UK which is addressing this important area of plant gene expression and in addition to investigating plant post-transcriptional processes, it provides innovative approaches to evolutionary comparisons. It aims to elucidate the mechanisms, components and molecular interactions involved in plant pre-mRNA splicing and includes basic investigations on alternative splicing, intron-enhanced expression and the differences in splicing mechanisms between monocotyledonous and dicotyledonous plants. Several snRNA, snoRNA and snRNP protein and helicase genes have been cloned and various techniques developed to analyse their genomic organisation. The potato U2B" and U1A clones which have been identified are the only full-length plant spliceosomal complex genes that have been isolated to date and are particularly significant since they are highly conserved in comparison to the human U2B" and U1A proteins and hence provide experimental models for studying RNA-protein interaction. Elegant studies involving *in situ* hybridisation and immunofluorescent labelling of antibodies against U2B" have been used to study the sub-nuclear distribution of plant spliceosomal components (see p. 69).

The molecular characterisation of plant-pathogen interactions has advanced considerably in recent years. For example, the first resistance gene participating in a gene for gene interaction has been described using map-based cloning strategies[1]. Many plant pathogens produce cell wall degrading enzymes that cause tissue disintegration and recently a polygalacturonase inhibitor protein (PGIP) that inhibits endopolygalacturonase activity by the pathogen has been isolated from resistant plants. In collaboration with the University of Auckland, a cDNA clone encoding PGIP has been isolated from kiwifruit (see p. 65).



Microsatellite locations superimposed on genetic maps of plant chloroplasts (Powell *et al.*, Current Biology in press).

The next phase will be to attempt to express this gene in kiwifruit and other soft fruit crops to determine the potential of PGIP expression to confer resistance to fungal attack.

Work on the molecular biology of carbohydrate metabolism has revealed a family of invertase genes with individual members exhibiting tissue- and development-specific expression patterns. Research is now focused on the function of acid invertases encoded by this family in source-sink interactions and low temperature sweetening. These topics are being addressed at several levels. Promoter studies are examining the sequence architecture of invertase gene promoters and, by promoter fusion to reporter genes, offering a higher resolution to the localisation of invertase gene expression. The potential for post-transcriptional regulatory mechanisms, mediated by alternative splicing events around a 9 bp mini-exon which encodes a highly conserved region of the enzyme, is under investigation. Sequences encoding proposed intracellular targeting signals have been identified and cloned with a view to their application in the targeting of the products of heterologous gene expression in potato. They are being used to stabilise heterologous protein in tubers of potato plants engineered to give high level expression of genes from maize and Brazil nut which encode proteins with high methionine content. A wide range of transgenic potato lines, carrying antisense constructs against various invertases expressed from constitutive and tuber-specific promoters, has been produced in the first phase of the low temperature sweetening programme (*Ann. Rep. 1991, 40-42*) and, in the second phase, these will be entered into extensive field trials at SCRI with promising transgenic lines from the other groups throughout Europe involved in the initial research. The second phase, funded by industry, will also include the generation of novel promoter-gene constructs and transgenic potato lines in which the expression of multiple genes is manipulated. The results from this work may aid the future design of potatoes which have been genetically

manipulated both for altered carbohydrate metabolism and other traits.

Somatic hybridisation offers an alternative method to sexual hybridisation for the re-synthesis of potato breeding lines, by combining two different dihaploid lines possessing complementary agronomic traits. This method has been used for the production of hybrids with resistance to nematodes and late blight in addition to good tuber shape and yield properties.

New information on the *Ty1-copia* group of retrotransposons in plants is described on p. 53 and has provided the statistic that this class of retroelement occupies 5-10% of the genome of *Vicia faba*. The chromosomal distribution of the *Ty1-copia* element has also been studied using *in situ* hybridisation and revealed that it is absent from heterochromatic regions of the *Vicia faba* genome. Transposon tagging provides a powerful alternative tool for the cloning of plant genes. This method is being used to isolate the *Hero* gene of tomato which provides resistance against all known pathotypes of the potato cyst nematode, *Globodera rostochiensis.*

Technological developments have made possible the development of high resolution genetic linkage maps in a range of organisms. These provide new opportunities to analyse complex phenomena such as the genetical basis of polygenic (quantitative) forms of variation, comparative genome mapping, genome evolution and studies of gene flow. Repetitive DNA sequences form a substantial fraction of the genomes of most eukaryotes. One particular class of repetitive DNA termed microsatellites or simple sequence repeats (SSRs) has attracted considerable interest for two main reasons: firstly, they have been used in conjunction with PCR to generate highly polymorphic DNA markers, and, secondly, the observation that length expansion of tri-nucleotide repeats has been shown to be associated with a number of human genetic diseases. Evidence is now available that SSRs exist in plants and that they are highly polymorphic. However, considerable effort is required to identify flanking primers to amplify the repeat motif and generate length polymorphism. In order to reduce the number of clones to be screened, various enrichment techniques are being investigated to increase the frequency of SSRs. A comparison of the utility of various polymorphic assay procedures undertaken in barley has confirmed the high information content of SSRs but also identified amplified fragment length polymorphism (AFLP) as a generic technology with considerable potential for use in plant breeding and genome research.

New insights into the ecological genetics of two important tropical tree species (*Gliricidia sepium* and *G. maculata*) have been revealed by the use of a range of polymorphic assay procedures (see p. 52). Both uni- and bi-parentally transmitted markers were deployed to provide complementary information on gene flow. These results have considerable implications for the conservation, management and utilisation of *Gliricidia* genetic resources in the Tropics.

Barley dominates Scotland's arable production contributing significantly to national wealth creation and offering considerable export opportunities. Research in barley genetics has been strengthened by the integration of the Cereals group within the Cell and Molecular Genetics Department thereby creating a comprehensive research base to sustain Scotland's primary agricultural commodity. This brings new opportunities to link research in molecular genetics with barley genetics and breeding. Two new laboratories have been formed: an accelerated breeding laboratory which will help to establish a bridge between classical and molecular breeding and an abiotic stress group which will investigate the genetics of stress tolerance in cereals. These new initiatives reflect the importance of barley in our planning and the reorganisation will provide an effective, inter-disciplinary environment for cereal research.

[1] Martin, G.B. *et al.* (1993). *Science* 262, 1432-1436.

Cell & Molecular Genetics Research Highlights

Genetic markers & genome organisation

Development of 400 point map for barley cross using PCR based markers.

Demonstration of utility of microsatellites in potato and barley.

Exploitation of microsatellites in the nuclear and chloroplast genomes of trees for use in molecular ecology and population genetics.

Retrotransposon organisation in plant genomes and gene isolation by transposon tagging.

Plant breeding

High yielding winter barley lines in trials.

Detection of QTLs reproducible across seasons for a range of characters.

Spring barley line in National List trials - high yield potential with good malting quality and disease resistance.

$\delta^{13}C$ discrimination and abiotic stress.

Molecular breeding programme for barley.

Somatic hybridisation in potato.

Gene expression

RNA splicing: intron signals
snRNA genes
snRNP protein genes
polycistronic snoRNAs
snRNA-snRNP interactions
nuclear organisation studies

Ribozyme and antisense RNA technology.

Promoters: constitutive and tissue specific.

Genetic manipulation

Invertase gene family characterised:

Modification of carbohydrate metabolism by anti-sense RNA in transgenic potato tubers - field trials in 1995.

Constructs to modify expression of multiple enzymes of carbohydrate metabolism in potato tubers.

SAMDC and modulation of polyamine metabolism.

Molecular ecology of tropical tree species: detection of interspecific gene flow between *Gliricidia sepium* and *G. maculata* using PCR

I.K. Dawson, R. Waugh & W. Powell

Gliricidia sepium is a medium-sized leguminous tree which forms an important component of tropical dry forest in Meso-America. The species has a multitude of present or potential uses, including wood for fuel and construction, leaves for fodder and mulching, and live trees for fencing, shade in plantations and the prevention of soil erosion. Natural populations of *G. sepium* in Guatemala and Mexico form an important source of seed for distribution to farmers and other users, but concerns centre on mechanisms which may lead to their genetic erosion, including man-mediated range expansion and subsequent interspecific gene flow (introgression) from the related species, *G. maculata*. Therefore, an assessment of interactions in the genus is important in order to define appropriate conservation and collection strategies for *G. sepium*. Traditional taxonomic approaches are limited in their ability to assess interactions among species due to phenotypic plasticity and the lack of sufficient markers. Consequently, we employed molecular markers based on the polymerase chain reaction (PCR) to test for interspecific gene exchange in *Gliricidia*. PCR is particularly amenable for ecological studies of tropical tree species because only very small quantities of low quality leaf material are required. Apart from facilitating collection in the field, leaf fragments from previously collected herbarium specimens can be analysed. In addition, PCR provides a variety of methods appropriate for the detection of genetic variation in ecogeographical studies, including random amplified polymorphic DNA (RAPD)[1], restriction fragment length polymorphism (RFLP-PCR)[2] and single strand-conformation polymorphism (SSCP)[3] analyses. All three of these methods were used to assess nuclear and organellar variation at a macrogeographic level among 15 populations of *Gliricidia*. The sampled area included

Figure 1 Geographic distribution of sampling localities for *Gliricidia* in Meso-America.
A (Gs), B (Gs/Gm), C (Gm), D (Gm), E (Gm), F (Gm), G (Gm), H (Gs/Gm), I (Gm), J (Gs), K (Gs), L (Gs), M (Gs), N (Gs), P (Gs). Codes in parentheses indicate the designation of populations based on morphological descriptors at the time of collection. Gs = *G. sepium*, Gm = *G. maculata*, Gs/Gm = characteristics of both species. Populations K and L from the Pacific coast represent important natural resources of *G. sepium* for distribution to farmers and other users.

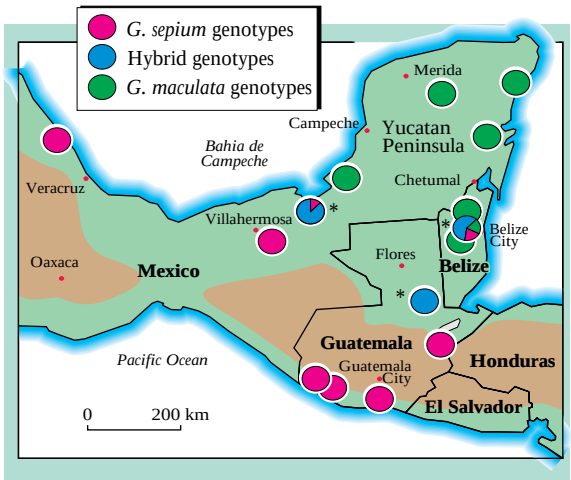


Figure 2 Macrogeographic distribution of *G. sepium*- and *G. maculata*-diagnostic polymorphisms in populations of *Gliricidia* sampled from Meso-America. Data shown represents polymorphism at a single RFLP-PCR locus, and is indicative of variation detected at other loci. Three populations resultant from interspecific gene exchange are indicated *.

'pure' populations of *G. sepium* and *G. maculata*, as well as sites of apparently mixed ancestry (Fig. 1). Results from each method revealed species-diagnostic polymorphisms which could be used to identify populations resulting from interspecific gene flow. In all, three sites revealed combinations of markers consistent with interspecific gene exchange (Fig. 2). Taken together, these sites defined a hybrid zone between the species, the presence of which is consistent with range-wide extension of *Gliricidia* under the influence of man. The occurrence of introgression in the genus indicated a potential mechanism for the genetic erosion of important resource populations of *G. sepium* on the Pacific coast. Significantly, however, these populations displayed no evidence of interspecific gene exchange and appeared genetically intact (Fig. 2).

Apart from increasing our biological understanding of the genus, the data indicated potential management strategies for the collection and conservation of genetic resources within *Gliricidia*, which is an essential pre-requisite for its future development and utilisation. Field testing of *G. maculata* in Pacific coastal regions should be discouraged in order to maintain the integrity of *G. sepium* populations, while 'pure' populations of each species positioned close to the hybrid zone should receive priority for collection. Further analysis of the transition zone will allow a more complete description of the extent and consequence of introgression within *Gliricidia*. Finally, the work reported here has indicated the overall applicability of molecular marker based strategies to the ecology of tropical tree species.

References

1 Dawson, I.K., Simons, A.J., Waugh, R. & Powell, W. (1995). *Heredity* 74, 10-18.

2 Karl, S.A. & Avise, J.C. (1992). *Science* 256, 100-102.

3 To, K-Y., Liu, C-I., Liu, S-T. & Chang, Y-S. (1993). *The Plant Journal* 3, 183-186.

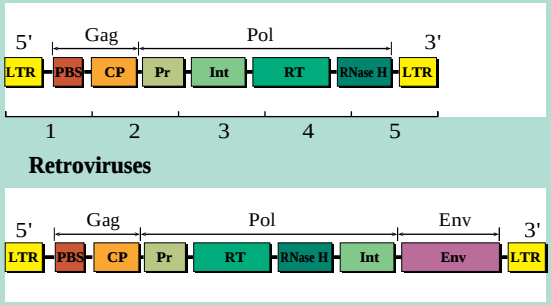
The *Ty1-copia* group retrotransposons in plants

A. Kumar, S.R. Pearce, A.J. Flavell[1], G. Harrison[2] & J.S. Heslop-Harrison[2]

Mobile genetic elements, initially discovered in maize by Barbara McClintock in 1951, have since been discovered in a wide variety of organisms from microbes to higher eukaryotes. Although united in their ability to insert at different positions in the genome, individual transposons achieve this goal by different means, and have been classified according to their mode of propagation[1]. Class I elements such as Ac and En/Spm use DNA transposition intermediates, while Class II elements or retroelements transpose via an RNA transcript which is reverse transcribed to DNA prior to re-insertion. Retroelements are the most common class of eukaryotic transposable elements. They include the retroviruses and the LTR (long terminal repeats) retrotransposons and the non-LTR retrotransposons (Fig. 1a and 1b). The best understood group of LTR retrotransposons is the *Ty1-copia* group, named after the most studied elements isolated in yeast (*Saccharomyces cerevisiae*) and fruit fly (*Drosophila melanogaster*). *Ty1-copia* group elements are characterised by the presence of long terminal repeats and a unique gene order (Fig. 1a). A number of these elements have been fully sequenced including *Ta1* of *Arabidopsis thaliana*, *Tnt1* and *Tto1* of tobacco, *Tst1* of potato, and *Bare-1* of barley.

Ty1-copia group retroelements are ubiquitous and heterogeneous in plants There has been considerable interest in the evolution of retrotransposons in plants. To find out whether *Ty1-copia* group retroelements are ubiquitous in the angiosperms and whether they are present in the primitive non-flowering plants, the polymerase chain reaction (PCR) has been used to isolate fragments of the reverse transcriptase (RT) gene of retrotransposons (Fig. 1b) from a wide variety of members of the higher plant kingdom. Fifty-six species from as diverse families as bryophytes (e.g. *Sphagnum capillifolium*), gymnosperms (e.g. *Ginkgo biloba*) dicotyledons (e.g. potato and pea) and mono-

[1] University of Dundee [2] John Innes Centre, Norwich



LTR - Long terminal repeats (300- 600bp)
CP - capsid or structural proteins
PBS - Primer binding site
Pr - Protease
Int - Integrase or endonuclease
RT - Reverse trancriptase
RNaseH - ribonuclease H
ENV - envelope proteins

b)

5'ACNGCNTTPyPyTNCAPyGG3' 3'ATPuCANCTPuCTPuTACPuA5'
T A F L H G Y V D D M L

Figure 1 a) structural features of the *Ty1-copia* retrotransposon and its comparison with the retroviruses. b) nucleotide and peptide sequences of primers used in this study.

cotyledons (e.g. barley and rice) produced an amplified fragment of the size expected for the reverse transcriptase gene of *Ty1-copia* group retroelements. Sequence analysis of subclones shows that PCR fragments display varying degrees of sequence heterogeneity. For example, sequence analysis of 31 subcloned fragments from potato reveals that each is different from the others, with predicted amino acid diversities between individual fragments varying between 5% and 75%. Therefore, sequence heterogeneity seems to be a general property of *Ty1-copia* group retroelements of higher plants, in contrast to the limited diversity seen in retrotransposons of yeast and *Drosophila*. Phylogenetic analysis of all these RT sequences shows, with some significant exceptions, that the degree of sequence divergence in the retrotransposon populations between any pair of species is proportional to the evolutionary distance between those species. For example, most of the RT sequences from plants belonging to the *Solanaceae* family including tobacco, tomato and potato tend to show some degree of homology (Fig. 2). This implies that RT sequence divergence during vertical transmission of *Ty1-copia* group retroelements within plant lineages has been a major factor in the evolution of *Ty1-copia* group retroelements in higher plants and that the retrotransposons are from a very ancient lineage. However, there are some cases in which the RT sequences show more homology to the RT sequences from diverse species than to their closely related species. For example, the RT sequences of barley and potato, which diverged from each other approximately 200 million years ago, have homology as high as 60% suggesting that horizontal transmission of this retrotransposon group between different species has also played a role in the process.

Ty1-copia group retroelements are major components of plant genomes The copy number estimates of *Ty1-copia* elements within plant species has shown that the number of elements is highly variable between species. In *A. thaliana*, which has a small genome size (1C = 0.2 pg), there are 10 elements, each with a copy number of about two, giving a total number of about 20 elements in the genome. In contrast, rice (*Oryza sativa)* which has also a relatively small genome size (1C = 1.2 pg) has been found to contain three subgroups of retrotransposons (*Tos1*, *Tos2* and *Tos3*), each of which has a copy number of around 1000. However, the copy number of retrotransposons in potato (*Solanum tuberosum*) which has a relatively small genome size (1C =1.9pg), but bigger than rice, was only 400. Therefore from the data available to date, it is not clear whether there is a relationship between *Ty1-copia* retrotransposon copy number and genome size in plants. A study of the *Ty1-copia* group of retrotransposons within the genus, *Vicia* which contains species with widely differing

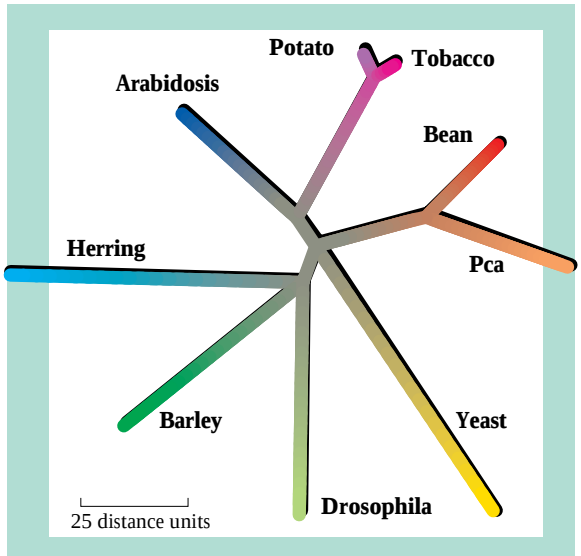


Figure 2 Phlogenetic tree for selected Ty1- copia groupmembers. Divergences in distance units are indicated by branch lengths.

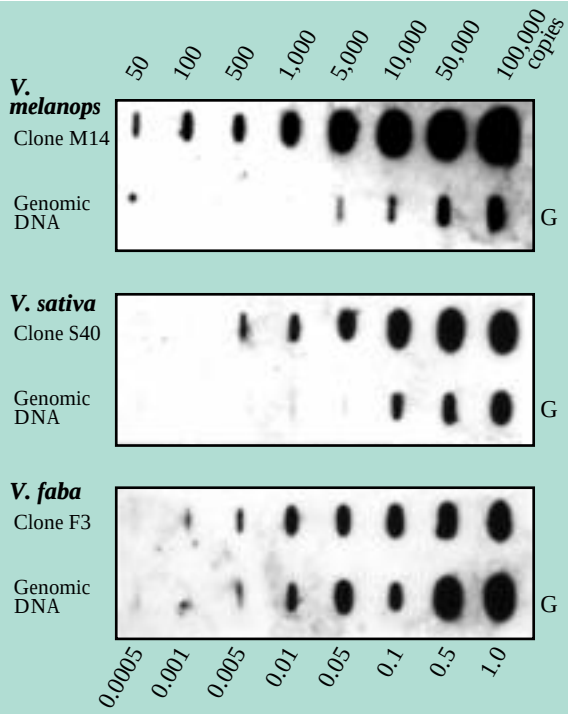


Figure 3 Total copy number of *Ty1-copia* retrotransposons in *Vicia* species. Cloned reverse transcriptase fragments from *V. faba*, F3; *V. melanops*, M14; and *V. sativa*, S40 were loaded in columns 1 to 8 corresponding to 50, 100, 500, 1000, 5000, 10,000, 50,000, 100,000 copies per genome respectively. Row G contains genomic DNA from the appropriate species loaded in columns 1 to 8 containing 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1 genome respectively. Filters were probed with a heterogeneous probe containing the complete population of sequences from within a species.

genome sizes, examined the numbers and sequence heterogeneities of these genetic elements in three diploid species chosen to represent large (*Vicia faba* 1C = 13.3 pg), medium (*V. melanops*, 1C = 11.5 pg) and small (*V. sativa* 1C = 2.3 pg) genomes within the genus. The copy numbers of the retrotransposons were all high but varied greatly, with *V. faba* containing 1,000,000 copies, *V. melanops* with 1,000 copies and *V. sativa* 5,000 copies (Fig. 3). The sequence heterogeneity of *Ty1-copia* group elements correlated with their copy number within each genome, but neither heterogeneity or copy number were related to the genome size of the host. Approximately 5-10% of the *V. faba* genome constituted *Ty1-copia* group retroelements and therefore represents by far the most numerous *Ty1-copia* group elements found to date in any organism.

Distribution of *Ty1-copia* retrotransposons in the genome To determine the chromosomal distribution of *Ty1-copia* elements in *V. faba*, metaphase and prophase chromosome spreads were hybridised with biotin-labelled PCR probe representing the entire population of elements (Fig. 4, Spread A). Spread B shows the same chromosomes of Spread A with DAPI staining. The probe hybridised almost uniformly along with all *Vicia* chromosomes, as visualised by bright red fluorescence (Fig. 4a). It appears that these retroelement sequences were present in almost all regions of all chromosomes of *V. faba* but were absent from most heterochromatic regions near metacentric centromere, acrocentric centromere, and acrocentric intercalary heterochromatin (Fig. 4a and 4c, marked with arrows). It may be absent also at centromeres and NOR loci regions (Fig. 4a).

Retroelement evolution in plants *Ty1-copia* group retrotransposons appear to be highly successful in invading and proliferating in the genomes of plants and our *in situ* data suggests that they are dispersed throughout the genome. Retrotransposons are the only sequences known to be distributed in this way.

Retroelements have become a major component of the euchromatic regions of plant chromosomes and their proliferation can not only vary between plant species but can also vary between the closely related species (i.e. among *V. faba, V. melanops* and *V. sativa*). The large fluctuations in *Ty1-copia* element copy numbers which are apparently confined to different sub-groups in different species may arise because only a proportion of the elements are transpositionally active at any one time and these active elements respond during periods of copy number expansion. This would explain why the populations of sequences differ so much, even between closely related species. Plant retrotransposons in many species are defective, for example, the 10 different types of *Ty1-copia* retrotransposons of *A. thaliana* are all incapable of transposition and the large majority of potato elements are similarly crippled. Another factor which limits activation is the transcriptional specificity of the retrotransposition. Not all retrotransposons are activated under the same conditions. In tobacco, a specific subset of *Ty1-copia* retrotransposons is transcriptionally activated by microbial stress, the same sub-group in potato is activated under similar conditions, while the other major potato sub-groups are not. Differences in retrotransposon activity would lead to significant changes in the copy numbers of these populations within a relatively short time. The dispersed nature of the ele-

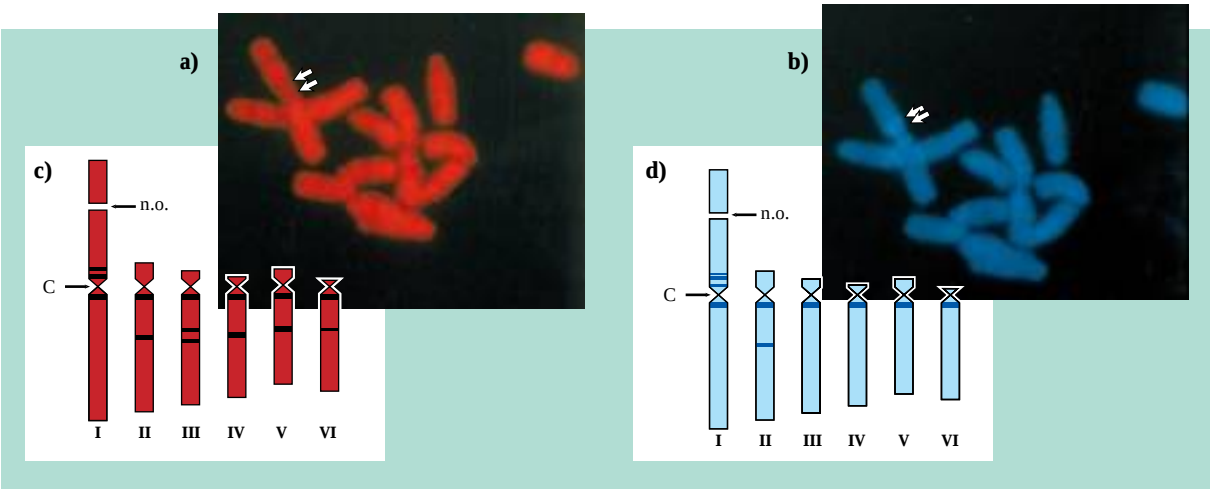


Figure 4 *In situ* hybridisation using biotinylated labelled PCR-fragments of *Ty1-copia* retroelements of *V. faba* metaphase chromosomes. a) Sites of hybridisation on metaphase chromosomes detected using streptavidin conjugated CY3 which fluoresces red when excited with green light. b) Chromosomes as a) stained simultaneously with DAPI (fluorescing blue when excited with UV light). Strongly stained blue bands (b) (arrowed), representing AT-rich heterochromatic regions, and the centromeric regions show almost no hybridisation signal from the retroprobe (a) (arrowed). Figs 4c and 4d show diagrammatic representations of the chromosomes shown in a and b.

ments indicates that the elements have moved to new locations by replicative transposition via reverse transcriptase. The lack of any correlation between the genome sizes and the retroelement copy number means that it is unlikely that the high copy number of elements is a result of them being involved in genomic amplification events. It is rather surprising to find that these elements are absent from heterochromatic regions. It is not known whether these elements have specificity for target sequence and therefore are unable to invade highly repetitive sequences such as heterochromatic regions, or that they are eliminated soon after insertion. Factors which may limit retroelement invasion in heterochromatin may include: unique repetitive sequence arrangement; highly condensed and supercoiled state; its late replication during cell cycle; and its recent origin in plants.

Interestingly, *in situ* hybridisation studies on the distribution of retroelements in *Drosophila* have provided rather different results to our work on *V. faba*. *D. melanogaster* retroelements are present in both euchromatic and heterochromatic regions of chromosomes, but they strongly predominate in heterochromatic regions. It is unclear at present why *Ty1-copia* retroelements behave differently in the genomes of *D. melanogaster* and in plants.

Data from our research and others show that a significant proportion of plant genomes are composed of retrotransposon sequences, for example, 5-10% of *V. faba* genome, 5% of barley genome, and 5% of rice genome[1]. Furthermore, it is likely that other types of transposable elements also inhabit the genome of plants. Why should a large fraction of the plant genome be composed of these sequences, sequences which have the potential to transpose? To explain this Barbara McClintock (1987) proposed the genome stress hypothesis: plants may have specifically evolved to adapt themselves to the genomic stresses such as activation of transposable elements by chromosome breakage. This may lead to a variety of genetic alterations, on which natural or artificial selection may be imposed.

Reference

[1] Flavell, A.F. Pearce, S.R. & Kumar, A. (1994). *Current Opinions in Genetics and Development* 4, 838-844.

Molecular marker techniques for barley genome analysis and breeding

W. Powell, N. Bonar, E. Baird, J. Russell & R. Waugh

The detection and exploitation of polymorphism in plants and animals represents one of the most significant developments in biology. The concept of using genetic markers to identify specific regions of the genome is well established but initially was hampered by the lack of appropriate markers. Developments in molecular biology have increased the repertoire of polymorphic assay procedures available for use in genetic analysis, DNA fingerprinting and plant breeding.

The greater utility of molecular markers arises from six inherent properties that distinguish them from morphological markers:

- The phenotype of most morphological markers can only be determined at the whole plant level, whereas molecular loci can be assayed at the whole plant, tissue, and cellular levels.
- Allele frequency tends to be much higher at molecular loci compared with morphological markers.
- In addition, morphological mutants tend to be associated with undesirable phenotypic effects.
- Alleles at morphological loci interact in a dominant-recessive manner that limits the identification of heterozygous genotypes.
- Molecular loci exhibit a co-dominant mode of inheritance that allows the genotypic identification of individuals in a segregating population.

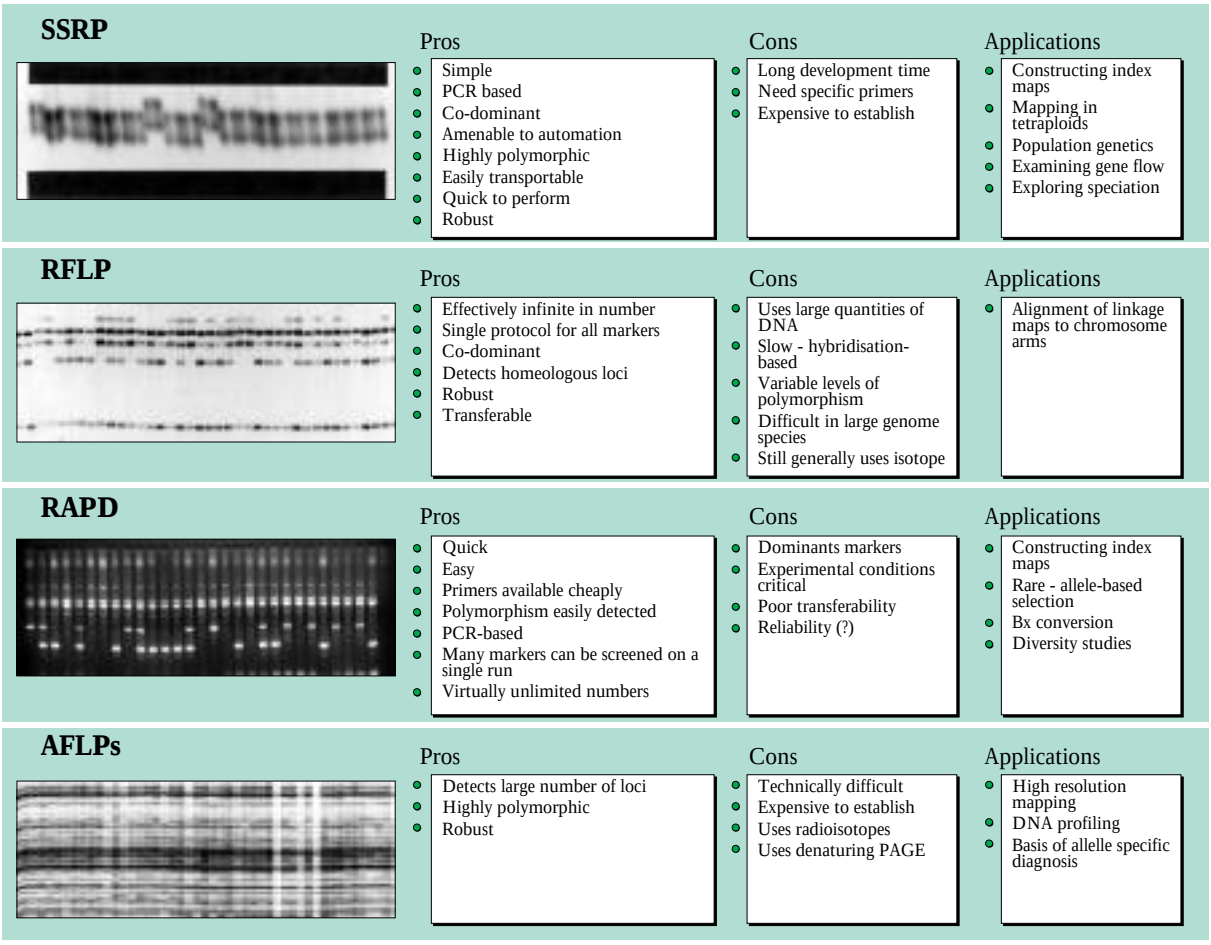


Figure 1 Technologies for molecular breeding. The choice of marker system depends on a number of factors including technical considerations; the goals of the project; and the genetics and biology of the species being studied.

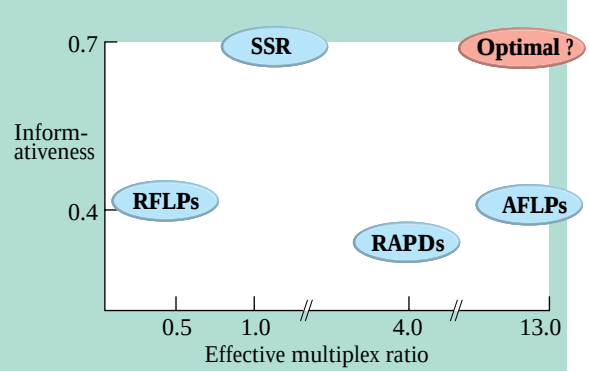


Figure 2 Graphical representation of how marker index may be derived from informativeness and effective multiplex ratio for four marker systems in barley.

• Fewer epistatic or pleiotropic effects are observed with molecular markers than with morphological markers. Hence, a large number of polymorphic markers can be generated and monitored in a single cross.

The purpose of this article is to compare the utility of different marker systems for genetical analysis in barley and to establish a rational basis for choice of marker assay.

Comparison of marker systems The principles involved in detecting polymorphism with four different technologies: Restriction Fragment Length Polymorphism (RFLPs), Randomly Amplified Polymorphic DNA (RAPDs), Simple Sequence Repeats (SSRs) and Amplification Fragment Length Polymorphism (AFLPs) are given in Figure 1. Many factors determine the utility and choice of marker system but the two most important biological parameters are informativeness and ease of genotyping. Expected heterozygosity ($\hat{H}$) provides a good estimate of informativeness and is the probability that two alleles taken at random from a population are different, $\hat{H} = 1 - \sum p_i^2$ where p_i is the allele frequency of the i^{th} allele. A second factor is the number of loci simultaneously analysed per experiment i.e. multiplex ratio. Marker systems vary greatly in their multiplex ratio e.g. SSRs are characterised by a multiplex ratio of one whereas AFLP assays can reveal up to 100 products on an acrylamide gel.

Estimated values for the multiplex ratio and expected heterozygosity are given in Table 1 for the four polymorphic assays. These may be used to derive the marker index which is a measure of the utility of a given marker system for barley. This is shown graphically in Figure 2 where two parameters: informativeness and effective multiplex ratio are quantified. This clearly demonstrates that AFLPs provide a distinctive technology which has great potential for use in barley genome research and breeding. Furthermore it emphasises that the unique properties of the AFLP assay arise from its relatively high effective multiplex ratio. There are therefore two important challenges for the future: to harness high multiplex assays for use in breeding and to devise strategies that will combine the high multiplex ratio of AFLPs with the high information content of SSRs.

	β	n	E	$\hat{H}$	MI
Amplified Fragment Length Polymorophism	0.235	55	12.35	0.32	4.14
Randomly Amplified Polymorphic DNA	0.302	13.75	4.15	0.40	1.66
Simple Sequence Repeats	1.00	1.00	1.00	0.700	0.70
Restriction Fragment Length Polymorphism	0.50	1	0.41	0.41	0.21

β = proportion polymorphic loci n = number of loci analysed per gel E = effective multiplex ration E = β x n
$\hat{H}$ = Diversity index 1 - $\sum p_i^2$ MI = marker index , product of H and E

Table 1 A comparison of various genetical parameters that determine the utility of different polymorphic assays in barley.

Genetic control of albinism in barley regeneration

B.P. Forster, M. Macaulay, R. Waugh, M. Folling[1] & S.B. Andersen[1]

The production of albino plants is a problem in regeneration systems of many monocotyledon species. It is particularly acute in anther and microspore cultures of cereals and other grasses where albino plants can greatly outnumber green regenerants (Fig. 1). In some systems, although thousands of plants can be regenerated, the majority are albino. The efficiency of green plant production can be improved by various modifications to culture conditions, but there is a large genetic component and some genotypes are more responsive than others. Despite these limitations, anther culture of barley has become an important technique in producing doubled haploids (*Ann. Rep. 1992, 36-40*) which are homozygous and can be exploited in both applied and basic research. Most plant breeding companies throughout the world have incorporated anther culture in their breeding programmes as the quickest method to produce pure breeding, homozygous lines. However, success is dependent on production of high numbers of green plants and the number of responsive genotypes. Similar constraints apply to doubled haploid production for genetic studies. Barley transformation systems are now being developed which exploit the embryogenic potential of cultured microspores but albinism has been identified as a major constraint. Tissues derived from microspores can be transformed using biolistic, electroporation and PEG techniques, but the success in producing viable transformed plants is restricted by the low numbers of green plants regenerated at the end of the process.

Figure 1 Albinism in regeneration from cultured barley anthers.

We have studied the genetic control of albinism in barley anther cultures. The anther culture response of barley doubled haploids generated from the cross Igri x Grit was scored and related to DNA profiles using randomly amplified polymorphic markers (RAPDs). Igri is a winter cultivar which responds well to anther culture (low frequency of albinos) and is often used as a model genotype for such work. In contrast, Grit is a spring cultivar which responds poorly to anther culture and shows a high frequency of albinos. The Igri x Grit doubled haploids were screened for their ability to produce green plants in anther culture. DNA from the six lines exhibiting the highest frequencies of green plants were bulked, as was the DNA from the six lines exhibiting the lowest frequencies. These bulks were then screened for RAPD polymorphisms using over 300 arbitrary sequence 10-mer primers. The use of RAPDs allowed polymorphic markers to be detected between the two bulks (Fig. 2). Co-segregant analysis of these markers in the doubled haploid population showed that the markers associated with green plant production were located on two linkage groups which together accounted for almost all the genetic variation for the character in this mat-

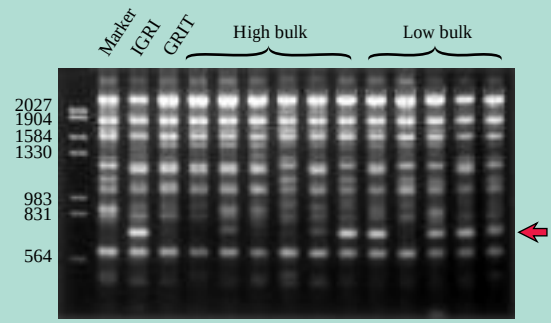


Figure 2 Bulked segregant analysis of green plant production using the RAPD primer OPK15. The RAPD product OPK15-H700 (arrowed) was found in the unresponsive parent Grit and in five out of the six doubled haploids which formed the low green plant bulk (high frequencies of albinos). In contrast, the responsive parent Igri did not possess this band which was also absent in the six most responsive (high frequencies of green plants) doubled haploid lines. This indicates that OPK15-H700 is associated with albinism in barley regeneration.

[1]Plant Breeding and Biotechnology Department, Royal Veterinary and Agriculture University, Copenhagen, Denmark

erial. The results are consistent with the hypothesis that two major unlinked genes control the capacity to form high percentages of green plants in this cross combination. Since the two parents, Igri and Grit, differ greatly in their response to anther culture it is possible that these two lines segregate for most, if not all, of the major genes controlling capacity to form green plants in anther culture.

No genetic map had been developed from the Igri x Grit doubled haploids, and consequently the RAPD loci associated with green plant production could not be assigned to specific linkage groups. Since the cross was made between a winter and spring barley, the doubled haploids segregated for vernalisation response and for markers linked to vernalisation genes. One marker, ß-amylase (*Bmy1*) which is tightly linked to the major vernalisation gene for spring habit, *sh* (*Ann. Rep. 1990, 25-28*) on chromosome 4H, segregated in the doubled haploids and was associated with vernalisation demand in the material but not with green plant production. In contrast, the RAPD marker, OPE17-H1300, was associated with vernalisation response, apparently independently of *Bmy1* and *sh* on chromosome 4H and with the allele from the responsive winter parent Igri favouring green plant production. OPE17-H1300 is therefore presumed to be linked to one of the other vernalisation genes, possibly Sh_2 or Sh_3 on chromosomes 7H and 5H respectively.

Mapping genes of economic importance in spring barley

W.T.B. Thomas, W. Powell, R. Waugh, B.P. Forster, K.J. Chalmers, U.M. Barua, J.S. Swanston, R.P. Ellis, P. Jack[1] & V. Lea[1]

Plant breeders attempt to improve a crop species by crossing parents that complement each other for economically important characters and selecting superior recombinant lines. Whereas some of the characters can be controlled by one or two genes of large effect, many are controlled by several genes, each of small effect. This poses a problem for breeders as genes of large effect generally produce distinct phenotypic classes but genes of small effect produce much smaller differences which can also be modified by the environment. As most of the genes controlling economically important characters are unknown, breeders select for phenotypic expression of a character rather than direct selection of the desirable genotype. Whilst there is information about the genetic architecture of characters that can be used to plan selection strategies, breeders are often frustrated by the need to produce sufficient seed to measure accurately important characters such as yield and quality. In conventional breeding schemes, this causes a delay in selection for such characters and resources often do not permit testing large populations. Breeders therefore desire a means of tagging important genes so that the desired phenotype can be recognised easily without the need for lengthy and expensive selection regimes. There are a number of morphological markers in barley which can be recognised easily in the field but few of them which have been placed on the genetic map of barley vary in the types of parents used in most breeding programmes. One exception is rachilla hair length, where the gene *S* gives long rachilla hairs. This gene has been associated with an increase in yield through increased thousand grain weight. However, most morphological markers have no application in breeding improved barleys.

The development of biochemical and, more especially, molecular markers has renewed interest in tagging economically important genes with markers. There are now a range of molecular markers available which can reveal considerable differences between parents that were not apparent with other markers and which have facilitated the creation of extensive genetic maps of many crop species. In addition, sufficient differences have often been found with molecular markers to create a genetic map of most of the genome with a single pair cross, rather than using statistical techniques to amalgamate data from disparate crosses and sources as was done for morphological markers. When combined with techniques to produce immortal lines, such as doubled haploidy, the benefit of

[1] Plant Breeding International, Maris Lane, Trumpington, Cambridge CB2 2LQ

being able to construct a map from a single cross becomes much more significant because the immortal lines can also be grown in trials to measure economically important characters. This has led to the real possibility of being able to pinpoint each individual gene or blocks of genes controlling economically important characters, termed a quantitative trait locus (QTL), by relating the mapping data to the character data. This information can then be translated into identifying suitable markers to tag genes controlling characters of interest. For such results to be applicable in plant breeding, it is important that mapping QTLs controlling important characters is carried out on germplasm typical of a breeding programme.

A spring barley cross between cv. Blenheim and an SCRI breeding line, E224/3, was selected to map QTLs controlling a range of important characters in barley. Blenheim was a popular malting barley cultivar in North West Europe but suffered from water sensitivity and susceptibility to mildew and leaf rust in the field. E224/3 was a high yielding line with good all-round disease resistance but only medium malting quality. Thus the parents complemented each other and it was hoped to obtain recombinant lines which were overall superior to either. A number of doubled haploid (DH) lines were produced from the cross and grown in replicated trials along with their parents from 1989 to 1992 and a range of agronomic, yield, grain quality, germination, disease and malting characters were recorded. In addition, the DH lines were scored for 144 genetic markers, mostly molecular, but also some biochemical markers, a morphological marker (the *denso* dwarfing gene, carried by Blenheim) and *Rhynchosporium* resistance, carried by E224/3. A genetic map of the cross was constructed from these markers (Fig. 1). The map falls into a number of linked segments, even for regions on the same chromosome arm, which may reflect some minor chromosomal rearrangements between the two parents which is a possibility because one parent has an X-ray mutant as one of its ancestors. However, the linked segments cover approximately half of the total genome. Bearing in mind that the cross was between two adapted parents, it is highly likely that there were few differences in the remaining unmapped half of the genome.

A search was made using a regression technique for QTLs controlling 10 characters presented in Table 1.

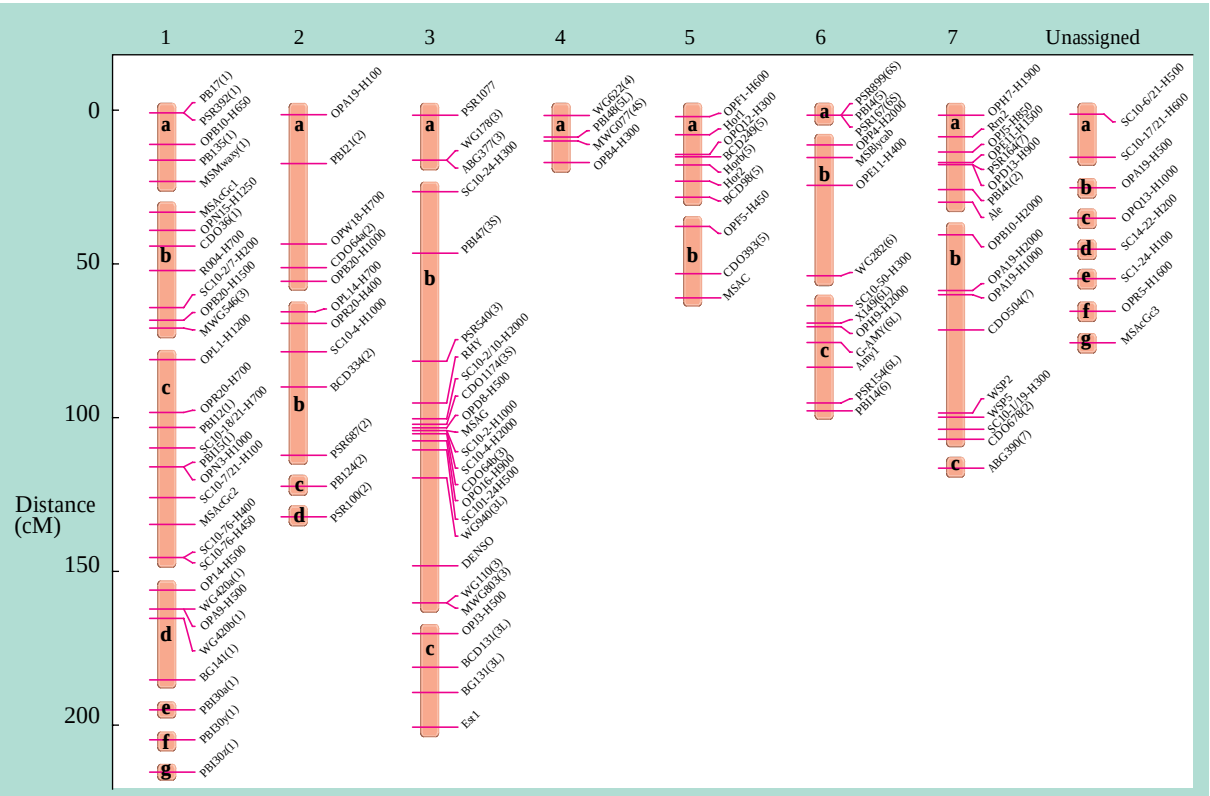


Figure 1 Chromosome maps for Blenheim x E224/3. Letters indicate separate linked segments of chromosomes. The gaps between segments represent no significant linkage for LOD = 3.0.

Character	Units
Heading date	days
Plant height	cm
Plot yield	t/ha
Thousand grain weight	g
Proportion of grain passing over a 2.5 mm sieve	%
Germinative energy - number of seeds germinating in 4 ml of water 72h after imbibition	%
Water sensitivity - number of seeds germinating in 8 ml of water 72 h after imbibition	%
Milling energy of a sample of grain	J
Nitrogen content of the dry grain	%
Hot water extract of a malted sample of grain	L°/kg

Table 1 Characters scored on doubled haploid lines from Blenheim x E224/3 over four years of trials and for which their means were examined to reveal significant QTLs.

The results of the QTL mapping for agronomic, yield and grain quality characters are summarised in Figure 2 and those for the germination and malting quality characters in Figure 3. Each bar on the graph represents the effect of the cv. Blenheim QTL allele expressed as a percentage of the overall mean of the DH lines. The *denso* dwarfing gene affects every character shown in Figure 2 apart from thousand grain weight. This is important as the *denso* dwarfing gene, which has been used as a source of short straw in European spring barley breeding, is contained in cultivars that compose some 85% of the UK spring barley area. The gene is associated with a semi-prostrate juvenile growth habit (Fig. 4), and resulted in an average 15% reduction in height to a mean of 61 cm over the four years. In contrast, there was an average increase of 8% in yield of the dwarf lines to a mean of 6.8 t/ha over four years of trials. Other sources of the *denso* dwarfing gene have been found to be associated with a yield decrease which, together with the finding of an association with a yield increase in this study, suggests that the association of the dwarfing gene with a yield QTL was due to linkage. The parents of the other resources of the *denso* dwarfing gene must have had a QTL allele reducing yield whereas cv. Valticky, the parent used in a mutation programme to produce the source of the *denso* gene in this study, had the alternative allele increasing yield. However, there is still room for improvement of the dwarf type as the gene is associated with a reduction in the proportion of grain passing over a 2.5 mm sieve and a delay in

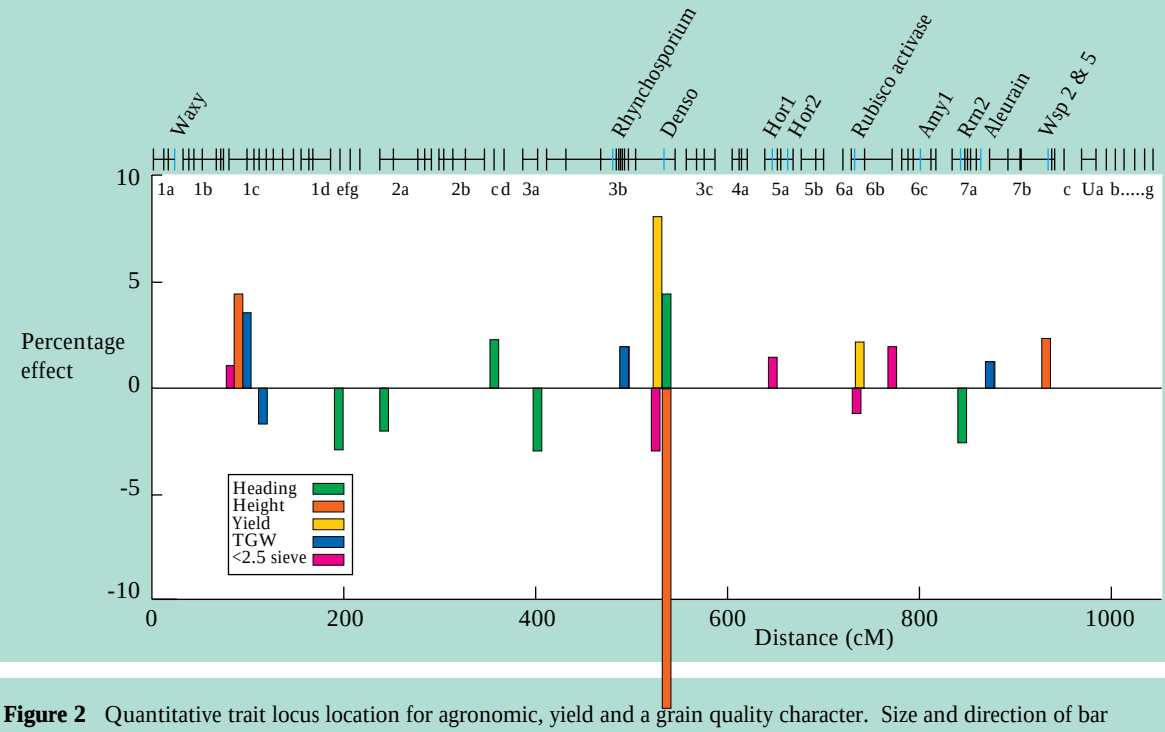


Figure 2 Quantitative trait locus location for agronomic, yield and a grain quality character. Size and direction of bar represents percentage effect of Blenheim allele compared to the population means. The mapped segments of Blenheim x E224/3 are displayed at the top of the chart with linked markers joined by a horizontal bar. Markers of interest are displayed in blue.

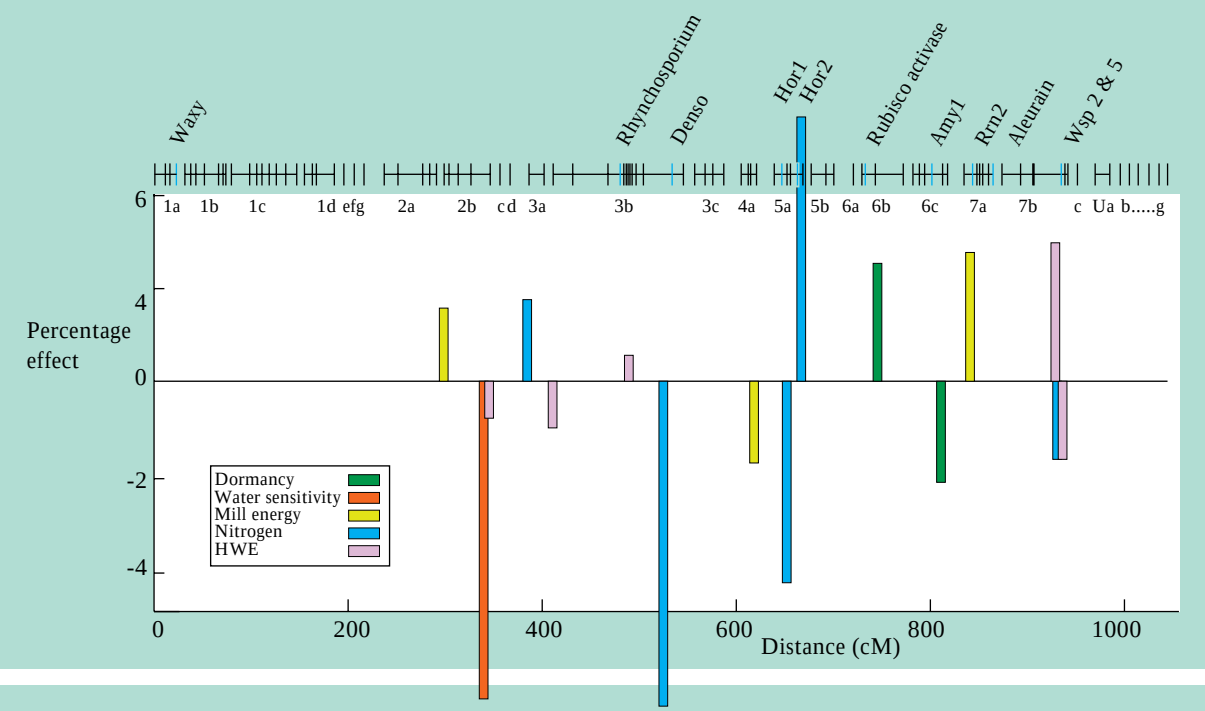


Figure 3 Quantitative trait locus location for germination and malting quality characters. Size and direction of bar represents percentage effect of Blenheim allele compared to the population means. The mapped segments of Blenheim x E224/3 are displayed at the top of the chart with linked markers joined by a horizontal bar. Markers of interest are displayed in blue.

heading date, both undesirable characteristics for a successful spring barley. In addition to the region around the *denso* locus, a region on chromosome 1 between the RAPD markers OPL1-H1200 and OPR20-H700 was important in the control of several characters. Cv. Blenheim alleles in this region produced an increase in height, thousand grain weight and the proportion of grain passing over a 2.5 mm sieve. The segment of chromosome 6 containing Rubisco activase contained QTL alleles from cv. Blenheim increasing yield and decreasing the proportion of grain passing over a 2.5 mm sieve, a similar relationship between the two characters to that around the *denso* locus. There was a further QTL for the proportion of grain passing over a 2.5 mm sieve on the same segment of chromosome 6 but, in this case, the Blenheim allele resulted in an increase. Of the characters shown in Figure 3, the *denso* dwarfing gene was only associated with a QTL reducing grain nitrogen content. The association of the dwarfing gene with an increase in yield means that the reduction in grain nitrogen content has a dilution effect because the total nitrogen yield of the dwarf and non-dwarf groups was the same. However, there were also QTLs affecting nitrogen content in the region of the *Hor1* and *Hor2* hordein loci on chromosome 5. Hordeins are the major storage proteins of barley and, as there were no QTLs affecting grain yield in this region, variation in nitrogen controlled by this region must result from changes in uptake or deposition of nitrogen in the grain.

Germination was tested at two water levels, optimum and excess, to give estimates of dormancy and of water sensitivity respectively. Two QTLs located on chromosome 6 affected germination in optimum water conditions. The Blenheim allele in the region of Rubisco activase led to an increase in germination whereas that in the region of *Amy1* led to a decrease. Both effects were small, indicating that there was little dormancy amongst the DH lines 4 weeks after harvest. Only one QTL on chromosome 2 affected water sensitivity; the Blenheim allele produced a reduction in germination of over 6% compared to the overall mean of the DH lines. This germination QTL was close to one controlling hot water extract which was also decreased by the Blenheim allele. Whilst the decrease in hot water extract was small in percentage terms, it represented a difference of 4L°/kg between the means of the Blenheim (291.3 L°/kg) and E224/3 QTL alleles and probably reflected the germination problems in malting due to the water sensitivity germination QTL. A QTL controlling milling energy was also found on the same segment of chromosome 2

Figure 4 Barley lines with (foreground) and without (background) the *denso* dwarfing gene. Note the short, spreading habit of the plot in the foreground compared to the more upright habit of the plot in the background. This characteristic is typical of genotypes with the *denso* dwarfing gene.

as that which affected water sensitivity and the Blenheim allele increased milling energy. Whilst this linkage was loose, its existence provided some evidence for the association of hard endosperm with reduced hot water extract. The other QTLs controlling milling energy were not associated with any other germination or quality characters, although QTLs affecting heading date and thousand grain weight were also found in the region of the milling energy QTL on chromosome 7. The water soluble protein loci, *Wsp2* and *Wsp5*, on chromosome 7 had a large effect on hot water extract but it was complex as two QTLs were found very close together with one allele from Blenheim and another from E224/3 which both increased hot water extract.

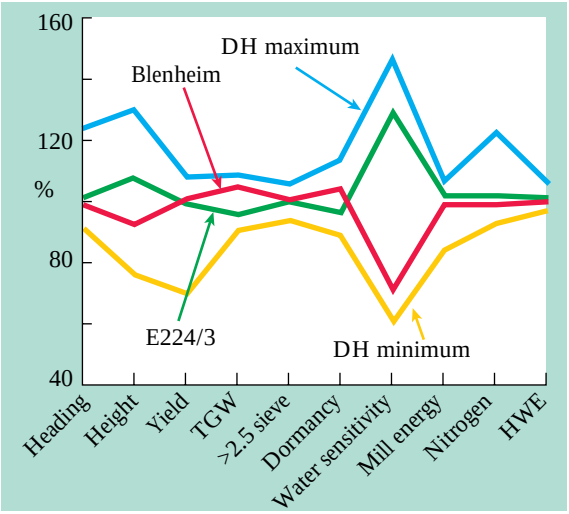


Figure 5 Four year means of 10 characters. Expressed as % of Blenheim & E224/3.

Other evidence from the DH lines showed that the highest scoring DH line exceeded the higher scoring parent and the lowest scoring DH line was less than the lower scoring parent for each character (Fig. 5). This means that the desirable QTL alleles for each character were dispersed between the parents and recombinant lines can therefore be derived that possess more favourable QTL alleles than the better parent and less than the worse parent. The data shown in Figures 2 and 3 confirmed that favourable QTL alleles were found in both parents for each character except water sensitivity.

There were a number of instances where favourable QTL alleles from different parents were closely linked and the linkage relationship would need to be altered for improvement in performance. In such cases, a large population would need to be raised to ensure that there were sufficient numbers of lines recombining both increasing alleles to give a high probability that one recombinant combined these attributes with other desirable characteristics. This is an area where the deployment of molecular markers in breeding would be particularly useful as the majority of lines that lacked both increasing QTL alleles could be screened out before raising populations in the field. The use of molecular markers has the undoubted potential to bring much more precision to the manipulation of characters in breeding and the QTL mapping described above is a necessary precursor to such a strategy.

Isolation of a cDNA clone encoding polygalacturonase inhibitor protein from kiwifruit

C.G. Simpson & R.C. Gardner[1]

Polygalacturonase Inhibiting Proteins (PGIP) are generating wide interest in plant pathogen interactions. A PGIP isolated from immature raspberry fruit has been found to directly inhibit endo-polygalacturonases (PGs) released by the fungus *Botrytis cinerea* during the breakdown of pectin in the cell walls of the plant host[1,2]. In addition, breakdown of pectin in the presence of the inhibitor leads to an accumulation of oligogalacturonides which may induce host resistance responses in some plants. Clearly this anti-fungal action could be used ultimately as part of plant breeding and biotechnology programmes, and to investigate the involvement of PGIP in signal transduction pathways in plants. At present, PGIP genes have been cloned from bean, pear and more recently tomato. Expression of the pear gene in transgenic tomato led to enhanced resistance of fruits to infection by *B. cinerea*, suggesting a contribution by PGIP to host defences. However, over-expression in transgenic tomatoes of the tomato PGIP gene had relatively little effect on resistance compared with the pear PGIP. The variation in the performance of transformants highlights the diversity of PGIP genes isolated from different dicotyledonous plants against fungal PGs. It is, therefore, important to isolate PGIP from various sources.

Kiwifruit in New Zealand is a commercially important crop which is susceptible to post-harvest fungal disease and therefore amenable to disease resistance strategies. During a visit to the University of Auckland, New Zealand, funded by a Bank of Scotland Waitangi Fellowship, The British Council and SCRI/SOAFD, attempts were made to identify and isolate a PGIP gene from kiwifruit. A cDNA library from unripe kiwifruit (*Actinidia deliciosa* cv. Hayward), prepared in Auckland, was screened with PGIP probes and eight potential cDNA clones containing PGIP were identified from 200,000 colonies screened. Each of the clones contained an insert of about 1300 bp, the size expected for an insert containing a full-length copy of the gene. Sequencing of one of the colonies revealed 26 nt of 5' untranslated sequence and 240 nt of coding sequence corresponding to the N-terminal 80 amino acids, which have 76, 75 and 57% similarity with tomato, pear and bean PGIPs respectively (Fig. 1). Sequencing of the 3' end revealed 226 nt of 3' untranslated sequence and 45 nt (15 amino acids) which show a high degree of similarity with the 3' end of the above PGIPs.

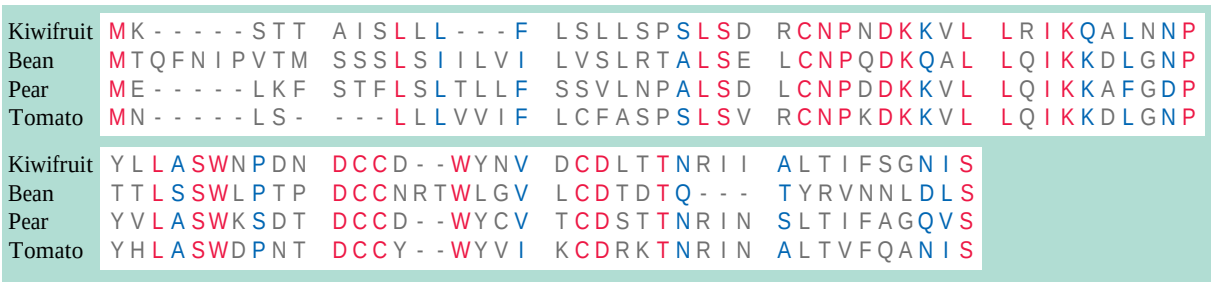


Figure 1 Sequence alignment of the first 80 amino acids of kiwifruit PGIP with those of tomato, pear and bean. Regions of identity and conservative substitutions are highlighted.

Kiwifruit is relatively easy to transform and fruit-specific promoters have been isolated in Auckland. Both of these will be important for development of PGIP-mediated disease resistance in kiwifruit and when it is fully sequenced the kiwifruit PGIP gene can be introduced into soft fruit and other crops at SCRI to determine its potential to confer resistance to fungal attack.

References

1 Johnston, D.J., Ramanathan, V. & Williamson, B. (1993). *Journal of Experimental Botany* 44, 971-976.

2 Johnston, D.J., Williamson, B. & McMillan, S.P. (1994). *Journal of Experimental Botany* 45, 1837-1843.

[1]University of Auckland, New Zealand

Synthesis of intraspecific somatic hybrid plants between dihaploid lines of *Solanum tuberosum*

A. Kumar, S. Cooper-Bland, M.J. De,Maine, M.L.M.H. Fleming, M.S. Phillips, H.E. Stewart & W. Powell

One of the major aims in potato breeding is to develop potato cultivars with improved agronomic traits such as disease resistance. Extraction of dihaploids from tetraploids following parthenogenetic crossing with *Solanum phureja* has greatly facilitated potato genetics allowing useful combinations of agronomic traits to be assigned to specific dihaploid lines. However, dihaploids are sometimes sterile or sexually incompatible and in these cases somatic hybridisation is the only means available for achieving hybridisation between dihaploid lines. Therefore, somatic hybridisation offers an alternative method to sexual hybridisation for re-synthesising tetraploid potato breeding lines by combining two different dihaploid lines each possessing complementary agronomic traits. Additionally, this method avoids the meiotic disruption of useful agronomic traits in hybrid plants that can occur with sexual hybridisation. The procedure thus brings greater precision to the resynthesis of tetraploid genotypes from previously selected donor dihaploids[1].

In recent years, potatoes have been found to be amenable subjects for somatic hybridisation because methods of protoplast culture, protoplast fusion and selection of hybrid cells have been improved (*Ann. Rep. 1990, 31-33*). Now the main emphasis is to assess agronomic traits of the hybrids and to evaluate their practical use in potato breeding. Although many somatic hybrid plants have been produced in the genus *Solanum*, there are only a few reports of pest and disease resistance genes having been successfully transferred and their expression studied in the hybrids. In this article, assessments of the agronomic traits of intraspecific somatic hybrid plants which were produced between complementary dihaploid lines are described.

Somatic hybridisation between dihaploid lines PDH 40 and PDH 727 Intraspecific tetraploid somatic and sexual hybrid plants have been resynthesised following protoplast fusion and by sexual crosses between two dihaploid potato (*S. tuberosum*) lines each possessing complementary agronomic traits. The dihaploid PDH 40 possesses good tuber shape and yield but its foliage is susceptible to late blight (*Phytophthora infestans*). On the other hand, PDH 727 possesses resistance to blight in the foliage but has a low yield of small and irregular shaped tubers. Since it was only possible to use a partial selection strategy based on culture media to facilitate recovery of somatic hybrid plants, further morphological and esterase isozyme based characterisations were performed to select somatic hybrids from the non-hybrid parents. When the blight resistance of both the intraspecific somatic and sexual hybrid plants was assessed, there was no significant difference in the mean resistance value and it was intermediate between those of their parents (Fig. 1). However, the range of resistance was much wider among the sexual hybrids than among the plants derived from somatic fusion. An assessment of tuber yield between tetraploid sexual and somatic hybrids showed no significant difference and it was higher than that of either parent (Fig. 1).

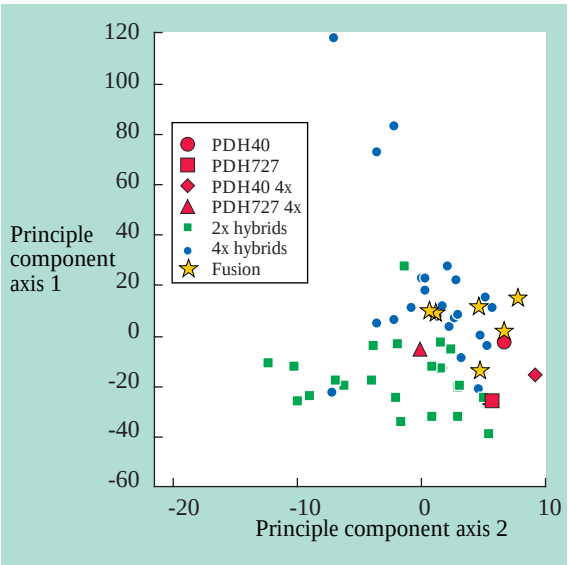


Figure 1 Comparison of the agronomic traits between somatic and sexual hybrids and their parents.

Somatic hybridisation between dihaploid lines PDH 40 and PDH 417 A number of intraspecific somatic hybrid plants have been produced following leaf mesophyll protoplast fusion between *S. tuberosum* dihaploid clones which possess good tuber shape and yield, and PDH 417 which has resistance to potato cyst nematode, *G. pallida*. PDH 417 protoplast-derived

Figure 2 Morphological characterisation of somatic hybrids of PDH 40 and PDH 417 and their parental lines. (2a) Flower morphology; left - PDH 40, centre - somatic hybrid, right - PDH 417. (2b) Tuber morphology; left - PDH 40, centre - somatic hybrid, right - PDH 417.

calli failed to regenerate plants from regenerated fusion products. Initially, somatic hybrid plants were selected on differential pigmentation in tuber sprouts and, on petal colour (Fig. 2a and 2b). Differential mobility of patatin bands in electrophoresed tuber extracts further confirmed their hybrid status. The hybrids showed different levels of resistance to *G. pallida* pathotypes Pa2 and Pa3 (Fig. 3).

Application of dihaploids in potato genetics and breeding There were significant differences between somatic hybrids of PDH 40 and PDH 417 for PCN resistance and the mean hybrid resistance is closer to the susceptible parent PDH 40 than the resistant dihaploid PDH 417. There are some clones which have a higher resistance than the mid-parent value. The evidence indicates that the expression of the resistance genes of PDH 417 are reduced by the presence of the non-resistance genes from PDH 40. A dilution of blight resistance traits has also been observed in the somatic hybrids produced between resistant dihaploid PDH 727 and susceptible PDH 40. The dilution effects of non-resistance genes are evident from these results. However, some of the variation in PCN and late blight resistance among the somatic hybrids could

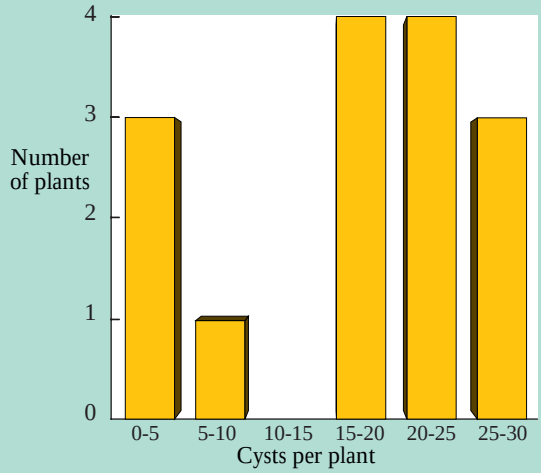


Figure 3 Frequency of cyst numbers in the 15 somatic hybrid plants tested for *G. pallida* resistance.

also be due to somaclonal variation generated during the callus phase of somatic hybrid growth.

These experiments have shown that some, but not all, of the quantitative PCN and blight resistance traits of the dihaploids can be incorporated into protoplast fusion hybrids and that quantitative resistance can be increased by an increment less than the full resistance of a dihaploid fusion partner. The value of resistant somatic hybrids will depend upon the genotype of both resistant and susceptible partners and the effects of somaclonal variation.

In conclusion, somatic hybridisation results in the production of a normal tetraploid hybrid with characteristics similar to those of the average of all the comparable sexual hybrids and can provide an alternative method to sexual hybridisation for combining useful combinations of agronomic traits. The major difference is that the somatic hybrid produces only one genotype ignoring any somaclonal variation, rather than a range of recombinants. The synthesis of intraspecific somatic and sexual hybrids also provides a useful tool for the study of how gene interactions affect agronomic characters in similar genetic backgrounds. For example, the hybrid vigour observed for tuber yield suggests epistatic gene interaction whereas the pest and disease resistance appears to be governed by additive genes. It is intended to use somatic and sexual hybrids in our breeding programme designed to improve the agronomic performance of potato cultivars.

References

1 Cooper-Bland, S., De,Maine, M.J., Fleming, M.L.M.H., Phillips, M.S., Powell, W. & Kumar, A. (1994). *Journal of Experimental Botany* 45, 1319-1325.

Molecular characterisation of the spliceosomal proteins, U1A and U2B"

G.G. Simpson, G.P. Clark & J.W.S. Brown

The major components of the spliceosome are RNA/protein complexes called small nuclear ribonucleoprotein particles consisting of snRNA molecules (U1, U2, U4/U6, U5) associated with a number of proteins (*Ann. Rep. 1990, 28-30*). As part of a research programme to study proteins and complexes involved in splicing in plants, we have isolated genes encoding the spliceosomal proteins U1A and U2B". Both proteins are RNA-binding proteins which contain RNP-80 RNA binding motifs. These consist of 80-90 amino acids with two conserved regions, RNP1 and RNP2, which contain aromatic amino acids. There are now numerous proteins containing RNP-80 motifs which can bind to very different RNA substrates and which are involved in many aspects of RNA processing. One of the major questions is how specificity of RNA recognition and binding is achieved by RNP-80 type proteins. The U1A and U2B" proteins are of particular interest because they allow a study of the determinants of specificity of RNA binding. The structure of the N-terminal RNP-80 motif of human U1A has been solved and places the RNP1 and RNP2 on the two central ß-sheets of a four anti-parallel ß-sheet structure from which the aromatic side chains of phenylalanine and tyrosine can protrude and interact with the bases of the RNA target. The amino acid sequences of U1A and U2B" and their target RNA sequences are very similar but U1A binds specifically to U1snRNA and U2B" binds specifically to U2snRNA. In addition, while U1A can bind on its own, U2B" requires the association of a second protein, U2A' for specific binding.

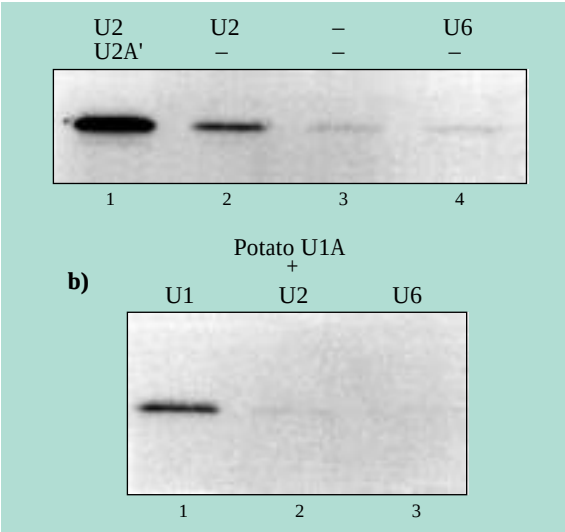


Figure 1 RNA-binding analysis showing the amount of labelled protein recovered after incubation with UsnRNAs. (a) Specific U2B" binding to U2snRNA is enhanced by the presence of human U2A' (lane 1). In the absence of U2A', U2B" binding to U2snRNA (lane 2) is minimal compared to the control (lane 3) or to U6snRNA (lane 4). (b) U1A binds specifically to U1snRNA (lane 1) and not to U2 or U6snRNAs (lanes 2 and 3).

Genes encoding both these proteins have been isolated previously only from human although U1A sequences are also available from some other animal species. The isolation of genes for U1A and U2B" from plants permits evolutionary comparisons among these highly conserved proteins to be made. We have shown that potato U1A, *Arabidopsis* U1A, obtained from the *Arabidopsis* expressed sequence tag programme, and potato U2B" exhibit specific binding to both animal and plant U1 and U2snRNAs respectively. In particular, specific binding of U2snRNA by plant U2B" also requires the second protein U2A'. In the absence of a plant U2A', human U2A' has been used with potato U2B", and demonstrated the conservation of this protein-protein interaction across a wide phylogenetic range (Fig. 1). Although mutational analysis of human U1A and U2B" has identified some determinants of binding specificity and the U2B"/U2A' interaction, other determinants, and in particular those governing U1A specificity, are yet to be described. The isolation of the plant genes allows comparisons of the amino acid sequences of all available isolated U1A and U2B" proteins to be made and have helped to identify amino acid residues, which may be involved in binding specificity, for further mutational analysis and functional characterisation.

Organisation of spliceosomal components in plant nuclei

G.G. Simpson, A.F. Bevan[1], P.J. Shaw[1] & J.W.S. Brown

In the mammalian nucleus, components involved in precursor messenger RNA (pre-mRNA) splicing are localised in three subnuclear structures: perichromatin fibrils, interchromatin granules and coiled bodies. Perichromatin fibrils may represent nascent pre-mRNA transcripts undergoing splicing. Interchromatin granule clusters may represent sites of storage or assembly of splicing factors while coiled bodies, larger structures often associated with nucleoli, may have a role in recycling spliceosomal components. The distribution of splicing factors in these structures is dynamic and changes under conditions of altered transcriptional activity such as viral infection, heat shock and during the cell cycle.

Plant pre-mRNA splicing and splicing components are less well characterised than animal or yeast systems. Although some aspects of splicing differ between plants and animals, spliceosomal snRNAs and some spliceosomal proteins are conserved. We have used *in situ* hybridisation with snRNA probes and immunofluorescence labelling with antibodies against the spliceosomal protein, U2B", and the coiled body protein, p80, coilin, to analyse the subnuclear distribution of plant spliceosomal components. These studies begin to address the structure and function relationships of nuclear components in plants, and permit an evolutionary comparison to be made to the mammalian system.

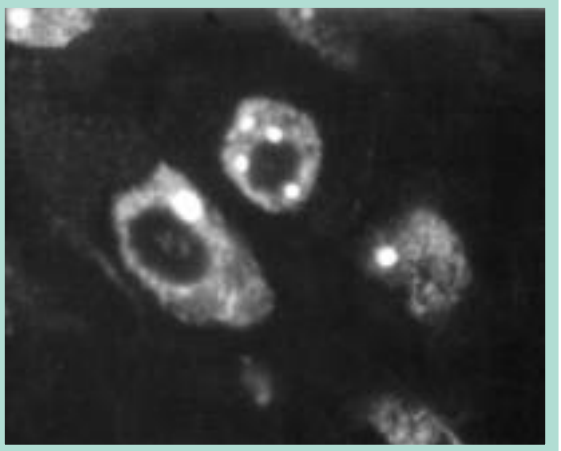


Figure 1 Nuclear localisation of U2snRNA by *in situ* hybridisation showing labelling of the nuclear interchromatin network and prominent coiled bodies.

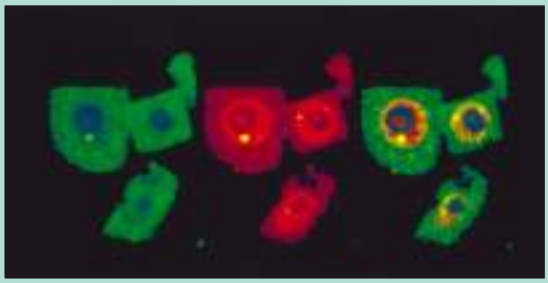


Figure 2 Double labelling with anti-p80 coilin (green) and anti-U2B" (red) showing that the nuclear bodies labelled by anti-U2B are coiled bodies.

Antisense snRNA probes and anti-U2B" labelled the interchromatin nuclear network and prominent round nuclear bodies (coiled bodies) (Fig. 1). Label was largely excluded from nucleoli and the labelling pattern of U1snRNA differed from those of U2, U6 and U2B" in that U1 did not accumulate in coiled bodies. Under heat shock conditions and during cell division the patterns of re-distribution of spliceosomal components altered dramatically and were similar to the reorganisation seen in mammalian nuclei. The identity of coiled bodies containing spliceosomal components in plant nuclei was confirmed for the first time immunologically, using anti-coilin antibodies (Fig. 2). The evolutionary conservation of these structures suggests that the organisation and composition of these subnuclear bodies is of fundamental importance in nuclear RNA processing.

[1] John Innes Institute, Norwich Research Park, Colney NR4 7UH

Novel genomic organisation of plant U14 small nucleolar RNA genes

D.J. Leader, P.J. Shaw[1] & J.W.S. Brown

Eukaryotic cells contain two major classes of small nuclear RNAs (snRNAs). Spliceosomal snRNAs are involved in precursor messenger RNA splicing (*Ann. Rep. 1990, 28-30*) while small nucleolar RNAs (snoRNAs) are involved in precursor ribosomal RNA processing and ribosome formation. One of the best characterised snoRNAs in animals and yeast is U14snoRNA. The genomic organisation of U14s in human, mouse, rat, *Xenopus* and rainbow trout is significant since copies of U14 genes are only found within introns of constitutively expressed heat shock genes and are released by processing of the excised introns[1]. More recently a number of other snoRNAs have been found in introns of other constitutively expressed genes in both animals and yeast, and the processing mechanisms and components are now the subject of intensive study.

We have cloned plant U14snoRNA genes from potato, maize and barley and have demonstrated their nucleolar localisation by *in situ* hybridisation to pea nuclei (Fig. 1). Initial PCR analysis showed a number of U14s to be closely linked, and a maize U14 gene cluster has been isolated which contains four intact genes in only 750 bp[2] (Fig. 2). Typical plant gene promoter elements are absent from the isolated plant U14 genes, and the clustered genes are too close to contain such elements suggesting transcription from a single, upstream promoter or as part of a large intron. In comparison, animals contain only a single snoRNA gene in any one intron indicating a novel genomic organisation for the maize gene cluster. This has been confirmed by the demonstration that plant U14 gene clusters are transcribed as polycistronic transcripts from which the individual U14snoRNAs presumably are processed. The consequences of this organisation are that processing must involve endonucleolytic cleavage between the snoRNAs, followed by exonucleolytic trimming (Fig. 2). In contrast, it has been shown for human intron-encoded snoRNAs that only exonucleolytic trimming of the lineared intron is required[3]. We are currently examining the sequences required for processing of plant U14snoRNAs, and whether the gene clusters are intron-encoded or expressed from novel promoters.

Figure 1 Nucleolar localisation of plant U14s. *In situ* hybridisation of potato U14 antisense RNA to pea root cell preparations showed exclusive labelling to the nucleolus. A field of cells is shown; the left hand image shows U14 labelling (green), the centre image shows chromatin labelling (red) and the right hand image is a superimposition.

1 Liu, J. & Maxwell, E.S. (1990). *Nucleic Acids Research* 18, 6565-6571.

2 Leader, D.J., Sanders, J.F., Waugh, R., Shaw, P. & Brown, J.W.S. (1994). *Nucleic Acids Research* 22, 5196-5203.

3 Kiss, T. & Filipowicz, W.(1995). *Genes and Development* 9, 1411-1424.

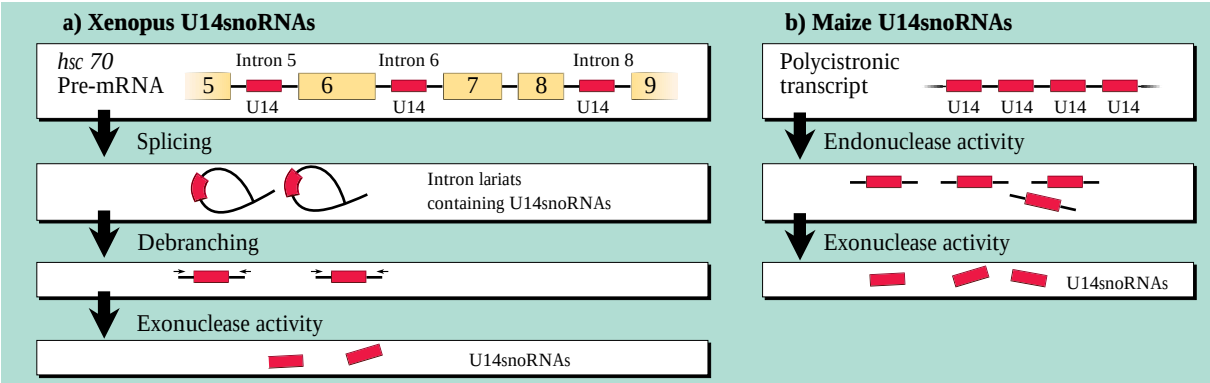


Figure 2 Models for processing of animal and plant U14snoRNAs.

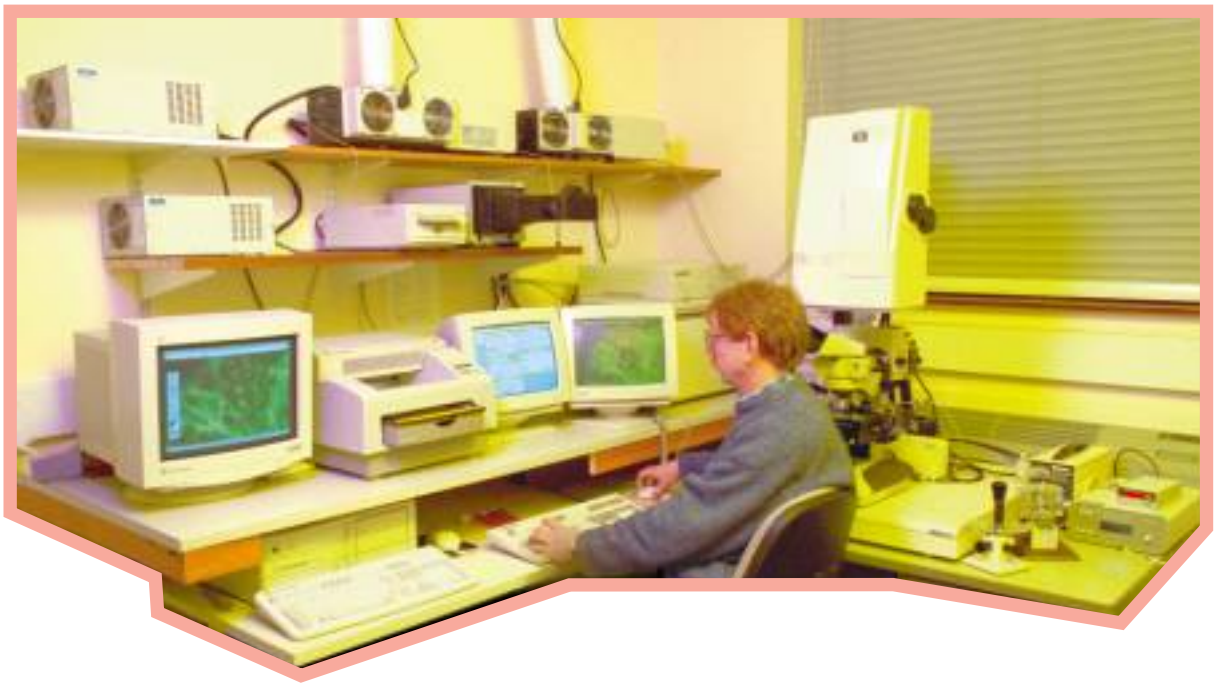
[1]John Innes Institute, Norwich Research Park, Colney NR4 7UH

Cellular and environmental physiology

Howard V. Davies

It is extremely gratifying that this overview can again include many new and significant research findings - testimony to the application and professionalism of the staff involved. The longer, accompanying articles, re-emphasise the positive impact of our activities in the wider scientific and agricultural communities. This is not a recipe for complacency, but a springboard from which to amend and adapt research strategies to address the scientific issues which will improve the quality of life and the competitiveness of the UK economy. New alliances will need to be formed, both within and outwith the Institute to achieve the critical mass needed to secure future success. Change can be uncomfortable, but the new challenges offered stretch the imagination and fuel the fires of scientific curiosity.

Carbohydrate research continues to assess the mechanisms responsible for the commercially important problem of low temperature sweetening in potato tubers. The approach uses a combination of biochemical and molecular avenues to complement activities of the geneticists. Supplementary to core activities, and in collaboration with four European research centres, considerable progress has been made in isolating genes believed to contribute to sugar accumulation at low temperature. The first phase of this programme was jointly funded by Industry and the EC ECLAIR initiative. It is gratifying that the next stage of this programme will be funded at the SCRI, and will involve the exploitation of existing technologies to improve the storage potential of a commercial cultivar. Relevant to this goal is ongoing work aimed at unravelling the role of pyrophosphate-dependent phosphofructokinase in plants. Theoretical work has been developed to estimate fluxes under both steady-state and non-equilibrium conditions. This is the first



time that such large scale analysis has been possible, thanks to a combination of novel experimental techniques and new powerful mathematical approaches. This work is the subject of one of the following research reports.

Our growing interest and impetus in soft fruit biochemistry and molecular biology has been strengthened by an industry-funded project in collaboration with the geneticists which is designed to determine the regulation of vitamin C content of blackcurrant fruit and to understand the basis for genotypic variation in vitamin levels. Methods for ascorbate purification and enrichment have been developed to assist experiments on fluxes of radiolabelled precursors and ascorbate transport. Molecular studies on blackcurrant ripening continue with the isolation of genomic clones for promoter analysis. Excellent candidates for tissue-specific promoters have been identified and a patent application filed. Work on ripening processes in raspberry is also well underway. Ethylene plays an important role in regulating pigment formation and softening in raspberry but increased ethylene evolution from ripening fruit is not accompanied by a respiratory burst. Enzyme studies have revealed that contrary to previous reports, the fruit does contain polygalacturonase activity. The relative roles of this enzyme and others such as pectin methyl esterase, ß-galactosidase and cellulase in the softening process will be the subject of continued investigations alongside modifications in cell wall chemistry. Efforts in this direction will be strengthened by MAFF funding obtained via competitive tendering.

In other developmental biology programmes, differential screening and PCR-based subtractive hybridisation have produced cDNAs associated with dormancy break in *Pseudostsuga menziesii* and tuber formation in potato. In the case of the former, sequence homologies with each of the three late embryo abundant (LEA) gene families have been isolated alongside other genes of unknown function. As far as tuberisation is concerned, several differentially expressed cDNAs have already been isolated but others have emerged recently, one with homology with a tomato gene induced by nematode infestation. The SAMDC gene isolated early in the tuberisation programme has been used to generate transgenic potato in which a stunted phenotype occurs with a constitutive promoter. Plants overexpressing the gene under a tetracycline inducible promoter have modified polyamine levels, but again constitutive expression induces a deleterious effect. The SAMDC promoter has been isolated and promoter/deletion analysis is currently underway with GUS as the reporter gene.

Confocal laser scanning microscopy (CLSM) has facilitated the imaging of intercellular transport processes *in vivo* within *Arabidopsis* roots, including the development of intercellular communications between primary roots and developing lateral primordia. Joint

work with the University of Kiel, Germany, has successfully used CLSM imaging to examine solute transfer between host and pathogen during infection of *Arabidopsis* roots with parasitic cyst nematodes. Work on the uptake and sequestration of xenobiotics has been set aside for the time being. Progress to date is the subject of a more detailed article in the following pages. Our expertise in cell biology and microscopy has been channelled into the production of a video, "The Living Cell", to be marketed by MRS using funds from a SMART award.

The stable isotope facility, now well established at the Institute, is used by environmental physiologists to quantify the effects of leaf position, ontogeny, genotype and water stress on carbon isotope discrimination in potato. In irrigated plants, the pattern of discrimination relative to air (Δ) appears to differ with leaf position and ontogeny. With well-watered plants of diploid clones Δ is largely determined by stomatal conductance, with no correlation between Δ and photosynthetic capacity. Total dry matter production is positively correlated with isotope discrimination only when differences in plant emergence are included in the regression model. Other studies confirm that stomatal conductance and leaf water potential are determined by the shoot, in both irrigated and droughted plants. Rates of water uptake (determined with $H_2{}^{18}O$ as a tracer) by wheat grown in nutrient solution are the same whether discrete root zones or whole root systems are employed. Uptake rates from soil are an order of magnitude lower, probably because increased localised resistance to water flow through soil occurs during soil drying around absorbing roots. It has also been revealed that cell division is decreased in mechanically impeded pea roots and may account for reduced elongation rates in compacted soils. Until now work on impedance has focused on the restriction of cell expansion. Data produced in collaboration with La Trobe University, Melbourne have revealed that sloughing root cap cells play a major role in reducing frictional resistance to growth of maize roots. In waterlogged soils, roots may form air passageways throughout the cortex but aerenchyma formation is inhibited in barley grown in these conditions. This is of considerable relevance to field grown crops where compacted soil becomes less permeable and therefore more prone to waterlogging.

The natural abundance of stable isotopes has also been used to determine the contribution of plant carbon to the CO_2 pool in the soil. This method allows plant-derived carbon to be distinguished from soil-derived carbon. Plant contribution is closely coupled with leaf carbon assimilation and during canopy expansion virtually all of the CO_2 in the soil can be traced back to the living plant. Canopy senescence or defoliation coincide with a reduced contribution. This implies that the capacity of the soil to store carbon will vary with the phenology and management of vegetation. A collaboration with the Universities of Dundee and York has determined that elevated CO_2 alters wheat root architecture but without affecting topology (branching frequency). The developmental changes will modify the effectiveness with which roots exploit soil and with which root-derived carbon is deposited in the soil.

A wide variety of volatile organic compounds (VOCs) are produced from incubated soils and may be involved in remote stimulation of other organisms. The VOC profiles produced by soil (determined by gas chromatography), are similar to those produced by pure cultures of micro-organisms and vary considerably in response to minor changes in soil nitrogen availability. They clearly provide a potential tool for qualitative mapping of changes in soil microbial population and functioning in response to specific inputs e.g. environmental contaminants. A suite of molecular techniques has also been developed to reveal large differences in soil microbial community structure among different soil types. These combined technologies are now being used to determine the effects of soil management strategies and environmental pollution. Indeed, the establishment and applications of novel mechanisms for the bioremediation of contaminated soils will feature largely in future activities. A collaboration has already been initiated with Glasgow University on the use of plants to remediate degraded and polluted soils. This will utilise our strengths in soil physics, microbial and plant ecology and environmental physiology. Processes will be examined at a range of scales with the aim of understanding functional relations between plants and associated bacteria and fungi.

Achievements over the past 10 years

An historic perspective of the research achievements in Cellular and Environmental Physiology over the past decade will, by its nature, be subjective since various criteria contribute to any overall assessment. Some may concentrate on the scientific citation index, others on commercial value, yet others on the extent of true innovation! The achievements referred to below are by no means exhaustive, but highly representative of what has been achieved in distinct research areas.

In cell physiology, potato has figured highly as the model experimental system over the past 10 years and together with programmes in environmental physiology has established SCRI as a world lead centre in many areas of potato physiology and biochemistry.

Carbohydrate transport and metabolism has been researched extensively. The importance of symplastic transport into the tuber storage parenchyma has been demonstrated, also the sensitivity of starch biosynthesis to both cell turgor and "sink" isolation. From these experiments the importance of specific enzymes in regulating starch and sugar metabolism, either in developing or stored tubers, has been researched. This work led for example to a major EC and Industry grant and a collaborative effort on the isolation of invertase proteins and genes and the generation of transgenics with modified sugar balance for commercial purposes. This work is now in the technology transfer phase. Other novel genes and/or proteins involved in carbohydrate metabolism have been isolated for the first time including fructokinase and alkaline invertase. These are currently exploited to modify primary carbohydrate metabolism, again in commercially important crop species. In parallel, NMR has been exploited to determine metabolic fluxes and to elucidate the transport of a six carbon compound into the amyloplast to support starch synthesis. The importance of alkaline (plastidic) pyrophosphatase as a regulator of starch formation has also been proven experimentally. Complementary studies, aimed at dissecting the molecular basis of tuberisation, have isolated several differentially expressed genes and we were the first to clone a higher plant S-adenosyl methionine decarboxylase (SAMDC) gene, involved in polyamine synthesis. Differential screening has also been successfully applied to ripening fruit and seeds undergoing dormancy break. As a result, tissue specific promoters have been isolated from *Ribes* and cold inducible genes from seeds of *Pseudotsuga menziesii*.

Unique work on transport mechanisms has shown that plasmodesmata function as pressure-sensitive 'valves', gating in response to applied pressure gradients across cell walls. These findings have implications for the regulation of solute transport through plasmodesmata and also for cell wounding, suggesting that one of the first responses during wounding is the isolation of the damaged cell(s) through closure of plasmodesmata. A successful collaboration with virologists has exploited microinjection techniques to demonstrate, for the first time *in vivo*, plasmodesmatal gating during virus infection. To advance this work a novel microinjection system has been developed in which turgor pressure can be monitored continuously during microinjection. This work received a SMART award (Small Firms Merit Award for Research and Technology) in 1994 and the micropressure probe is likely to become commercially available in 1996.

Extensive studies on plant cells undergoing osmotic shock have elucidated the membrane interactions which occur during plasmolysis. Work with a range of radiolabelled and fluorescent xenobiotics has been instrumental in demonstrating the role of the plant vacuole in xenobiotic sequestration. A novel discovery was that this system can be inhibited effectively by the mammalian drug probenecid, a known inhibitor of organic anion transport in humans. Vacuolar sequestration is likely to play a major role in herbicide resistance.

A simulation model of the potential development, growth and yield of potato has been produced and used both to assess the performance of field crops and to guide other experimental work. The model has been applied successfully to forecast yields of potato at regional and national levels for the UK. The results showed that the greatest effect on tuber yield was to improve the relation between leaf expansion rate and soil moisture status. The model of water-constrained potato growth has been enhanced by the development of sub-routines that

simulate the growth and distribution of roots with the soil volume. An important feature of the model for its eventual, general application is that it avoids the need to simulate, or measure, soil water potentials for the estimation of water uptake. A model of tuber-size distribution has also been developed and is in regular commercial use (ADAS) to support decisions on potato seed rates, and on assessing consequences of changing harvest date.

An understanding of the effects of water-stress on potato physiology has been crucial to the development of the model of water-constrained yield and has also provided a significant body of work in its own right. Drought tolerance has been shown to be influenced by the balance between root and shoot characteristics. In grafting experiments the scions have a clear dominant effect in determining dry matter partitioning under drought conditions. Potato cultivars differ in the level of relative water content that is lethal but those levels occur at the same points of insertion of leaves. Chlorophyll fluorescence has been shown to be an effective indicator of water-stress in potato. Carbon isotope discrimination ($\Delta 13$ values) techniques have also been applied but the use of $\Delta 13$ values as a valuable selection tool for potential yield has been discounted.

Research on crop water use has been complemented by field scale projects to determine ways of minimising nitrogen application to potato crops. Rapid methods of assessing plant N status in real time have been developed. The effectiveness of foliar applied urea in meeting crop demand whilst reducing the potential for nitrate leaching has been clearly demonstrated. The development of growth models and Advanced Information Systems/Decision Support Systems for crop management now follows.

Achievements in field crop nitrogen and water use have been supported by in-depth analysis of nutrient cycling in soils and the soil-plant surface. The importance of root induced modification in soil N cycling has been established as has the poor efficiency of plant root systems in N uptake from the soil. The discovery that the microbial biomass as a pool does not necessarily relate to its functional characterisation has led to the development of novel protocols to unravel the complexities of soil microbial populations. For example, DNA hybridisation techniques have been developed for broad scale analysis of soil community DNA.

Soil physicists have shown that mechanical impedance to root growth in soil causes anisotropic stiffening of cell walls, decreasing rates of cell production. A new approach to modelling soil strength and root growth has been devised and used to study implications for root distribution under various conditions. The combination of experimental and theoretical approaches has led to a systematic re-evaluation of conventional soil physics, adopting a new framework where the influence of soil structure on biophysical processes can be quantified for the first time. Theoretical biology has also led to the development of a quantitative framework for studying complex biochemical systems. The Institute takes an international lead in this arena. The establishment of the Theoretical Biology initiative instigated the formation of the Centre for Non-Linear Systems in Biology in collaboration with Dundee University.

The resourcing of a unit for stable isotope research has established the Department as a major European centre for the application of these techniques in ecophysiological and biochemical research. This has attracted EC funding and stimulated internal and international collaborations in diverse areas, including food webs research and the genetic/physiological mechanisms of stress tolerance.

Finally, in this historic perspective I would like to pay tribute to the scientists who have retired from the Department over the past 10 years. Most have operated in the near market sector but their efforts have bridged the interface between varied disciplines and have contributed to the fine tuning of commercial growing practices. This has been to the advantage of the grower, consumer and the environment. These agronomic experiments have determined in practice the potential yield of potato crops, the impact of inter-plant competition on nutrient acquisition, the efficacy of herbicides and desiccants, the basis for specific quality disorders of vegetables. In parallel, the qualitative and quantitative data obtained has led to commercial products such as the HERBREX database for herbicide usage.

Strategies for optimising nitrogen fertilisation of potato

D.K.L. Mackerron, M.W. Young & H.V. Davies

The 'optimum' rate of application of N fertiliser can be defined in several ways: It can be that level of N application that results in (a) the highest yield in a particular field and season; (b) the greatest marginal cash return between N applied and tuber yield; and (c) the lowest residual N consistent with an economic yield and the least risk of leaching of N. These options can be discussed in turn together with the characteristics of these optima.

In the first case, (a), the optimum application lies between low levels where N supply limits leaf canopy expansion or longevity and high ones where excess N supply delays the translocation of dry matter from the haulm to the tubers (Fig. 1). The principal difficulty with this concept is that the optimum differs from field to field and from year to year, and can only be determined retrospectively. The true optimum supply varies with the potential yield, which is determined by dates of planting, haulm destruction, and the weather in between these dates. The optimum rate of fertiliser application varies further because of variable rates of supply from the soil (influenced by soil type, previous crop, and the weather). Because N fertiliser is relatively cheap, farmers tend to rely on the response curve of yield versus fertiliser application having a broad, flat top, and tend to ensure they have applied at least enough, often using too much. In the event of drought, disease, or early harvest, crop growth and N uptake will both be limited and extra N applied is not used. An excess supply of N can alter the physiological balance of the plant and vegetative growth may be stimulated at the expense of reproductive growth and the formation of commercially important storage organs.

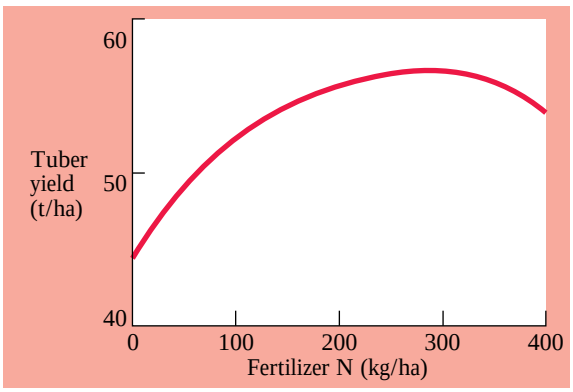


Figure 1 The average response curve of potatoes (cv. Bintje) to fertilizer N estimated from 98 experiments carried out in The Netherlands between 1973 and 1982. (Drawn from Neeteson and Zwetsloot[1]).

The important commercial and environmental aims are to match N supply with crop demand for N to avoid excessive fertiliser use. Predicting the availability of soil N would be invaluable, if it could be done well, as the supply of soil N governs the need to apply fertiliser. However, analysis of total N in the soil does not indicate N availability as a large proportion of soil N is in an organic form and is not immediately available to plants. Recommended rates of N fertiliser for potato range from 50 kg N/ha to 240 kg N/ha depending on soil type and N index and are based on the N requirement of the crop whilst making allowances for any residual N from the previous crop and fertiliser applications. Such estimates may be broadly correct, but are insufficiently accurate to avoid excess applications in some of the wide variety of conditions and requirements which may be encountered between individual fields, crops and combinations of weather conditions.

The crop as an indicator of N supply Analyses of the crop may provide an indicator of soil N supply but they must provide reliable data as early in the season as possible to allow additional N to be applied whilst the crop is able to use it. This is particularly important for potatoes as rates of N uptake slow considerably after approximately 60 days after planting[2].

In the north-western USA, petiole sap nitrate is measured routinely in the growing crop and is used to monitor the need for additional N applied in an overhead irrigation system. These crops are growing on light, free draining soils with low organic matter, almost in a 'hydroponic' system. Experiments at SCRI have shown that petiole nitrate levels vary within a plant, from plant to plant, and from plot to plot within the same field and change during the day. Furthermore, it was not possible to match the changing petiole nitrate concentration during the growing season to crop growth or rates of N uptake. These results cast doubt on the validity of estimating crop N

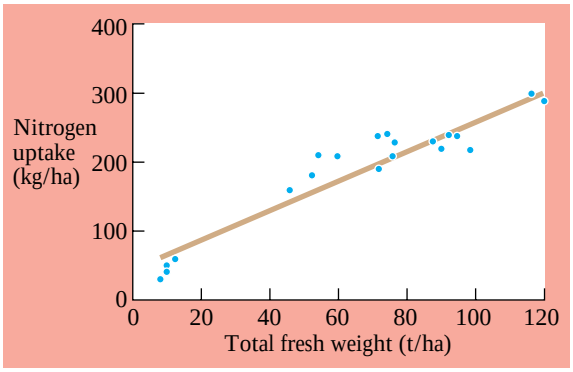


Figure 2 The relation between total fresh weight and N-uptake for cultivar Maris Piper given 160kgN/ha at planting. Experimental data (points) and fitted line (r^2 = 0.92).

status of potatoes by measuring a specific nitrogenous constituent in a specific region of the plant.

An alternative approach is to assess total plant N uptake. This involves measurements of growth and of N concentrations. Traditionally analysis for N has been done by either Dumas or Kjeldahl techniques but they are both expensive and slow and Kjeldahl analysis involves the use of hazardous chemicals. We have used near infrared reflectance (NIR) spectroscopy on dried and milled tissues. The technique is relatively quick and simple and there is good agreement between NIR and the standard N analyses by either Dumas or Kjeldahl techniques.

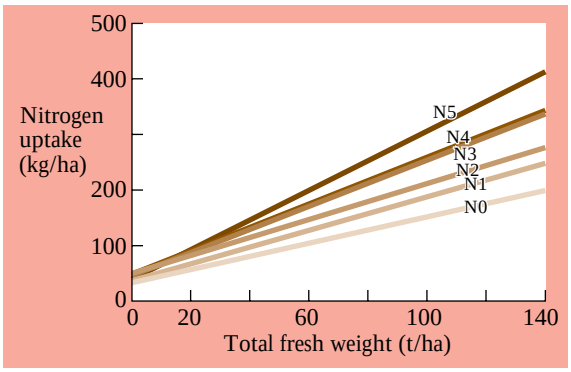


Figure 3 The relation between total fresh weight and N-uptake for cultivar Maris Piper given 0, 40, 80, 160, 80+80 and 240 kgN/ha (N0, N1, N2, N3, N4, N5 respectively). N4 represents a split application of 80kgN/ha at planting and 80kgN/ha at tuber initiation. Data points omitted for clarity.

An alternative approach for estimating plant and crop N status There is a stable relation between the N content of the whole plant and plant fresh weight (Fig. 2) that can be shifted from one level to another by supplementary applications of N at the time of tuber initiation and later (Fig. 3). The slope of this relation is equivalent to an average N concentration in the plant and, once the relation has been established, the crop grows in step with its N uptake so that there is another linear relation between that slope and the final N uptake by the crop (Fig. 4) that applies over a range of maturity classes. This relation and other techniques can be used to assess N uptake by potato crops in real time and to assist in the development of models to schedule N applications.

Development of theory into practice Mathematical models of crop growth can provide estimates of the potential yield of potato, and also the minimum, critical N concentration required, [N_r], to attain that yield[3]. Efficient use of nitrogen requires that the crop incorporates only sufficient nitrogen to attain its [N_r] and potential yield, thus avoiding excess uptake. By using a low initial application rate of N at planting and monitoring uptake rate by NIR combined with the N x fresh weight model, the contribution from the soil can be estimated. That information can be coupled with estimates of yield and the related [N_r] to give an estimate of the requirement for supplementary applied N. The supplement can be given either as granules or as foliar applications and should result in the optimal combination of the highest yield attainable in the particular field and season and a minimal residual N with the least risk of leaching of N

N application tailored to requirement The hypothesis outlined above was tested in an experiment in which the cv. Maris Piper was grown with nitrogen treatments comprising two levels of base dressing (40 and 80

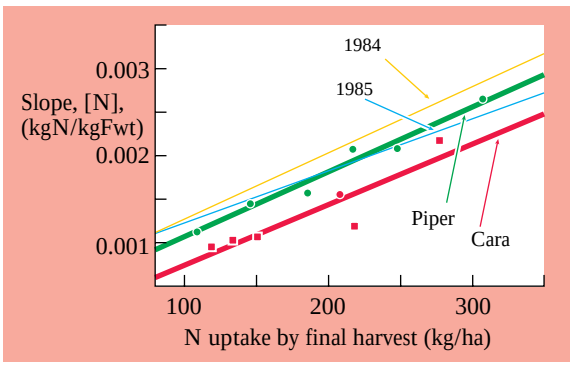


Figure 4 The relation between the average concentration of nitrogen in all the plant tissues (the slopes from Figs 2 & 3) in each treatment in 1990 and the N-uptake by final harvest. Each point corresponds to one N-treatment. (• = Maris Piper, ▪ = Cara). Thin lines are relations found in previous years with Maris Piper.

Base dressing (kgNha^{-1})	$[N_a]_F$ (gNha^{-1} per kgFWtha^{-1})	Standard error	Change required	Supplement required
40	1.02	1.24	1.029	174
80	1.46	2.37	0.588	100

Table 1 Values for the slope of the N-uptake x FWt relation, $[N_a]_F$, estimated following base dressings of 40 and 80 kgNha^{-1}, together with the change in slope required to provide a slope of 2.048 gNha^{-1} per kgFWtha^{-1} and a final uptake of 226 kgNha^{-1}.

kg N ha^{-1}), each followed by four levels of supplementary N (0, r-40, r, r+40 kg ha^{-1}) where r = the recommended rate estimated as described later. Irrigation was scheduled to maintain a soil moisture deficit not more than 30 mm. Samples were harvested at the end of June and in mid-July for analysis of N uptake and the calculation of fertiliser requirement. The crop was harvested in mid-September for determination of yields and final N uptake. The values for N uptake were regressed on the total fresh weight of the plants to give estimates of the actual N concentrations, $[N_a]$. The values calculated were 1.019 ± 0.124 g N/kg FWt and 1.46 ± 0.237 g N/kg FWt for the plants receiving 40 and 80 kg ha^{-1}, respectively.

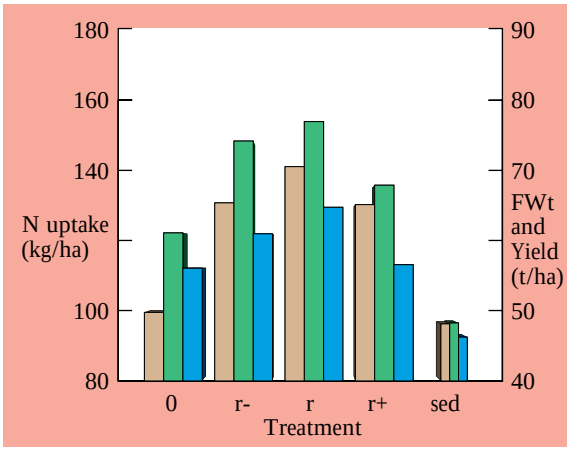


Figure 5 Nitrogen uptake total fresh weight (haulm and tubers), and tuber yield for a base dressing of 40 kgN/ha at four levels of supplementary N. 0 = no supplement, r = calculated optimum requirement (see text), r- = (r-40) kgN/ha, r+ = (r+40) kgN/ha, sed = standard errors of difference.

Calculation of the requirement for supplementary N Long-term average weather data for the site were used together with the SCRI model of potential yield[4] to predict the crop yield between planting time and an anticipated harvest date in mid-September. The estimated potential yield of crop was 60 t ha^{-1} and the requirement for supplementary N was calculated as follows:

Yield of 60 t ha^{-1} → Tuber DWt of 12 t ha^{-1}
(using tuber dry matter concentration, 20%)

Tuber DWt of 12 t ha^{-1} → Total DWt of 16 t ha^{-1}
(using harvest index, 75%)

Total DWt of 16 t ha^{-1}→ $[N_r]_D$ of 1.41% of dry matter[3]
→ Total N uptake of 226 kg N ha^{-1}
(calculated as $[N_r]_D$ x total DWt)
→ The required slope of the relation N uptake x FWt, $[N_r]_F$ of 2.048 g N ha^{-1} per kg FWt ha^{-1} (Fig. 4).

Where the symbol → represents 'corresponds to' and where $[N_r]_D$, $[N_r]_F$ represent the minimum N concentration required in the dry weight and the fresh weight, respectively, for the potential yield.

The value for $[N_r]_F$ was compared with the actual slopes, $[N_a]_F$, estimated for the plants receiving each level of base dressing. The changes required in the values of $[N_a]_F$ were calculated by simple difference and these were related to a required level of supplement by a regression between the values of $[N_a]_F$ and N application rates from earlier experiments. The estimated values are given in Table 1. The values for the treatments, r-40, r, r+40 kg ha^{-1}, were calculated accordingly. In this experiment, the required levels of supplement were calculated on 29 July and the supplements were applied as granular NH_4NO_3 on 6 August.

Final values of total N uptake, total fresh weight and tuber

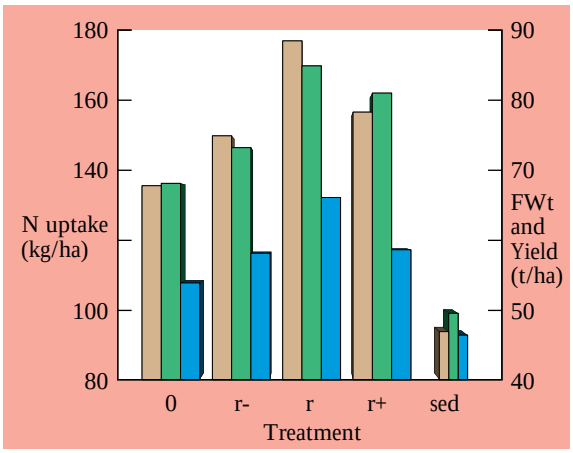


Figure 6 Nitrogen uptake total fresh weight (haulm and tubers), and tuber yield for a base dressing of 80 kgN/ha at four levels of supplementary N. 0, r, r-, r+ as in Fig. 5.

yield were calculated following the final harvest. The data (Figs 5 & 6) show that, with each level of base dressing, the greatest fresh weight production, tuber yield, and N uptake were all found in the plots that had received the recommended supplement.

The results of this experiment showed that our method had successfully produced the highest yield and, because the uptake of N was greatest, it minimised the residual N that could have been leached from the soil. An earlier estimate of the required supplement and the possibility of giving the supplement as a foliar application of urea solution are being investigated.

Supplementary application Supplementary applications of N have kept the crop effectively supplied with sufficient N to maintain growth but there can be difficulty getting supplementary N into the plants. Supplement(s) applied to the soil can only become available if there is adequate soil water available and irrigation would be necessary to ensure uptake of the applied N. Alternatively the supplementary N can be given as foliar applications (Fig. 7). Our experiments showed that the method of estimation worked and that foliar applications of N could maintain an adequate uptake of N by the crop even when it had been supplied initially with only a low level of soil-applied N fertiliser.

Conclusions The proposed method for determining the optimum application of N gave recommendations for the supplements that differed by more than the original applications of fertiliser, so that the total application on the treatment with the low base dressing was higher than would normally be recommended. This almost certainly reflects the lapse of time while early growth was made and the rates of uptake were being established, followed by the need to take up the required nitrogen at a faster rate if the requirement were to be met in the time available. Where the base application was around half the 'normal' rate, the sum of base and supplementary applications was close to normal. This emphasises the importance of giving an adequate initial dressing for early growth.

Total uptake of N in the two 'recommended' N treatments was much lower in the plants given a base dressing of only 40 kg/ha. Those plants did not take up all the fertiliser that was available, regardless of any soil mineralisation. In contrast, the plants given the higher base dressing took up the equivalent of all the applied N fertiliser. This result shows the importance

Figure 7 Potatoes, Maris Piper in mid-July and early September, given conventionally estimated N-requirement (left of the pictures) and foliar-applied N following a low, basal application of N (right of the pictures).

of giving the supplementary applications early in the growing season. In deriving an estimated value for potential yield the long-term average weather and a simulation model of potato growth and yield were used[4]. In more general applications, where either a suitable simulation model or records of long-term average weather are not available, or where yields are not expected to reach the potential values it may be better to chose yield levels for a particular farm or field on the basis of past experience. The annual variation in potential yields is generally less than 20%[5]. Even allowing for extreme conditions the yields will probably lie within 20% of the estimates.

Our work has shown that the growing crop can be used to monitor the rate of supply of N from the soil whether from applied fertiliser or mineralised organic matter. That information can be coupled with values for expected yield and the related [N_r] to give an estimate of any requirement for further applied N. The method provides a rational basis for using split applications of fertiliser and a means to determine the size of a second or subsequent part of a split application.

It avoids much of the uncertainty over N levels in soil and should enable a closer match between application and requirement for N. Furthermore, it should offer growers the double benefits of economic use of fertiliser and of minimising environmental impact through leaching losses.

This work has been supported by the Potato Marketing Board, Great Britain and the Scottish Office Agriculture and Fisheries Department.

References

1 Neeteson, J.J. and Zwetsloot, J.C. (1989) *Netherlands Journal of Agricultural Science*, 37, 129-1141.

2 Millard, P. & Marshall, B. (1986). *Journal of Agricultural Science* 107, 421-429.

3 Greenwood, D.J., Neeteson, J.J. & Draycott, A. (1985). *Plant and Soil* 85, 163-183.

4 MacKerron, D.K.L. & Waister, P.D. (1985). *Agricultural and Forest Meteorology* 34, 241-252.

5 MacKerron, D.K.L. (1985). *Agricultural and Forest Meteorology* 34, 285-300.

Nitrogen transformations in cultivated soils

R.E. Wheatley & K. Ritz

Almost 80% of the earth's atmosphere is molecular nitrogen. However in all ecosystems the amount of nitrogen present in combined forms that can be assimilated by organisms is small. This combined nitrogen, either as ammonia, ammonium, nitrite, nitrate or organic compounds originates from the atmosphere and is a transient fraction characterised by rapid gains and losses of each nitrogen species. Ammonium-nitrogen and nitrate-nitrogen are the forms most commonly used by plants. Transformations between nitrogen species result from a variable sequence of biological activities such as, nitrogen-fixation, immobilisation, mineralisation, nitrification and denitrification. The interaction of these individual nitrogen transforming processes in the soil leads to a pattern of nitrogen pools connected by biochemical pathways, known as the nitrogen cycle (Fig. 1).

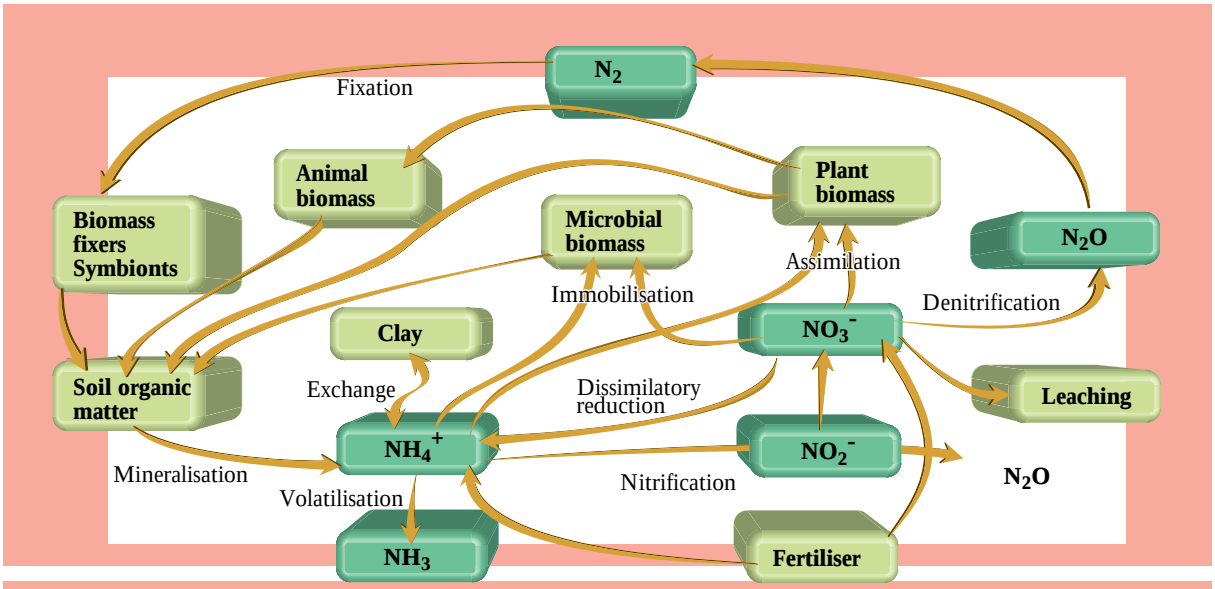


Figure 1 The nitrogen cycle.

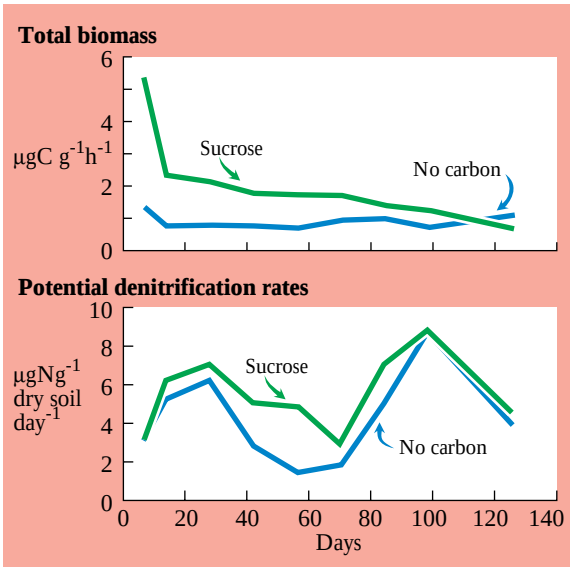


Figure 2 a) Total biomass, by substrate induced respiration, and
b)Potential denitrification rates, with glucose amendment prior to incubation in the laboratory, in soils growing potatoes, that received fertiliser only (no carbon) or were further amended with sucrose.

Nitrogen supply is a major factor in crop production, and the rate of supply can be increased by the addition of fertiliser. Uptake of this additional nitrogen by crops is generally inefficient, and there is a need to improve efficiency and also to limit losses of nitrogen to the environment. Immobilisation of this added mineral-nitrogen, and any produced by mineralisation, is obviously important with regard to nitrogen availability.

Some microorganisms are capable of biological nitrogen fixation, i.e. the conversion of molecular nitrogen to ammonia. The root nodule forming symbiosis between *Rhizobia* spp. and legumes is the most important agriculturally, fixing about 90 x 10^6 tonnes of nitrogen annually. This represents approximately twice the amount of nitrogen applied as chemical fertiliser and more than half of all the annual global biological fixation.

Mineralisation is a key process in the nitrogen cycle, in which heterotrophic organisms convert the nitrogen in organic residues into one of the inorganic nitrogen species, and obtain energy. These microorganisms, as well as respiring, are also multiplying and growing, and so a small proportion of the mineralised nitrogen will be immobilised in new microbial biomass. Thus the two processes of mineralisation and immobilisation are opposed, one breaking down, the other building up organic nitrogen. The dominant net effect is normally mineralisation, as energy is the main requirement. This interaction between autotrophic and heterotrophic biological activity is a major feature of the nitrogen cycle. Green plants use, and store, solar energy as plant tissue, via photosynthesis and, when incorporated into the soil after death, this tissue is used as an energy source by heterotrophic microorganisms, so releasing the inorganic-nitrogen compounds originally taken up by plants. Some of the mineral nitrogen will be used by plants and some will be nitrified to nitrate. Nitrification is the oxidation of any reduced form of nitrogen to a more oxidised form. It is an exclusively biological process, in two steps, carried out by members of the bacterial family *Nitrobacteraceae*, frequently referred to as nitrifiers. The first step is the oxidation of ammonium to nitrite, and the second the further oxidation of the nitrite to nitrate, and distinct groups of bacteria are responsible for each of these steps. In both cases, mineral-nitrogen is used as the sole energy source and carbon dioxide as the main carbon source. Nitrifying bacteria fix carbon dioxide via the Calvin cycle and the fixation of one molecule of carbon dioxide requires the oxidation of 35 ammonium molecules, or 100 nitrite molecules, which accounts for about 80% of their energy budget. Nitrifiers thus have an energy source denied to more conventional bacteria, but use up most of it in a perverse insistence on fixing their own carbon from carbon dioxide. However nitrifying organisms therefore will never be carbon limited, unlike the heterotrophic organisms in soils.

Denitrification is a respiratory process used by some aerobic bacteria in the absence of molecular oxygen. As a result, oxides of nitrogen are reduced to a less-oxidised state and ultimately to molecular nitrogen. Suitable electron donors, such as organic carbon-compounds, reduced sulphur-compounds or molecular hydrogen, are also required.

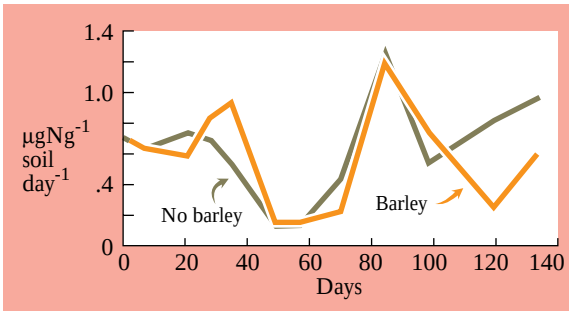


Figure 3 Average nitrification rates in poultry manured soil, either with or without barley plants.

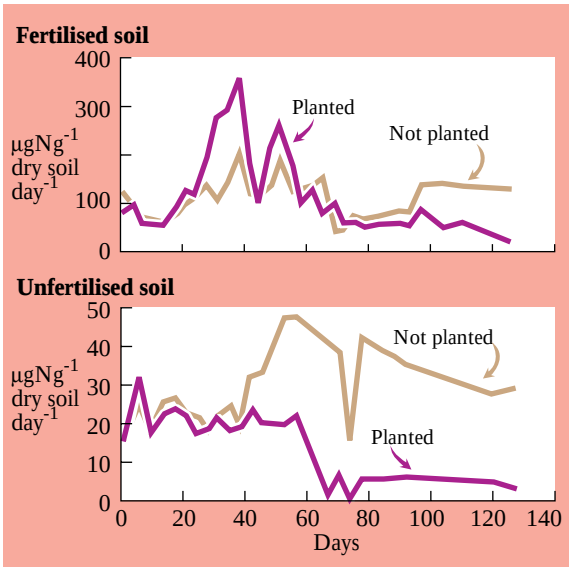


Figure 4 Average mineral nitrogen concentration in soil a) fertilised with 187kg nitrogen ha^{-1}, or b)not fertilised, that was either planted or not planted.

In most natural terrestrial ecosystems the nitrogen released by mineralisation forms the main fraction of the total annual flux of mineral nitrogen through the pool of soil nitrogen available to the plant. The net effect of the two opposing processes of mineralisation and immobilisation will determine the nitrogen supply to, and thus the productivity of, non-nitrogen fixing plants, under non-fertilised conditions. In agricultural systems this natural pool is frequently supplemented by the addition of other, usually inorganic, nitrogen sources.

Microbiological activity in soils is limited by the availability of carbon and plant roots are a significant source of carbon. The addition of different carbon sources to field soil at SCRI showed that large sustained inputs are required to increase, then maintain the microbial biomass and that the amount of carbon released by growing plants is only a fraction of that required for maximum microbiological activity. Short-term increases in available carbon only result in transient increases in microbial biomass.

Temporal patterns observed in assays of both nitrification and denitrification activities, from field and microcosm studies, suggested that plants may influence systems in other ways than simply by the gross input of carbon. In denitrification assays that were designed to remove the possibility of any carbon limitations on the system, very distinct seasonal variations in activity levels still occurred, suggesting that plants may be capable of more subtle effects.

This concept was further supported by the pattern of nitrification rates revealed in organically manured soil where microbial activity was much greater than in inorganically fertilised soils. Increases in activity rates were too rapid to result from changes in the numbers of nitrifying organisms, and plants did have a slight positive effect on nitrification rates early in the season and a negative effect later on.

In a field experiment studying the dynamics of soil nitrogen under a potato crop, concentrations of mineral nitrogen differed markedly from those anticipated, assuming uniform incorporation of the nitrogen as fertiliser. For example, concentrations of ammonium and nitrate were about one third of those anticipated 6 days after application, and two to three times greater between 12 and 32 days after application.

In the unfertilised planted soils nitrogen concentrations declined, due to plant uptake, whilst in the fallow plots they did not. Rates of nitrogen mineralisation and immobilisation were minimal compared with the apparent rates in the fertilised plots.

So these processes cannot be responsible for the rapid changes in mineral-nitrogen concentrations observed in fertilised soils. Neither can the rapid and unexpected changes be due to microbial osmoregulation or nitrogen assimilation, as both these would result in unrealistic carbon to nitrogen ratios in the microbial biomass. Another possible explanation is that the cultivation methods did not result in a homogeneous spatial distribution of the pelleted fertiliser so that when capillary action in the soil was re-established and the fertiliser dissolved, incomplete mixing resulted in a soil-solution of a concentration much greater than that anticipated, based on uniform incorporation. This solution then moved up the soil ridge as a front of concentrated soil-solution. Mixing between this band of concentrated solution and the rest of the soil water was poor, but eventually the effective strength of it was reduced as the ions dispersed. Many models of nitrogen cycling in soil take no account of heterogeneity in mineral nitrogen pools, but our work has demonstrated that it is very important. Although mineral nitrogen dynamics were not so extreme in organically manured soils, relatively rapid changes still occurred, and nitrogen transformation rates were only marginally affected by plants.

Plants also had apparently little effect on short term variations in total microbial biomass when grown in unfertilised soils. However there was a suggestion that the activities of specific components of the biomass,

such as nitrifying or denitrifying organisms showed significant temporal variation.

Thus temporal patterns of total soil microbial biomass appear to remain relatively stable throughout the growth of the crop but rates of specific activities such as nitrification and denitrification show very dynamic changes that can be both rapid and widespread throughout the bulk soil. Elucidation of whether the changes are due to extant populations switching on and off, or to compositional changes in community structure requires further research. This is being approached using techniques such as community DNA analyses and volatile organic compound and phospholipid profiling.

The continual input of relatively large amounts of nutrients appears to be required to produce any persistent significant effects on total biomass in soil. The input of carbon from plant roots does not appear to be sufficient to cause significant short term perturbations in total microbial biomass. However, it is possible that plant inputs may be responsible for more subtle effects. The temporal patterns of rapid changes in the rates of specific processes against a background of a relatively stable total microbial biomass suggests that although population numbers may remain relatively constant throughout the season, the degree of activity of specific portions of the biomass may be affected in some way.

A large variety of volatile organic compounds are present in soil atmospheres and many of them are probably microbial in origin. Environmental factors that influence either population dynamics or microbial activities will probably also influence production of volatile organic compounds. As chemical control mechanisms occur in a wide range of biological systems, it is feasible that volatile organic compounds act as signals between microorganisms in both the rhizosphere and the bulk soil, and promote the rapid dynamic changes in activity levels seen in specific nitrogen transformation processes in soils. This is being investigated at the moment.

Examination of the nitrogen cycle shows that frequently each nitrogen species can be produced by different transformation routes from different nitrogen sources. The ratio of the naturally occurring forms of the stable isotopes of nitrogen, ^{15}N and ^{14}N, can be used to allocate the source and transformation route of a particular product, since the different transformations cause differential fractionation, i.e. the ratio between the two isotopic forms is changed slightly. Although such changes are small, they can be reliably measured with high performance mass spectrometers. The partitioning of the production of nitrous oxide between the processes of nitrification and denitrification can be determined by analysing the stable isotope signatures of nitrogen and oxygen produced during each process and then applying the results to field measurements.

Uptake and compartmentation of xenobiotics in plant cells

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

There has been increasing public concern about the environmental impact of pesticides and the possible contamination of food crops. This, along with the substantial economic costs involved in the manufacture and application of agrochemicals, emphasises the need for a better understanding of the mechanisms involved in movement of agrochemicals within plants. In designing systemic chemicals, the agrochemical industry synthesises molecules with properties to enable their maximal penetration into, and movement within, the plant. It has frequently been assumed that, since these molecules are foreign to the plant (termed xenobiotic), their behaviour is governed solely by their physicochemical properties. However, this view ignores physiological aspects of chemical transport within plant cells. The work described in this article casts doubt on the solely physicochemical nature of xenobiotic movement and addresses the mechanisms by which xenobiotics are transported by plant cells.

The two main physicochemical properties believed to influence the movement of chemicals through plant cell membranes are the lipophilicity and the electronic

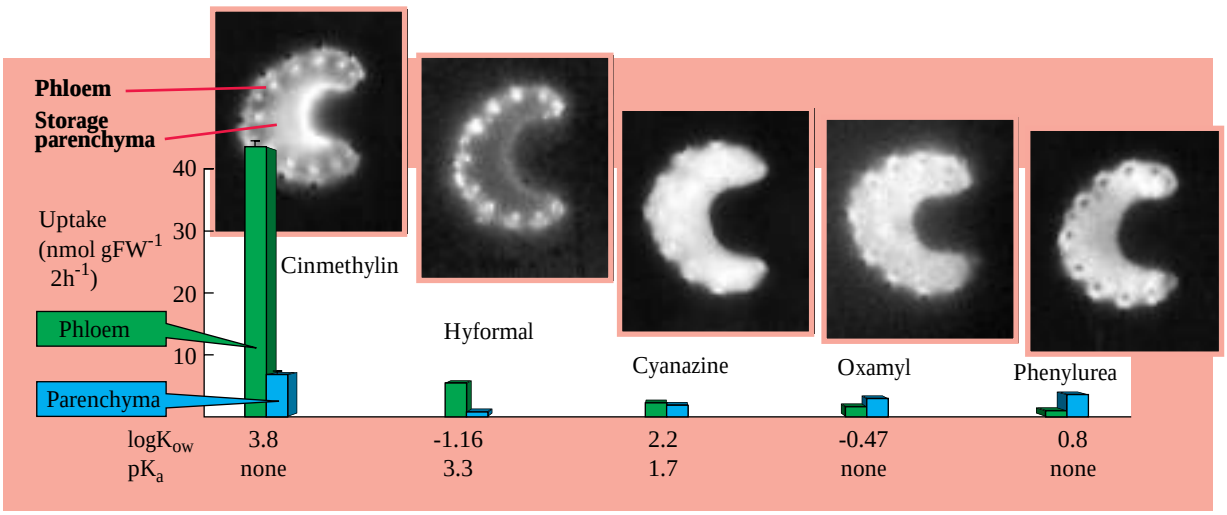


Figure 1 Autoradiographs of celery petiole slices incubated with cinmethylin, hyformal, cyanazine, oxamyl and phenylurea showing radiolabel distribution in the phloem and storage parenchyma. The bar chart shows the net uptake by phloem or parenchyma tissue following 2h incubation in 10µM xenobiotic. Below are the physicochemical properties, the lipophilicity ($logK_{ow}$) and the dissociation constant (pK_a).

charge. The lipophilicity of a molecule is its affinity for lipid as opposed to water and is measured as the oil:water partition coefficient (log K_{ow}). The higher the log K_{ow} value for a molecule, the greater is its affinity for lipid, and thus the greater its ability to pass through the lipid areas of plant membranes. The lipophilicity of a molecule is decreased if the molecule is electrically charged. Membranes are therefore much less permeable to molecules carrying a charge than to those that are uncharged. Certain molecules dissociate, and become negatively charged, depending on the pH or acidity of the environment they encounter. The pH at which 50% of a molecule is dissociated is defined as the pK_a or the dissociation constant. If a molecule is dissociated at physiological pH, i.e. the environment normally encountered by plant cells (pH 5-8), then it is classed as a weak acid. It is believed that weak acids are able to permeate membranes in the undissociated state and then dissociate at the higher pH encountered inside the cell. The resulting, negatively charged, anions are trapped within the cells since they are less able to penetrate the lipid membranes.

Uptake of radiolabelled xenobiotics by celery tissue In order to determine whether the movements of xenobiotic chemicals can be predicted by their physicochemical properties alone, a number of experiments involving radioactively labelled agrochemicals were carried out on two types of celery tissue. The two tissues investigated were the phloem which is the pathway by which sugars are transported throughout the plant, and the parenchyma tissue (Fig. 1), which makes up a large proportion of the celery petiole and has a storage function. The phloem tissue was of particular interest to examine whether or not xenobiotic chemicals could move via the same mechanism as endogenous sugars. However, there was no evidence to suggest that the xenobiotics were being actively transported via a carrier(s) into phloem tissue since the uptake of the chemicals was linear with respect to their concentration. This is consistent with movement via diffusion and contrasts with the uptake of sucrose into the phloem tissue that showed two phases, a linear phase, and a saturable phase indicative of carrier mediated transport.

The ability of five radiolabelled agrochemicals to be taken into, and retained by, the celery tissue did not appear to be related to their physicochemical properties (Fig. 1). Uptake of the chemicals was not related to either the lipophilicity (log K_{ow}) or related to the ionisation state (pK_a). Further, markedly different uptake and accumulation rates of the five agrochemicals were observed for the two tissues (Fig. 1). For example, cinmethylin (herbicide) and hyformal (fungicide) accumulated preferentially in the phloem whereas oxamyl (nematicide) and phenylurea (herbicide) accumulated in the parenchyma tissue.

The lack of a relationship between uptake and log K_{ow}, and the different partition characteristics between phloem and parenchyma tissues, suggest that factors in addition to the physicochemical properties of these molecules were involved in their uptake. However, this type of work suffers from the deficiency

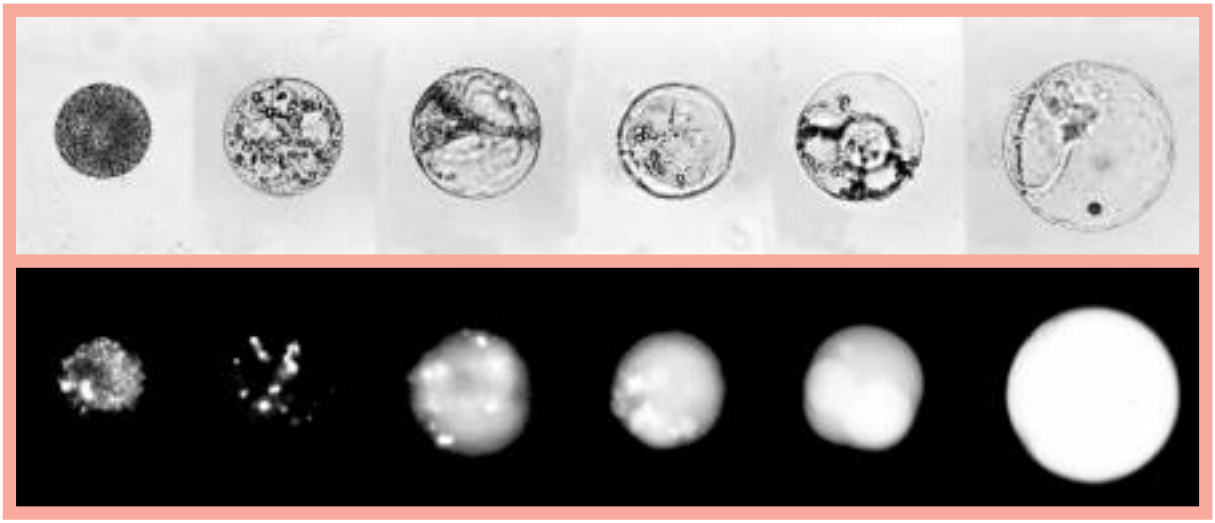


Figure 2 Appearance of oat aleurone protoplasts cultured for 0 to 5 d (days) and then incubated with LYCH. The upper micrograph shows a bright field, the lower micrograph the corresponding fluorescent image.

that the location, or compartmentation of the chemical within the plant cell cannot be identified. However, this problem may be overcome by investigating the compartmentation of fluorescent xenobiotics, the uptake of which can be both visualised and quantified.

Uptake and compartmentation of fluorescent xenobiotics by oat aleurone protoplasts Protoplasts, individual cells from which the wall has been digested, may be isolated from a variety of plant tissues and grown in sterile liquid culture. Protoplasts isolated from the aleurone layer of oats show an interesting sequence of vacuole development making them a valuable system in which to study the role of the vacuole in xenobiotic compartmentation. The vacuole is a membrane bound body, sometimes making up the majority of the cell volume. The densely cytoplasmic oat aleurone protoplasts, when placed in sterile culture, develop into highly vacuolate protoplasts over a period of about 5 days (Fig. 2). During this time the numerous protein bodies break down and the protein body membrane reorganises to form the tonoplast, the membrane surrounding the vacuole.

Oat aleurone protoplasts of varying ages were incubated with the fluorescent xenobiotic Lucifer Yellow (LYCH) which has a log K_{ow} of -10.2 and a pK_a less than 0.7. According to these physicochemical properties one would predict that this molecule would be completely impermeant to plant membranes. However, aleurone protoplasts show a developmental sequence with respect to their ability to take up LYCH, the xenobiotic being accumulated within the developing vacuoles (Fig. 2). This dye uptake was prevented in the presence of the drug probenecid, a known inhibitor of organic anion transport in animal cells. Since LYCH was not observed in the cytoplasm

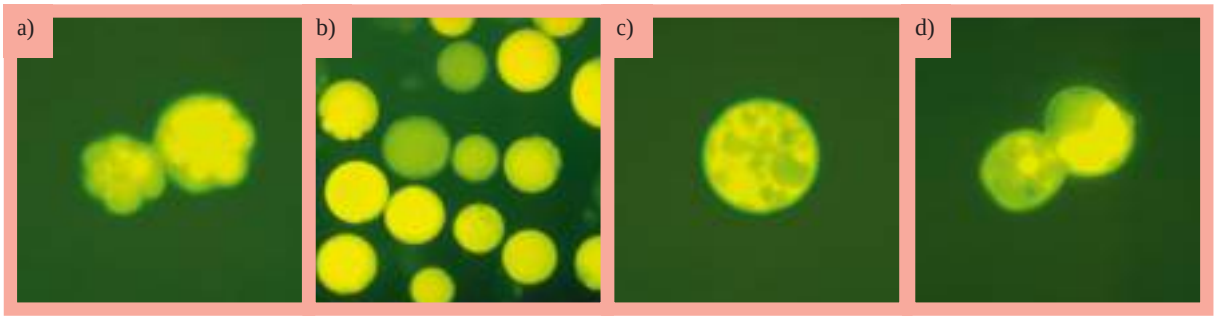


Figure 3 Oat aleurone protoplasts incubated with CF in the absence (a,b) or presence (c,d) of probenecid. The uptake of CF into the protein bodies of Day 1 protoplasts (a) is inhibited in the presence of probenecid (c) restricting the dye to the surrounding cytoplasm. Similarly Day 5 protoplasts accumulate dye in the vacuoles (b) or in the cytoplasm in the presence of probenecid (d).

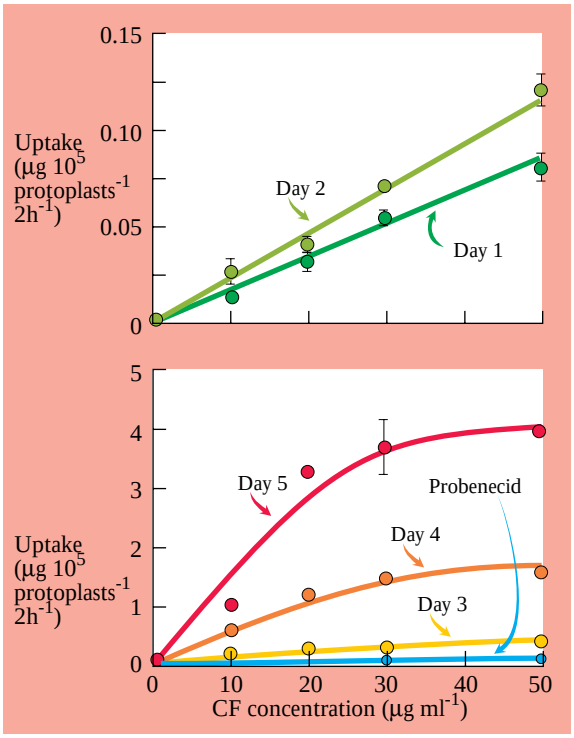


Figure 4 The uptake of carboxyfluorescein into oat aleurone protoplasts with respect to substrate concentration. Protoplasts were incubated for 2h in the designated concentrations of CF. The protoplasts were used at day 1 - 5. Day 5 protoplasts were also incubated in the presence of 0.125 mol m^{-3} probenecid.

surrounding the vacuole it would appear that between day 3 and day 5, the aleurone protoplasts develop two highly co-ordinated transport mechanisms capable of transporting LYCH across both the plasmalemma and the tonoplast. The former transport mechanism, at least, is inhibited by probenecid.

In an attempt to identify when, during this developmental sequence, the tonoplast transport mechanism is operational and whether it is also affected by probenecid, the protoplasts were incubated with another fluorescent xenobiotic, carboxyfluorescein (CF), which has pK_as of 3.4, 4.4 and 6.3 and a reported log K_{ow} of -1.5. From these pK_a values it can be calculated that a small proportion (one molecule in every 794) of CF will be in the undissociated form at the external pH used in these experiments (pH 6.3). This contrasts with LYCH where less than one in 398,197 molecules will be undissociated. CF was able to enter all ages of aleurone protoplasts. At the early stages of development it entered presumably by diffusion of the small amount of undissociated molecule present (Fig. 3a & b). However, during development, the protoplasts showed an increasing capacity for CF uptake that was sensitive to probenecid and, like LYCH uptake, was saturable with increasing substrate concentration (Fig. 4). This suggests that CF, probably as the mono- di- or tri-anion (CF^-, CF^{2-} or CF^{3-}), like LYCH, is transported across the plasmalemma via a probenecid-sensitive mechanism. Once inside the protoplasts, the CF accumulated within the protein bodies or the developing vacuoles via a process which was also inhibited in the presence of probenecid (Fig. 3c & d). This indicates that, in contrast to the plasmalemma, the transport mechanism on the tonoplast was operational throughout development.

The wrong compartment? Since CF is a weak acid, it has been suggested that the undissociated molecule could diffuse across the plasmalemma, encounter a higher pH, dissociate and then become trapped within

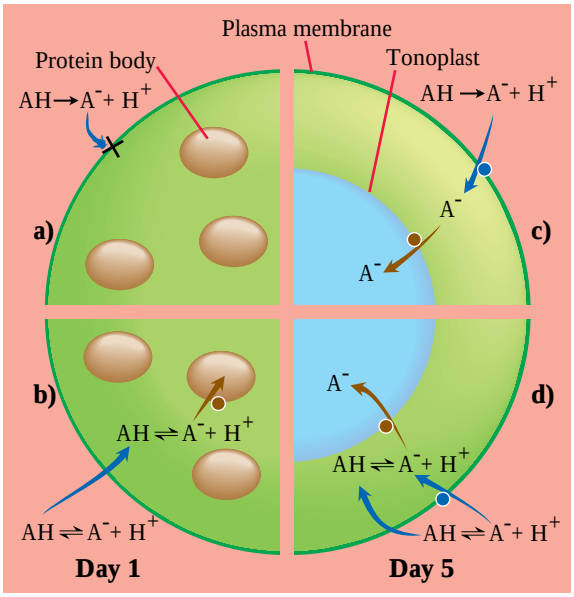


Figure 5 Overview of the possible mechanisms involved in the uptake of fluorescent xenobiotics. (a,c) Molecules with low pKas which are fully dissociated at physiological pH. The anion is not able to diffuse across the plasma membrane of day 1 protoplasts (a) but may be transported across both the plasma membrane and tonoplast of day 5 protoplasts (c) by probenecid-sensitive systems. (b,d) Probes with higher pKas are sufficiently undissociated at physiological pH to allow their diffusion into both day 1 (b) and day 5 (d) protoplasts. Once inside, the molecule dissociates in response to the cytoplasmic pH and the anion formed is transported via probenecid-sensitive mechanisms into the protein bodies of day 1 protoplasts (b) or the vacuoles of day 5 protoplasts (d). In day 5 protoplasts, carrier-mediated transport of the anions may also occur across the plasma membrane (d).

the cell. According to this model, the dye would be expected to accumulate in the compartment of the highest pH, i.e. the cytoplasm. However, this only occurs in the presence of probenecid thereby confirming that additional transport mechanisms are present to sequester CF into the vacuole. These different uptake mechanisms are illustrated diagrammatically in Figure 5 where A^- represents all the ionisation states for the dissociated molecules.

Size limit for plasmalemma transporter In addition to their ability to take up LYCH and CF, aleurone protoplasts were also able to accumulate a number of other fluorescent probes via probenecid-sensitive routes. These probes, PTS, HPTS, SR-G, SR-101, Calcein and LYAB, have a variety of structures and molecular weights in the range 376 to 637. However, the non-fluorescent Trypan Blue (MW 961) was excluded, possibly indicating an upper limit to the size of molecule that can be transported across the plasmalemma via the probenecid-sensitive route.

Conclusions It is clear from the use of radiolabelled agrochemicals that their movement is not simply related to physicochemical properties alone. There are numerous reports that demonstrate that the majority of a foliar applied agrochemical does not move from the site of its application, suggesting that the molecules may have become trapped within the plant cells. Our studies with fluorescent xenobiotics have demonstrated the existence of mechanisms able to transport xenobiotics into plant cells and subsequently sequester them within the vacuole at high concentrations. Since the vacuole is isolated by the tonoplast from the majority of the enzymatic machinery located in the cytoplasm of the cell, this might provide a mechanism of preventing damage by foreign molecules.

A number of questions remain, not least of which are the following: (1) How do these plant membrane transporters recognise such a diverse range of 'foreign' molecules and do they also transport metabolites during the growth of the plant cell? (2) Is the movement of fluorescent xenobiotics analogous to that of agrochemicals? The linear uptake kinetics suggest that the route across the plasmalemma might be via a diffusion-like process, but the possibility remains that once inside the cell the agrochemicals are sequestered from the cytoplasm via a probenecid-sensitive transport mechanism.

It is clear that inhibition of the vacuolar sequestration mechanism would result in a greater accumulation of xenobiotics in the cytoplasm. This would lead to an increased possibility for movement between cells, as well as a means of targeting xenobiotics to the 'correct' compartment. Thus, any useful future strategies for the rational design of systemic xenobiotics should include the successful elimination of vacuolar sequestration.

The role of pyrophosphate:fructose 6-P, 1-phosphotransferase in plant metabolism

R. Viola, J. Liu, J.A. Crawford & B.A. Goodman

The glycolytic pathway Glycolysis plays a central role in the metabolism of almost all cells. The universal function of the pathway is the generation of energy and the production of substrates required for oxidative phosphorylation. Most of the reactions involved in the glycolytic pathway are also shared with other important metabolic processes (e.g. the generation of organic acids and amino acids). Furthermore, some metabolic processes (e.g. gluconeogenesis, photosynthetic sucrose synthesis) require that the overall flux through the glycolytic system is reversed. The pathway consists of a set of thermodynamically irreversible reactions (key reactions) joined up by a set of highly active enzymes catalysing near equilibrium reactions *in vivo*. Such reversible enzymes can be described as the biochemical "stirrers" within the glycolytic reactor serving the purpose of rapidly interconverting the intermediates. The activity of the reversible enzymes is usually not regulated and the relative concentrations (mass action ratios) of their substrates will determine the net flux they catalyse. Important regulated steps are located at the entry in

the pathway, the phosphorylation of fructose-6-phosphate (Fru6P) catalysed by phosphofructokinase (PFK), and at the exit, the de-phosphorylation of phosphoenolpyruvate catalysed by pyruvate kinase (PK). An important regulatory site for the gluconeogenic pathway is the de-phosphorylation of fructose-1,6-bisphosphate (Fru16BP) catalysed by fructose bisphosphatase (FBPase; Fig. 1).

Regulation of glycolysis A large amount of work has been done on the regulation of glycolysis in microorganisms and animal cells. In particular, much attention has been devoted to the study of the glycolytic system in yeast. Here, PFK activity is inhibited by ATP and citrate and strongly stimulated by the allosteric regulator fructose-2,6-bisphosphate (Fru26BP) which simultaneously inhibits the activity of the FBPase. As the synthesis of Fru26BP is repressed by ATP and C_3 glycolytic products, and stimulated by hexose-P, it is clear that metabolic requirement for glycolytic products will fine-tune the entry of hexose-P in glycolysis. A detailed picture of the interaction between glycolytic and gluconeogenic enzymes has been obtained by using an approach based on nuclear magnetic resonance (NMR) spectroscopy. This technique enables the rapid detection

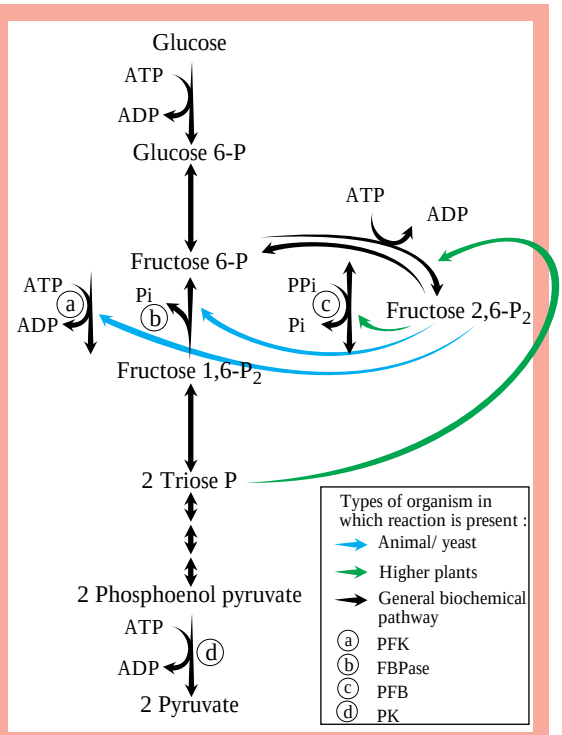


Figure 1 Schematic representation of Glucose catabolism in the glycolytic pathway. The mechanisms of Fructose 6-P conversion to Fructose1,6-P_2 are shown.

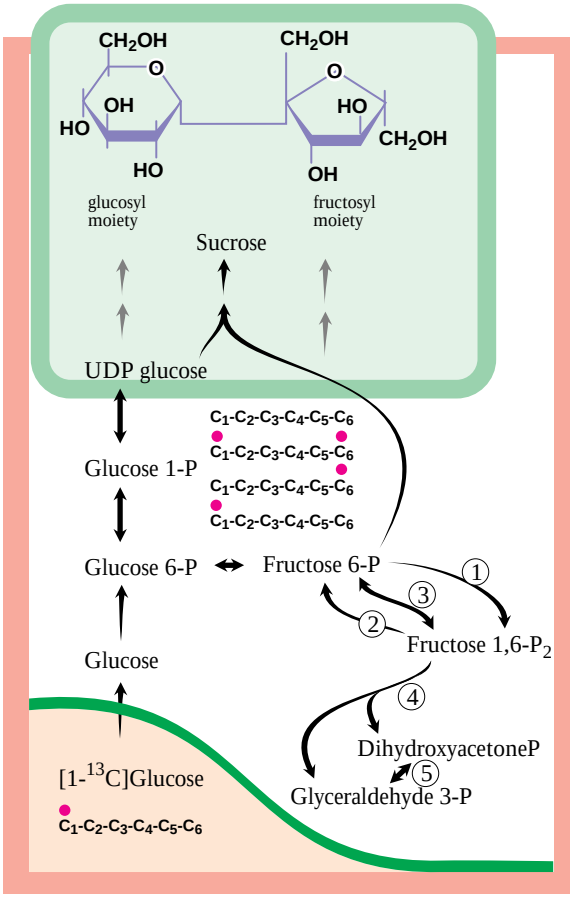


Figure 2 Schematic representation of label (●) distribution in hexose-P following metabolism of [1-^{13}C] glucose. The pathway of conversion of hexose-P into sucrose in plants is also illustrated. Enzymes utilised in hexose-P/triose-P interconversion are 1) PFK; 2) FBPase; 3 PFP (plants only); 4) aldolase; 5) triose-P isomerase.

of inter- and intramolecular label distribution following the incubation of yeast cultures with specifically labelled ^{13}C-glucose. For example, when [1-^{13}C]glucose is supplied, ^{13}C-NMR analysis of yeast extracts shows that enrichment is rapidly distributed to the C_6 position in hexose-P. This occurs as a result of the combined reactions catalysed by PFK and FBPase ("futile" cycling) which gives rise to isotopic exchange between hexose-P and triose-P (Fig. 2). Thus, the degree of isotopic redistribution from the C_1 of substrate to C_6 in hexose-P can be taken as an indication of the extent of metabolic cycling between PFK and FBPase. The feed-back regulation on the pathway can be induced by subjecting yeast to anoxia. Under these conditions, the energy charge rapidly declines and C3 glycolytic products are turned over rapidly. This results in a direct stimulation of the glycolytic flux via activation of PFK and a repression of the gluconeogenic flux via inhibition of FBPase. These direct effects are amplified by stimulation of the

synthesis of Fru26BP. Indeed, when [1-^{13}C]glucose is supplied to yeast cells under anoxic conditions, no labelling of the C_6 position in the hexose-P pool is observed[1]. Thus, the "futile" cycle of metabolites between PFK and FBPase can be described as an overflow mechanism for unutilised glycolytic products. Such mechanism is apparently widespread and described also in prokaryotes (*E. coli*), animal tissues (muscle, liver) and higher plants.

Regulation of glycolysis in plants Glycolysis in plants has been much less investigated. In addition to PFK and FBPase, plant cells contain also unique enzyme in their cytosol which they share only with some specialised bacteria. This enzyme, pyrophosphate:Fru6P,1-phosphotransferase (PFP) catalyses the conversion of Fru6P into Fru16BP similar to PFK. However, the phosphoryl donor in this case is inorganic pyrophosphate (PPi) instead of ATP and also the reaction is readily reversible *in vitro*, i.e. PPi is generated during the formation of Fru6P. Furthermore in plants, unlike in yeast and animal cells, Fru26BP is a strong allosteric effector of PFP whilst it has no effect on PFK. The involvement of PFP in this crucial aspect of glycolytic metabolism in plants has obviously attracted considerable interest. The widespread distribution of PFP amongst plant species and plant tissues has been attributed to a possible 'housekeeping' function. However, the enzyme appears to be also regulated by "coarse" mechanisms as its extractable activity, unlike that of PFK, appears to respond specifically to certain environmental stimuli (i.e. anaerobiosis, Pi deficiency). This has generated the hypothesis that PFP functions as an adaptive enzyme which is activated under specific conditions.

The role of PFP It is difficult to ascribe a precise role for PFP, an enzyme which catalyses the ready interconversion of Fru6P and Fru16BP in the same cell compartment as PFK and FBPase both of which catalyse irreversible conversions and supposedly regulate the glycolytic and gluconeogenic fluxes. Indeed, a proposal has been advanced which ascribed the maintenance of PPi homeostasis in the cell cytosolas a main function for the enzyme. Inorganic pyrophosphatases are absent from the cytosol of plant cells allowing for the establishment of pools of PPi, a by-product of biosynthetic reactions. For example, PPi is generated during the conversion of glucose-1-phosphate (Glc1P) into UDPglucose (UDPGlc) which is required for sucrose biosynthesis. As the reaction is close to equilibrium a build-up of PPi would result in an inhibition of UDPglucose production and, ultimately, of sucrose biosynthesis. According to this hypothesis, PFP would remove the PPi generated by utilising it as a phosphoryl donor for the glycolytic conversion of Fru6P. On the other hand, during the mobilisation of sucrose via the sucrose-synthase pathway in storage organs, a massive conversion of UDPGlc into Glc1P takes place. This process requires PPi which could be generated by PFP catalysing in this case a net gluconeogenic flux (Fig. 3). This proposal, albeit removing PFP from a direct involvement in the glycolytic sys-

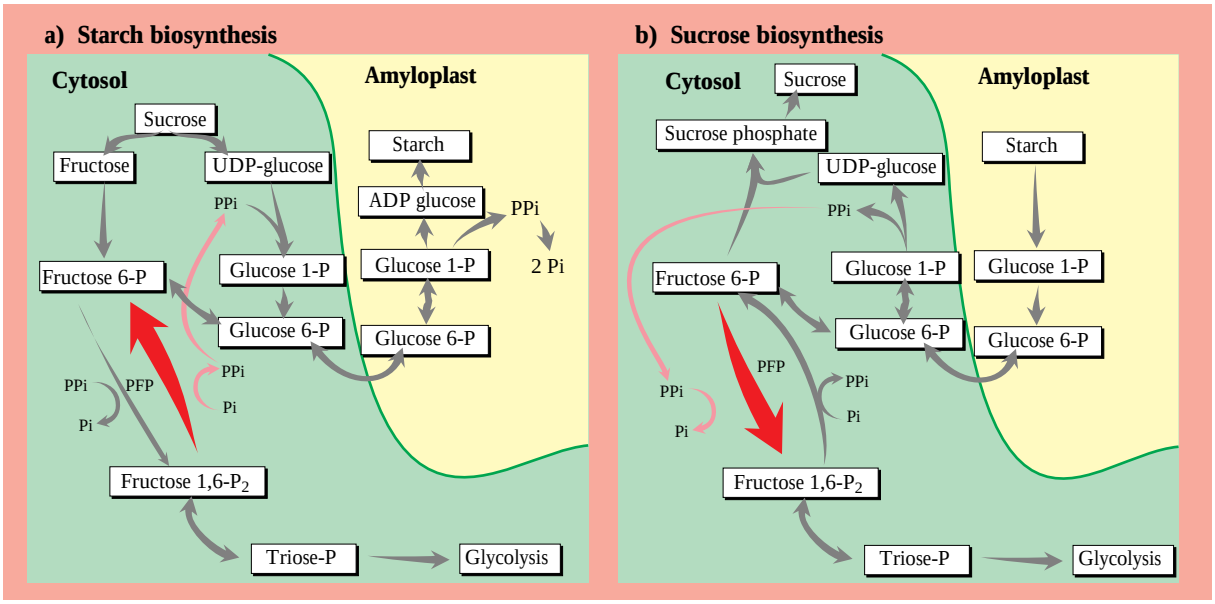


Figure 3 Purported involvement of PFP in the regulation of cytosolic PP content in higher plant cells and its role in sucrose-starch interconversion.

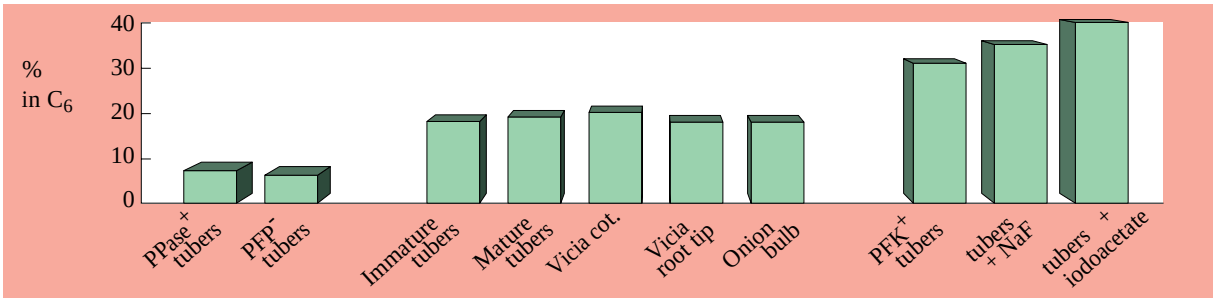


Figure 4 ^{13}C enrichment in C_6 of the fructosyl moiety of sucrose (relative to total enrichment in moiety) isolated from various plant tissues following incubation with [1-^{13}C] glucose.

tem, attributes a key role in the regulation of sucrose-starch interconversion in plant cells to the enzyme and, ultimately, in the regulation of sink strength.

Assessment of PFP activity *in vivo* In order to examine the reaction catalysed by PFP *in vivo*, we have adopted the same approach based on the supply of specifically labelled glucose used in yeast. Potato tuber cells, in common with other starch-storing organs, lack FBPase. Thus, it may be assumed that scrambling of enrichment from C_1 of [1-^{13}C]glucose and C_6 of hexose-P would result from the reaction catalysed by PFP. Thus, in this system, the degree of label redistribution from the C_1 position in glucose to the C_6 position in hexose-P could be attributed directly to PFP activity. Unlike experiments with yeast which established enrichment distribution in hexose-P, intramolecular ^{13}C distribution in sucrose following the supply of [1-^{13}C]glucose was determined by ^{13}C-NMR spectroscopy. Sucrose is synthesised from hexose-P and the approach overcame the problem of the much smaller size of the hexose-P pool in plants compared to yeast. Figure 4 shows the percentage label recovered in C_6 in the fructosyl moiety of sucrose in a number of plant tissues incubated with 50 mM [1-^{13}C]glucose]. The hypothesis that in potato tubers the gluconeogenic flux via PFP operated as a mechanism for the generation of PPi was not confirmed as the degree of label transfer from C_1 to C_6 was almost identical in developing tubers, which rapidly mobilise sucrose and require PPi, and dormant tubers which have a restricted metabolic activity. Moreover, labelling of C_6 in hexose-P was not limited to tissues which interconvert sucrose and starch, or which require PPi generation for sucrose mobilisation as it was also detected in onion bulbs (which lack starch) and *Vicia faba* hypocotyls which most likely metabolise sucrose via the PPi-independent invertase pathway. The data suggest that PFP catalyses a readily reversible reaction *in vivo* in all tissues investigated which results in rapid interconversion of triose-P and hexose-P. Such exchange could provide an overflow mechanism which would enable plant cells to recycle unused glyclolytic products in a similar way as that described earlier for yeast where FBPase replaces PFP. The hypothesis was supported by results obtained when glycolytic flux was manipulated by the use of metabolic inhibitors or by genetic manipulation of enzyme activities. The glycolytic inhibitors, sodium fluoride an inhibitor of enolase and iodoacetate an inhibitor of glyceraldehyde-3-phosphate dehydrogenase were effective in reducing the output of glycolytic products in developing tubers. Such products accumulated and were actively recycled into hexose-P through PFP. As expected, the labelling of C_6 of hexose-P relative to that of C_1 was enhanced compared to untreated tissue (Fig. 3). More detailed evidence was provided by experiments on transgenic potato tubers. Isotopic transfer from C_1 to C_6 was increased in transgenic potato tubers expressing *E. coli* PFK (PFK^+ tubers). This result was surprising as the unidirectional conversion of Fru6P to Fru16BP was enhanced in the mutant leading to the expectation of a decline in the rate of recycling through PFP. However, although no increase in the rate of respiration was observed in the mutant relative to the wild type, an accumulation of glycolytic products was observed in the PFK^+ tubers[2]. Furthermore, NMR analyses revealed that the biosynthesis of lactate and alanine was enhanced in discs excised from PFK^+ tubers compared to discs from wild type tubers. Under these circumstances, and similarly to what observed with glycolytic inhibitors, the glycolytic products were recycled to hexose-P via PFP. On the other hand, isotopic redistribution to C_6 in hexose-P was almost completely absent in potato tubers where PFP was markedly down-regulated (<95% compared to the wild type) by antisense transformation (PFP^- tubers). This finding provides direct confirmation that PFP activity is indeed responsible for the recycling of triose-P in this

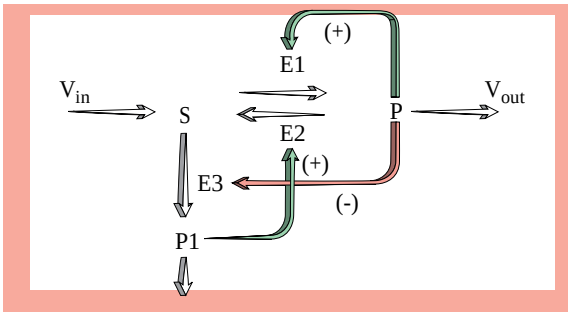


Figure 5 Schematic diagram of the central part of glycolysis illustrating the positive(+) and negative (-) feedbacks between products P and P1, and enzymes E1, E2 and E3.

tissue and validates the criteria used for the assessment of PFP activity *in vivo* by the ^{13}C-NMR technique[3]. Again, in spite of the marked alteration in triose-P recycling, no change in PPi content was observed in the transgenic PFP$^-$ tubers. Isotopic redistribution to C_6 in hexose-P was also substantially reduced in potato tubers expressing *E coli* PPase (PPase$^+$ tubers). In this case PPi content was reduced by more than 50% compared with wild type tubers[4]. These findings suggest that: (1) the main function of PFP is to provide elasticity to the glycolytic pathway by enabling the recycling of excess glycolytic products; (2) entry of hexose-P into the glycolytic pathway is unlikely to represent an important site of control for respiratory metabolism; and (3) the recycling of C3 glycolytic intermediates catalysed by PFP is independent from the concentration of PPi in plant cells. This all but eliminates the possibility of PFP involvement in the regulation of PPi content in the cytosol.

Mathematical analysis of PFP and PFK interaction The complex interaction between PFP and PFK which may play an important role in the efficiency of the glycolytic flux has been studied by developing a novel theoretical approach. Control theory is routinely applied to represent the experimental observations of the changes which occur in one part of a complex biochemical system, which result from the perturbation in another part. Perturbations which result in only a small change in flux at a particular step may reflect the fact that the perturbed part does not `control' the flux through that part. The change is assumed to be proportional to the perturbation, making control theory a linear theory. Such an assumption means that feedback cannot be explicitly treated using control theory. These experiments therefore address a narrow question concerning the influence of one part of the system on the steady state conditions of another part in the neighbourhood of a particular operating point. Broader questions, concerning the dynamical role of PFP both near to and far from equilibrium, at any operating point, are beyond the intended scope of control theory. To address these questions, as well as to develop a more general theoretical framework for studying complex biochemical systems, we have employed a non-linear systems theory.

To begin the analysis, a model for the couplings and feedbacks present in glycolysis which directly influence the behaviour of PFP and PFK must be constructed. The complexity is so large that the whole glycolytic system cannot be represented in detail and the central features are extracted from the rest which are treated as perturbations. There are three important observations: (1) the downstream, triose-P and C3, products positively regulate PFK (in yeast and animals, although the situation may differ in plants) and inhibit the production of Fru26BP; (2) Fru26BP positively regulates PFP; (3) the input to the PFK/PFP system is from the single Fru6P. Taking these observations together, the abstract scheme shown in Figure 5 was constructed. The role of PFP can be examined by studying the dynamics of the metabolite pools as the activity of PFP (V_{max}) is increased and was shown to be critically important whether the reaction catalysed by the enzyme was in equilibrium or not (i.e. whether the forward and back fluxes are equal or not). When it was in equilibrium, the coupling to Fru26BP was weak, and the dynamics were controlled by the Fru6P-triose-P/C3 compound interaction. Experiments with animal cells and yeast where PFP is absent (zero activity), have shown that the system produces oscillations which were con-

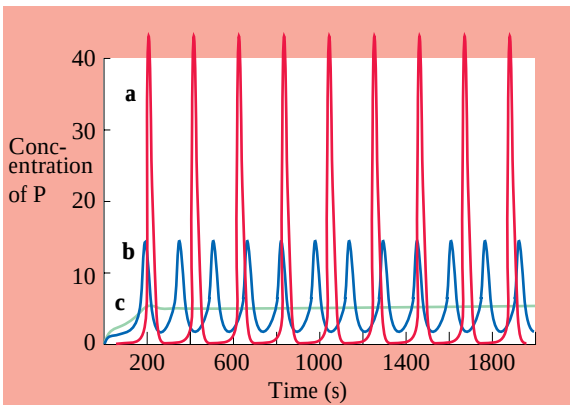


Figure 6 Concentration of product P, as a function of time, 3 different values for the activity (V_{max}) of enzyme PFP. Curve (a) shows the behaviour when V_{max} =0. The curves (b) and (c) illustrate the behaviour as V_{max} is steadily increased.

firmed when PFP V_{max} = 0 in our model system (Fig. 6). However as PFP is switched on, the amplitude of the oscillations decreases and above a threshold value for the activity, the oscillations are critically damped, producing a steady state behaviour. Under more general conditions, this property of PFP is constant and acts to stabilise the conversion of Fru6P to triose-P. Furthermore, the V_{max} value acts like a switch, turning off the oscillatory behaviour above a certain threshold. This switching behaviour may have implications for other reactions which are coupled downstream and if the system is subjected to an environmental shock such that the value of V_{in} is increased, or the product Fru26BP rapidly removed, then PFP will be shifted out of equilibrium. The coupling to Fru26BP becomes significant, and the dynamic behaviour changes. In this case, complex switching behaviour is possible with different kinds of organisational behaviour (oscillations and chaos) being controlled at different values for V_{max} (Fig. 7). The implications of this behaviour for the functioning of glycolysis in plant cells are not yet understood, although it is possible that a type of thermodynamic efficiency is greatest when the system is oscillating.

This analysis shows, that communication via feedback and coupling, is central to the organisation of metabolites and enzymes in cells. Varying the strengths of the communication can change not just the quantitative, but also the qualitative behaviour of complex non-linear systems. Another important point is that the nature of the self-organisation observed is not arbitrary, but is linked to the functioning of the system. In this case, PFP acts to stabilise an important link in the glycolytic chain. This fact presumably conveys a selective advantage which acts to reinforce its presence in organisms which cannot move from a stressful environment.

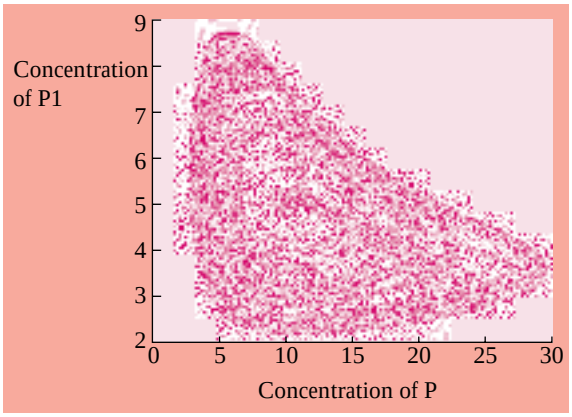


Figure 7 An example of chaos resulting from non-equilibrium behaviour of the enzyme PFP.

Work on transgenic potato tubers was carried out in collaboration with Prof. M. Stitt, Dr U. Sonnewald and Dr M. Hajirezeai (PFP^-, $PPase^+$) and Dr M. Burrel and P. Mooney (PFK^+).

References

1 Den Hollander, J.A. & Shulman, R.G. (1983). *Tetrahedron* 39, 3529-3538.

2 Burrell, M.M., Mooney, P., Blundy, M., Carter, D., Wilson, F., Green, J., Blundy, K.S. & ap Rees, T. (1994). *Planta* 194, 95-101.

3 Hajirezeai, M., Sonnewald, U., Viola, R., Carlisle, S., Dennis, D. & Stitt, M. (1993). *Planta* 192, 16-30.

4 Jelitto, T., Sonnewald, U., Willmitzer, L., Hajirezeai, M. & Stitt, M. (1992). *Planta* 188, 238-244.

Chemistry

William W. Christie, Bernard A. Goodman & Ian M. Morrison

This section reviews the progress achieved through the chemical expertise of the Institute, encompassing the investigations of the Chemistry Department, and the Spectroscopy and Fibres Groups. Some topics are covered directly by the Institute's commissioned remit, and some are assistance for this remit through other disciplines or are supported financially by outside agencies.

The Chemistry Department has a tradition of developing novel analytical methodology to meet the needs of the Institute's research programme. It has devised new chromatographic methods for the analysis of a number of antinutritional compounds that may be involved in plant-pathogen interactions. For example,

the levels of toxic glycoalkaloids in potatoes were shown to exhibit considerable genetic variation and these changed in different ways in response to light. Methods for entrainment of plant volatiles for analysis by gas chromatography-mass spectrometry (GC-MS) are increasing our understanding of how plants respond to insect pests. In addition, the department has an international reputation for the structural analysis of lipids, using high-performance liquid chromatography (HPLC) and GC-MS. New methods for separating complex lipids have been devised, advances have been made in both the practise and theory of silver ion chromatography, chiral chromatography has been applied to determine positional distributions of fatty acids in glycerolipids, and location of structural features in fatty acid chains has become a relatively simple task.

Lipids are key building blocks of plant membranes. This year, novel HPLC methodology has been developed for the separation of all the lipid classes, including simple lipids, glycolipids and phospholipids, in a plant extract in a single chromatographic run. The elution system employs a ternary gradient and evaporative light-scattering detection. As only microgram amounts of lipid are required, it is possible to analyse the lipids in small leaf samples or membrane preparations from plant tissues. The methodology will be adapted for HPLC-MS.

Waxes coat the external surfaces of leaves and are the interface between the plant and the environment. The composition of leaf epicuticular waxes of genotypes of kale and swede grown indoors and outdoors at SCRI and Wädenswil in Switzerland were determined by GC-MS, and were found to exhibit considerable variation. Differences in leaf surface wax ultrastructure, between species and between different growth conditions, were detected by scanning electron microscopy. The studies suggest a possible role for leaf surface waxes in the antixenotic resistance to the turnip root fly, *Delia floralis*.

Novel methodology for stable isotope analysis, using continuous flow-isotope ratio mass spectrometry, continues to be developed. ^{13}C can be measured with higher precision than previously, using the Europa Scientific 20-20 system installed early in the year. This has also been developed for the analysis of ^{34}S; it has been established for minerals and offers similar through-put to that possible for ^{13}C and ^{15}N. We have now devised methods for direct analysis of sulfur in plant material, avoiding the large samples and wet chemistry required conventionally. Plant samples of 5 to 10 mg can be analysed, and inter-laboratory comparisons will establish accuracy.

A study of the effect of light on the chlorogenic acid content (the compounds responsible for after-cooking blackening) of potato tubers revealed a cultivar-dependent response. The magnitude of the effect after 48 h was significantly correlated with the initial concentration in tubers and with glycoalkaloid levels. Time course studies indicated the greatest rate of increase of chlorogenic acid between 8 - 16 h exposure to light. A maximum was reached in 2 days with some cultivars, but in others the content increased steadily over 5 days.

A low dead volume entrainment system has been devised to monitor volatiles given off by live insects. This was used in conjunction with the Zoology Department to investigate the aggregation pheromone of the raspberry beetle, *Byturus tomentosus*. A volatile compound detected from both male and female beetles was identified, and will be tested by bioassay. The data base of plant volatiles has been extended by entraining the headspace of a series of essential oils, for information on chromatographic and MS characteristics. Thermally labile terpenes were successfully analysed.

The Spectroscopy Group was established in October 1989 and has built up a powerful array of magnetic resonance instruments. Indeed, with the possible exception of a few of the major American Universities, the combination of state-of-the-art facilities for EPR (Bruker ESP300E) and ENDOR (Bruker ESP350) spectroscopy with NMR microscopy (Bruker AMX300/SWB) is probably not matched in any other scientific establishment. These magnetic resonance techniques are supported by a Finnigan SSQ710C HPLC/mass spectrometer, with APCI and electrospray ionisation interfaces. Collectively, they have provided the infrastructure necessary for major advances in a wide range of problems in the agricultural sciences. These have included the development of methodology based on EPR spectroscopy for the detection of irradiated food products of plant origin, pioneering work on the application of NMR microscopy to agricultural problems (in collaboration with Dundee University), an understanding of the roles of free radicals in plant senescence processes and in plant-derived foods and food products, identification of mobile elements in the coat proteins of intact tobravirus particles using ^{1}H NMR spectroscopy, and

the demonstration that electrospray MS can identify low molecular weight adducts and the sites of free radical damage in macromolecules such as proteins.

This year has seen major developments in the facilities of the Spectroscopy Group in addition to extensive laboratory refurbishments, which allow the mass spectrometry facilities of the Chemistry Department and the Spectroscopy Group to be housed together. The new LC-MS is the final item of equipment for the Flexible Funded project for the development of methodology for the detection of irradiated foodstuffs. It will have applications to a wide range of problems at SCRI.

The temperature range of operation of the electron paramagnetic resonance (EPR) and ENDOR facilities has been extended by the acquisition of a liquid helium variable temperature accessory, which will now allow samples to be investigated down to 5°K. Low temperatures will be of particular value for the characterisation of metal centres in paramagnetic metalloproteins.

A Bruker AMX300SWB nuclear magnetic resonance (NMR) spectrometer, which was provided through a joint developmental agreement with MRS Ltd and Bruker Spectrospin Ltd, was also commissioned. The large probe sizes that can be used with this model will allow the study of specimens with diameters up to about 65 mm. With such state-of-the-art equipment, SCRI is in a unique position to exploit this non-invasive methodology in the investigation of a wide range of developmental processes. NMR micro-imaging procedures for the generation and presentation of complete 3-dimensional data sets, which are essential for extended studies on developmental processes, have been devised. They have been applied in investigations of the vascular architecture of the fruit receptacle of raspberry and to the progressive development of disease processes in strawberry fruits.

EPR spectroscopy is providing new insights into both normal and induced senescence processes as a result of increased understanding both of the mechanisms of free radical generation and the chemistry of plant-derived antioxidant molecules under oxidative stress. Such reactions have wider nutritional relevance for the consumption of uncooked plant material. Physical damage to plant-derived foods that are consumed in biologically viable states appear to exhibit three types of spectral response: (i) the ascorbyl radical is generated, (ii) a cocktail of at least two unstable free radicals is produced and these can be detected by using spin traps, or (iii) no EPR signal is seen. The results are of particular interest because the unstable free radicals that are observed with many 'healthy' foodstuffs resemble those that have been implicated in cellular damage during disease processes.

Procedures have been developed to evaluate the $O_2^{\cdot -}$-scavenging capacity of food products through competitive reactions with chemical spin traps. In non-aqueous systems, potassium superoxide is a good source of $O_2^{\cdot -}$ ions, but in aqueous systems, enzymatic generation using xanthine/xanthine oxidase appears to be the most satisfactory approach. We have concentrated on investigating free radical generation in potato tissue as a result of biotic or abiotic processes. Methodology using the spin trap phenyl-*N*-*t*-butylnitrone (PBN) has also been developed for monitoring the generation of free radicals in heated cooking oils *in situ* within the EPR spectrometer cavity. It is being used to investigate the oxidation of a range of commercial vegetable oils and the roles of added antioxidants on the decomposition reactions.

The Director's Fibres Group (now the Unit for Industrial Crops) was set up in 1989 to explore the potential for manipulating suitable crops for the production and utilisation of plant fibres. It has confirmed that annual and perennial crops, particularly those suitable for growing under northern European conditions such as the brassicas, can be used as sources of fibre especially for the pulp and paper industries, while the role of non-carbohydrate components, lignin and substituted cinnamic acids, in the structure of cell walls of graminaceous species and their potential effect on processing methods have been identified. The application of peroxy compounds, especially peroxymonosulphate in combination with alkali, has been proposed as a more environmentally acceptable delignification/bleaching agent for the pulping industry. It is most effective on the Gramineae and flax. The role of peroxidases in the lignification of plant cell walls has been explored and evidence has been obtained that a polyphenol oxidase is involved and is a more likely target enzyme for genetic manipulation.

In the current year, diffuse Reflectance Fourier Transform Infra-red (DRIFT) and Raman spectroscopies were used to investigate changes in composition and structure of oak sawdust and barley straw subjected to chemical and biochemical treatments (in collaboration with Drs H.M. Wilson and P.J. Hendra, University of Southampton). Both types of

spectroscopy were used since symmetric bonds usually give strong Raman bands and weak IR bands while the converse is normal for non-symmetric bonds. Oxidative delignification was seen most clearly in the Raman spectra while the changes due to acidic treatments were best observed in the DRIFT spectra. Changes due to alkali treatment of oak samples could be seen in both DRIFT and Raman spectra but fluorescence in the Raman spectra of the barley straw samples was decreased, probably due to the covalently bound cinnamic acids.

FT-IR microspectroscopy was applied to changes in four different cell types (xylem, fibre bundles, epidermis and gland cells) of flax (*Linum usitatissimum*) during development of the hypocotyl. The spectra of 5-day-old cells were poorly resolved but, by day 7, much greater order was established that was retained through to day 20 for each cell type. For the fibre bundles, the 5-day sample showed a strong pectin absorbance absent from the older samples. Ester bonds from acetyl groups were seen in more mature samples.

Peroxidase isozymes can be either ionically or covalently attached to the plant cell wall and are implicated in lignification. When the ratio of activity against coniferyl alcohol (a lignin precursor) to that against *o*-dianisidine (a universal substrate for peroxidase activity) was calculated, the covalently bound forms had a much greater activity for the lignin precursor. After separation, specific but comparable isozymes from both the ionically and covalently bound fractions showed similar selectivity.

Investigations on another lignin-specific enzyme, a laccase-type polyphenol oxidase, were extended to Sitka spruce (*Picea stichensis*). The enzyme activity arises in synchrony with initiation of lignification in the developing xylem. Cell wall preparations retain the activity and can oxidise coniferyl alcohol, simultaneously increasing the consumption of molecular oxygen. Partial purification suggested a number of isozymes in the neutral to cationic range.

To assist in the immunological detection of cell wall components, polyclonal antibodies were raised to conjugated arabinoxylans. Tissue prints are being examined as a means of immunologically probing cell wall components which can be transferred to nitrocellulose from a cut stem. Immunization *in vitro* was used to produce antibodies to a model protein but not to xyloglucan oligosaccharides.

Using a specific MAB raised against the starch granule surface protein from wheat, friabilin, variations in friabilin content were detected in barley cultivars. Most significantly, a low concentration was found in Hyproly, a cultivar with a very vitreous endosperm. This further confirms the role of friabilin in the prevention of bonding between endosperm proteins and starch granules.

Automated analysis of stable isotopes of sulfur in plants

C. M. Scrimgeour

Sulfur is present in a number of biologically important molecules, including vitamins, co-factors and amino acids which control protein conformation and enzyme function, and is an essential element for plant growth. Sulfur occurs naturally as a mixture of stable non-radioactive isotopes, ^{32}S (~95%), ^{33}S (~0.75%) and ^{34}S (~4.25%). The proportions of these isotopes vary depending on the source and history of the sulfur-containing material, with the ratio of $^{34}S/^{32}S$ ranging from 4.25 to 4.70%. Geochemists and soil scientists have exploited this variation to study sulfur cycling on a global scale and in agricultural systems. ^{34}S signatures may also be used to trace sources of pollution such as acid rain. However, sulfur stable isotopes are under-exploited in physiological and metabolic studies when compared to those of carbon, nitrogen and oxygen (*Ann Rep 1991, 59; 1993, 70*). There are two main reasons for this; the lack of readily available (and affordable) tracer compounds, and the lack of simple automated methods for isotope analysis at or near natural abundance.

The first automated continuous-flow system coupling an elemental analyser to an isotope ratio mass spectrometer (CF-IRMS) was reported in 1983 by Preston and Owens[1]. This was designed for measuring the nitrogen stable isotope ^{15}N, used as a tracer in studying nitrogen uptake by plants and for clinical measurement of nitrogen metabolism. The longest lived radioactive isotope of nitrogen has a half-life of only 10 min, making the stable ^{15}N the only tracer suitable for most purposes. The potential of CF-IRMS for carbon measurement was realised shortly after, and while radioactive ^{14}C is widely used as a tracer, the stable isotope ^{13}C is preferable for clinical use. Thus CF-IRMS for ^{15}N and ^{13}C was developed for a variety of tracer applications. Improved instrument design subsequently allowed these systems to be used to study natural variation with adequate precision for many applications in ecology, plant physiology, crop genetics and food web studies. CF-IRMS systems have become the norm for most clinical and biological applications of carbon and nitrogen stable isotopes, and of oxygen in carbon dioxide and water, where high throughput is required.

A CF-IRMS system for sulfur isotope analysis was described in 1988[2], but CF-IRMS systems are still little used for sulfur isotopes, despite the potential benefits that they offer. Why is this? Firstly, there is little tracer work done with ^{34}S as it is expensive (almost 20x the cost of ^{13}C per mole), in short supply, and available in only a few chemical forms. Radioactive ^{35}S is a suitable tracer for many applications, and this has further inhibited the use of stable isotopes. Tracer studies, other than the limited use of natural minerals with distinct isotopic signatures, have not been the driving force in developing sulfur isotope analysis in the way that they were for nitrogen and carbon.

Technically, the isotopic analysis of sulfur is more difficult than that of carbon and nitrogen. Analysis of carbon and nitrogen requires conversion of the sample material to carbon dioxide and nitrogen gas respectively, a well tried and robust process. Isotopic analysis of sulfur is usually carried out on sulfur dioxide (SO_2), and reliable sample conversion to SO_2 is more difficult. Additionally, SO_2 is less easy to handle in mass spectrometer inlet systems, especially in the presence of trace amounts of water. While elemental analyser manufacturers have developed systems capable of quantifying sulfur by conversion to SO_2, the relative difficulty of this procedure appears to have inhibited the development of CF-IRMS systems for sulfur.

The objective of development work at SCRI is to analyse sulfur directly in plant samples in the same manner as is routine for carbon and nitrogen. This is difficult, as the amount of sulfur in plant samples is generally low (≤1%), while that of carbon and hydrogen is very high. As a result, the product from sample conversion is rich in CO_2 and water and low in SO_2. Further, sulfur is present in whole plant material as a mixture of organic compounds and inorganic salts, and complete conversion of these different species to SO_2 is essential.

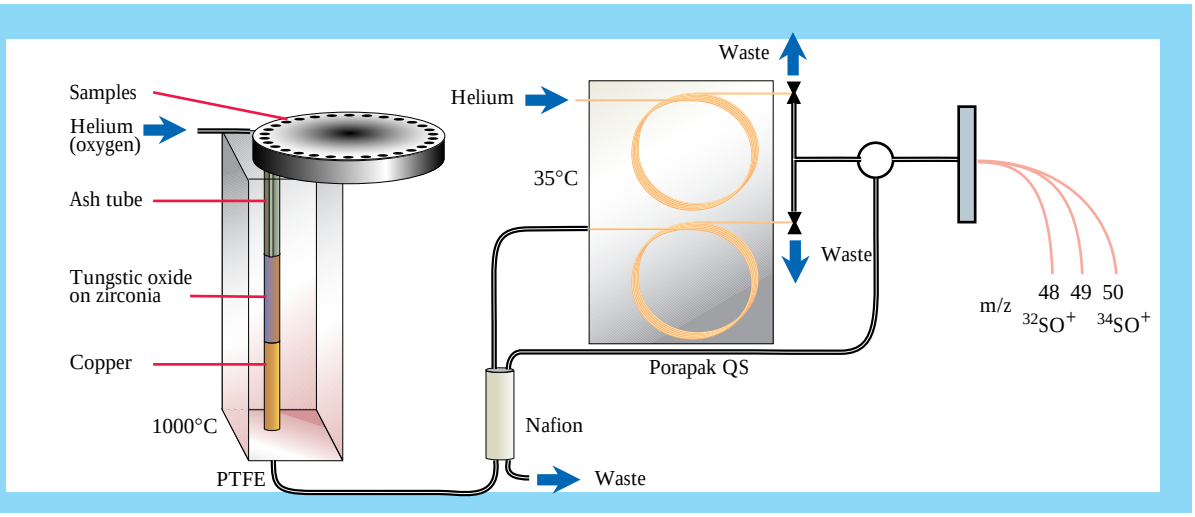


Figure 1 Schematic diagram of the CF-IRMS system used for sulfur analysis. The equipment used is a Europa Scientific SL sample converter with dual switchable GC columns for use with sulfur or carbon and nitrogen, and a Europa Scientific 20-20 mass spectrometer. Isotope ratio measurements are made on the SO^+ fragment.

The Europa Scientific CF-IRMS system used for sulfur isotope analysis is of novel design, with switchable GC columns to change from carbon and nitrogen mode to sulfur mode. The system configuration for sulfur is shown in Figure 1, and there are some important differences between this and standard carbon and nitrogen systems. The single tube combustion/reduction step is most obvious. Tungstic oxide is used as oxidant rather than chromium trioxide and is immediately followed by a copper reduction stage to convert any sulfur trioxide to SO_2 and remove excess oxygen. Water is removed from the combustion products using a Nafion™ tube connected by PTFE tubing to the combustion tube. Nafion™ is a sulfonated Teflon that has a high affinity for water, but is inert to most gases, and can be used as an active drying system by purging the exterior of the tubing with dry helium. The exhaust gas from the preparation system was found adequate for purging, as water is only produced in short pulses during each analysis. The effluent from the GC column is only allowed to enter the mass-spectrometer while SO_2 elutes from the column, and great care is taken to ensure complete separation of the large CO_2 pulse which elutes before the SO_2.

The performance of the sample conversion step degrades much more quickly because of ash accumulation than is the case for carbon and nitrogen, and the removable ash tube is changed more frequently than normal. The accumulation of ash is also reduced if the sample size is kept as small as possible compatible with sufficient analyte entering the mass-spectrometer. Using this approach up to 500 samples can be run from one combustion tube packing. Plant samples should be <10 mg to reduce ash build up, and have vanadium pentoxide added to them to ensure complete conversion to SO_2. Individual runs of up to 120 plant samples containing 25 µg sulfur are possible without degradation of performance during the run.

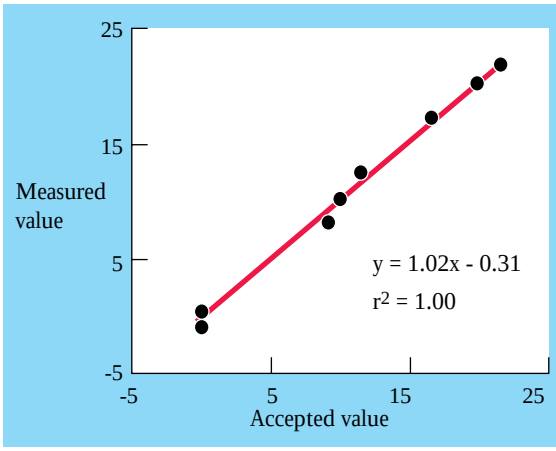


Figure 2 Graph showing the correspondence between observed and accepted values for a range of mineral sulfide and sulfate standards. Values are expressed as δ‰ relative to the CDT standard.
$\delta = (R_S/R_R - 1) \times 1000‰$ where R_S is the isotope ratio for the sample and R_R is the isotope ratio of the standard.

Establishing the accuracy of such systems requires the use of standard materials that have been tested previously on a number of instruments to obtain an accepted value. This reliance on standards is an inherent problem in stable isotope analysis, particularly when new techniques dealing with different sample matrices are developed. The available sulfur isotope standards are a range of heavy metal sulfides and sulfates, and in the first instance they were used to establish the reliability of the system. Sulfate minerals are only converted completely to SO_2 when vanadium pentoxide is added to the sample. The graph (Fig. 2) shows the correspondence between observed and accepted values and is entirely satisfactory. All samples are run against a laboratory standard of ammonium sulfate which is easily prepared in the required amounts by freeze drying solutions directly into the tin sample cups used to hold the samples. Acceptable precision for this is obtained for samples containing 20 to 200 µg of sulfur.

An important feature of these test results is the lack of noticeable memory effects when samples of very different isotopic composition are run sequentially (Fig. 3).

Ensuring the accuracy of plant sulfur analysis is more difficult as no calibrated standards for plant material

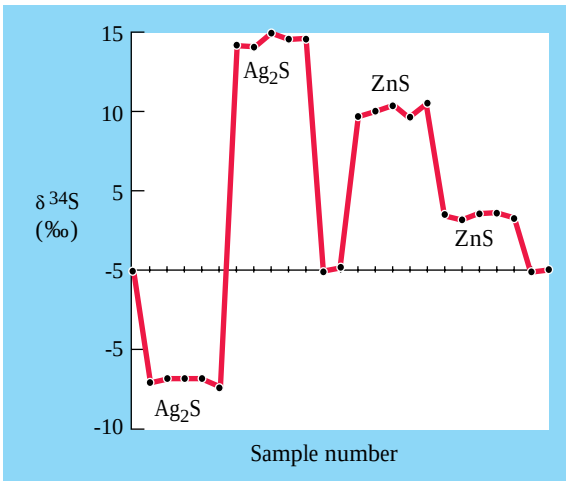


Figure 3 Replicate analyses of sulfur minerals of very different δ values, showing that there is no significant memory between samples.

are available. Collaborative analyses with other laboratories using alternative analytical methods are being carried out to establish the accuracy of the CF-IRMS method when applied directly to plant material. The high throughput with minimal sample preparation ensures that CF-IRMS methods for sulfur will greatly expedite the use of this isotope in a number of applications. Ready application of the technique to food-web analysis and studies of genetic and environmental effects on glucosinolate production in brassicas is now a possibility. While many of these studies will examine natural isotopic variation, access to simple analytical systems should also encourage the use of stable isotope tracers in biological systems.

References

1 Preston, T. & Owens, N.J.P. (1983). *Analyst* 108, 971-977.

2 Pichlmayer, F. & Blochberger, K. (1988). *Fresenius Zeitschrift fr Analytische Chemie.* 331, 196-201.

Advances in the theory and practice of silver ion chromatography as applied to lipids.

W.W. Christie & G. Dobson

Silver ion chromatography is a technique that utilizes the property of silver ions to form polar charge-transfer complexes reversibly with unsaturated centres in organic compounds such as lipids. It enables separation according to the number, geometrical configuration and position of double bonds in molecules. In practise, it is carried out in conjunction with one of the established chromatographic procedures, *e.g.* thin-layer chromatography (TLC) in much of the earlier work but more often in recent years with high-performance liquid chromatography (HPLC). The latter technique enjoys an advantage in that it is controllable to a high degree. Such parameters as solvent composition, flow-rate and column temperature can be fixed accurately and reproducibly, so that the retention behaviour of analytes can be determined accurately. In contrast, TLC is highly susceptible to temperature and humidity effects, retention values are not reproducible and compounds obtained by the method are frequently contaminated with the complexing agent. Accordingly, silver ion chromatography has developed as a useful *ad hoc* micro-preparative method for fractionating unsaturated lipids, but has never had a sound theoretical foundation. Over the last five years, we have been able to develop HPLC methodology based on this principle, not only to improve substantially the efficiency of separations but also to establish the underlying physicochemical mechanisms that govern the separation process. Dr Boryana Nikolova-Damyanova from Bulgaria has been an important partner in the work (Figure 1).

Initially, silver ion chromatography proved difficult to adapt to HPLC, but we solved the problem by using cation-exchange columns as a means of immobilising silver ions[1]. Our approach was to load a silica-based ion-exchange medium (*i.e.* with chemically bonded phenylsulphonic acid groups) with silver ions. Preparation of the column involved merely taking a standard pre-packed column with the appropriate stationary phase and introducing the silver ions *via* the sample injection valve while pumping water through the column. Finally, the aqueous phase was replaced with organic solvents.

An important use for such a technique is in the fractionation of triacylglycerols, the main constituents of commercial oils and fats[2]. In the analysis of molecular species of triacylglycerols, the simplest elution scheme was a gradient of acetone into dichloroethane-dichloromethane, but this is suitable only for fats with a relatively small proportion of linoleic acid, such as palm oil or cocoa butter. The

Figure 1 Dr Boryana Nikola-Damyanova, a visiting scientist from Bulgaria, operating the silver ion HPLC system.

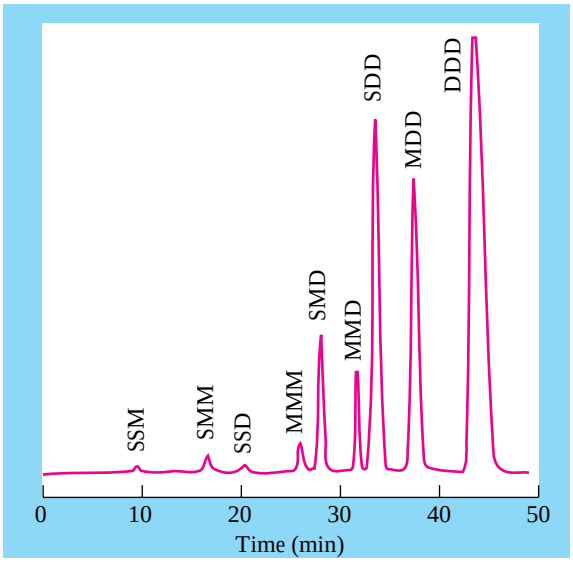


Figure 2 Molecular species separation of triacylglycerols of safflower seed oil by silver ion HPLC with evaporative light scattering detection.

trisaturated (SSS) species are eluted first followed by disaturated-monoenoic (SSM), saturated-dimonoenoic (SMM) and so forth. Indeed, it was possible to separate not only the usual fractions with saturated and *cis*- monoenoic residues but also those with *trans*-double bonds. The procedure may therefore have some potential for the analysis of partially hydrogenated fats such as those in margarines. Most samples of potential interest contain a higher proportion of linoleic acid, and this can be accommodated with the ternary gradient system simply by introducing acetonitrile into acetone after the first fractions are eluted, as was demonstrated with safflower seed oil illustrated in Figure 2. The retention time of one dienoic (D) acyl residue appears to be equivalent to about 2.5 monoenes. One triene (18:3(*n*-3)) is exactly equal to two dienes (18:2(*n*-6)), so there is some overlap of dienoic and trienoic fractions when ∝-linolenic acid is present in a sample. An important practical point is that very pure fractions are obtained when the technique is used in a micro-preparative mode.

The value of this technique in structural studies of olive oil was very apparent when silver ion HPLC was used in combination with a stereospecific analysis procedure, *i.e.* a method for the determination of the relative fatty acid compositions of each of the three positions on the glycerol moiety of triacyl-*sn*- glycerols. In olive oil, the proportion of oleic acid in each of these positions is close to 70%, tending to suggest that the triacylglycerols are not in fact asymmetric. When molecular species isolated from olive oil by silver ion HPLC were subjected to the stereospecific analysis procedure, however, marked asymmetry was observed in some fractions[3]. Not surprisingly, the trimonoenoic species (MMM - half of the total) was symmetrical, but other species were not. In most fractions, oleic acid was predominantly in position *sn*-2, but in the dimonoenoic-dienoic (MMD) fraction there was more in position *sn*-3 followed by *sn*-2 and then *sn*-1. In the SSM fraction, there was more oleate in position *sn*-3 than in position *sn*-1, but the opposite was true of the SMM and SMD fractions. Such results could not have been predicted from the results of the stereospecific analysis of the total triacyl-*sn*-glycerols. It is evident therefore that the structures of seed oils may be more complex than has been believed hitherto. There are important biosynthetic implications and the means are now available to investigate them.

The silver ion HPLC column has been used extensively for the separation of fatty acid derivatives, especially of positional and configurational isomers of derivatives of unsaturated fatty acids. When used in combination with gas chromatography-mass spectrometry, it has proved possible to identify all the fatty acid components of some complex mixtures of natural origin including some fatty acids new to science[4]. This methodology has also helped us to understand the complicated reactions occurring in vegetable oils heated to high temperatures, as when they are used to fry food. In order to obtain a better understanding of the quantitative nature of the interactions between the double bonds in lipids and silver ions, the chromatographic behaviour of simple derivatives of isomeric fatty acids was investigated[5]. For example, base-line separation of the three common naturally occurring *cis*- (*Z*)-octadecenoic acids, *i.e.* the 6-, 9- and 11-isomers (as phenacyl esters), were obtained as illustrated in Figure 3. The mobile phase was 1,2-dichloroethane-dichloromethane (1:1, v/v) with acetonitrile (0.025%) as a polar modifier. The corresponding *trans*-(*E*)-isomers were equally well separated from these and from each other. A separation of this quality cannot even be approached by any other means.

To explain this positional specificity we have evidence to suggest that there may be some form of dual interaction with a single silver ion and both the *pi* electrons of the double bond and the unpaired electrons of the carbonyl moiety of methyl esters, or the additional oxygen moiety of phenacyl esters, as shown

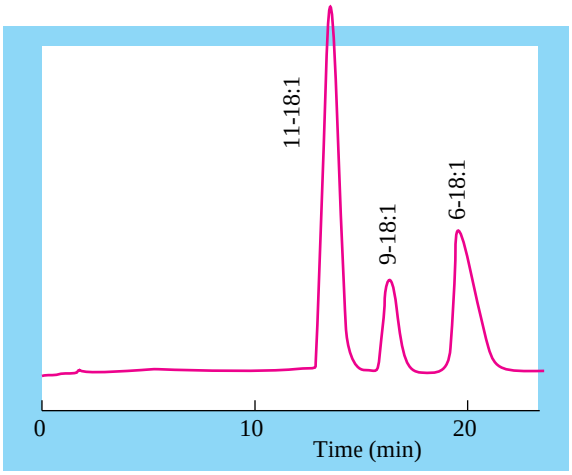


Figure 3 Separation of petroselenic, oleic and vaccenic acids (6-, 9- and 11-octadecenoic acids, respectively) as the phenacyl ester derivatives by silver ion HPLC.

schematically in Figure 4. Electron-rich esters, such as the phenacyl derivatives, are held much more strongly than are methyl esters when the double bond is within about 8 carbons of the carboxyl group, and the elution patterns of series of isomers are very different. From 9-18:1 onwards, when the possibility of such a simultaneous interaction would seem to be less likely, there is no significant difference between methyl and phenacyl esters. Experiments with esters with a variety of different electron-donating and electron-withdrawing substituents now provide firm evidence for this hypothesis (Nikolova-Damyanova and Christie, unpublished work). They have also allowed us to develop greatly improved silver ion TLC procedures[6].

The data and the above hypothesis are consistent with X-ray crystallographic studies which show that one silver ion can interact with two unsaturated molecules simultaneously, or with two double bonds in a single molecule. An interaction between one silver ion and two double bonds at the same time may explain the chromatographic behaviour of fatty derivatives with two double bonds in the acyl chain on silver ion HPLC. When the distance between the double bonds is optimum, *i.e.* with a 1,5-*cis*,*cis*-diene system, fatty acids are very strongly retained, and the effect diminishes as the number of methylene groups between the double bonds is varied. If the double bonds interacted singly with silver ions, it might have been anticipated that the kinetics of the system would be such that retention would be comparable in magnitude to the sum of the individual parts, but this is clearly not so. This theory of complexation between silver ions and *bis*-double bond systems could potentially be applied to polyenoic fatty acid derivatives. It would predict that a triene would be held twice as strongly as a diene, a tetraene three times as strongly and so forth. Such a simple relationship is not in fact found (the degree of the complex formation is even greater than anticipated), possibly because interactions with the ester moiety have to be taken into consideration and because the conformations of polyenes may permit some interactions between silver ions and double bonds that are remote from each other, *via* the formation of pseudo-cyclic structures. Analogous physicochemical studies of the behaviour of triacylglycerols on silver ion chromatography suggests that a dual interaction is important in this instance also. For example, highly unsaturated triacylglycerols are retained especially strongly; a species with nine double bonds is held 10,000 times as strongly as one with a single double bond. It is the strength of this interaction rather than the efficiency of the column *per se* that is responsible for the quality of the separations.

Although silver ion columns are very easily prepared using standard HPLC equipment, Chrompack Nederland BV manufacture and sell commercially a ready-silvered column ('ChromSpher Lipids™') prepared according to our methodology. The technique is being used widely in laboratories around the world, both in agricultural and medical research, and it now has a sound theoretical basis.

References

[1] Christie, W.W. (1987). *Journal of High Resolution Chromatography; Chromatography Communications* 10, 148-150.

[2] Christie, W.W. (1988). *Journal of Chromatography* 454, 273-284.

[3] Santinelli, F., Damiani, P. & Christie, W.W. (1992). *Journal of the American Oil Chemists' Society* 69, 552-556.

[4] Christie, W.W. (1993). *INFORM*, 4, 85-91.

[5] Nikolova-Damyanova, B., Herslöf, B.G. & Christie, W.W. (1992). *Journal of Chromatography* 609, 133-140.6.

[6] Nikolova-Damyanova, B., Christie, W.W. & Herslöf, B.G. (1994). *Journal of Planar Chromatography*, 7, 382-385.

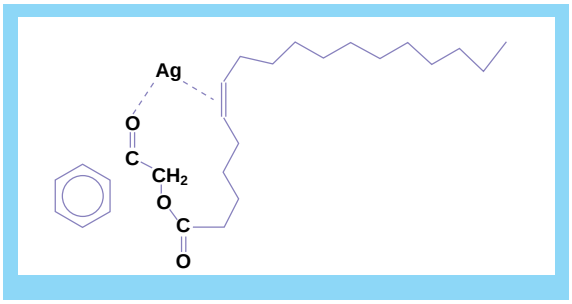


Figure 4 Proposed charge-transfer complex involving simultaneous interaction of a silver ion with a double bond and an oxygen moiety.

Spectroscopic Analysis of Plant Cell Walls

D. Stewart, I. M. Morrison & B. A. Goodman

Plant cell walls are a major raw material for many industries, ranging from paper to food production. Fibres are specific types of plant cell walls and they are subjected to a variety of treatments to remove all cell contents and the majority of the non-cellulosic wall components before they are transformed into paper. In the food industry, cell walls are an integral part of many foods, contributing to its sensory properties and its dietary fibre content but for some products, such as juice, oil and starch, the cell wall is not required.

Regardless of whether the plant cell wall is removed or used, its structure and chemical properties are extremely important and dictate the most appropriate processing methodology. For example, unforeseen changes in the properties of fibres during pulping could lead to the production of paper with inferior performance characteristics. Similarly, changes in the cell walls of foodstuffs may alter the taste, mouth-feel and digestibility. Understanding plant cell wall chemistry is, therefore, vital and the use of effective methods for characterisation is of paramount importance. Plant cell walls are complex three-dimensional networks comprising cellulose, non-cellulosic polysaccharides (NCPs), proteins and, sometimes, lignin (Fig. 1). Due to the intractability of cell walls, analyses have relied on destructive chemicals and derivatization, whereby the native cell wall structures have been altered and artefacts generated. This may cause misinterpretations of the properties of the cell walls. More recently, non-destructive methods of analysis have been developed and some alternative techniques being used for fibre analysis are described below with particular reference to cereal straw and oak sawdust.

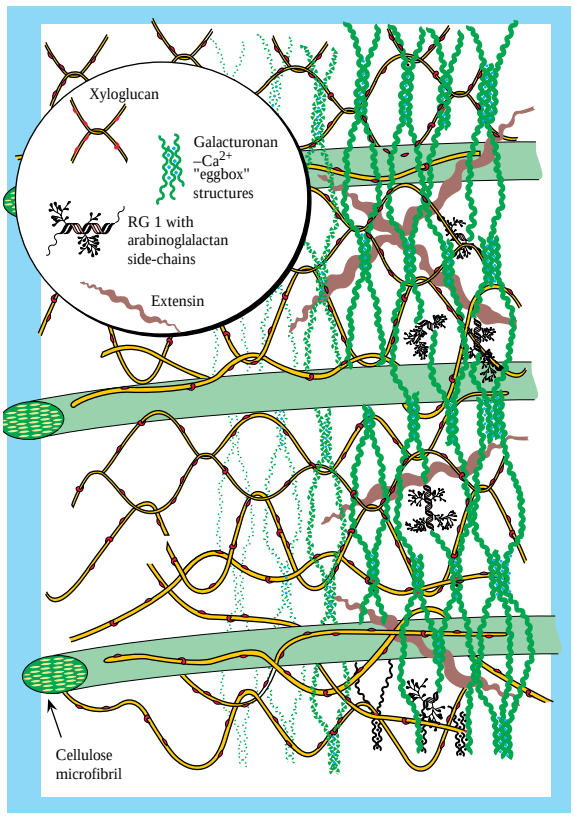


Figure 1 Diagram of plant cell wall showing the arrangements of the different polymeric structures within the wall network.

Nuclear Magnetic Resonance (NMR) Spectroscopy

^{13}C NMR spectroscopy using a combination of cross polarization and 'magic angle' spinning (CP/MAS) is now an established method for analysing solid samples. Resonances due to polysaccharides dominated the spectra of both barley straw (Fig. 2a) and oak sawdust (Fig. 3a) and were found in the region 62-105 ppm. The peaks at *ca*. 62 ppm were attributed to C-6 of amorphous cellulose and, to a lesser extent, NCPs, while the resonance at 65 ppm was that of C-6 in crystalline cellulose. This was poorly resolved from the most intense peaks at 72-76 ppm from C-2, C-3 and C-5 of cellulose (crystalline and amorphous) and NCPs. The accurate assignment of resonances within this region was very difficult but useful information could be obtained when they were used in combination with the polysaccharide resonances from C-1, C-4 and C-6. The peak at 105 ppm, the second most intense in all the NMR spectra, corresponded to C-1 of crystalline cellulose. The shoulder on the upfield side of this peak was associated with the C-1 of amorphous cellulose and NCP, principally the β(1-4) xylan backbone of arabinoxylan from barley straw and glucuronoxylan from oak. The corresponding C-4 was seen at 82-84 ppm. In general, the sharper the peak, the greater was the proportion of crystalline cellulose. The spectra of both barley straw and oak have poorly defined resonances suggesting low levels of crystallinity, which agrees with the previous reports of 30-35 and 20-25% crystalline cellulose in woods and grasses,

respectively. Confirmation of the presence of crystalline cellulose in the barley straw sample was found in the X-ray powder diffractogram (Fig. 4a) which showed peaks at 2θ equal to 22.5° and 16° which were representative of native, type I, cellulose.

With the exception of the aromatic methoxyl resonance at 53 ppm, lignin had resonances with shifts greater than 105 ppm. In the spectrum of the untreated oak residue, there was a relatively large group of resonances centred around 132 ppm which is the region associated with aromatic carbons directly involved in a 5-5' linkage, a common linkage in wood lignins. These resonances were present at reduced intensities in the barley straw spectrum. The resonance at 137-138 ppm in the oak spectrum corresponded to the C-4 of an etherified syringyl aromatic ring. The corresponding resonance for a guaiacyl aromatic ring occurred at 149 ppm, but was masked by the large peak from the resonances of the C-3,5 and C-3,4 carbons of etherified and non-etherified syringyl and guaiacyl rings, respectively. The barley straw spectrum also had these resonances, but at a reduced intensity; the lower intensity of the syringyl C-3,5 resonance (~150 ppm) was in agreement with both the smaller lignin content and decreased proportion of syringyl aromatic rings in graminaceous lignins.

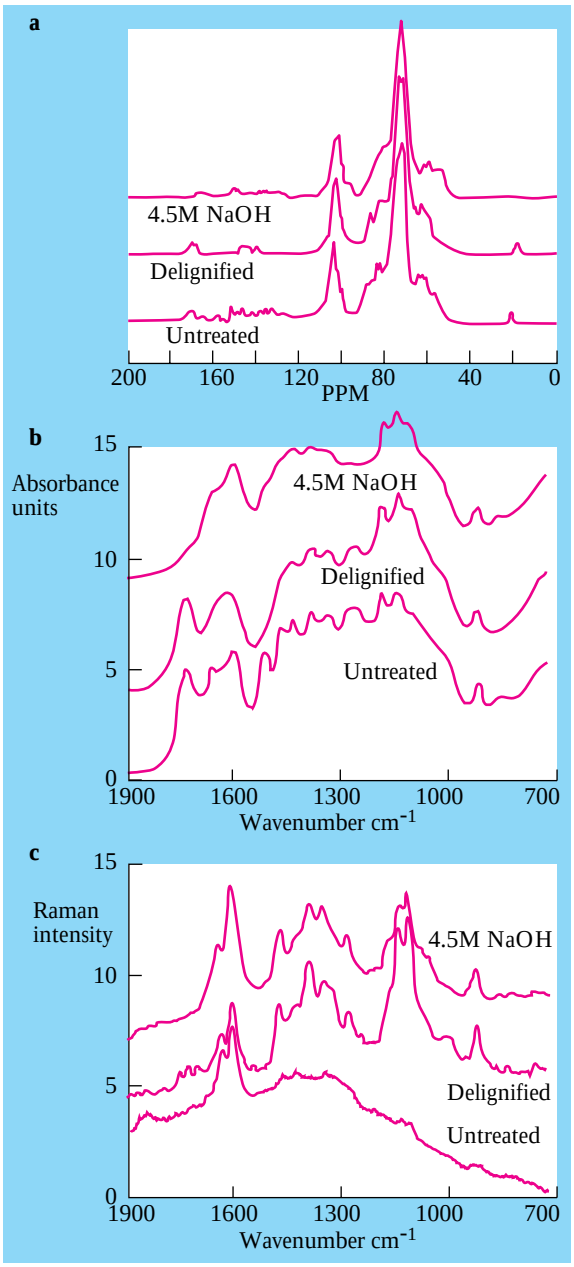


Figure 2 Spectra of barley straw cell walls. (a) ^{13}C CP/MAS NMR, (b) DRIFT and (c) Raman. Each section contains the spectra of the untreated, delignified and 4.5M sodium hydroxide treated samples.

Ester-carbonyl groups were centred on different regions, 170 ppm for barley straw and 165 ppm for oak. The oak resonances peaked at 166 ppm and decreased upfield to 160 ppm; they were almost certainly due to the acetyl and cinnamic acid esters on the corresponding NCPs. The different shifts for these resonances compared to barley straw (172 ppm) suggested differences in their chemical environment, possibly due to hydrogen bonding. The corresponding resonance from acetyl -CH_3 was at 22 ppm.

Delignification with acid chlorite reduced the lignin content of barley straw and oak by 88 and 60% respectively and caused alterations to the NMR spectra of both residues (Figs. 2a and 3a) The lignin methoxyl peak at 53 ppm virtually disappeared and the aromatic carbon resonances over 132-149 ppm were reduced in intensity, particularly the syringyl resonances (150 ppm) in the spectrum of the oak residue.

The effect of 4.5 M NaOH on the lignin was relatively subtle. In the spectrum of the alkali extracted oak residue (Fig. 3a) the intensity of the peaks had been reduced, reflecting the reduced residual lignin content, but their distribution appeared to be similar to those in the untreated sample. However, the peak at 148 ppm had shifted slightly to 149-150 ppm, suggesting the preferential extraction of some syringyl lignin substructures. Although reduced in intensity, this pattern of resonance loss was reflected in the corresponding barley straw spectrum (Fig. 2a).

The most significant change accompanying treatment with 4.5 M NaOH was the reduction in cellulose crys-

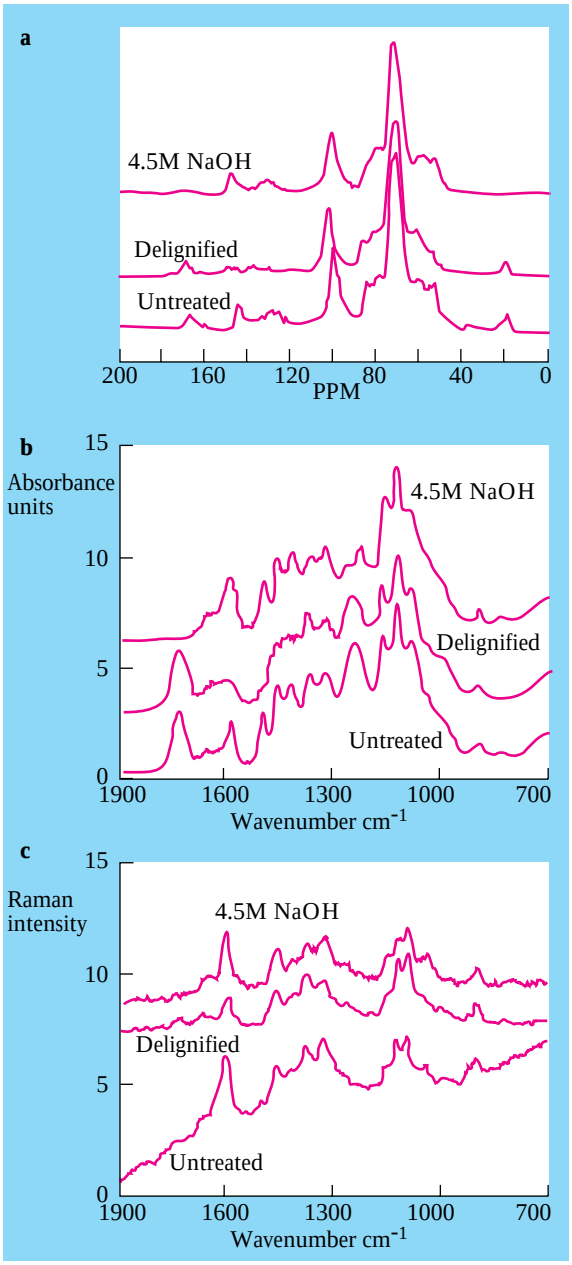


Figure 3 Spectra of oak sawdust. (a) ^{13}C CP/MAS NMR, (b) DRIFT and (c) Raman. Each section contains the spectra of the untreated, delignified and 4.5M sodium hydroxide treated samples.

tallinity. All the resonances representing crystalline cellulose had collapsed, and those representing the amorphous state had increased in intensity. Particularly noticeable was the disappearance of the crystalline C-6 and C-4 resonances (67 and 89 ppm) and the increased intensity of the corresponding amorphous resonances at 62 and 82-84 ppm respectively. Similarly, the crystalline cellulose C-1 (105 ppm) had shifted to 101 ppm, the position of the amorphous cellulose resonance. This reduction in cellulose crystallinity was confirmed in the X-ray powder diffractogram (Fig. 4b) where the crystalline cellulose I peaks at 2θ equal to 22.5 and 16.0° were reduced relative to the amorphous cellulose background and changed to the more thermodynamically favoured type II.

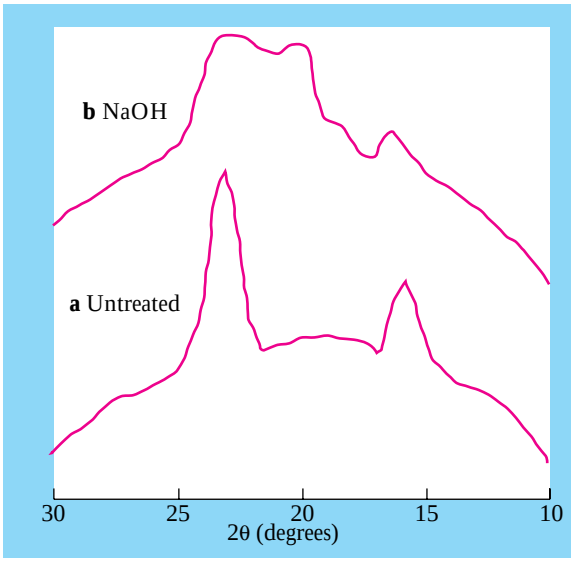


Figure 4 The X-ray powder diffractograms of a) untreated and b) 4.5M NaOH treated barley straw.

Fourier Transform Infra-red (FT-IR) and Raman spectroscopy

Although FT-IR and Raman spectroscopy both involve vibrational transitions in the infra-red region of the spectrum, FT-IR depends on changes in the permanent electric dipole during molecular vibrations, whereas Raman spectroscopy is a light scattering process due to the oscillation of an induced dipole moment. The practical consequences are that non-symmetric vibrations produce stronger bands in the IR, while Raman spectroscopy is superior for symmetric vibrations.

The Diffuse Reflectance Infra-red (DRIFT) and Raman spectra of barley straw and oak are shown in Figures 2b,c and 3b,c respectively. The DRIFT spectra from both samples contained distinct ester carbonyl absorbances at 1740 and 1260 cm^{-1} which were present with much reduced intensities in the Raman spectra. The esters derived from the substituted cinnamic acids, ferulic and *p*-coumaric, were responsible for the poorly resolved Raman spectrum of untreated barley straw. The fluorescence phenomenon was caused by the presence of highly coloured, conjugated compounds which, in the pres-

ence of the Raman laser, became excited and emitted a broad band of radiation in the IR/Raman frequency range. Both ferulic and *p*-coumaric acids showed extended conjugation when covalently attached to lignin thereby increasing the fluorescence which was seen as a broad absorbance hump. Significantly, fluorescence was more prevalent in the spectrum of untreated barley straw than in the spectrum of the more highly coloured, untreated oak sample where the substituted cinnamic acids were present only in trace amounts.

Absorbances representative of lignin were prominent in all spectra. In the DRIFT spectra, these were seen at 1595 and 1510 cm^{-1} although only the former was present in the Raman spectra. Polysaccharide-related absorbances at 1162, 1130, 1098 and 900 cm^{-1} were clearly evident in all the spectra, the latter two absorbances being representative of crystalline and amorphous cellulose respectively.

Delignification of barley straw and oak sawdust with acid chlorite resulted in a reduction in intensity of the absorbances at 1595 and 1510 cm^{-1} and an increase in intensity of the general carbonyl absorbances at 1780-1640 and 1260 cm^{-1}. In the DRIFT spectra (Fig. 2b and 3b), the absorbances became broader while, in the Raman spectra (Fig.2c and 3c), the bands, although weak, increased in intensity and became resolved into peaks at 1730, 1670 and 1640 cm^{-1}, representing conjugated esters, aldehydes and ketones, respectively. This was in agreement with the proposed mode of action of sodium chlorite in acid, whereby the chloronium ions (Cl^{+}) reacted with lignin to form carbonyl-bearing moieties. Significantly, delignification increased the resolution of the Raman spectra since the majority of the fluorescent (conjugated and/or coloured) compounds were degraded or removed.

Extraction of straw and oak sawdust with 4.5 M NaOH produced better resolved Raman spectra (Fig. 2c and 3c) due to the removal of fluorescent compounds and the principal NCPs, xylan and glucuronoxylan. The loss of the latter was reflected in the reduction in intensity of the absorbances at 1740 and 1260 cm^{-1}. The Raman and, to a lesser extent, DRIFT spectra of the 4.5 M NaOH treated oak residue (Fig. 3c and 3b) showed increasing intensity at 1660 cm^{-1}, which was due to reaction of lignin with NaOH to produce benzylic carbonyl compounds. Other carbonyl absorbances in the DRIFT spectra arose from alkaline degradation products from polysaccharides.

Both types of oak spectra show evidence of a reduction in the proportion of crystalline cellulose after treatment with 4.5 M NaOH. The absorbances over the region 1200-1090 cm^{-1} were reduced in intensity and, in the Raman spectrum (Fig. 3c), the peaks were poorly resolved whereas the amorphous cellulose peak (900 cm^{-1}) increased in intensity in the DRIFT spectrum (Fig. 3b).

FT-IR Microspectroscopy

The acquisition of an FT-IR microspectrometer has allowed more precise and detailed analysis of plant cell walls. As outlined on page 175, the microspectrometer projects an IR laser beam with a diameter of >8µm, thereby allowing compositional and structural information from the chosen area to be compared with that from distant or different tissues.

The FT-IR spectra of potato tuber cell walls which had been infected by *Erwinia carotovora* ssp. *carotovora* (Ecc) are shown in Figure 5. The spectra of the uninfected and aerobically infected tissues (Fig. 5a,b) were similar although the absolute intensities of the maxima differed. The protein peaks at 1650 and 1550cm^{-1} were strongest in the spectrum of the tissue infected under aerobic conditions, possibly due to intracellular degradation during infection. However these absorbances were less prominent in the spectrum of the tissue infected under anaerobic conditions (Fig. 5c). The enhanced protein absorbances in the spectrum of the tissue infected under aerobic conditions may have been due to cell wall hydrolases secreted by Ecc.

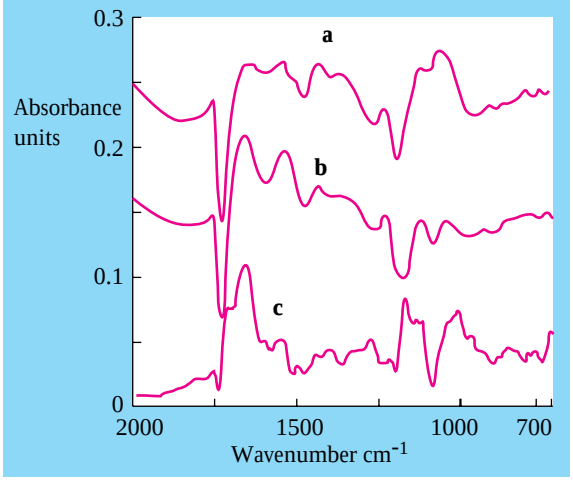


Figure 5 FT-IR spectra of typical cell wall regions in sections of potato tubers that were (a) uninfected and infected in (b) aerobic and (c) anerobic conditions.

The spectrum of the uninfected cell wall (Fig. 5a) contained distinct polysaccharide absorbances which were reduced in the spectra of the infected tissues confirming cell wall degradation. The spectrum of the tissue infected under anaerobic conditions (Fig 5c) differed from those of both the uninfected and aerobically infected tissues, suggesting more extensive degradation. Further examination of the spectra pointed to the presence of fatty acids and esters and, therefore, cell membrane degradation which had been reported by other studies, while there was little evidence for increased lignification.

Mass spectroscopy (MS)

Unlike the other techniques described, MS, either alone or in combination with gas chromatography (GC), is a destructive process although only minute quantities of material are required. Two applications which have proved invaluable in the elucidation of fibre composition and structure are the characterisation of substituted cinnamic acid dimers by GC-MS and the characterisation of chemically-treated straw by pyrolysis (Py)-MS.

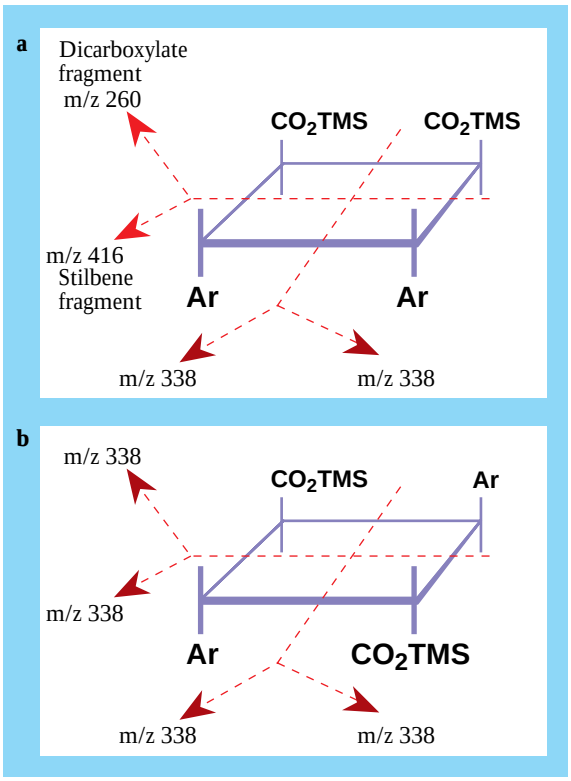


Figure 6 Major fragmentation patterns of a) truxinic acid and b) truxillic acids showing the generation of dicarboxylic and stilbene fragments from (a) and not (b). (Ar = 3-OCH_3,4-OTMS-Phenyl).

Ferulic and *p*-coumaric acids are constituents of the cell walls of certain plant families, particularly the Gramineae, and two types of dimers produced from these acids have been identified. One group is the photodimers which are derivatives of cyclobutane produced by either head to head (truxinic) or head to tail (truxillic) reactions about the side chain double bond. GC-MS has been used to characterise the products from the photodimerisation of both naturally occurring cinnamic acids and synthetic analogues. The truxillic and truxinic acids gave characteristic fragmentation patterns, particularly stilbene and dicarboxylic fragments which were produced from the truxinic acids and not the truxillic acids (Fig. 6). These fragments were used to demonstrate that the ability to form hydrogen bonds was a major factor in determining which type of dimer predominated. Both truxillic and truxinic acids derived from ferulic acid were confirmed in the cell walls of barley stems grown in the light throughout all growth stages but not in those grown in the dark.

GC-MS has shown the presence of six dehydrodiferulic acid dimers in barley stem cell walls. The frag-

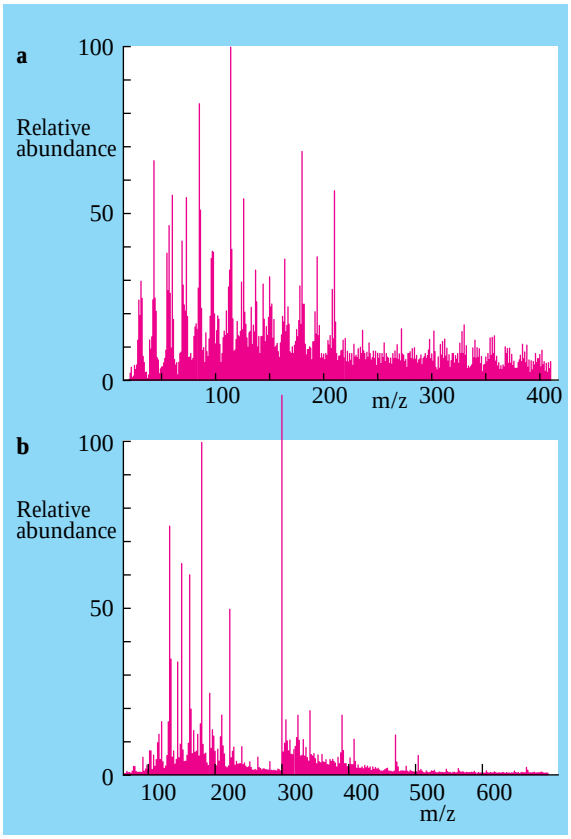


Figure 7 Py-MS of oat straw under (a) electron impact and (b) ammonia chemical ionisation conditions.

mentation patterns suggested that three major components were the *cis-cis*, *cis-trans* and *trans-trans* forms of the 5,5' dimer while the other three were probably similar combinations from the previously unreported 6,6' dehydrodiferulic acid. There was no consistent pattern in the particular dimers detected during growth stages but most were detected in etiolated barley straw cell walls. GC-MS also identified another class of previously undetected dimers based on hydroferulic acid although their concentration was low and their significance unclear.

Py-MS, in both electron impact (EI) and chemical ionisation (CI) modes, has been applied to the analysis of treated oat straw cell walls (in collaboration with Dr M M Mulder, FOM Institute for Atomic and Molecular Physics, The Netherlands). EI gave more information on the non-carbohydrate constituents while CI was preferred for polysaccharides (Fig. 7). From the spectra, the proportion of syringyl to guaiacyl constituents, a measure which is laborious by other methods, was directly obtained from the *m/z* at 210 and 180 respectively, and the proportion of ferulic and p-coumaric acid was available from the peaks at *m/z* 150/194 and 120/164 respectively. For most samples, different chemical treatments could be analysed from the changes in the mass spectra, particularly in the removal of lignin. Significantly, two delignification treatments, which gave virtually identical data by traditional chemical analyses, gave Py-MS which were quite distinct. Although a full explanation is not yet confirmed, these differences were due to changes in the fine structure of the polysaccharide components caused by the distinct modes of action of the two treatments and has major consequences in the processing of these fibres.

Electrospray mass spectrometry: application to the plant sciences

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

The routine application of mass spectrometry for the analysis of large biomolecules in the biochemistry laboratory has been facilitated in recent years by the introduction of the mild ionisation processes MALDI (matrix-assisted laser desorption ionisation) and ESI (electrospray ionisation). The latter process in particular, due to its simplicity and picomole-to-femtomole sensitivity has found diverse applications in carbohydrate, protein and nucleic acid analysis.

ESI mass spectrometry

Prior to the pioneering work on ESI mass spectrometry of several groups in 1984, the mass range of spectrometers was governed merely by the quadrupole or magnetic sector mass analyser used. The utility of ESI lies in its ability to produce both singly and multiply charged ions by creating a fine spray of highly charged droplets in the presence of a strong electric field. Dry gas and heat are applied to these droplets causing evaporation of the solvent, and the surface charge on the droplet consequently increases. Ions are then transferred to the gas phase as a result of their expulsion from the droplet and directed into the mass analyser through a series of lenses. A schematic representation of an ESI interface is depicted in Figure 1.

An intrinsic property of mass analysers is that they separate ions according to their mass-to-charge ratio (m/z). The ions produced are $[M+H]^+$, $[M+2H]^{2+}$...*etc* for positive ion and $[M-H]^-$, $[M-2H]^{2-}$...*etc* for negative ion modes, where M represents the molecule of interest and H is a proton. A typical spectrum shows a series of peaks with charges

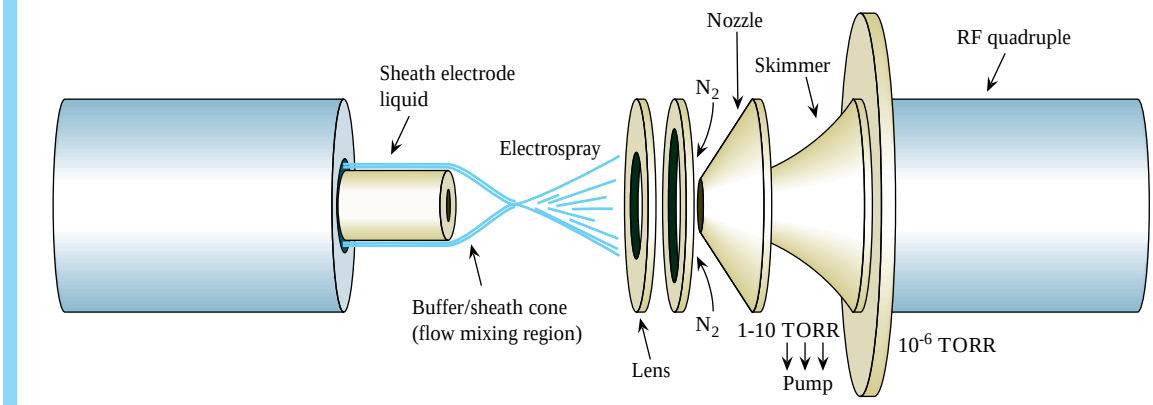


Figure 1 Detail of the electrospray ionisation interface tip and nozzle region to the quadrupole mass spectrometer. Ions are sampled through the focusing lens and nozzle-skimmer openings to the radio frequency (rf) ion focusing quadrupole lens. Typically, +450 V is applied to the focusing lens and +200 V to the nozzle (V_n), while the skimmer is at ground potential. The analysis quadrupole mass spectrometer has a range of *m/z* 2000.

differing by 1 in a simple geometric progression that allows calculation of the mass-to-charge ratio of the singly charged ion, *i.e.* the molecular weight ± 1. A further advantage of multiple charging is that more accurate molecular weight information can be obtained from the distribution of the peaks. An example of this is provided in Figure 2 for egg white lysozyme.

Peak position	Charge	Apparent M_r
1193.113	12+	12(1193.113-1.0078) = 14,305.50
1301.534	11+	11(1301.534-1.0078) = 14,305.79
1431.534	10+	10(1431.534-1.0078) = 14,305.26
1590.562	9+	9(1590.562-1.0078) = 14,305.99
1789.160	8+	8(1789.160-1.0078) = 14,305.22
		Average = 14,305.55

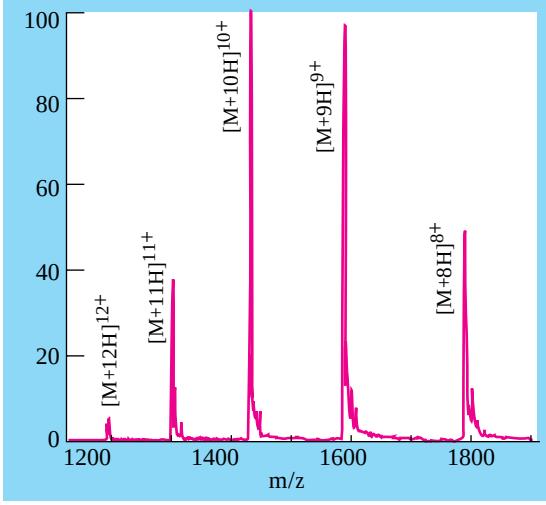


Figure 2 ESI mass spectrum of egg white lysozyme with calculated molecular weight.

Protein modifications characterised by ESI

A particular strength of the technique is the ability to discriminate between large molecules of differing molecular weights. This has been exploited in a variety of ways in our current research program. At present we are investigating the possibility of studying molecular weight changes of the three principal egg white proteins lysozyme (14,300 Da), ovalbumin (44,300 Da) and conalbumin (77,500 Da) as a means of detecting an irradiation history. All three proteins are ionised with ease from a solution of water:methanol:acetic acid. At irradiation doses of 1 kGy (which is considerably less than that recommended for elimination of *Salmonella*), we have been able to detect specific increases in the apparent molecular weights of two of these proteins. Further work is currently underway to couple HPLC to the ESI interface to allow for greater sensitivity and a higher throughput of samples.

Another area of SCRI research that has profited from the ability of ESI mass spectrometry to analyse mixtures of differing protein moieties has been the work on alleged allergy to oil seed rape. A number of volatiles released by the flowering crop in Spring were identified and the ability of dilute aqueous solutions of these chemicals to form covalent adducts with a number of proteins that may be involved in a human allergic / irritant response were investigated. Human serum albumin (66,440 Da) and haemoglobin (∝ = 15,126 Da; ß = 15,866 Da) were both found to be adducted by a number of the volatiles released from the crop; notably a series of isothiocyanates, isobuty-

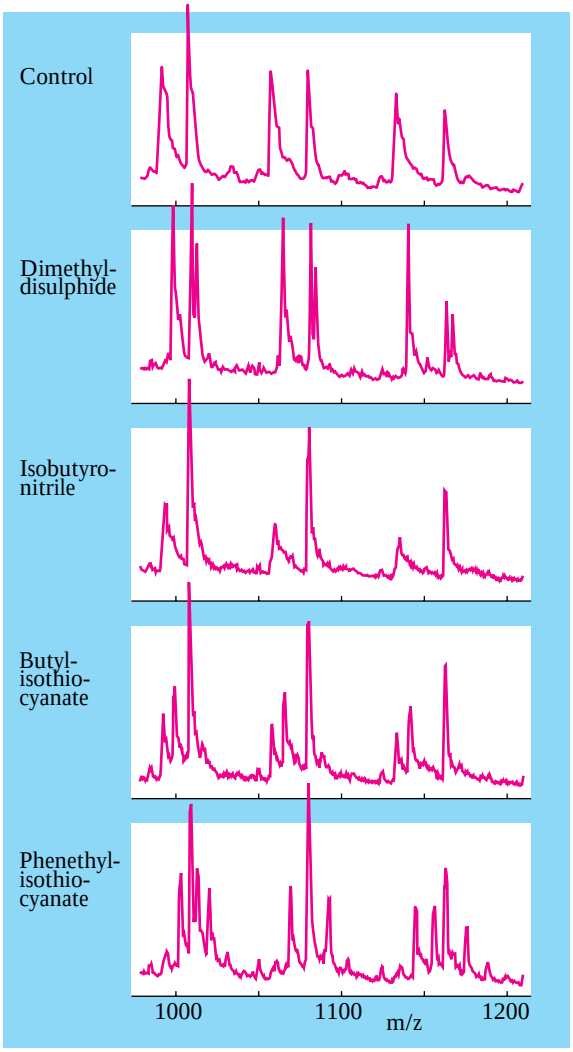


Figure 3 ESI mass spectra of haemoglobin adducted with volatiles released from oilseed rape.

ronitrile and dimethyl disulphide (Fig. 3). In the case of phenethyl isothiocyanate up to six different adducted forms of the two haemoglobin subunits were discriminated. It is intended that the next stage of the study will include proteins isolated from human lung surfactant, and hopefully a biomonitoring study during the flowering season.

An added bonus with the ESI interface is that, due to the relatively high pressure in the nozzle-skimmer region (Fig. 1), a process known as collision-induced dissociation (CID) can be utilised to gain further structural information. This process has been used extensively in the characterisation and sequencing of peptides and many smaller species. An example of partial C-terminal sequencing of a short peptide is depicted in Figure 4.

Recent applications of ESI mass spectrometry

Initially, the technique was used almost blindly as a method for obtaining the accurate masses of large biomolecules with the inevitable competition to obtain a mass spectrum of the largest. More recently, greater structural information has been the chief target, in part due to the evidence suggesting that the gas phase structure is essentially the same as that in solution. The biomedical sciences have led the way in the application of ESI mass spectrometry applying the technique to neonatal haemoglobin monitoring for genetic abnormalities such as sickle cell anaemia and thalassaemia, drug discovery and design by characterising drug-receptor complexes and a host of other areas throughout pharmacology. The principal efforts of biochemists seem to have concentrated on solution structures, refolding intermediates and enzymology. To date, there are only a small number of reported cases where plant scientists have used the method.

Likely future applications of ESI mass spectrometry

Increased efforts to elucidate reaction pathways and to use the technique in the general area of enzymology are inevitable. To this end it might be expected that non-competitive, competitive and suicide inhibition will all feature strongly. The direct observation of an enzyme-substrate complex has long been considered science fiction, but with a system with a low turnover number such a complex might be amenable to study by ESI. Non-covalent protein:protein and protein:nucleic acid complexes are also likely to fea-

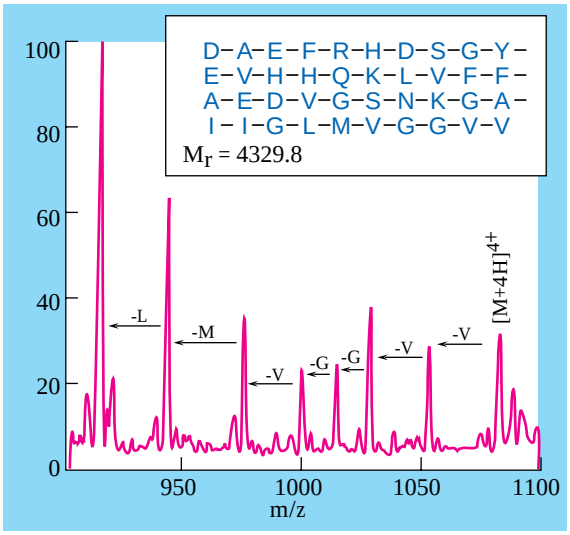


Figure 4 Collision induced dissociation ESI spectrum of the $[M+4H]^{4+}$ ion of a short peptide.

ture in future work. Given the sensitivity of the technique, interactions between nuclear proteins should also be open to investigation. Post-translational modifications, phosphorylation and iodination all result in clearly-defined changes in molecular weight. With the potential for using CID and trypsin digests, location of the sites of modification will soon be routine. In addition, by using ESI as the tool to detect or characterise the protein of interest, the need for certain radiolabels (*eg* ^{125}I) might be eliminated.

The future of ESI at SCRI

The capabilities of mass spectrometry, like many of the other powerful analytical tools available to the biochemist (*eg* NMR), are limited only by the imagination of those wishing to exploit them. To make proper use of the technique it is imperative that researchers understand the processes that ESI mass spectrometry can analyse. Once this has been accomplished, this and related techniques are likely to figure strongly in our research program for some time to come.

Applications of NMR microscopy to the non-invasive resolution of internal details of Lepidopterous pupae

S.C. Gordon, J.A. Chudek[1] & B.A. Goodman

Conventional approaches to the study of the internal structure of insects rely heavily on destructive sampling, involving killing the organisms, chemically fixing them and then sectioning and staining so that the structures can be examined by light or electron microscopic techniques. The production of artefacts associated with fixation, dehydration and embedding of biological tissues is well-known. The study of developmental changes is extremely time consuming, involving the preparation and examination of many specimens and several man-months of labour.

In recent years the development of nuclear magnetic resonance (NMR) microscopy (also known as NMR microimaging) has provided a new non-invasive method for examining the internal structures of a wide range of biological subjects. The technique measures physical and chemical characteristics of protons (hydrogen nuclei) in water and other abundant fluid molecules, such as lipids, within a specimen. The method, which was described earlier (*Ann. Rep. 1990, 57; 1993, 80*), is closely related to the magnetic resonance imaging (MRI) technique that has made a major contribution to clinical diagnostics in recent years. However, by working with much larger magnetic fields, NMR microscopy is able to achieve the spectral sensitivity that is necessary for the high degree of spatial resolution needed for examination of small specimens with dimensions ranging from a few millimeters to several centimetres. Images are generated either as a series of 2-dimensional slices through the specimen or as complete 3-dimensional reconstructions. This latter approach may, in the near future, provide a powerful tool in the study of developmental processes in small biological structures, especially since measurements can be made over extended periods of time on the same specimen. Examples obtained from the early stages of our work on lepidopteran pupae are presented in this article to illustrate an application of the NMR microscopic technique.

Lepidopteran pupal studies

Initial investigations carried out in collaboration with the University of Dundee involved pupae of the double dart moth (*Graphiphora augur*), a new pest of raspberry in eastern Scotland, and the large cabbage white butterfly (*Pieris brassicae*), a common pest of brassica crops[1]. The observations using a Bruker AM300/WB Fourier Transform NMR spectrometer were performed at ambient temperatures on specimens that were immobilised by exposure to vapours of either tetrachloroethane or chloroform. The images were collected using a pulse sequence which displayed separate water and lipid distributions through the specimens. A typical spectrum is shown in Figure 1, with a water peak at 4.6 ppm and a lipid resonance at 1.2 ppm. The relative brightness of tissue in images is determined by a combination of the concentrations of

[1]Department of Chemistry, University of Dundee

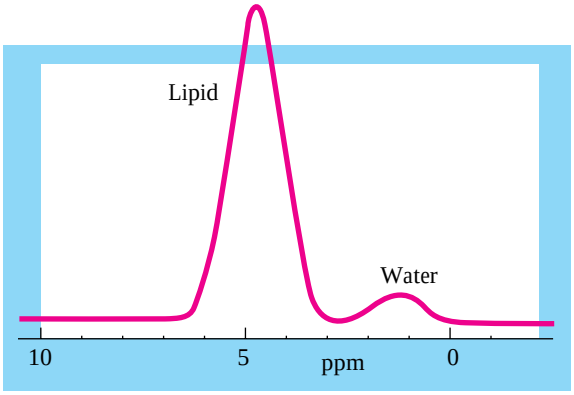


Figure 1 NMR spectrum of Lepidopteran pupa showing the lipid 1.2ppm and water (4.6ppm) peaks.

protons in their respective chemical forms and their relaxation properties. In the pupa of the cabbage white butterfly, the regions of high water content, e.g. the parts of the alimentary canal, wings and heart, are shown in Figure 2a, whereas Figure 2b shows that the lipids are concentrated mainly in the head and posterior regions.

The development of the organisation of internal structures of double dart moth pupae was compared using this technique. The internal structure of a 'young' pupa showed no evidence of organisation, whilst restructuring was complete in an older animal 2 days prior to emergence as an imago. Although these observations were made on separate animals, such images could conceivably have been acquired from the same animal in a time course study. The later specimen showed only one peak from water in the NMR spectrum, presumably because most of the lipid reserves had been exhausted during metamorphosis.

The recent introduction of more powerful computing capacity makes possible the routine production of

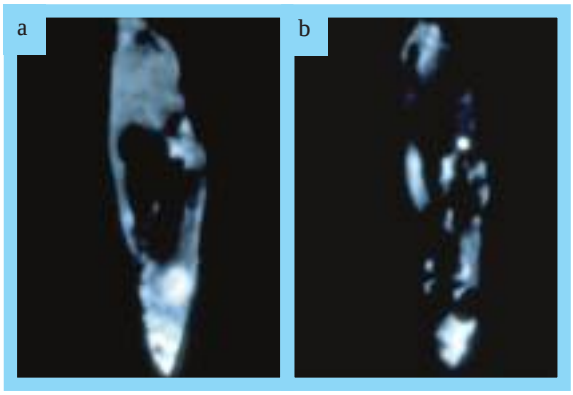


Figure 2 (a)Water image of Cabbage White Butterfly pupa. (b)Lipid image of Cabbage White Butterfly pupa.

Figure 3 Adult Owl Butterfly recently emerged from pupal case (photo courtesy of Edinburgh Butterfly World).

complete 3-dimensional images and examples of applications to fruit were presented previously (*Ann. Rep. 1993, 80*). The technique has now been used to view the internal structure and surface of pupae of the tropical owl butterfly (*Caligo memnon*) (Fig. 3 & 4). Further refinement of the procedures for generating images will permit us to 'map' the development of the internal structures of this butterfly as pupae mature.

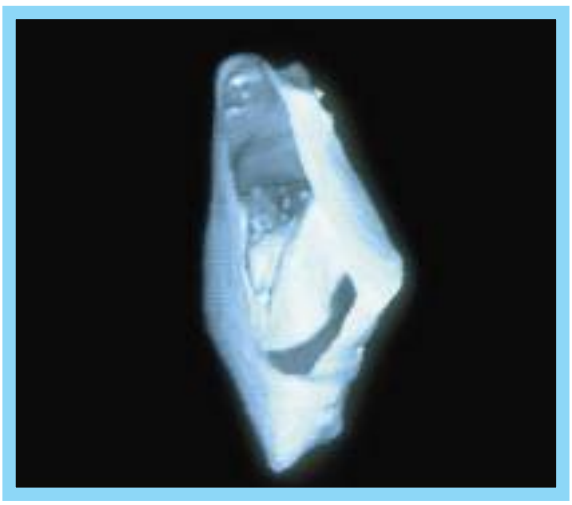


Figure 4 NMR 3-dimentional reconstuction of Owl Butterfly pupa electronically 'sliced' open to show internal detail.

Currently, investigations are being conducted with the object of developing an understanding of the process of diapause in the cabbage white butterfly. Structural and chemical changes can be seen within the animals as a result of the breaking of diapause, and particularly noteworthy is the observation that the quantity and distribution of lipids appear to differ between diapausing and non-diapausing animals. Both the location and relative concentrations of lipids can be identified in the various tissues within the pupae. With the development of suitable cold stages for retaining insects within the NMR spectrometer, the technique has the potential to assist developmental entomologists to understand a variety of internal processes in many arthropods. The principal limitations to applications are the need to completely immobilise the specimen for the duration of the measurements (which may take hours) and the resolution of the equipment, which at present is of the order of a few tens of micrometers (depending on the overall size of the specimen).

Conclusions

Although still in its infancy, NMR microscopy has great potential in entomology research and is completely complementary to the techniques that are currently in use. Chemical shift selective imaging allows the distributions of the major fluid components in a specimen to be visualised separately and, because of the non-invasive nature of the technique, they can be monitored over extended periods of time. Complete 3-dimensional image reconstructions, which are necessary for the study of developmental processes, are now possible with the dedicated computer facilities at both SCRI and Dundee University. In addition, it is also possible, in principle, to produce NMR spectra from defined regions of the NMR image (image directed spectroscopy) and these procedures should provide further insight into the biochemical processes that accompany developmental changes in a wide variety of organisms, including insect pupae.

References

[1] Goodman B A, Gordon S C, Chudek J A, Hunter G & Woodford J A T (1995). *Journal of Insect Physiology* 41, 419-424.

Fungal & bacterial diseases

James M. Duncan

'Lanarkshire Disease' was the name given originally to a devastating root disease of strawberries that appeared first in the Clyde Valley in the County of Lanark near Glasgow in the early 1920's and was largely responsible for decimating the lively market garden industry in strawberry in that area. Later it was recognised that this disease, now called red core, was due to a root-infecting species of Phytophthora that was subsequently named P. fragariae. SCRI has had a long association with this disease, through its strawberry breeding programme. It was first in the world to produce varieties with useful levels of resistance to the disease and it also clarified the epidemiology. The latter led to the realisation at the end of the 1970's, that despite the best efforts of inspectors and certification systems to control red core in planting material, spread in infected runners was still occurring. A sensitive bait test, now applied routinely to all strawberry stocks certified in Scotland, was introduced and this has helped prevent further spread and the contamination of previously unaffected land. Unfortunately, for reasons unconnected with its sensitivity and value in certification, the test has had little impact on outbreaks elsewhere in Europe. Changes in the trading and movement of young plants within Europe have led to an increased number of outbreaks on fresh land and in countries such as Sweden, Switzerland and Germany where the disease previously did not occur.

Many important horticultural crops including ornamental shrubs, flowers, vegetables and fruit trees are similarly attacked by *Phytophthora* spp. and in almost all cases the problem starts in the nursery where young plants are produced. Chronic levels of infection in the nursery and routine treatment with fungicides that control but do not eradicate the fungus mean that infected plants are distributed to producers. There is a clear need for tests that can determine the health status of plants and can be used to pinpoint sources of infection in water and growing substrates. The tests should be sufficiently sensitive to detect very small amounts of infection; they should discriminate between different pathogens, even closely related ones; and should be rapid and operator friendly.

A possible basis for plant health tests has arisen through molecular biology and knowledge about vari-

Phytophthora infestans

ation in the pathogen. The strawberry breeding programme inevitably led to an interest in variation within the fungus and thence to the first system of race classification within Europe. Similar studies undertaken at SPBS Pentlandfield on *P. infestans*, cause of late blight of potato, pioneered the gene-for-gene concept in which genes for resistance in the host are matched by genes for virulence in the pathogen. Interest in fungal variation, especially in pathogenicity and host range, continues to this day at SCRI, and expanded recently by a widening interest in all *Phytophthora* spp., especially after the discovery that the fungus responsible for root rot disease of raspberry was a new strain of *P. fragariae* which also had races.

Traditionally, the pathologist interested in pathogen variation was restricted to correlating morphological and to lesser extent, physiological characters with pathogenicity, but the development of sophisticated molecular techniques has led to the increasing use firstly of whole protein profiles, then isozymes and now variation in the fungal DNA to characterise pathovars and races. Work supported jointly by SOAFD and the Horticultural Development Council under the LINK scheme began in 1993 to develop rapid tests for *Phytophthora* infection in horticultural crops using DNA amplification techniques. Considerable advances have been made and several highly specific primers for individual species have been produced and are at an advanced stage of testing. Others are planned so that a range of diseases can be diagnosed by this approach.

Ribosomal DNA (rDNA) is a major target of molecular diagnostics and its value in identifying species is described in a later article (p. 121). Species can usually be distinguished from one another by comparing particular sections of rDNA and there are many copies of it. Thus the method is ideal for meeting two of the important criteria for health tests, sensitivity and discrimination. It also provides information about evolutionary relationships within and among fungal species.

Information on molecular relationships gathered in the LINK project is being used in studies on the movement of red core and similar phytophthora diseases across field sites in collaboration with mathematicians who are developing models for soil-borne disease. Diseased strawberry plants were planted among healthy plants in a random or regular distribution and the spread of disease, and the potential for new strains of the pathogen to arise in the field under host selection, will be monitored by characterising the pathogenicity of isolates and with RAPD markers. Earlier studies showed that the fungus persisted in another field for more than 10 years in the absence of

its natural host and that disease outbreaks initiated after this period of time were closely correlated with low lying parts of the field, while plants remained healthy in higher parts of the field.

Work has also continued on gene structure and organisation in *Phytophthora* spp. and although not ostensibly concerned with phylogeny, it has produced results with interesting phylogenetic implications. A form of gene organisation that has not been reported previously within eukaryotes has been found in *Phytophthora* spp. and throughout the Class Oomycetes. The work is reported more fully in an article on p. 119.

RAPD markers have been used in basic studies of pathogenicity. Two strains of *P. cactorum* that differ in their host range, one attacks strawberry but not apple and the other does *vice versa*, have been genetically transformed for resistance to hygromycin and geneticin. Oospores from the transformed strains have been germinated and segregation ratios for antibiotic resistances are now being determined. Eventually, the aim is to produce hybrids between strains differing in RAPDs, antibiotic resistance and host range to investigate the inheritance of host range and link it to some of the molecular markers.

Resistance to another phytophthora disease, late blight of potato caused by *P. infestans* can be conferred by several major R-genes. Previously, it was thought that R-genes were expressed strongly almost regardless of the environment in which plants were growing, but now it has been shown that this is not so. Using an ELISA that measures the biomass of the fungus to estimate host resistance, some R-genes which were strongly expressed in long days were only weakly expressed when the plants are grown in short days, while others were photoperiod-insensitive. R-gene resistance that was strongly expressed only in long days was readily overcome by challenging the leaves with large numbers of spores of *Phytophthora infestans*, but the photoperiodic-insensitive R-gene resistance did not break down under similarly high inocula.

Combining both molecular or immunological techniques may help achieve levels of detection normally unobtainable by either on its own and work on erwinias on potato supported by the EU AIR programme has used the polymerase chain reaction (PCR) to detect the plant-pathogenic bacterium *Erwinia carotovora* subsp. *atroseptica* (*Eca*) in peel extracts of potato tubers. By incorporating an immunomagnetic separation step (IMS) in the procedure and thereby separating the target bacteria from PCR-inhibitory compounds in the sap, the limit of PCR detection was lowered from 10^6 to 10^3 bacteria ml^{-1}, i.e. to the threshold level at which *Eca* may cause economic damage in a subsequent crop. The same IMS also greatly improved the consistency of determining numbers of *Eca* by the older method of colony counting on a selective medium, by reducing overcrowding by saprophytic bacteria.

Studies on the role of cell wall degradation in pathogenesis of *Erwinia spp.* have continued with the purification to homogeneity of pectin methyl esterase, one of the key enzymes involved in cell wall degradation. This enzyme is inhibited in a non-competitive manner by a heavily side-branched carbohydrate of 100 kDa consisting mainly of galacturonic acid residues with low levels of methylation. Concentrations of the inhibitor material were also higher in resistant than in susceptible potato cultivars. In addition, pectin lyase, the production of which previously was associated only with damage to the DNA of *Eca*, has now been shown to be produced constitutively at low levels, as well as probably being induced by methylated forms of galacturonic acid.

Cell wall fragments released by the action of pectic enzymes from the host and pathogen, are important in the elicitation of defence mechanism by plants. Previous reports have described how these fragments could be the basis of a novel disease control system in plants and this work has now attracted commercial support from Scotia Pharmaceuticals Ltd, a rapidly developing biotechnology company, with the aim of producing a commercially viable product.

Finally, no report for 1994 from the Mycology & Bacteriology Department, especially one which refers to *Erwinia* spp., could ignore the retirement of Michel Pérombelon in June of that year. Michel, who was recognised nationally for his contributions to plant pathology and bacteriology by being awarded with an MBE in the New Years Honours List, has made an outstanding contribution to erwinia research in all areas ranging from epidemiology to host resistance. As one would expect of him, he continued working actively until his retirement; he is still a regular visitor as he continues his supervision of the Concerted Action mentioned above!

Achievements of Mycology & Bacteriology since early 1980's

Clarified the etiology and epidemiology of raspberry root rot and determined the relative importance of nine species of *Phytophthora* in causing root rot in Britain, Europe and N. America.

Described a new variety, *rubi* of *Phytophthora fragariae*, major cause of raspberry root rot.

Isolated and described the pathogenicity, morphology and affinities of the new species *Phytophthora idaei* that commonly attacks raspberry.

Screened and developed fungicides for raspberry root rot control; three have obtained off-label registrations.

Developed fungicides for the control of red core in strawberry.

Identified raspberry germplasm and potential cultivars with valuable levels of resistance to raspberry root rot.

Developed a sensitive bait plant assay for detecting low levels of infection in strawberry propagation beds. Now used routinely in Scotland and Sweden and regularly elsewhere in Europe, this test has largely eliminated red core from strawberry stocks certified under the Scottish scheme.

Revised the race concept in *P. fragariae* var. *fragariae* and demonstrated the lability of race in single-zoospore isolates. Helped harmonise different international systems.

Developed new systems for generating protoplasts of *Phytophthora* for genetical transformation and transformed *P. infestans* and *P. cactorum.*

Isolated and sequenced several genes from *Phytophthora infestans.*

Developed RFLPs and other molecular markers (zinc finger protein genes) characteristic of *Phytophthora fragariae* and PCR primers that selectively amplify the DNA of this species.

Elucidated the aetiology & epidemiology of important fungal diseases of canes, leaves, flowers and fruits of raspberry and developed control strategies.

Identified cane blight (*Leptosphaeria coniothyrium*) as a major cause of yield loss in machine harvested raspberries and devised control strategies based on improved machine design, biennial cropping, fungicide spray programmes and resistance.

Determined role of early symptomless infections of flowers through stigma by *Botrytis cinerea* in post-harvest grey mould of raspberry and strawberry.

Related early symptomless infection of blackcurrant carpels by *Botrytis cinerea* and production of ethylene from infected fruit with premature abscission of fruits (running off).

Discovered threat to health of blackberry and red raspberry x blackberry hybrids posed by micropropagation which makes plants extremely susceptible to downy mildew.

Showed that bark-splitting in some important raspberry cultivars was a serious disadvantage because of their susceptibility to the disease complex midge blight.

Purified and characterised four pectin-degrading extracellular enzymes from *Botrytis cinerea* and established that two endo-polygalacturonases were constitutively expressed, whereas two exo-polygalacturonases were induced in the presence of pectin.

Found polygalacturonase-inhibiting protein (PGIP) in immature raspberry fruits. This protein, a non-competitive inhibitor of pectic enzymes, declines rapidly with the onset of ripening and increased susceptibility to *Botrytis cinerea*.

Developed NMR microscopy techniques for non-invasive histological examination of fruit tissues of several plants. Published the first use of NMR microscopy for observation of progress of a fungal plant disease, and also first published the structure of the vascular system of a botanical specimen using NMR with 3-D reconstruction and surface rendering techniques.

Revised concepts in blackleg epidemiology following the recognition of widespread latent infection by the pathogen from different sources before and after harvest.

Established the relationship between seed contamination and blackleg incidence that led to the devel-

opment of improved immunological methods for quantifying the bacterium.

Developed hot water treatment of potato stocks as an environmentally sound system of disease control.

Related tuber resistance in erwinias to the methylation of pectin in cell walls in potato.

Discovered a series of pectic enzymes induced *in planta* that were not induced *in vitro*.

Applied spatial modelling of epidemics to cultivar mixtures with stochastic and wavelet theory.

Determined the genetic basis of tolerance of barley to powdery mildew and developed methods for detecting and assessing tolerance.

Developed a rapid HPLC technique for the preparative purification and quantification of unsaturated oligogalacturonides released from cell walls by polygalacturonic acid lyase, a pectic enzyme from *Erwinia carotovora*. The oligogalacturonides elicit phytoalexins and their activity is affected by methylation of the original pectin.

Showed that the outer surface of *Erwinia carotovora* ssp. *atroseptica* was modified by the phytoalexin rishitin and that potato lectin binds to rishitin-treated erwinias but not to non-treated bacterial cells. Bacteria in rotting potato tubers incubated aerobically appeared damaged in a manner characteristic of that caused by rishitin, providing evidence that rishitin is acting on bacterial cells *in planta*.

Detected variation among isolates and mutants in non-pathogenic mutants of *Erwinia carotovora* and *Erwinia atroseptica* in their sensitivity to the phytoalexin rishitin that could be related to changes in bacterial wall structure.

Purified an extracellular metalloprotease from *Erwinia carotovora*, determined its N-terminal sequence and raised polyclonal antibodies to it. The metalloprotease degrades potato lectin *in vitro* and is not inhibited by protease inhibitors found in potato.

Using a range of kinase and phosphatase inhibitors, proved that protein phosphorylation was critical for phytoalexin accumulation in soybean cotyledons through the regulation and transcription of phenylalanine ammonia-lyase activity and other enzymes.

Raised polyclonal antibodies to isozymes of polygalacturonic acid lyase, cellulase, and polygalacturonase from *Erwinia carotovora*, and two isozymes of phenylalanine ammonia-lyase from potato using Multiple Antigenic Peptides.

Resistance of *Solanum tuberosum* tubers to soft rot erwinias has been shown to be multi-component involving a combination of inhibition of bacterial growth by sesquiterpene phytoalexins and phenolics, enzyme inhibition by phenolics and plant cell wall methylation. Resistance is also associated with the formation of free radicals.

Many similarities were shown to exist between incompatibility in pollen/stigma interactions and incompatibility in plant/pathogen interactions. Low molecular weight inhibitors of pollen germination produced in incompatible pollen/stigma interactions in *Brassica* spp., and detected by a novel TLC bioassay developed at SCRI, were absent in non-pollinated stigmas.

Developed novel disease control system based on elicitor-active yeast extracts. These give a high level of powdery mildew control on detached barley, oat and wheat leaves and field applications to barley reduce mildew and enhance yield compared to unsprayed plants. Control results from a stimulation of host defence mechanisms: increased phenylalanine ammonia-lyase activity and increasing papillae diameter. Yeast extracts also controlled *Botrytis cinerea* and *Rhizoctonia solani* on lettuce.

Demonstrated importance of interactions between ambient humidity and air speed in determining numbers of sporangia of *Phytophthora infestans* formed on infected potato leaves.

Devised an ELISA-based system for the quantification of resistance of potato leaves to colonisation by *P. infestans* that is now used routinely by many researchers to assess resistance to the disease.

Showed that polygenic resistance of potato leaves to colonisation by *P. infestans* depends on an interaction between potato genotype and environment.

Developed an ELISA-based system for the quantification of potato tuber-borne *Spongospora subterranea*.

A unique form of gene organisation in the Oomycetes

Shiela E. Unkles

Phytophthora infestans is the causative agent of potato late blight, the most devastating disease of potatoes throughout the world. Although considerable advances have been made in control of blight by the introduction of agrochemicals and disease resistant cultivars, it remains a major problem. The genus *Phytophthora* is an important member of the Oomycetes, a Class which contains many important plant pathogenic species but which is generally poorly characterised in molecular terms.

Investigations into the basic molecular biology of *P. infestans* commenced several years ago at SCRI as part of studies on the molecular mechanisms of late blight disease. In this early work, we isolated and characterised a number of *P. infestans* genes, one of which was *gpd*A encoding the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH)[1]. The DNA sequence of this gene was published and although not unique among fungi, the initiation codon was GTG instead of ATG. Recently it became apparent that the published DNA sequence upstream of the GAPDH coding region was homologous to the sequences of triosephosphate isomerase genes (*tpi*) from other organisms. Further sequencing of the original *gpd*A clone, pSTA33, confirmed the presence of the entire *tpi* coding region with an ATG initiation codon in the expected position (Fig. 1). Upstream of the ATG is a conserved motif found at the transcriptional start site of all *Phytophthora* genes investigated thus far. Linking the two coding regions is a 21 bp sequence which allows in-frame translational read-through from *tpi* to *gpd*.

Family	Genus	Species	Positive fragment
Pythiaceae	*Pythium*	*ultimum*	300 bp TP15-GAP1R
		violae	750 bp TP15-GAP2R
Saprolegniacae	*Saprolegnia*	*turfosa*	550 bp TP17-GAP2R
			750 bp TP15-GAP2R
	Achyla	*radiosa*	550 bp TP17-GAP2R

Table 1 Presence of *tpi-gpd* gene fusion in other oomycetes. Fragments were obtained by PCR amplification using the primer pairs indicated (see Fig. 1C), and positively identified by DNA sequencing.

The possibility remained, however, that the apparent gene fusion was actually a cloning artefact and a reverse transcriptase PCR reaction was performed using RNA extracted from two strains of *P. infestans*, the original strain from which the gene library had been constructed (48720) and another from our collection (89/AF1). PCR primers were chosen from sequences either side of the link, i.e. from within the *tpi* and the *gpd* sequences (positions of primers T1 and G1 respectively, shown in Fig. 1C). The results show a fragment of the expected size in PCR reactions with, but not without, reverse transcriptase (Fig. 2), indicating that the fusion is transcribed as a single messenger RNA. Further evidence was obtained from

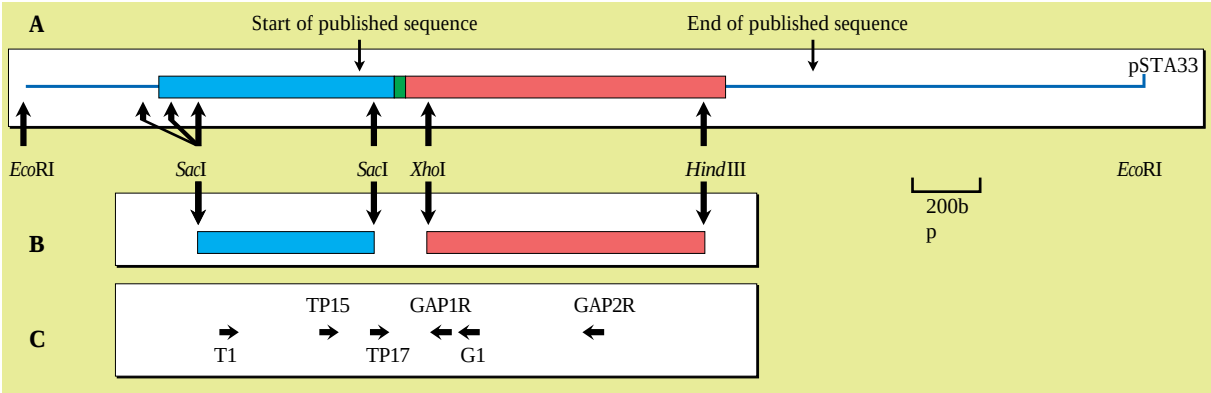


Figure 1 Organisation of the *tpi-gpd* gene. A) Locations of the coding regions within the restriction map of clone pSTA33. B) Fragments used as *tpi*- and *gpd*-specific probes for hybridisations. C) Positions of primers used in PCR reactions relative to the restriction map (A).

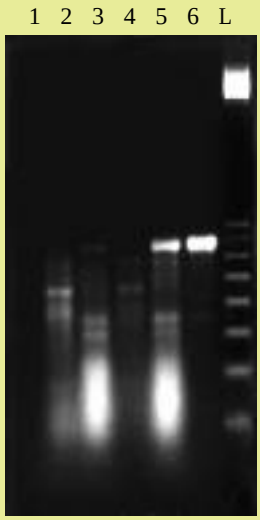


Figure 2 Amplification products using primers T1 and G1 with RNA from *P. infestans* 48720 (lanes 2 and 3) or 89/AF1 (lanes 4 and 5). PCR amplification was performed without reverse transcriptase (lanes 2 and 4) or with reverse trancriptase (lanes 3 and 5). Lane 6 is a control using pSTA33, L shows molecular size standards, and lane 1 is without RNA template.

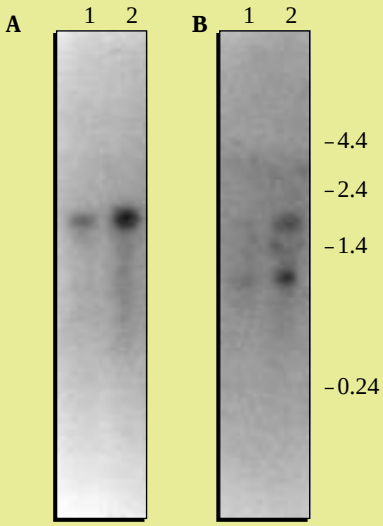


Figure 3 Northern blot of RNA from *P. infestans* strains 48720 (lane 1) and 89/AF1 (lane 2). Probes specific to the *tpi* (A) or *gpd* (B) coding region, shown in Fig 1B, were radioactively labelled and used in hybridisation to reveal the corresponding transcript.

Northern blots in which RNA from the two strains was hybridised with probes from either the *tpi* or *gpd* regions of the fusion. With the *tpi* probe, a single transcript of around 2 kb was observed, the size expected for the fusion (Fig. 3). With the *gpd* probe, a transcript of the same size was seen, but a second transcript of around 1 kb was also visible. This is the size expected for a transcript of *gpd* alone, and subsequent Southern blots indicate that there is probably one copy of *tpi* (found as a fusion with *gpd*) but more than one copy of *gpd* (data not shown).

The finding of a gene fusion in *P. infestans* prompted a search among related species for a similar arrangement. Representative members of the six groups within the genus *Phytophthora*, separated on the basis of biochemical and morphological traits, were analysed by Southern blots using either *tpi*- or *gpd*-specific probes. From the overlapping patterns of the hybridising bands obtained with several restriction enzymes, it

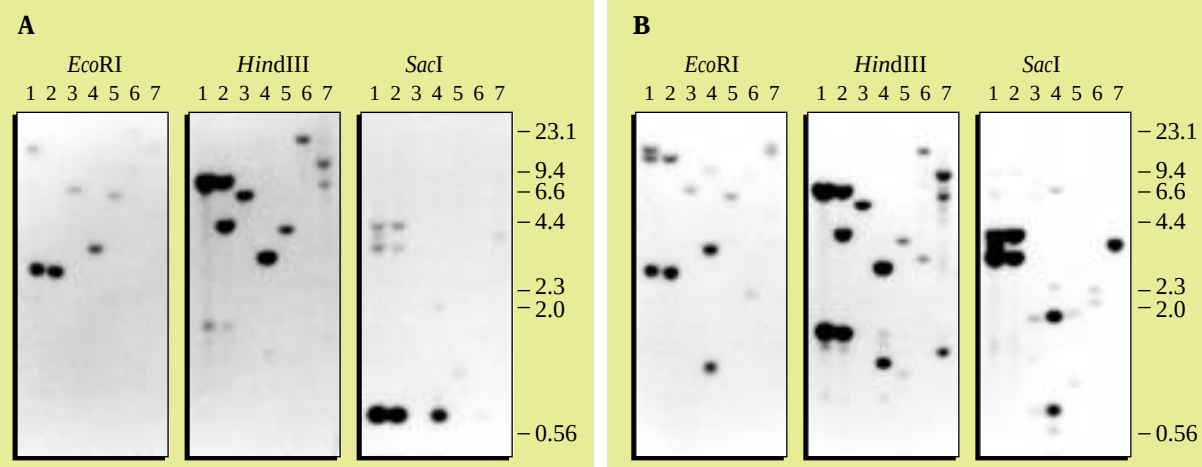


Figure 4 Southern blots of DNA fom different species of *Phytophthora* - *P. infestans* (two isolates lanes 1 and 2), *P. cactorum* (lane 3), *P. nicotianae* (lane 4), *P. citricola* (lane 5), *P. fragariae* (lane 6), *P. cinnamomi* (lane 7). DNA was digested with *Eco*RI, *Hin*dIII, or *Sac*I, electrophoresed and Southern blotted. Panel A was hybridised with the *tpi*-specific radioactive probe and Panel B with the *gpd*-specific radioactive probe shown in Figure 1B.

was clear that this gene fusion exists throughout the genus (Fig. 4). Similar hybridisation experiments were successful in demonstrating a *tpi-gpd* fusion in three species of a related genus, *Pythium*, but cross-hybridisation to other Oomycetes was not possible even under conditions of low stringency.

A PCR approach was then adopted in order to investigate other genera. The genes encoding TPI and GAPDH have been isolated and sequenced from many species ranging from bacteria to man. Degenerate PCR primers TP15, TP17, GAP1R and GAP2R (positions shown in Fig. 1C) were synthesised to highly conserved regions of both genes (positions shown, Fig. 1), and used in reactions containing DNA from several oomycete species. Reactions were attempted with different combinations of primers and a range of conditions for each template DNA. Bands identified on agarose gels as being the size expected for the particular primer set were sequenced either directly, or following cloning into a pCR-SCRIPT vector. DNA sequences were compared to the known *P. infestans* sequence and results are shown in Table 1. That PCR products have been obtained from a number of oomycete genera, suggests the gene fusion is typical of this group of organisms. In evolution, the closest relatives of the Oomycetes are probably the chrysophytes (the golden-brown algae)[2], but no specific PCR products were obtained using DNA from the diatom *Phaeodactylum tricornutum* as template.

The enzymes GAPDH and TPl catalyse sequential steps in the glycolytic pathway. In all species studied to date, GAPDH is a tetrameric protein, which may be homomeric, as in the prokaryotes, fungi, mammals and plant cytosol, or heteromeric, composed of two types of subunit, as in the plastid-localised enzymes of algae and higher plants. Chicken muscle TPI was crystallised in the early 1970's and its structure determined as a homodimeric protein. The DNA sequences of these subunits have provided the basis for extensive evolutionary research. In no case so far, however, has there been any indication of fusion of these genes. Currently, we are attempting to determine if the protein is also fused, which could raise interesting questions on the processing and activity of these enzymes.

For both enzymes, therefore, there is a wealth of information, biochemical, molecular and evolutionary. However, this is the first demonstration in any organism, bacterial, fungal, plant or mammalian, that a fusion has occurred between the genes encoding the two enzymes. Comparison of DNA sequences will provide valuable information on the evolutionary position of this important group of organisms. On a more practical note, the unique nature of this phenomenon to Oomycetes, offers the possibility of developing extremely specific probes or PCR primers allow rapid and accurate diagnosis of commercially significant members of the group.

References

[1] Moon, R. P., Unkles, S. E., Duncan, J. M., Hawkins, A. R. & Kinghorn, J.R. (1992). *Plant Molecular Biology* 18, 1209-1211.

[2] Gunderson, J. H., Elwood, H., Ingold, A., Kindle, K. & Sogin, M. L. (1987). *Proceedings of the National Academy of Sciences USA* 84, 5823-5827.

Detection of *Phytophthora* species in horticultural crops

D. Cooke

The genus *Phytophthora* (meaning 'plant destroyer') represents an important group of plant pathogenic fungi which are responsible for large scale losses of tropical and temperate crops and species in natural communities. The primary sites of damage caused by most species are roots, stem bases, bark and fruit. *Phytophthora infestans* is an exception in its ability to thrive in an aerial environment and attack foliage. *Phytophthora* spp. typically have a very broad host range: *P. cinnamoni* attacks more than 950 species and cultivars of mainly woody plants, and *P. nicotianae* at least 72 genera of plants.

Although traditionally grouped in the Mastigomycotina, a subdivision of the kingdom fungi, *Phytophthora* are now recognised to be more closely related to the brown and golden algae. As "water fungi", they rely on abundant moisture for much of

their life cycle. The infective agent is a motile zoospore which is attracted to host material where it is able to encyst and infect. Sporangiophores bearing the asexual sporangia grow out from the infected material and release more zoospores. Sexual crosses within a single strain in the case of homothallic species and between different strains (termed A1 and A2) in heterothallic species result in the production of oospores. Despite the lack of aerial dispersal mechanisms, the ability to spread locally in drainage or irrigation water and persist as long-lived oospores in soil or planting material has resulted in a worldwide distribution of many *Phytophthora* spp.

The development of an accurate and reliable detection and identification system is particularly relevant for Phytophthora diseases for the following reasons:

- early detection of root, crown or stem base Phytophthora diseases is not easy as the symptoms are non-specific and may only be seen after destructive sampling
- in a vegetatively propagated crop, clean planting material is vital to prevent disease introduction and spread on a local or national scale.
- if the presence of a *Phytophthora* spp. is confirmed, the identity of the pathogen may not be apparent until exhaustive laboratory studies have been carried out
- several species are often associated with a disease e.g. root rot of raspberries has been shown to be a complex of several species, predominated by the most pathogenic species *P. fragariae* var. *rubi* (Ann. Rep. 1991, 89-92)
- some species are quarantine organisms requiring stocks to be guaranteed "disease-free" before export or sale.

This article reviews the current status of detection and diagnosis of *Phytophthora* spp. and reports on progress at SCRI.

Identification and detection

It is important to distinguish between detection and diagnosis. The aim of a detection procedure is to test plant material, soil or drainage water for the presence of *Phytophthora* spp. where no symptoms are yet visible whereas diagnosis is the confirmation of the identity of the organism responsible for existing disease symptoms. In both cases it would be ideal to be able to discriminate at the species level.

The fungus may be detected directly by examining plant material microscopically for oospores or sporangia. However it takes a 'trained eye' to distinguish between the many morphologically similar *Phytophthora* species and the method is time consuming and therefore impractical and expensive on a large scale. By necessity few samples of roots can be examined making it a relatively insensitive test.

Phytophthora spp. may be isolated from infected soil or plant material by flooding with water to promote the release of zoospores which are then trapped on bait material such as lupin cotyledons or pine needles floating on the water surface. The fungus may then be isolated from the bait on selective media and identified in pure culture. Again, a reliable but lengthy procedure.

Bait tests have been used successfully for many years. In the case of *Phytophthora fragariae* var. *fragariae*, highly susceptible strawberry cultivars are grown alongside the potentially infected material under conditions conducive to root infection. The presence of characteristic red core symptoms on the bait plants is indicative of the presence and pathogenicity of the *Phytophthora* spp. present.

Serological methods have been used with some success in disease diagnosis but to date the antibodies developed have shown little species specificity.

Recently, DNA-based methods utilising the sensitivity and discriminatory powers of the Polymerase Chain Reaction (PCR) have been developed at SCRI to detect *Phytophthora* spp.

PCR involves the *in vitro* amplification of strands of DNA, typically less than 3 kb. Short (c. 20 bp) oligonucleotide primers of a specific DNA sequence initiate the amplification and are therefore critical to the success of PCR-based detection schemes. A successful primer should result in the amplification of an unambiguous product from only the target *Phytophthora* spp. A single nucleus of *Phytophthora* contains 2×10^8 bp or approximately 0.2 pg of DNA providing many potential sites for specific primers. Regions specific to the species to be identified therefore have to be defined. A number of approaches can be employed to search for PCR primers depending upon the level of phylogenetic resolution required.

Random clones or RAPDs

The selection of regions at random allows selection at the finest scale of resolution which should allow the selection of primers to differentiate between sub species. The screening of random genomic clones for

cross reaction with other *Phytophthora* spp. and the cloning and sequencing of RAPD bands are two possible approaches. The application of RAPDs to *P. cactorum* has revealed a fine scale resolution, different pathotypes were identified by the range of DNA fragments produced. However the technique has the disadvantages that the screening procedure must be repeated for each target species, no information on the rates of evolution of the target sequence would be known, and a region present as a single copy would suffer a lower sensitivity compared to a repeated sequence.

Ribosomal DNA sequences

Ribosomes, which are vital for protein synthesis, are composed of RNA subunits and many ribosomal proteins. Ribosome architecture is governed by the secondary structure of the RNA molecule which is a function of the sequence of bases. Because protein synthesis is so fundamental to life, the DNA that codes for ribosomal RNA (rDNA) is highly conserved, sequence homology in some rDNA regions is found in all forms of life. Despite the highly conserved "core sequences", there is some variation in other rDNA which allows phylogenetic separation at many levels from kingdoms through to genera. Such regions have been the focus of much work to construct a global phylogenetic tree and elucidate the origin of mitochondria and chloroplasts. There are many advantages to using rDNA sequences as target sites for PCR primers: there is an abundance of publications on rDNA sequence variation, sequences are rich in informative regions, mutation rates are known and many copies are present in each nucleus thus increasing the sensitivity of detection. Because the region is tightly defined, species can be added to the analysis at any time resulting in an expanding sequence database. Although the 18S and 28S subunits themselves reveal little variation within a genus there are other regions which are transcribed to RNA but removed in subsequent processing and because these internal transcribed spacers (ITS regions) do not form part of the ribosome, their sequences are not so highly conserved.

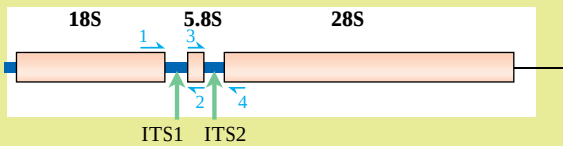


Figure 1 Part of the ribosomal repeat unit showing the arrangement of the internal transcribed spacer regions and the position of the PCR primers (ITS 1-4) used for their amplification.

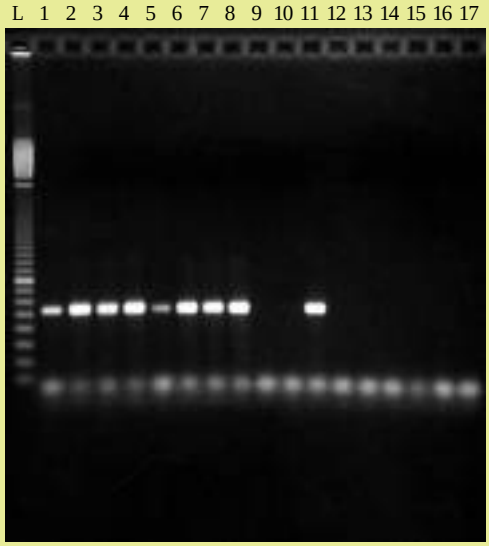


Figure 2 The results of agarose gel electrophoresis of the products of PCR using *P. fragariae* specific primers. A 550 bp DNA fragment is amplified in only those reactions containing *P. fragariae* DNA. Lane 1-8 *P. fragariae* var. *fragariae* races A1, A2, A3, A4, A6, A7, A8 and A9; Lane 9 *P. cambivora*, Lane 10 *P. cinnamomi*, Lane 11 *P. fragariae* var. *rubi*, Lane 12 *P. cryptogea*, Lane 13 *P. drechsleri*, Lane 14 *P. megasperma*, Lane 15 *Phytophthora* spp., Lane 16 Control reaction with no template DNA. L 100base pair ladder (Life technologies).

There are two such spacers, ITS1 lying between the 18S and the 5.8S gene and ITS2 between the 5.8S gene and the 28S gene (Fig.1). Evidence from studies on plants (pine and sorghum) and other fungi (rusts and *Colletotrichum*) suggests that the level of resolution offered by these regions is suitable for the purposes of molecular detection. Little variation has been detected within a species.

Using a set of PCR primers designed for the amplification of fungal spacer regions[1], *Phytophthora* ITS 1 and 2 regions were amplified and found to be 220 and 400 bp long, respectively. The double stranded PCR products were manually sequenced and aligned using multiple sequence alignment software on Seqnet

P. fragariae var. *fragariae*	*P. cryptogea*	*P. pseudotsugae*
P. fragariae var. *rubi*	*P. citricola*	*P. cactorum*
P. cambivora	*P. drechsleri*	*P. idaei*
P. megasperma	*P. infestans*	*P. nicotianae*
P. cinnamoni		

Table 1 The ITS1 and ITS2 regions of the above *Phytophthora* species have been sequenced and are being used for the design of specific PCR primers.

(Daresbury Laboratory). The species sequenced to date are shown in Table 1. Both ITS1 and ITS2 regions were sufficiently conserved to allow an accurate alignment, some regions were conserved for all species and others showed considerable variation. This rich source of defined sequence allowed the design of PCR primers specific for a particular species. Primers were designed for *P. fragariae*, *P. cambivora* and *P. nicotianae* and when tested against pure DNA from other *Phytophthora* spp. an amplification product was obtained from only reactions containing the DNA of that species. Figure 1 shows an agarose gel of the PCR amplification products using a *P. fragariae* specific primer on DNA from six races of *P. fragariae* var. *fragariae*, one isolate of *P. fragariae* var *rubi* and seven other *Phytophthora* spp. Amplification products were only produced in reactions containing DNA from *P. fragariae* and even the closest known species (*P. cambivora* and *P. cinnamoni*) showed no DNA amplification. Detectable PCR products were obtained from PCR reactions using as little as 100 fg of pure target DNA which is approximately half the amount of DNA found in a single *Phytophthora* zoospore. This demonstrates the power of PCR to detect the fungus sensitively and selectively. Further work is underway to optimise the efficacy of the test on zoospores in water samples, infected plant material and ultimately the long-lived oospores in field soil.

The refinement of the prototype test into a practical detection system is essential and will be of immediate benefit to growers, plant propagators and regulatory authorities. An ability to detect diseased planting material will reduce introduction of the disease into hitherto 'clean' fields, regions or countries and an ability to rapidly diagnose a disease problem will result in more timely and effective fungicide treatment. The resultant reduction in fungicide requirement provides a clear environmental benefit as well as economic advantages.

References

1 White T. J., Bruns T., Lee S. & Taylor J. (1990). *PCR protocols*: *A guide to Methods and Applications*. Academic Press: San Diego.

Understanding plant disease epidemics through mathematical modelling

A.C. Newton, G. Gibson & D. Cox

The dominant features of an epidemic, such as its overall severity, its distribution over areas of land, and its evolution through time are the result of the dynamic interactions of the host and pathogen systems, both of which are influenced by numerous complex biological processes. If successful disease-control strategies are to be designed, it is important that the pathologist has an understanding of what are the most important processes, and how they combine to define the dynamics of an epidemic. Recently, there has been an increase in the use of mathematical models in biology to develop quantitative, or qualitative, predictions of the dynamics of biological systems. At SCRI, pathologists and mathematicians are working together in order to bring mathematical modelling to bear on problems in plant epidemiology.

In this approach, the main biological interactions between host and host, host and pathogen, or pathogen and pathogen are represented in the form of mathematical functions specified by parameters which quantify the relative strengths of interactions or the rates at which processes evolve. Examples of processes and interactions which are typically represented in models of plant epidemics include gene-for-gene interactions between host and pathogen whereby a host only suffers attack by pathogens with a specific genotype, the development of pathogen populations on hosts, e.g. sporulation and lesion growth, and the spatial movement of pathogens from one host to another.

There are many mathematical modelling techniques which can, in theory, be used to construct models of

plant epidemics. These include deterministic models which assume that events occur according to strict rules and do not allow for the working of chance. Thus, if an epidemic is simulated many times from a deterministic model with a fixed set of initial conditions and parameter values, it will always produce the same outcome. While deterministic models have been extremely successful in some applications, there is now a growing appreciation that in other cases random, or stochastic, processes must be modelled in order to reproduce the behaviour encountered in the real world.

Spatial modelling is an approach which is particularly relevant in plant epidemiology. In contrast to animal populations, plant populations are unable to mix freely, and whether an individual plant is healthy or diseased is often closely related to the state of other plants in its immediate neighbourhood. The spatial nature of plant epidemics can be appreciated from Figure 1 which shows recorded levels of mildew infection in an experimental plot of barley.

Figure 1 Contour map of mildew severity on barley showing 'hot spots'.

Mathematical models which seem particularly appropriate to represent the spatial nature of plant epidemics are those based on cellular automata. In a cellular model of a plant epidemic, the host population is divided into small-scale units, each of which is located at a site on a rectangular lattice. For example, in studying the spread of an aerially disseminated foliar disease in a mixed cultivar crop such as mildew in barley, one candidate for the basic unit would be the minimum area planted by one genotype, be this a single plant, small plot or large plot. A model for the spatial interactions between units, via spore dissemination in this case, is then chosen. One decides how many spores are produced by the lesions, how they disperse, and how many lesions then result given the resistance or susceptibility of each cultivar, etc. The resulting model can be implemented as a computer programme and allowed to run for an appropriate length of time, showing the general evolution of the larger model. The model can be used to investigate the likely sensitivity of the epidemic dynamics to, for

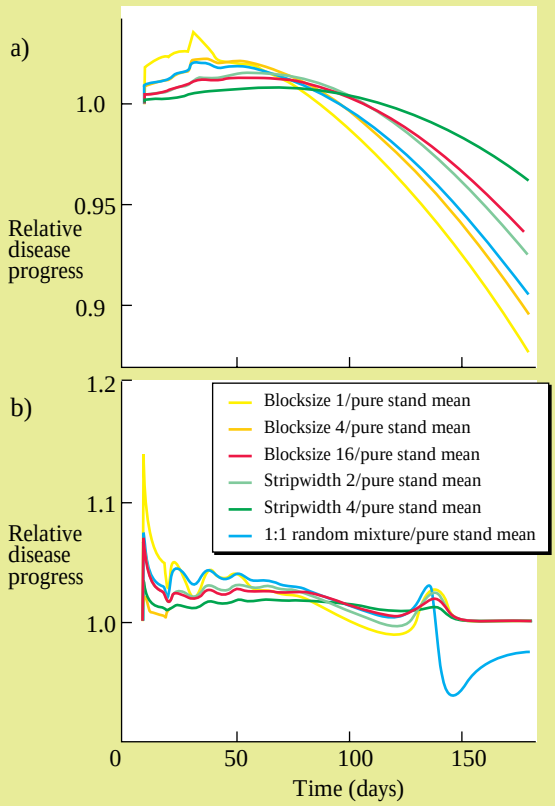


Figure 2 a) Relative disease progress curves (standard disease progress curves normalized by mean pure stand disease levels) computed from a spatial average of a deterministic model with spatially uniform initial infection for a range of block and strip geometries and the 1:1 random mixture. The block and strip patterns are based on smaller subunits of varying size and have a regular structure. Allodeposition parameter = 0.0001, autodeposition parameter = 0.001.
b) Relative disease progress curves computed from a spatial average of a model averaged over 20 random initial infection patterns for a range of block and strip geometries and the 1:1 random mixture. Allodeposition parameter = 0.001, autodeposition parameter = 0.01.

example, the way that units of different genotypes are arranged on the lattice. This has been done at SCRI using a deterministic cellular model for the spread of a fungal disease. Figure 2 shows examples of disease progress curves for one such model.

Although mathematical models for plant epidemics are proving to be useful investigative tools, there are considerable difficulties in evaluating models critically in the light of data from actual epidemics. There are several reasons for this. Because the biology of the host-pathogen interactions is so complex, there is a tendency to allow models to become increasingly complex, making them computationally intensive, with many parameters, and difficult to analyse. It is extremely difficult to fit such models to real data and parameter estimation techniques which can be applied to spatio-temporal data sets are not generally available. BioSS and SCRI are currently tackling this problem and bridging the divide between models and experiments by focusing attention on much simpler spatio-temporal models for the spread of a disease where stochastic effects are included. In these models, complex biological processes are not treated explicitly but are summarised in terms of a few relationships describing, for example, the rate at which an infected individual infects a susceptible individual, and how this varies with the distance between them. Techniques for estimating model parameters from disease maps recorded at different times have been developed and applied to data sets in the literature. At present these techniques are limited to diseases which are characterised by presence or absence, such as a virus disease, and as yet they cannot be applied directly to the study of fungal pathogens. Nevertheless, it has been shown that they can be used to fit spatio-temporal stochastic models in other areas of plant pathology. Figure 3(a) shows the spread of a real epidemic of citrus tristeza virus in an orchard taken from the literature. Figure 3(b) is an epidemic simulated from a simple spatio-temporal stochastic model whose parameters were estimated from the data set of Figure 3(a).

A further difficulty which arises in the modelling of plant epidemics is the impact that spatial heterogeneity in the environment can have on epidemic dynamics, which may invalidate any model which fails to take account of it. For example, in the case of soil-borne pathogens, e.g. red core in strawberries, the initial inoculum distribution in the soil can have an overwhelming effect on the development of an epidemic, as the soil environment is far more heterogeneous than the aerial environment. Where these effects are present, the investigator may be tricked into attributing spatial heterogeneity in patterns of disease to the internal dynamics of an epidemic rather than to heterogeneity in an extrinsic factor. In the work at SCRI, emphasis is being placed on the standard statistical analysis of epidemiological data in conjunction with physical data, such as elevation or soil moisture, to determine where such dependencies might be driving the pattern of disease. Figure 4 (a,b&c) shows the distribution of red-core in one field of strawberries annotated with height contours. In this case it can be seen that there is good correlation between the site elevation and disease.

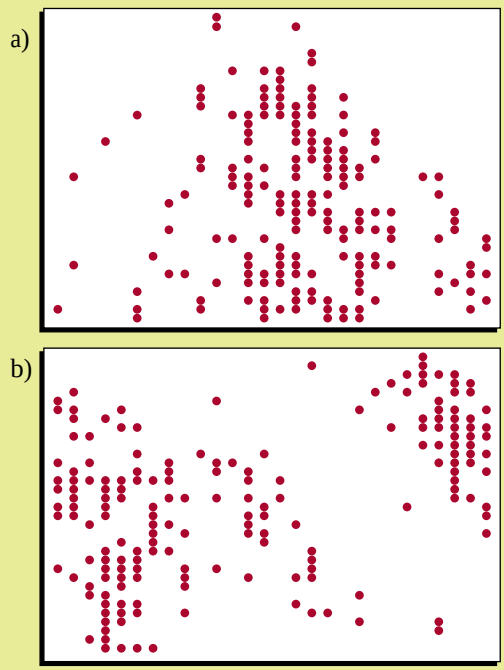


Figure 3 a) Location of trees infected with citrus tristeza virus in an orchard. b) Epidemic of CTV of same size as (a) simulated using a stochastic spatio-temporal model with parameters estimated from (a). It is assumed that the epidemic spreads from 3 randomly placed initial infections. Both patterns exhibit similar degrees of aggregation.

The methods outlined above represent a shift of emphasis in the approach to mathematical epidemiology. Rather than constructing complex models which include many biological processes and parameters but which cannot be easily validated we are focusing on much simpler models which, nevertheless, have the ability to reproduce the behaviour of real systems. Because of their simplicity, they can be more easily

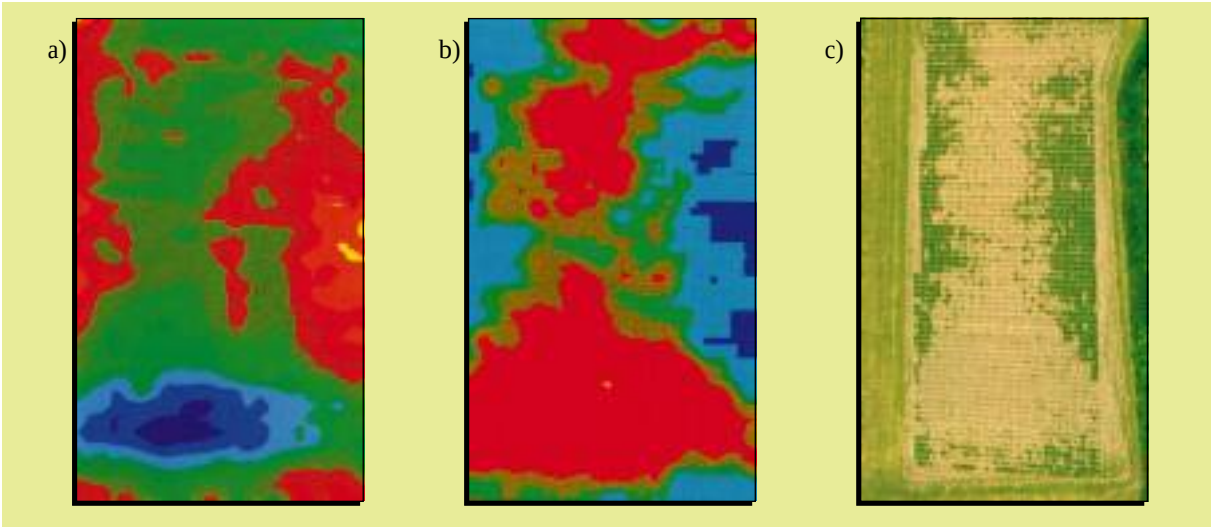


Figure 4 a) is an elevation map of a field of strawberries. b) is a map of red core severity, with bluer areas denoting greater disease. c) is an aerial view of the site, showing the dead plants. The maps seem similar.

related to real data. Another feature of the approach is the emphasis on field data and the use of standard data analysis techniques, often developed in other areas of science, to draw out as much information as possible. These can yield much insight into what processes determine the dynamics of an epidemic and the scale at which these processes should be treated, which can guide the subsequent development of mathematical models. It is hoped that through this approach we can help to bridge the gap between theory and observation, increasing the impact of mathematical modelling on the development of genetic and agronomic methods to manipulate epidemiological processes and minimise the threat which they present.

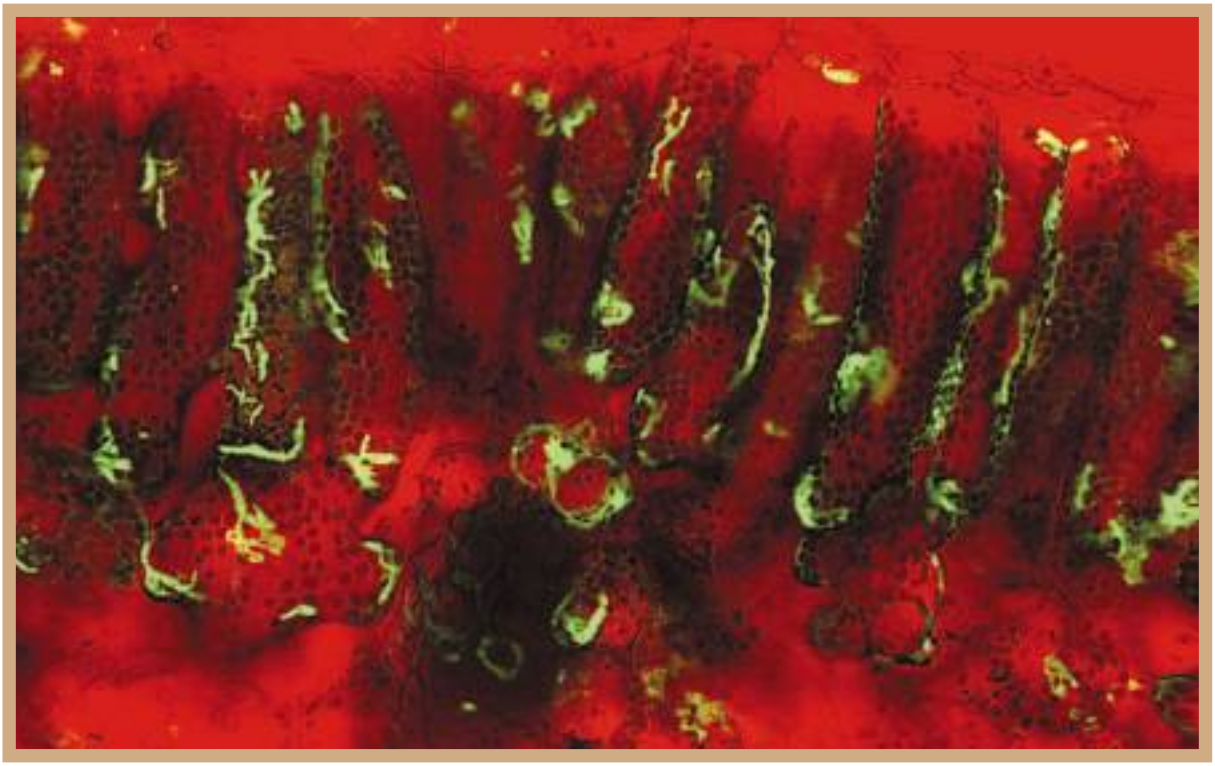
Plant viruses

T. Michael A. Wilson

Plant virus research at SCRI remains committed to long-standing basic and strategic objectives: to understand the molecular basis for interactions between important viruses, their plant hosts and their natural insect, mite, nematode or fungal vectors; to investigate novel approaches to diagnose, characterise and control these ubiquitous agents of disease; to exploit the relatively simple genetics and efficient biological activities of plant viruses to improve our understanding of plant processes and plant cell biology and to contribute to wealth-creating biotechnology in general. To achieve this we must first dissect and comprehend structures and functions responsible for the replication, pathology and transmission of appropriate and convenient model viruses, or viruses of commercial importance.

The overall virus research programme has seen further focusing and fine-tuning during 1994: SOAFD-funded geminivirus work was discontinued, electron microscopy was assimilated into the appropriate RNA virus-based projects, and our soft-fruit virus disease work has been focused on the serious and real threats posed by raspberry bushy dwarf virus (RBDV) and blackcurrant reversion disease. We have welcomed record numbers of new staff, visiting scientists, collaborators and research students. Several major externally funded contracts have been awarded in 1994 to expand existing programmes in recombinant antibody techniques as well as to improve post-harvest virus testing of potato tubers, to provide novel virus-derived resistance genes against groundnut rosette disease and to develop virus vectors in cell engineering for biomedical and veterinary applications. In each case, we have added to the critical mass of expertise and staff effort, which is vital to improve our competitiveness, and depth of commitment to innovation and productivity. The cumulative total of external grant income for currently active projects in plant virus research is £2.88m. We have also gained full support for 10 Ph.D. students.

Strategic links between plant virus research and crop breeding for virus resistance have been strengthened with the appointment (*vice* Murant), in 1994, of Dr Simon Santa Cruz from the Sainsbury Laboratory, Norwich. Timely programmes of molecular genetic research on potato and *Brassica* resistance genes to potato viruses X and Y, and to turnip mosaic potyvirus, respectively, have begun. In addition, new initiatives using virus-based expression vectors with visible reporter proteins to probe plant cell biology and physiology have arisen through synergistic collaborations involving Dr Santa Cruz, Dr Karl Oparka and the new confocal laser microscope facility. Some of this work is described in the article on page 173.



Confocal image, with false colour background, showing viral inclusions of PVX in which GFP is fused to the coat protein, within the palisade mesophyll of *Nicotiana benthamiana.*

SOAFD policy-led work on virus-tested (VT), certified SCRI stock of soft fruit crops has continued. Six selections from the SCRI *Rubus* breeding programme and 144 VT stock cultivars, species or selections were confirmed to be free from RBDV. Thirty-eight of these VT cultivars were also shown to be free from other known viruses. More than 700 *Rubus* plants received from ADAS were tested and many were found to contain RBDV; some also contained arabis mosaic virus (ArMV), tomato black ring virus and aphid-borne viruses. Two SCRI strawberry cultivars were virus-tested and VT stock provided for propagation. Basic research work has also continued on the isolation, cloning and sequencing of double-stranded (ds)RNA molecules associated with soft fruit crops, especially blackcurrant (*Ribes* spp.) showing disease symptoms. A 0.5 kilobase pair (kbp) DNA clone of a 5.3 kbp dsRNA found in the blackcurrant cultivar Baldwin has been sequenced and used as a probe to detect related molecules in several other *Ribes* cultivars. Four DNA clones derived from a 4.4 kbp dsRNA found in cultivar Ojebyn are also being characterised. Thread-like closterovirus particles have been found in some diseased *Ribes* plants and 25 DNA clones, derived from several clostero-associated dsRNA species (1.3-4.0 kbp) are being assessed. The value of native *Ribes* resistance genes against the agent of blackcurrant reversion disease and its mite vector (*Cecidophyopsis ribis*) are being assessed by SCRI zoologists and virologists in field trials here and in Finland.

Two groups of soil nematode-transmitted viruses, spherical nepoviruses and rod-shaped tobraviruses, are the subject of major interdisciplinary, SOAFD core-funded research programmes. Transgenic tobacco plants expressing the coat protein gene of arabis mosaic nepovirus (ArMV), or either the large (L), or small (S), or uncleaved precursor form of the coat protein of strawberry latent ringspot nepovirus (SLRSV) were provided by our collaborator Dr J.I. Cooper (NERC, Oxford). All plants, except the non-transformed controls and SLRV L-CP transgenic lines, displayed strong resistance to the homologous virus transmitted by the vector nematode, *Xiphinema diversicaudatum*. Because coat protein-mediated transgenic resistance had failed to work against nematode-transmitted tobacco rattle tobravirus (*Ann. Rep. 1993, 97*), this is the first positive report of effective transgenic resistance to nematode-transmitted plant viruses (ArMV & SLRSV). In the field of virus detection and diagnosis, short and specific DNA primers have been devel-

oped for reverse transcription-polymerase chain reaction (RT-PCR)-mediated detection of two important pathogens, grapevine fanleaf nepovirus (RNAs 1 and 2) and ArMV (RNA 2). An important 'first' in tobravirus research has arisen through sequencing and mutagenesis of a full-length, infectious clone of a nematode-transmissible isolate (TPA56) of pea early browning virus (PEBV). Elegant work targeted on PEBV RNA 2 has shown, unexpectedly, that another virus-coded protein (of size 29 kDa) is required, in addition to the coat protein, for nematode transmission. These experiments and related studies on mutations in other TPA56 PEBV RNA 2 genes (for proteins of 9 kDa and 23 kDa) are described further on page 140. Epidemiological studies have shown that six out of 13 potato cultivars challenged with nematode-borne tobacco rattle virus were tolerant (infected but symptomless) and, in cv. Wilja, the TRV infection was systemic and persistent through two tuber generations.

In our work on fungus-transmitted furoviruses and bymoviruses afflicting cereals, RT-PCR techniques have also been used to follow rapid intraplant movement of virus and to map spontaneous deletion events in soil-borne wheat mosaic furovirus (SBWMV) RNA 2, following either mechanical inoculation of foliage or *Polymyxa graminis* infection of roots. RNA deletions occur exclusively in the extended, coat protein-readthrough domain. A conserved 5'-boundary and a predicted stem-loop structure in the negative-strand RNA 2 template suggest that deletion events occur during virus replication at the stage of positive-strand RNA synthesis. High growth temperatures, rapid serial transfers or prolonged growth of infected plants encourage RNA 2 deletions. Subsequent mechanical transfer to healthy wheat plants then results in severe-to-devastating disease symptoms. To undertake this study, the complete sequence of SBWMV RNA 2 (Oklahoma isolate; 3593 nucleotides) was obtained. There were 65 nucleotide differences from the earlier sequence of a Nebraska isolate. Only seven amino acid changes were predicted in coding regions, of which five lay in the coat protein-readthrough domain implicated in fungus transmission. Four monoclonal antibodies and one polyclonal antiserum to SBWMV have also been produced during these studies. Extensive RT-PCR, cDNA cloning, sequencing and restriction enzyme analyses refute an alleged correlation between the identity of a 600-nucleotide region of barley yellow mosaic bymovirus RNA 2 and the ability of the virus to overcome ('break') an important resistance gene (*ym4*) present in many UK and European cultivars of winter barley.

Two other furoviruses, which affect broad-leafed plants, potato and peanut, are also studied at SCRI. The complete sequence of Indian peanut clump furovirus (Hyderabad isolate; IPCV-H) RNA 1 (5843 nucleotides), and over 50% of IPCV-H RNA 2 (approx. 4000 nucleotides) have been determined. Portions of IPCV-H RNA 1 show 60-90% homology to West African peanut clump furovirus (WA-PCV) sequenced by a group at the CNRS IBMP, Strasbourg. However, the RNA 2 sequences, especially the coat protein gene, differ sufficiently for the viruses to be considered distinct furoviruses. IPCV RNA 2 (Ludhiana isolate; IPCV-L) has been almost fully sequenced (95%). IPCV-L, IPCV-H and WA-PCV RNA 2 gene sequences differ from one another by equivalent amounts and resemble the RNA 2 of potato mop top furovirus (PMTV) more than SBWMV or beet necrotic yellow vein virus. Further details can be found in the article on page 135.

Potato mop top furovirus (PMTV) is important in Scotland as an agent of spraing symptoms in potatoes. The variable size of PMTV RNA 2 molecules has been mapped by RT-PCR techniques to the coat protein-readthrough domain. A direct correlation exists between the length and integrity of this region and the efficiency of virus transmission by the powdery scab fungus, *Spongospora subterranea*. Two Scottish, five Scandinavian and one Japanese isolate of PMTV were compared in ELISA with polyclonal and monoclonal antibodies. Only the Scottish T isolate showed any serological difference. RT-PCR cloning and sequencing showed that the coat protein gene of PMTV-T also differed in some amino acids (including positions 4, 9 and 74) from eight South American isolates of the virus. Importantly, three independently transformed lines of PMTV coat protein transgenic *Nicotiana benthamiana* plants, previously reported to be highly resistant to mechanical or graft inoculation with PMTV (*Ann. Rep. 1993, 104*), were also found to be immune to virus transmitted by zoospores of the natural fungal vector, *Spongospora subterranea*. Following this novel result, we are transferring the technology to commercial PMTV-susceptible potato cultivars at risk because no natural PMTV-resistance gene is known.

A major new initiative in recombinant antibody repertoire cloning, selection and protein engineering has begun at SCRI, and is focused primarily on applica-

tions to plant pathology, plant virus detection and diagnostics. To this end, RNAs from cells expressing monoclonal antibodies (MAbs) against potato leafroll luteovirus (anti-PLRV MAbs 1, 6 and 8) were used to clone and amplify antibody gene fragments (IgG scFv fragments) in bacterial expression vectors [pDuck (from IAM Vienna) and pHen (from MRC, Cambridge)]. The 'Nissim' library (MRC) of human antibody gene variable segments (in pHen) has also been panned with plant viral antigens. Further details are presented in the article on page 136.

To further our studies on virus-plant interactions, the rapid, long-distance movement of potato leafroll virus (PLRV) through grafted stem sections in potato plants was studied using immunological assays (ELISA) and tissue printing (blotting) techniques. The results showed that neither host gene-mediated, nor PLRV coat protein transgene-mediated resistant stem grafts caused any retardation in the rate of movement of PLRV from susceptible tubers and rootstocks to upper parts of the potato plant. The effect was also independent of the number of internodes grafted. These results conflict with some earlier studies by Monsanto scientists on transgenic tobaccos, and are especially important because PLRV is confined to the plant's vascular system (phloem) from which it is spread by aphids. Additional studies on the susceptibility to PLRV of potato clones exhibiting host gene- or coat protein transgene-mediated resistance were made with an Andean isolate of the virus (EA 29) or with the Scottish isolate from which the coat transgene came. Both forms of resistance functioned against both isolates of PLRV, whose coat protein genes differed by only 2.4%. The relatively conserved nature of PLRV coat protein genes suggests that transgenic resistance will be effective against a wide range of isolates. However, the efficacy and breadth of host gene-mediated resistance is unpredictable.

In a new programme of work, 50 ecotypes (out of 300) obtained from the Nottingham *Arabidopsis* stock centre have been screened for resistance to an English strain of turnip mosaic potyvirus (TuMV, UK4), the most important viral pathogen of *Brassica* crops. Two ecotypes showed some host gene-mediated resistance.

An inducible bacterial expression vector system for producing TMV-like pseudovirus particles *in vivo* has been used to perform site-directed mutagenesis on the TMV coat protein gene. The coat protein subunits or ribonucleoprotein particles thus created are predicted to show altered binding affinities to monoclonal antibodies specific for particular protein surface features (a collaboration with the CNRS IBMC, Strasbourg). Using this bacterial system, we have also obtained the first evidence for the involvement of cellular chaperone proteins in the correct folding of plant viral coat protein subunits *in vivo*. Chaperones do not appear to be required, however, for the assembly of helical ribonucleocapsids within cells. Thus we have developed the first easily manipulated, plant virus assembly system for further related studies *in vivo*. We also expect to use this US Patented system for a variety of general biotechnological applications.

Plant viruses and biotechnology

T.M.A. Wilson, H. Barker, S.N. Chapman, B. Reavy & S. Santa Cruz

Plant virus research at SCRI has traditionally incorporated a strong biotechnological component in many programmes, both SOAFD-supported and externally funded. Broadly, these biotechnological aspects can be classified under three sub-headings:

(i) Crop plant protection by transgenesis

(ii) Plant viruses or virus-derived sequences as molecular toolboxes

(iii) Design and deployment of molecular detection and diagnostic systems

Under each sub-heading, a variety of plant, virus and vector systems are being studied directly, using authentic cultivars of the appropriate crop species, or in convenient model systems to optimize experimental parameters, to provide evidence of technical utility more rapidly, or because appropriate methods have not yet been perfected for a particular partner in the pathological triangle between virus, vector and crop plant.

Several 'plant viral technology systems' have been described in recent SCRI Annual Reports. This arti-

cle presents an update and overview of these as well as more recent, novel work with some comments about possible future applications and their commercial, intellectual or humanitarian value in providing new knowledge, a better understanding of virus-plant-vector interactions, and greater quantities of higher quality crops to meet increasing consumer demand for reduced inputs of agrochemicals, pesticides and other environmentally damaging, crop 'protectants'.

Increasingly, our competitive funding environment requires that we remain alert to strategic and applied aspects arising from our work, in order to 'realize our potential' and to secure commercial co-sponsorship. The challenge is therefore to sustain intellectual novelty, academic quality, impact and significance while exploring these new opportunities.

Crop plant protection by transgenesis:

Past and present agriculture and horticulture have relied on high health status planting material and stringent phytosanitary controls to minimize losses caused by viral pathogens. The devastating effects of failing to apply such a regime is clearly evident in many developing countries. Once a field crop is growing, host-coded resistance (R) genes, introgressed over decades of selective breeding, and/or pesticide application to control virus vectors such as aphids, nematodes or fungi, are the only lines of defence available to the grower. Unfortunately, for many devastating viruses, either no R gene has been identified or R gene-breaking strains of the virus have evolved because of the high selection pressure created through our reliance on a single R gene to protect extensive areas of susceptible crop monoculture. The detrimental environmental effects of over-use of pesticides are familiar to everyone.

How can biotechnology assist in protecting crops against viruses?

The concept of a pathogen-derived, dominant resistance 'gene' against a virus was first conceived by R.I. Hamilton in 1980, while on sabbatical leave at SCRI (personal communication). In 1986, confirmatory evidence was obtained by R.N. Beachy from work on tobacco plants which had been transformed, using the DNA-transfer mechanism from *Agrobacterium tumefaciens*, to express the coat protein (CP) gene of tobacco mosaic virus (TMV). Numerous parallel studies followed and eventually coat protein-mediated resistance (CPMR) was shown to function with greater or lesser efficiency against one or more members from each of the important plant RNA virus groups[1,2]. In the early days, only dicotyledonous plants could be transformed easily and there seemed to be a general, but not exclusive, rule that the higher the level of coat protein expressed, the better the protection provided against the homologous virus, or very closely-related viruses. Huge investments were made in this technology by most of the multi-national agrochemical and seed companies targeting all the world's major crops and their important viruses. Commercial scale releases are now planned or underway in many countries.

Since about 1990, other portions of viral genomes and even some intentionally defective or non-expressing 'gene' constructs have been shown to confer protection against the source virus. Most national environmental safety agencies and similar interest groups have tried to assess the real or imagined hazards and risks associated with large-scale releases of transgenic crops expressing plant viral sequences. SCRI produced the first transgenic model plants *(Nicotiana tabacum)* and potatoes *(Solanum tuberosum)* expressing the coat protein of potato leaf roll luteovirus in the UK[3]. The primary effect of this transgene is to reduce the accumulation of PLRV. In closely monitored field and gauzehouse release experiments, the effects of transgenesis on agronomic performance and the efficacy of CPMR against aphid-vectored spread have been assessed. The results showed that PLRV spread much less from infected transgenic potato plants than from non-transformed plants. Transgenically expressed PLRV coat protein restricted virus to the inner phloem cells but did not affect long distance virus movement within the plant. Some *S. tuberosum* cultivars contain host-coded R genes whose effects seem to mirror those of the PLRV CP transgene in transformed potato lines. Most recently, it has been shown that combining host-coded R genes with CPMR results in PLRV replication that is about 1% the level in susceptible cultivars, and demonstrates that there is an additive effect against the virus. This level of resistance could result in almost complete protection in the field. Studies in this area at SCRI have been extensive, and have focused particularly on persistent, secondary, tuber-derived infections. In the future, other non-structural or defective gene constructs from PLRV will be examined for their beneficial and potentially additive effects in interfering with virus infection.

It was not known, until recently, whether resistance was expressed against PLRV strains that are distinct from those used to obtain the transgene sequence. However, the coat protein amino acid sequences of PLRV isolates are highly conserved and experiments

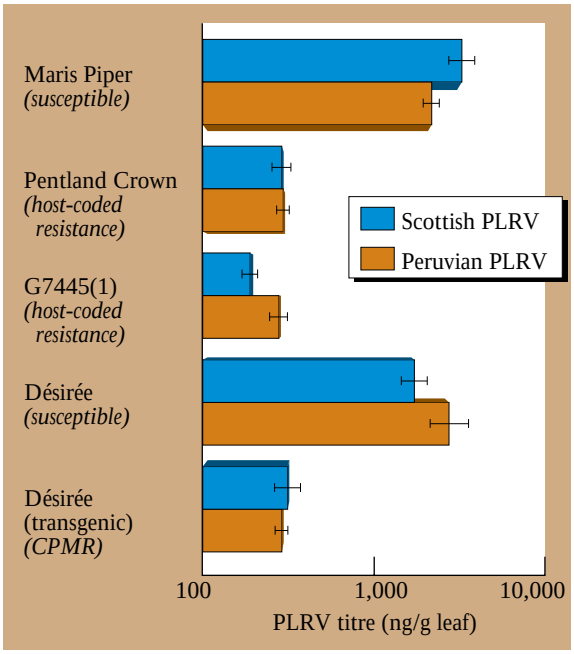


Figure 1 Host gene- and Scottish PLRV coat protein transgene-mediated resistance are equally effective against geographically distant isolates of the virus.

showed that the Scottish PLRV coat protein transgene conferred equally good resistance against an Andean isolate of the virus. It was also found that host-coded resistance bred into SCRI germplasm was effective against the Andean isolate of PLRV.

Potato mop-top furovirus (PMTV) has been studied at SCRI for many years. The complete tripartite RNA genome has been cloned and only part of RNA 1 remains to be sequenced. PMTV is transmitted persistently by the fungal pathogen *Spongospora subterranea* and causes 'spraing' symptoms in tubers of susceptible potato cultivars. There is no known host R gene against this virus. Some potato cultivars are tolerant, showing mild or no symptoms, while others are sensitive to infection and develop severe symptoms. A single-spore culture of *S. subterranea* which is able to transmit PMTV has been isolated, and transformed lines of *Nicotiana benthamiana* which express a PMTV coat protein transgene have been developed. Three of these lines are immune to mechanical and graft inoculation with PMTV (*Ann. Rep. 1993, 103*) and, more importantly, they are also immune to fungus-transmitted virus (Fig. 2). This important result augers well for effective field protection against PMTV in potato crops of several cultivars of major commercial value throughout the world for which, until now, there has been no practical method of conferring resistance.

Line	Infectivity assay * (no. infected/ no. tested) 3-4 weeks	6-8 weeks
W1	0/23	0/23
W2	0/23	0/23
W7	0/22	0/22
W16	1/18	2/18
W25	0/13	0/13
wt (control)	60/60	60/60

* Infectivity assays were performed using leaf tissue 3-4 weeks after start of fungal inoculation, and leaf and root tissue 6-8 weeks after start of fungal inoculation.

Figure 2 PMTV-S inoculation by *Spongospora subterranea* to coat protein-transgenic lines of *Nicotiana benthamiana.*

Viruses afflicting raspberry pose a serious threat to this specialized, high value crop. In recent years, problems of rapid detection, diagnosis and eradication of raspberry bushy dwarf virus (RBDV) have been exacerbated by extensive plantings of primocane cultivars and of the susceptible cv. Autumn Bliss. RBDV is transmitted by pollen and infects both the maternal plant and the progeny. There are no realistic or effective measures to prevent the spread of viruliferous pollen except early grubbing-out of affected source plants. To compound the current risk to raspberry growers who rely on planting resistant varieties to control RBDV, the natural R gene (*Bu*) which conferred resistance to RBDV has been compromised by a resistance-breaking (RB) isolate of the virus. Work at SCRI is underway between virologists, raspberry breeders and molecular biologists to transform germplasm with a variety of gene sequences from RBDV to achieve useful and durable field resistance against this threatening virus. A rapid nucleic acid-based screening method to distinguish common from RB isolates of RBDV is also being developed.

Work at SCRI and Leiden University showed several years ago that CPMR failed to operate against nematode-transmitted tobacco rattle tobravirus (TRV). The molecular mechanism responsible for this result is now under study using alternative sequences from TRV RNA to investigate their ability to confer protection against virus introduced by the natural vector. In contrast, analogous studies have shown that two nepovirus coat protein transgenes have conferred good resistance against nematode-delivered virus. Further work in this area is directed towards producing useful resistance against important viruses of woody perennial crops such as raspberry and grapevine.

In future work, it should be possible to introgress and combine multiple plant virus sequences (functional, defective or chimaeric), from one or more target viruses, into potato and soft fruit clones with native host R genes, to enhance the virus resistance of the germplasm produced in SCRI breeding programmes.

Groundnut is a vital subsistence crop throughout Africa and India and it is affected by two serious virus diseases, African groundnut rosette and Indian peanut clump, caused by an aphid-transmitted complex of two viruses and a satellite RNA, and a fungus-transmitted furovirus, respectively. Research at SCRI seeks to identify and design virus-based transgenes for crop protection by genetic engineering methods. The programmes are funded largely by the UK ODA in collaboration with ICRISAT. Non-commercial deployment of effective, pathogen-derived, dominant R genes in the appropriate germplasm is intended to improve the nutrition, security and quality of life of local farming populations.

Although at a preliminary stage, work at SCRI is also targeted to designing novel antiviral transgene strategies by expressing the active domains of bacterially cloned antibody genes which recognize and bind to essential virus-encoded proteins, either structural (coat protein) or non-structural (catalytic enzymes).

Plant viral toolbox:

Concurrent with a greater understanding of the molecular biology of viral and sub-viral plant pathogens, a number of short sequence elements have been identified and exploited as functional modules for generic applications in biotechnology. The best known examples are the highly active, constitutive promoters derived from cauliflower mosaic virus (CaMV) for RNA synthesis in plants. The CaMV 35S or 19S promoter is used in most plant DNA transformations. Self-cleaving RNA sequences from viral satellite RNA molecules are the basis for *trans*-acting ribozyme molecules or 'gene shears'; and enhancers of protein synthesis have been derived from efficiently translated genomic or sub-genomic viral RNA leader sequences (especially the TMV leader 'omega', the tobacco etch potyvirus leader and the alfalfa mosaic virus (AlMV) coat protein sub-genomic RNA 4 leader). The TMV 'omega' and AlMV RNA 4 leader sequences, which are used extensively for many applications, have been patented and are a potential source of revenue. The TMV RNA leader can enhance native or foreign messenger RNA expression in bacteria, yeast, plants and animals[4]. Another short sequence from TMV RNA signals the initiation of single-stranded RNA packaging into stable virus-like particles *in vitro*, in plants and in bacteria. The discovery that co-expression of TMV coat protein (CP) and RNA molecules with this packaging signal leads to the assembly of virus-like particles has been patented and is being exploited as a flexible and easily-manipulated presentation or purification system for peptide vaccines, antibiotics (Fig. 3) or other foreign peptides, as well as being a protective RNA storage and delivery system[5] (*Ann. Rep. 1992, 94*).

There are also three areas where plant viruses can be used as high copy number, cytoplasmic expression vectors without necessitating stable transformation of plant DNA. First, a number of full-length infectious clones of well-characterized plant viruses have been

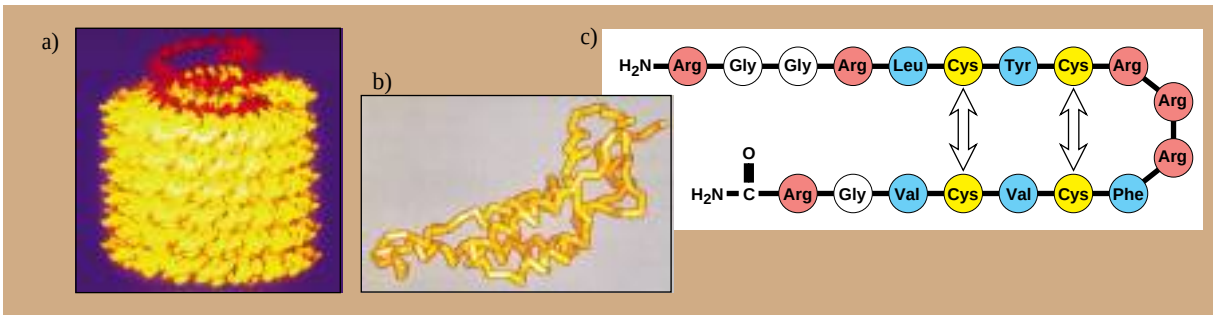


Figure 3 a) Computer graphic simulation (courtesy of E. Stubbs, Nashville,TN) of a segment of the tobacco mosaic virus particle structure (from X-ray fibre diffraction data) showing the single-stranded RNA (red) and the helical array of identical coat protein subunits (yellow).
b) Ribbon diagram of the polypeptide backbone of a single TMV coat protein subunit, tilted as in the virus. The free amino- and carboxy- termini are located close together on the outside of the virion surface, to which we can attach foreign peptides through genetic manipulation.
c) a schematic diagram of an antibiotic peptide (protegrin) which can be cloned onto the N- or C- terminus of each TMV coat protein subunit.

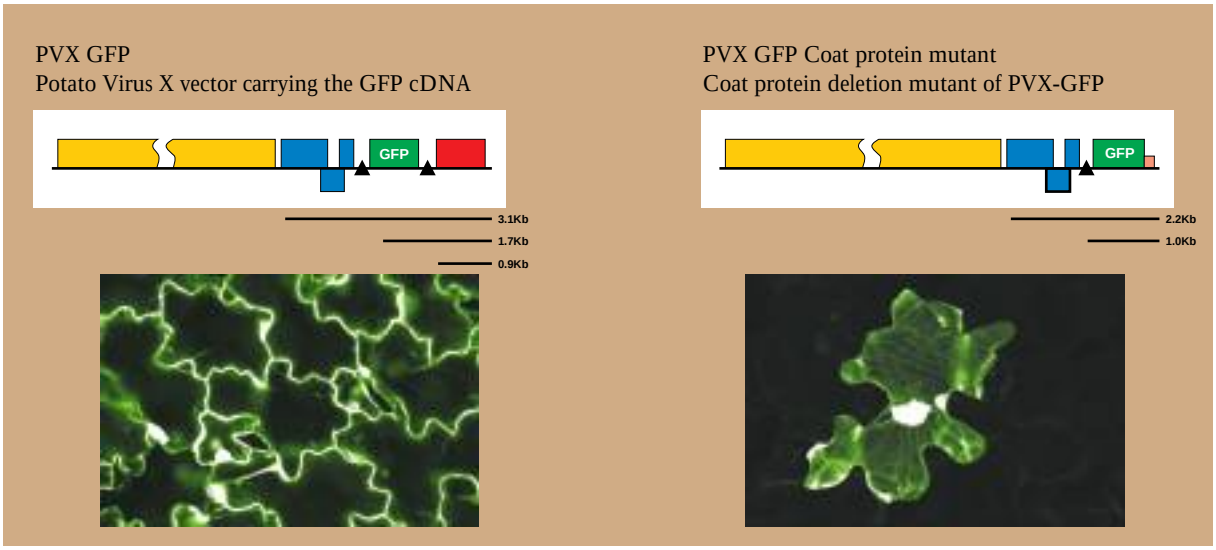


Figure 4 Schematic genetic maps of potato virus X vectors containing the jellyfish green fluorescent protein gene (GFP) and either an intact (left) or dysfunctional (right) PVX coat protein gene. The effects of infection by these PVX-GFP vectors on epidermal cells of tobacco shows that the functional coat protein is required for effective cell-to-cell movement (left) from the primarily infected single cell. Defective CP limits PVX-GFP to the primary site (cell) of infection (right). The sizes and positions of PVX-derived sub-genomic RNAs are also shown.

modified to carry and express a convenient foreign reporter gene. Such chimaeric viruses can be used to observe virus movement directly inside infected plants (Fig. 4). Currently, the levels of gene expression and stability of modified genomes of potato virus X, TMV and some other viruses which contain a green fluorescent protein (GFP) reporter gene from a jellyfish are being optimized. GFP can be detected by non-invasive techniques, in real time, at the sub-cellular level or in whole plant tissues using the confocal laser scanning microscope or conventional epifluorescence. The behaviour of a reporter gene-tagged virus mutated in one of its own genes can be compared with non-mutant tagged virus to unravel the functions of viral genes *in vivo*. For example, GFP-tagged PVX (Fig. 4) with a mutation in its coat protein gene failed to move from cell-to-cell, directly confirming an hypothesis that there is a requirement for more than the viral 'movement proteins' in this process.

Second, as an alternative to conventional, stable DNA transformation techniques, plant virus-based gene delivery vectors can direct high levels of foreign protein expression. This allows genes to be screened for function far more rapidly than is possible using conventional transformation procedures.

Finally, the extremely high level of expression possible with some virus vectors allows synthesis and accumulation of valuable proteins or CP-peptide fusions in commercial quantities. This offers non-food applications of crops as bioreactors to produce biomedical therapeutics or diagnostics. At present, several rod-shaped viruses are being used as infectious vehicles to express antibiotic peptides, as an alternative to the non-replicative bacterial system described above.

Diagnostics

Resistance breaking (RB) isolates of both RBDV and barley yellow mosaic virus (BaYMV) must be distinguished from their more benign forms to assist in phytosanitary control. Because they are serologically indistinguishable, work on the common or RB strains of both viruses involves reverse transcriptase-polymerase chain reaction (RT-PCT) methods, followed by DNA fingerprinting. RBDV has been mentioned already. BaYMV threatens winter-sown cereals, especially in northern Europe. In collaboration with IACR Rothamsted, Plant Breeding International, Cambridge and the Max Planck Institute, Cologne, several regions of the genome have been sequenced and fingerprinted in attempts to provide a reliable diagnostic marker.

The incidence of soil-borne wheat mosaic virus (SBWMV), transmitted to winter cereals by a soil fungus, will be examined in the south of England using several SBWMV-specific monoclonal antibodies made at SCRI. The ability of two of our MAbs to distinguish between strains of SBWMV has been mapped by sequencing to a single amino acid change near the amino-terminus of the coat protein (Glycine$_6$ →

Serine$_6$), a region predicted to lie on the outer surface of the virus (G.J. Stubbs, Nashville, TN. Pers. comm.). Further biotechnological advances in plant virus detection and diagnosis using recombinant antibodies and cloned fragments of antibodies are described below.

Potato virus Y is common in post-harvest stored tubers and accurate VT certification relies upon efficient sampling and virus detection methods. Although still infected and able to produce infected shoots, stored tubers lose their PVY-specific molecular signals, thus acting as foci of infection in the progeny crop. The sensitivity of the triple-antibody sandwich (TAS) ELISA method for PVY, using four different substrate systems, has been compared systematically using leaf or tuber saps. The most sensitive TAS ELISA method (using a fluorescent chemical) detected 3 ng PVY per ml leaf sap and 9 ng PVY per ml tuber sap, while standard RT-PCR techniques were 10x less sensitive. Further studies and sampling strategies are essential to increase the reliability of virus-free seed potato stocks.

References

1 Wilson, T.M.A. (1993). *Proceedings of the National Academy of Sciences USA* 90, 3134-3141.

2 Fitchen, J.H. & Beachy, R.N. (1993). *Annual Review of Microbiology* 47, 739-763.

3 Barker, H., Reavy, B., Kumar, A., Webster, K.D. & Mayo, M.A. (1992). *Annals of Applied Biology* 120, 55-64.

4 Wilson, T.M.A., Saunders, K., Dowson-Day, M.J., Sleat, D.E., Trachsel, H. and Mundry, K.W. (1990). *NATO ASI Series* 49, 261-275.

5 Hwang, D-J., Roberts, I.M. & Wilson, T.M.A. (1994). *Proceedings of the National Academy of Sciences USA* 91, 9067-9071.

New immunological reagents from recombinant DNA technology

L. Torrance, M.A. Mayo, A. Ziegler, G. Himmler[1] & F. Rüecker[1]

Antibodies play an important part in the detection and diagnosis of virus diseases as well as in the characterisation and classification of viruses. Work at SCRI over many years has resulted in the establishment of a collection of more than 200 antisera and monoclonal antibodies specific for many of the viruses that induce economically important diseases of potato, soft fruit, bulbous ornamentals, cereals, grasses and tropical crops such as cassava and groundnuts. Recent work with monoclonal antibodies has provided knowledge of the antigenic structure of virus particles, the identity of surface-located amino acid residues, and the roles of capsid proteins in the transmission of viruses by vectors.

Monoclonal antibodies are produced from mammalian cell cultures and all of the antibody molecules in a preparation have identical binding specificities and affinities. Although the application of monoclonal antibodies has provided useful results, the techniques for their production and maintenance are expensive, inefficient and time consuming. In recent years, alternative reagents have been devised which are

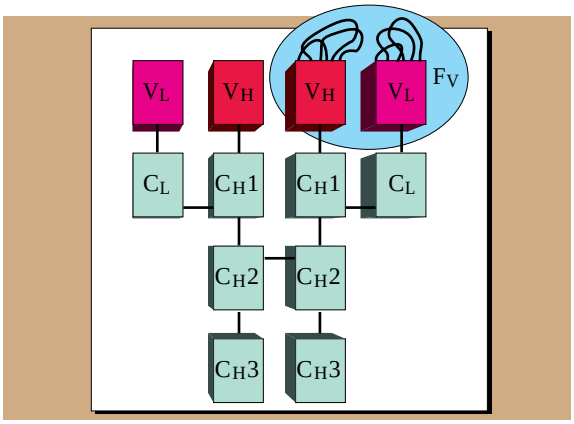


Figure 1 Diagram of IgG molecule. The loops at the tip of the V_H and V_L domains represent the complementarity determining regions.

[1]Institute of Applied Microbiology, University of Agriculture and Forestry, Vienna, Austria.

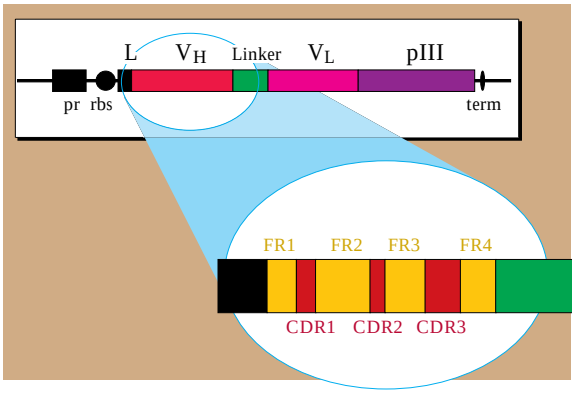


Figure 2 Structure of scFv gene. The framework (FR) and complementarity determining regions (CDRs) of the V_H gene are highlighted.

produced by cloning fragments of antibody genes and expressing them in bacterial cells. The potential application of these reagents in plant virology is now being explored.

Structure of antibodies

Antibody molecules are composed of different functional domains (Fig.1). Immunoglobulin G (IgG; the most common serum globulin), has two identical heavy chains consisting of one variable (V_H) and three constant (C_H1, C_H2, C_H3) domains, and two light chains, each with one variable (V_L) and one constant (C_L) domain. Different antibodies differ principally in the V_H and V_L domains and these regions determine the specificity of binding to antigen. Most variation is concentrated in three regions called CDRs (complementarity determining regions) and they are located at the tips of the Fv region.

Recombinant DNA technology

Using appropriately designed primers, the V_H and V_L fragments encoding the CDR and framework regions of the Fv can be amplified in a polymerase chain reaction (PCR) and the resulting cDNA is cloned into an appropriate vector (Fig. 2). In the vector, the V_H and V_L genes are linked by DNA encoding a short peptide. Expression of the linked cDNAs produces a single chain Fv (scFv) molecule. The scFv is cloned so that when expressed in an appropriate strain of *Escherichia coli*, it will be produced either as part of a fusion protein with the product of gene III

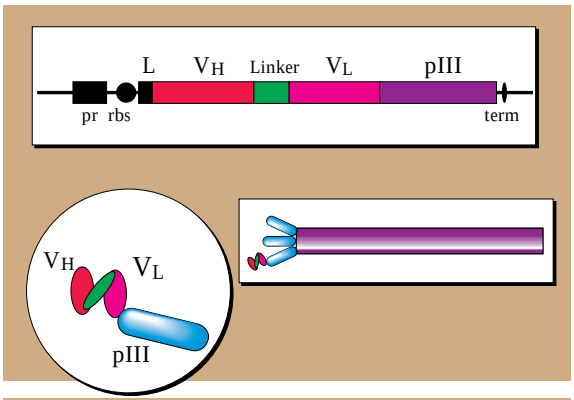


Figure 3 Expression of scFv molecules on phage.

(the minor coat protein pIII of a filamentous phage, phage-display, Fig. 3), or as a soluble molecule. Because antibody fragments are displayed on the surface of infective bacteriophage, it is possible to select and clone an individual phage-antibody from a large population, or combinatorial library of phage-antibody genes. Furthermore, it is possible to change the binding affinities and specificities of scFv fragments using techniques such as site-specific mutagenesis and the exchange of V_H and V_L segments between different scFv molecules (chain shuffling).

Phage-antibodies against potato leafroll virus (PLRV)

We have cloned antibody genes from existing hybridomas that secrete monoclonal antibodies codenamed SCR 6 and SCR 8. The nucleotide sequences of the V_H and V_L domains have been determined, and comparisons of the nucleotide and amino acid sequences with a database of V gene sequences showed that they contain variable and constant (framework) regions that are typical of the variable region of antibody genes. The comparisons revealed that SCR 6 has a long CDR 1 in the light chain (Fig. 4), that the V_H genes of SCR 6 and SCR 8 belong to the same V gene family, and that they were more similar (89% identity) than the V_L genes (62% identity). Most of the variability between the V_H genes was in the CDRs, whereas the V_L genes varied in the CDRs and in framework regions 1, 2 & 3, indicating that they belong to different V_L gene families. These differences in sequence reflect the

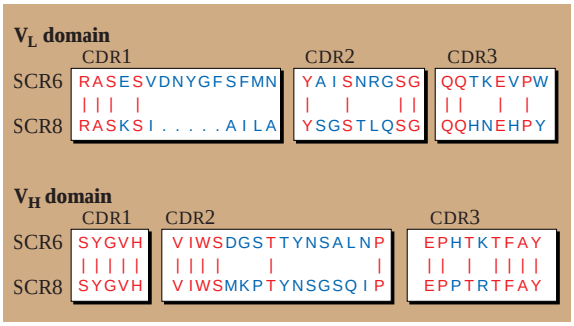


Figure 4 Comparison of the amino acid sequences of the CDR regions of SCR 6 and SCR 8.

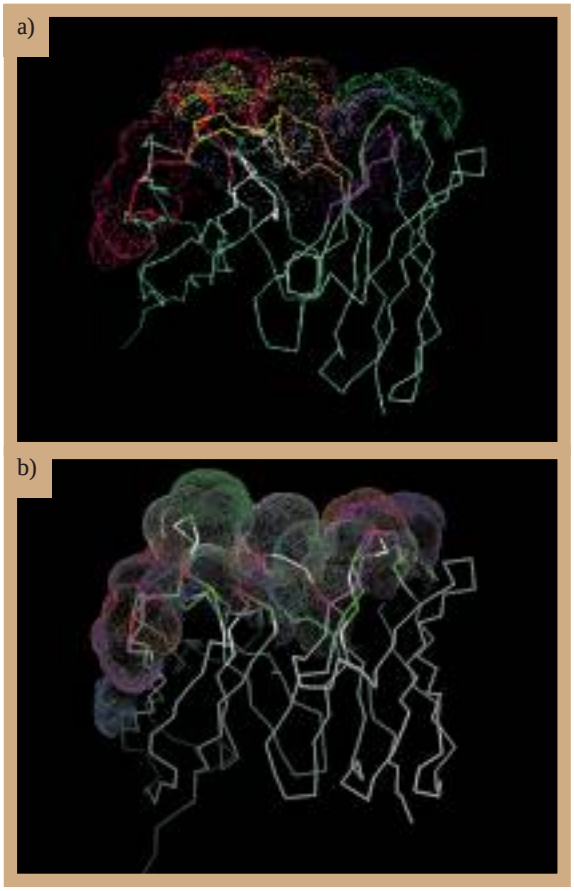


Figure 5 Models of SCR 6Fv. The amino acids of the CDRs are represented as space filling models whereas only the α-carbon backbone of the framework regions are shown.
a) V_L domain: CDR1 = red; CDR2 = yellow; CDR3 = white. V_H domain: CDR1 = green; CDR2 = purple; CDR3 = brown.
b) The amino acids constituting the CDRs are coloured according to the properties of their side chains.
Red = negatively charged; blue = positively charged; green = hydrophobic; purple = hydroxyl group; white = others.

differences found in biological activity, since although these two antibodies react with PLRV, SCR 6 reacts with a poorly aphid-transmissible isolate of PLRV, whereas SCR 8 does not.

The V genes of SCR6 and SCR 8 were assembled and cloned into the pDUCK vector and expressed on phage. The phage preparations readily detected PLRV particles in ELISA, and there were no non-specific reactions with the other viruses or peptides tested. Soluble scFv, produced in *E. coli* were shown to have the expected molecular weight in immunoblots.

Computer programmes to predict protein structure are based on algorithms that take account of structures obtained by X-ray crystallography and amino acid sequences of molecules. There are reasonably large databases of such information for antibodies and consequently the programmes can produce quite accurate models. The sequence data of SCR 6 and SCR 8 were analysed by an antibody structure prediction programme (AbM, Oxford Molecular). Figure 5a shows the predicted structure for the binding site of SCR 6 to be a planar surface and the CDR1 of V_L appears to extend away from the main binding site down one side of the molecule. Figure 5b shows the same view with the different amino acids that make up the CDRs coloured according to the properties of their side chains. Similar models of antibody binding sites will be used to predict the consequences of changes to the binding site by substitution of different amino acid residues.

Future prospects

Monoclonal antibodies are sensitive and specific tools that can be used to help answer questions in both fundamental and applied plant pathology. In the future, selection of scFv fragments from antibody gene libraries has the potential to increase the efficiency of production of useful antibodies, to decrease costs, and to provide a way of making antibodies with novel specificities that are difficult to produce by conventional methods.

Cloned antibodies may be used to produce: 1. reagents with modified binding specificities tailored to particular needs; 2. cheap standardised reagents for disease detection; 3. genetically engineered crop plants to develop novel forms of resistance to plant disease; 4. recombinant antibodies selected from large expression libraries which may eventually replace conventional methods of antibody production involving animal immunisation, and culture of hybridoma cells. Current research is pursuing each of these objectives.

Investigation of the viral determinants of nematode transmission of pea early browning virus

S.A. MacFarlane, D.J.F. Brown, M.M. Swanson, J.F. Bol & D.J. Robinson

Tobraviruses, which include pea early browning virus (PEBV), tobacco rattle virus (TRV) and pepper ringspot virus (PRV), infect a wide range of plants including many common weed species. The viruses become important when they move from weed species and infect cultivated crops such as pea, in eastern England, and potato, in Scotland. The transmission of tobraviruses from weeds to crops, and within fields of crop plants, is mediated by ectoparasitic nematodes belonging to the family Trichodoridae. These nematodes feed on the roots of plants by puncturing the epidermis with a stylet, injecting saliva into the plant and pumping the contents of damaged plant cells back into the nematode digestive system. Using the electron microscope tobravirus particles have been seen attached to the inner surface of the nematode oesophagus and, during feeding, the virus is transferred into the root system through the stylet, together within nematode secretory products. Nematodes denied access to plants, and therefore unable to reacquire virus, have been shown to retain viable TRV for as long as 3 years. The extreme persistence of the viruses, combined with their maintenance in wild plants in cultivated fields, makes effective control of them very difficult.

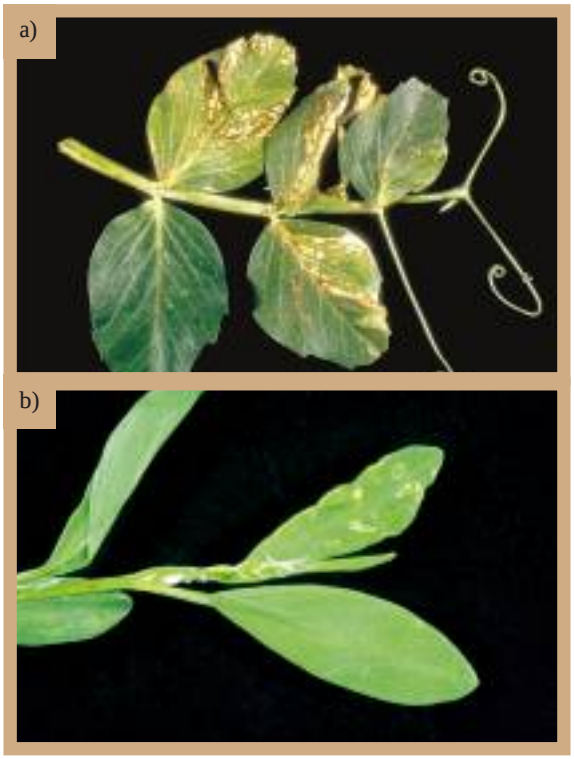


Figure 1 a) Field grown pea infected with PEBV. b) Redshank (*Polygonum* sp.) infected with TRV.

Studies on TRV at SCRI have shown that the interaction between the virus and its vector nematode is highly specific. Generally, different tobraviruses are transmitted by different species of nematode and, as an example, the nematode *Paratrichodorus pachydermus* is only able to transmit isolates of TRV belonging to a single serotype [1,2]. The aim of our work is to discover the virus and nematode determinants which are involved in the interaction between them, and to understand how the specificity of the interaction is maintained. This knowledge will enable the rational design of control strategies which are precisely targeted at the virus and its nematode vector. Such an approach will overcome some of the environmental problems associated with current methods of control that employ highly toxic but relatively non-specific chemical treatments.

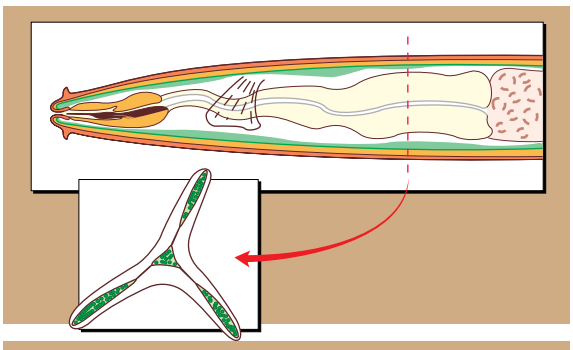


Figure 2 Mouthparts of a Trichodorid nematode and (inset) oesophageal region of *Paratrichodorus pachydermus* showing particles of TRV.

Recent work has focused on the transmission of PEBV by its vector nematode *Trichodorus primitivus*. We have constructed full-length cDNA clones of both genomic RNAs (1 and 2) of the virus, which enable us to synthesize viral RNA *in vitro* for infectivity testing on plants and transmission testing with nematodes.

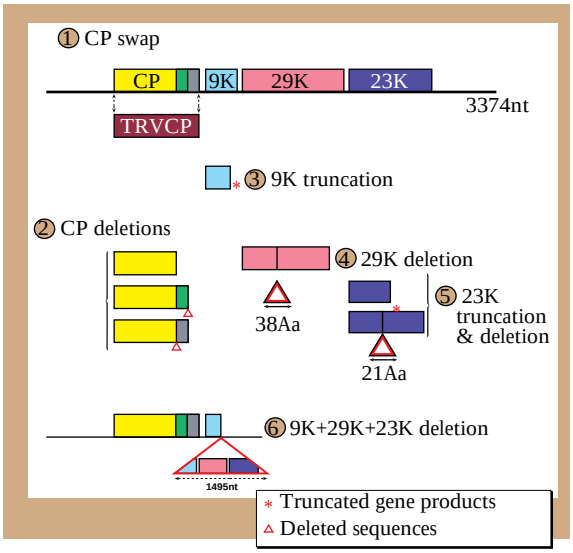


Figure 3 Genome Organisation and Location of Mutations introduced into PEBV RNA2

Studies have progressed in three directions: 1) construction of a PEBV/TRV hybrid, 2) sequencing of different isolates of PEBV, 3) mutagenesis of a transmissible clone of PEBV.

Initial studies resulted in the sequencing and construction of infectious clones of the SP5 isolate of PEBV, which is the type isolate of this virus and which has been maintained under glasshouse conditions since its isolation in 1964. Originally, SP5 was shown to be transmitted by the nematode *Trichodorus primitivus*. However, current stocks of this isolate and virus derived from cDNA clones of this isolate cannot be transmitted by nematodes although the virus is fully infectious following mechanical inoculation to plants. This situation probably has arisen by the introduction of one or more mutations into the virus genome during the many years of glasshouse propagation, as has been reported also in several other virus/vector systems. We replaced the coat protein gene from the SP5 clone with that from the PPK20 isolate of TRV, which is transmitted by *P. pachydermus* at high frequency. Although this hybrid virus was able to infect plants and form virus particles, it was not transmitted by either *T. primitivus* or *P. pachydermus*. This result suggested that the non-transmission of PEBV SP5 was not the result of a mutation in the virus coat protein and that at least one other virus gene is involved in nematode transmission.

Recently we constructed a cDNA clone for the smaller RNA (RNA2) of a new isolate (TPA56) of PEBV. Virus containing RNA2 derived from this clone together with RNA1 from the cDNA clone of the SP5 isolate was transmitted by *T. primitivus*, showing that the mutation responsible for preventing transmission of the SP5 isolate is located within RNA2. Sequencing of the TPA56 cDNA clone revealed eleven nucleotide differences from the SP5 clone. However, only three of the base changes result in an alteration of the amino acid sequence of any of the virus gene products. A single change occurs in the virus coat protein which, however, introduced a chemically similar residue that would not be expected to affect the physical properties of the coat protein. The two other amino acid changes both occurred in the gene encoding a 29K non-structural protein and are probably responsible for the lack of transmission of the SP5 isolate.

In addition to the genes for the virus coat protein and the 29K protein, both of which are known to be expressed in infected plants, PEBV RNA2 carries two other open reading frames (ORFs) which encode a 9K and a 23K protein. However, neither of these proteins has yet been detected in virus-infected plants and codon analysis has suggested that the 9K ORF might not be expressed. Using restriction enzyme digestion and oligonucleotide site-directed mutagenesis we have introduced mutations separately into each of the genes, and tested the mutants for transmission by *T. primitivus*. A mutant which retained the coat protein gene but lacked all three of the other genes was not transmitted. This is the first direct demonstration that genes in addition to the coat protein gene are required for nematode transmission of a plant virus. A mutant producing an internally deleted 29K protein also was not transmissible, which corroborates the conclusions drawn from the sequencing experiments described above. A mutant which produced a truncated 23K protein was not transmitted, although on one occasion a mutant producing an internally deleted 23K protein was transmitted. In the latter mutant only 21 amino acids were deleted from the protein and it may retain some of its activity. A mutant which alters the reading frame of the 9K ORF and would produce a truncated protein was transmitted, but at a much reduced frequency. This finding is surprising in view of the doubts concerning the expression of this ORF.

Work at SCRI on TRV and PRV showed that a region at the carboxy terminus of the coat proteins of these viruses is highly immunogenic, mobile and is exposed at the outer surface of the virus particle [3,4]. These features make this region a good candidate for maintaining an interaction with other molecules, perhaps nematode or other viral proteins, and offers the

possibility that this part of the virus coat protein is involved in nematode transmission. To test this hypothesis we made three mutants, which were deleted for 28 amino acids at the carboxy terminus of the coat protein, or for the first 15 amino acids, or the last 12 amino acids from this region. The mutant which was deleted for the entire carboxy terminal region was unable to form virus particles, while the two mutants which were partially deleted for the carboxy terminal region were able to form virus particles and are currently being tested for nematode transmission.

We are also currently preparing antisera to the three non-structural proteins encoded by PEBV RNA2, which will allow us to determine the localisation of the proteins in infected plants and perhaps also in the mouthparts of the nematode. We will then screen nematode cDNA expression libraries and 2-D blots for nematode proteins which bind to the virus transmission proteins. This work will be complemented by the construction of more PEBV-TRV hybrids which will identify which combination of virus proteins is responsible for maintaining the specificity of the interaction between the virus and its vector nematode.

References.

1 Ploeg, A.T., Brown, D.J.F., & Robinson, D.J. (1992). *Neth. J. Plant Path.* 98, 291-300.

2 Ploeg, A.T., Brown, D.J.F., & Robinson, D.J. (1992). *Ann. Appl. Biol.* 121, 619-630.

3 Mayo, M.A., Brierley, K.M., & Goodman, B.A. (1993). *Biochimie.* 75, 639-644.

4 Legorburu, F.J. (1993). PhD. Thesis, University of Dundee.

Development of potential control measures for Indian peanut clump virus from molecular biological studies

J.S. Miller, S.V. Wesley[1], R.A. Naidu[1], D.V.R. Reddy[1] & M.A. Mayo

Groundnut (*Arachis hypogaea* L.) is grown in semi-arid regions throughout the world in which the resulting peanut crop is an important source of both oil and protein. Groundnut is one of the mandate crops of the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) which has a centre in India. One of the diseases which significantly constrains the growing of groundnut crops in India is clump disease which is induced by Indian peanut clump virus (IPCV). Infection causes the plants to become bushy and stunted and the leaves to turn a darker green. The symptoms can be very severe if a plant is infected in early stages of its growth (Fig. 1), and then the yield of peanuts is drastically decreased. The virus is transmitted by the soil-inhabiting fungus *Polymyxa graminis* which can persist in soil for several years. The disease is a serious problem because there are no effective control measures against the fungus and no source of resistance was found when over 10 000 lines of groundnut germplasm were screened at ICRISAT. A further complication is that diagnosis of the disease by standard serological tests is uncertain

Figure 1 The effect on groundnut plants of early infection by IPCV. Photograph taken in February near Ludhiana, Punjab. The plant in the centre is healthy, those on either side have clump disease. There are few or no young peanuts among the roots of the diseased plants.

[1]ICRISAT Asia Center, Patancheru, Andhra Pradesh, India

Figure 2 Map of India showing the approximate origins of the Hyderabad (H), the Ludhiana (L) and the Talod (T) isolates.

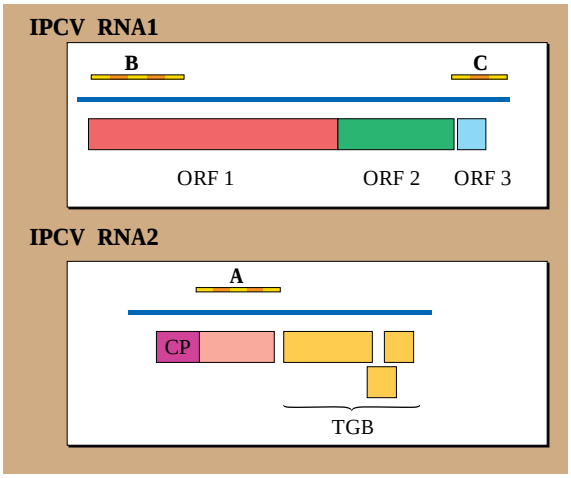


Figure 3 Diagram of the genome of IPCV. The solid lines represent the two RNA species, the open boxes indicate the open reading frames (ORF) in the RNA sequences and the shaded bars indicate the region of the sequences represented in the probes refered to in Table 2. CP indicates the ORF encoding the coat protein, TGB indicates the three ORFs that make up the 'triple gene block'.

because IPCV is known to occur as three distinct serotypes which exist in geographically separate areas of India. These are typified by the Ludhiana (L) isolate from Punjab, the Talod (T) isolate from Rajasthan and the Hyderabad (H) isolate from Andhra Pradesh (Fig. 2).

Early work in the 1980's on the virus demonstrated the extent of its serological variation and also resulted in sufficient biochemical characterisation for IPCV to be classified into what is now the genus *Furovirus*. More recently, work has centred on the molecular biology of IPCV. The genome of IPCV comprises two RNA molecules, RNA-1 and RNA-2, and the results can be conveniently described for each RNA in turn.

RNA-2

The region of the genome which encodes the virus coat protein (CP) was located at the 5'-most open reading frame (ORF) of RNA-2 (Fig. 3). The nucleotide sequence of the coat protein gene was determined and a cDNA copy of it was inserted into a plant transformation vector. To test the potential of this vector for making groundnuts resistant to IPCV, it was transferred to *Agrobacterium tumefaciens* which was then used to transform tissue from *Nicotiana benthamiana* plants. Regenerated plants have been obtained which express IPCV coat protein and resistance tests are currently underway.

In RNA-2, the coat protein gene is followed by four other ORFs (Fig. 3). The three ORFs at the 3'-end form a 'triple gene block' of overlapping genes (TGB). A similar arrangement of genes is found in RNA of a number of viruses and the encoded proteins are believed to be involved in the spread of virus infection between cells.

RNA-1

The nucleotide sequence of RNA-1 showed that it contains three ORFs (Fig. 3). Comparisons with the sequences of other virus proteins suggest that ORF 1 encodes a protein with methyl-transferase and helicase-like activities, and that ORF 2 encodes an RNA-dependent RNA polymerase. As with several other viruses, the 'polymerase' gene is probably expressed when ribosomes ignore the termination codon of ORF 1 to produce a protein comprising the translation products of ORF 1 and ORF 2 fused together. The protein encoded by ORF 3 does not contain any motifs which might suggest a function.

An alternative to inducing resistance by transformation with virus coat protein genes is to use sequences from the virus polymerase gene. Work is in progress to construct a transformation vector containing ORF 2 sequences to test this possibility for obtaining resistance to IPCV infection.

Virus*	ORF† 1	2	3	CP
PCV	88	95	75	59
SBWMV	38	56	33	18
IPCV-L	-	-	-	65
BSMV	-	-	-	34

†see Fig. 3.
*PCV : peanut clump virus; SBWMV : soil-borne wheat mosaic; IPCV-L : Ludhiana serotype of IPCV; BSMV : barley stripe mosaic virus

Table 1 Percentage sequence identity of proteins encoded by IPCV-H RNA with corresponding proteins of other viruses.

Sequence comparisons with other viruses

The availability of the sequences of functionally equivalent proteins from different viruses makes it possible to infer relationships between the viruses by measuring the percentage identity between pairs of sequences. Table 1 shows the results of some such comparisons. The translation products of ORFs 1, 2 and 3 of IPCV RNA-1 were relatively similar (between 75% and 95% of amino acids being identical) to the corresponding proteins encoded by RNA-1 of peanut clump virus (PCV) which is biologically similar to IPCV but which occurs in West Africa. The RNA-1 translation products are also similar (33-56% amino acids identical) to corresponding proteins encoded by RNA-1 of soil-borne wheat mosaic virus (SBWMV), which is the type species of the genus *Furovirus*. However, there was no significant similarity between the sequences of the coat proteins of IPCV and SBWMV. Also, the coat proteins of the H and L isolates of IPCV were identical in only 65% of the amino acids, which is close to the value of 59% found between the coat proteins of IPCV-H and PCV. Unexpectedly, the coat protein of IPCV-H was significantly similar in sequence to the coat protein of barley stripe mosaic virus, although this virus is not fungus-transmitted and belongs to a different genus (genus *Hordeivirus*).

Diagnosis of IPCV infection

Serological variation between isolates of IPCV is a reflection of the variation between the sequences of the coat proteins and has caused difficulties in the efficient detection and diagnosis of IPCV infections in the field. However, comparisons among nucleotide sequences of RNA of different isolates of IPCV and of

Virus	Probe * A	B	C
IPCV-H	+	+	+++
IPCV-L	–	–	+++
IPCV-T	+/–	–	+++
PCV	–	–	+++

†see Fig. 3.

Table 2 Reaction of probes of IPCV-H cDNA in dot blot assays.

PCV suggested that assays based on hybridization between RNA extracted from infected plants and cloned cDNA could have a broader specificity than serological assays. Table 2 shows that, of the three cDNA probes tested, which corresponded to different regions of the genome of IPCV (Fig. 3), only that corresponding to the 3'-terminus of RNA-1 readily detected all three IPCV isolates and PCV. This probe reacted with both RNA-1 and RNA-2 of IPCV-H, which may account for the strong reactions observed.

Another outcome of this sequencing work is that it makes possible the use of an assay based on reverse transcription of virus RNA followed by polymerase chain reaction amplification of the resulting cDNA for very sensitive detection of infection by IPCV. Moreover, by using oligonucleotide primers which contain, or are complementary to, sequences identical in the RNA-1 molecules of H-IPCV and PCV, it may prove possible to detect a wide range of isolates with great sensitivity. The DNA produced would identify infected plants and, by its sequence, could also be used to identify the isolate present.

Conclusions

The work on the molecular characterization of IPCV illustrates how, with technologies currently available, it is possible to develop strategies for the control of a troublesome pathogen. Methods are now in place which should yield a resistance gene for incorporating into groundnut germplasm and will allow sensitive and effective methods of diagnosing infection by all serotypes of IPCV. A further unexpected outcome of the work has been the detection of apparent relationships between IPCV and other viruses which will be useful for taxonomists seeking to rationalize affinities among furoviruses.

Nematode & Insect Pests

David L. Trudgill

Consumers throughout Europe have benefited from agricultural research through increases in agricultural productivity and decreases in 'farm gate' food costs. However, there is increasing awareness of possible damage to the environment due to modern agricultural practices. Consequently, current research is involved in developing new methods of pest management to minimise dependence on synthetic pesticides. The application of molecular techniques as diagnostic tools, in population genetic studies and for targeting new pest and plant genes hold great promise for progress in many areas of our research. These new approaches need to be integrated with crop production and require knowledge of the biology, physiology and ecology of the pests and their host plants. To achieve this requires integration of a range of skills from molecular biologists to ecologists and laboratory to field studies. It also requires greater collaboration, both with colleagues within the UK and internationally, which has been assisted by the award of external grants and contracts.

Research on the mechanisms of plant resistance to nematodes, insects and mites and related studies on the management of pest-resistant crop varieties to minimise selection of virulent pest populations is a priority area. Nematode studies are focussed on two species of potato cyst nematodes (PCN), introduced with potatoes from South America into many countries. Major gene resistance to the yellow species (*Globodera rostochiensis*) was found some years ago and has been incorporated into many cultivars. Initially, resistant cultivars were used in combination with very toxic, systemic nematicides to enable potato production to continue on infested land. However, the widespread use of resistant cultivars has progressively favoured the incidence of the white species of PCN (*G. pallida*) which is more virulent and difficult to control than the yellow species. Sources of resistance to *G. pallida* have been found in wild *Solanum* spp. but they are polygenic and the progeny of crosses with commercial cultivars of potato are only partially resistant. Molecular marker studies have revealed that *G. pallida* is genetically more variable than *G. rostochiensis* and that there have been at least three different gene pools introduced into the UK resulting in a wide range of virulence on partially resistant potatoes. *G. pallida* also has a more prolonged period of hatching than *G. rostochiensis* and hence is more difficult to control with nematicides. To cope with these problems an expert system for the integrated management of *G. pallida* could be developed based on data from a

New Zealand Flatworm *(Artioposthia triangulata)*

combination of laboratory, glasshouse and field trials. These trials led to the development of mathematical equations which describe the population dynamics of *G. pallida* and the effects of various control measures and environmental factors on potato yield.

Prospects for integrated pest management in raspberry crops have been investigated in collaboration with ecologists at St Andrews University. In an experimental plantation where insecticides had never been used, a range of parasitoids and predators decreased the numbers of the large raspberry aphid, *Amphorophora idaei*, but did not consistently prevent re-colonisation. No effective natural enemies were found for raspberry beetle, *Byturus tomentosus*, but infestations were reduced by increasing cane densities. Olfactometer tests showed that the beetles were strongly attracted to volatile compounds produced by raspberry flowers and, in collaboration with IACR-Rothamsted, these compounds have been identified in ether extracts of raspberry flowers and their efficacy in attracting *B. tomentosus* to lures is being tested in infested crops.

Various recognition and biochemical interaction processes are initiated in plants attacked by nematodes and insects. In collaboration with Glasgow University, we are studying PCN gland-cell secretions that initiate the changes in the root in susceptible potatoes on which the developing nematode relies to become adult. These secretions probably also elicit the resistant response in resistant cultivars. Plant products which stimulate secretion of these gland cell secretions *in vitro* have been identified. Transgenic plants which incorporate a “reporter gene” are being used in a collaborative project throughout Europe to identify at nematode feeding sites plant genes, and their promoters whose expression is modified.

Similar collaborative studies with the John Innes Centre are in progress on the genetics and chemical mechanisms of plant resistance, host interactions and chemoreception of host stimuli in the cabbage and turnip root flies (*Delia radicum, D. floralis*). DNA mapping techniques are being used to localise root-fly resistance genes in brassicas and to provide selectable molecular markers to accelerate breeding for insect resistance. In further cooperative work, the key chemical factors involved in recognition and behavioural selection by root flies of susceptible hosts have been characterised and a novel primary oviposition stimulus has been identified in leaf surface extracts. Damage to brassica roots caused by root fly larva can induce substantial compensatory root and shoot re-growth and shifts in host plant primary and secondary metabolism for up to 4 weeks after initial attack. These effects show how insects can manipulate their hosts to optimise their own development and also how damage-induced chemical signals from host plants are important in subsequent pest attacks.

Ecological studies are aimed at understanding complex interactions between different trophic levels and the impact of agricultural practices on populations of pests and beneficial organisms in natural and managed habitats. The spread of the New Zealand flatworm, a predator of earthworms recently introduced in the U.K., was monitored during 1994. There were over 200 new records from throughout Scotland and 20 new records from England ranging from the north to as far south as Dorset. Results from ecoclimatic data suggest that the flatworm could infest north-western Europe, parts of North and South America, Australia and Japan.

A MAFF-funded study is monitoring the changes in the nematode fauna of land under "set aside". Nematodes have been chosen because they are a particularly useful group of organisms in soil ecological studies. They are relatively easy to extract, identify and count; they comprise a range of trophic groups including bacterial, fungal and plant feeders; their rates of population change can be inferred from their population structure; and they provide specific indicators of habitat stability and vegetation types. Also, nematodes are relatively immobile so their species composition reflects previous conditions. In a related area of study ratios of naturally occurring stable isotopes of nitrogen in the soil fauna are being analysed as a means of elucidating food webs. As a precursor to studying terrestrial food webs, geostatistical techniques are being used to determine the spatial distribution of ^{13}C and ^{15}N in an upland pasture soil. Small scale fluctuations in isotope composition have allowed optimal sampling procedures for food web studies to be devised. Stable isotope ratios have also been used to study the effects of trophic level and an insect's metabolic state in *Amphorophora idaei* and *Byturus tomentosus*, together with two species of predatory ladybirds feeding on raspberry aphids. The ^{15}N and ^{13}C isotope levels in raspberry aphids and early instars of the raspberry beetle were similar to those of their host plant tissue, whilst levels in the aphid predator ladybirds were elevated compared with their prey. However, adult raspberry beetles that had recently emerged from overwintering sites in the soil had unexpectedly high ^{15}N levels compared with larvae or young adults that had just completed metamorphosis. These findings indicate that extensive amino acid recycling occurs during prolonged fasting in overwintering beetles.

Ecological studies are developing and using mathematical models as a basis for understanding developmental biology and to formulate future control strategies for pests. Thermal time relationships have been applied to nematodes and show that an inverse relationship exists between base temperature and the day-degree requirement (the thermal constant) for development. Tropical *Meloidogyne* spp. have higher base temperatures and lower thermal constants than comparable temperate species. Also, major differences in thermal constants have been shown between different trophic groups, indicating major differences in basic metabolic rates. NMR microimaging methods have been used in collaboration with the University of Dundee to obtain three dimensional imaging and optical sections which provide further information on the structural and metabolic changes during pupation of butterflies and moths.

Molecular techniques have been applied to study the population genetics and taxonomic relationships of several insect, mite and nematode pests and to assess the impact of environmental selection pressures on pest populations. For example, virulent populations of *Amphorophora idaei* which overcome the A_1 resistance gene in raspberry have been shown to be genetically heterogeneous, indicating extensive interbreeding between raspberry aphid populations and not the expansion of a small number of virulent clonal races. In contrast, initial isozyme and RFLP studies suggested that there were no differences within *Myzus persicae*, an aphid which reproduces largely or wholly by clonal means in the UK. However, more sensitive molecular techniques have now been developed and variation within populations of *M. persicae* has been demonstrated. The molecular genetics of *M. persicae* are complicated by the presence of two sibling species, *M. nicotianae* and *M. antirrhinii* which are morphologically very similar and individuals cannot be readily differentiated by light microscopy from *M. persicae*. Molecular techniques are also being used to elucidate the taxonomic relationships of a group of *Eriophyid* mites which attack a range of *Ribes* crops and wild plants. The care needed in the above types of investigations is emphasised by results which showed that one product of amplification of aphid derived DNA using the polymerase chain reaction (PCR) came, not from the aphid genome but from a fungal organism associated with the aphid cuticle. This necessitated the first reported use of *in situ* hybridisation to demonstrate that the origin of the ribosomal DNA under study was located on X chromosome of the aphid. Similar molecular biology studies on a range of root-knot nematode (*Meloidogyne* spp.) populations

have identified PCR markers associated with populations that are virulent on tomato cultivars containing the *Mi* resistance gene. They have also enabled *M. mayaguensis* to be separated from *M. incognita* with which it has been previously confused. This result is of particular importance because *M. mayaguensis* is extremely virulent and has recently been shown to be widespread in parts of west Africa.

Molecular studies are being used to examine the control of nematode moulting which, throughout the animal kingdom, is controlled by steroid hormones. The hormones bind to specific intracellular receptors which, in turn, control the expression of genes involved in moulting. Sequences of the steroid receptor genes have now been isolated from potato cyst nematode, the first time this has been achieved with a plant parasitic spp., and the putative regulatory regions are being isolated to learn more about control of gene expression. A similar approach is targeting specific binding sites for 'plantibodies' in aphid guts, in a new initiative with the Moredun Research Institute.

New nematode virus vector associations that have been identified recently in collaborative studies include *Paralongidorus maximus* as a vector of an atypical strain of raspberry ringspot nepovirus in German vineyards and *Longidorus arthensis* transmitting a new nepovirus, cherry raspleaf, to cherries in Switzerland. *Xiphinema diversicaudatum* was also shown to transmit a new strain of Arabis mosaic nepovirus causing yellowing in barley. Studies with the virologists have shown that the coat protein of tobraviruses is not the only factor influencing specificity of nematode transmission and that a second gene on the viral RNA-2 is involved, perhaps by producing a "helper factor".

Past Achievements

The integrated, collaborative nature of much of our research is demonstrated by many of our past achievements. Early studies with virologists established techniques and criteria for studying the efficiency with which nepoviruses were transmitted by nematodes and showed that *Xiphinema* spp. were generally much more efficient vectors than *Longidorus* spp. Similar studies demonstrated that there are various degrees of specificity between trichodorid spp. and the many serotypes of tobacco rattle virus that they transmit. The current focus is on the molecular basis of vector specificity, a search for natural and transgenic resistance to both tobra and nepoviruses, and studies on virus epidemiology and control.

Behavioural analysis of insects on resistant plants has shown that antixenosis (lack of preference) is a key factor for aphids and root flies, and involves chemoreception of plant surface chemical and volatiles.

Research with chemists on mechanisms of plant resistance to insects showed that differences in the attractiveness of brassica leaf-surface chemicals to egg-laying root flies was the major factor influencing host selection and recognition. With Swiss colleagues new, highly active oviposition stimuli have been identified and these form the basis for developing a new generation of insect resistant brassicas using marker-assisted techniques. With IACR, RES several host flower volatiles attracting raspberry beetle have been identified for field testing as trap attractants.

The ability of pests to overcome plant resistance genes has been a major concern. Molecular studies on raspberry aphids showed that virulence alleles to resistance in raspberry are distributed through the whole aphid population. Similar studies on potato cyst nematodes (PCN) have shown that there are considerable virulence differences between populations within the same species and that these are due both to the various introductions which contained different virulence gene-pools, and to fragmentation of these gene-pools as the introductions were spread within Europe. Selection for virulence against a potato cultivar with resistance derived from

Solanum vernei did not necessarily confer virulence against another cultivar with resistance from the same source. This information is now being used to target better efforts to breed for resistance. Poor growth due to PCN damage was shown to be associated with chronic nutrient deficiency and that, on sandy soils, it could be partially ameliorated by additional fertiliser. Modelling of yield losses due to PCN showed that there were major differences in tolerance of damage between cultivars, especially those with resistance. A model was developed with predictive potential in which soil type and cultivar tolerance differences could be incorporated.

The problems we study are continually changing. The 1970s saw an up-surge in pest problems associated with warmer weather and changes in agricultural practice. Direct damage to strawberry due to the virus vector nematode *Longidorus elongatus* was demonstrated. Raspberry cane midge became a major pest and control measures based on insecticides targeted to the first generation and cultural control measures were devised. Subsequently, the timing of control measures was improved by the development of a model to predict midge emergence. A new problem in raspberry caused by root lesion nematodes was identified and soil sterilants shown to provide good control. In potatoes PLRV became a major problem and a series of control measures were devised based on research to predict numbers and time of arrival of vector aphids. Granular insecticides and foliar sprays using the new pyrethroid insecticides were shown to be effective but the value of early and effective rogueing was emphasised. Recent research with the virologists has identified resistance genes to PLRV and demonstrated their effectiveness. Research is now focused on PVY which is an increasing problem! Yet another new problem, and again an introduction, is the New Zealand flatworm which preys on earthworms. Research at SCRI has shown that it is widely distributed in Scotland, is well adapted to the Scottish environment and is potentially a serious problem whose control is likely to require much more information on its natural enemies than we currently possess.

Other collaborative achievements include the identification of natural pesticides including a sugar analogue from tropical legumes and a lectin from snowdrop, both of which are effective against certain nematodes and insects. Collaboration with a German group has also shown that the lectin may also be useful against the AIDS virus. Several plant-derived chemicals have been patented as novel anti-nematode treatments. One (DMDP) is now being extracted from Costa Rican plantations and assessed by agrochemical companies. Genomic libraries have been produced from both cyst and root-knot nematodes and the latter used, in collaboration with a French colleague, to identify the widespread distribution in west Africa of a new, highly virulent species of root-knot nematode (*Meloidogyne mayaguensis*). In a related project on root-knot nematodes the bacterial parasite *Pasteuria penetrans* was also shown to be much more widely distributed throughout the world than had previously been recognised. The first reported *in situ* hybridisation on aphid chromosomes has revealed fundamental differences in rDNA assays associated with the X chromosome of different species. Molecular techniques were also used to separate related species of eriophyid mites in relation to transmission of reversion disease in blackcurrants. Similar techniques also identified previously undetected variation in the aphid *Myzus persicae* and are being used to differentiate this aphid from its sibling species. So far it has been possible to distinguish *M. certus* but not *M. antirrhinii* or *M. nicotianae*.

Ecological studies tend to be long-term but are of increasing importance. Geostatistical and other techniques have been applied to determine the degree of aggregation of various pests, and hence the most appropriate sampling strategy and nematodes have been shown to be useful indicators of environmental change.

Transmission of nepoviruses by longidorid nematodes

D.J.F. Brown, W.M. Robertson, A.T. Jones & D.L. Trudgill

Plant nepoviruses, many of which are transmitted by longidorid nematodes, cause economically important diseases world-wide in fruit, vegetable and ornamental crops (Fig. 1). Considerable progress has been made in understanding the transmission of the viruses by longidorid nematodes by the application of molecular biology methods and specialised techniques developed at SCRI. Current research is focused on determining the range of associations between nematodes and viruses and elucidating the complex and subtle mechanisms determining the specific recognition between the vectors and their associated viruses.

New virus and vector combinations are continually being discovered. Recently *Paralongidorus maximus* was identified as the vector of an atypical strain of raspberry ringspot nepovirus (RRSV) in German vineyards. This is the first record of a *Paralongidorus* species being a virus vector. *Longidorus arthensis* has been identified and was confirmed both as a new nematode species and as the vector of cherry rosette nepovirus (CRV), a previously uncharacterised virus causing a decline disease in cherry trees in north eastern Switzerland and a serologically distinguishable strain of arabis mosaic nepovirus (ArMV), naturally transmitted by *X. diversicaudatum*, was the agent causing a yellowing disease in barley in western Switzerland. This last example is the first evidence of a nematode transmitted nepovirus causing a disease in a graminaceous crop.

Figure 1 a) a grapevine infected with a chromogenic strain of grapevine fanleaf nepovirus transmitted by *Xiphinema index* showing characteristic yellowing of leaves and a predominance of small berries in fruit clusters. b) a raspberry crop infected with raspberry ringspot nepovirus transmitted by *Longidorus elongatus* showing patches of poorly growing and dead plants.

Currently, only one third of nepoviruses are known to have nematodes as their vectors although there is circumstantial evidence of a soil-borne mode of transmission, probably involving nematodes, for several others. With one exception in Japan, the vector and virus associations are indigenous either to Europe or North America. The association between longidorid vectors and their viruses appears to be highly specific

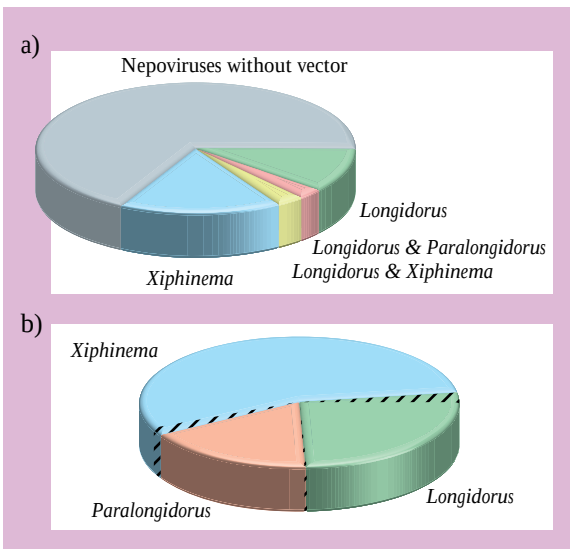


Figure 2 Pie chart showing a) nepovirus and the proportions transmitted by *Longidorus*, *Paralongidorus* and *Xiphinema* species, b) *Longidorus*, *Paralongidorus* and *Xiphinema* species with the proportion (hatched areas) of species in each genus which transmit the virus.

involving a recognition between the RNA-2 segment of the virus genome, which contains the coat protein gene, and inherited characteristics at the site of retention within the vector nematode. This concept of specificity of transmission is supported by only a small proportion of the almost 400 species belonging to the three virus-vector nematode genera, *Longidorus*, *Paralongidorus* and *Xiphinema*, that have been shown to be capable of transmitting viruses (Fig. 2).

The specificity between serologically distinct nepoviruses and longidorid species has been shown to extend to particular populations within a vector species and these populations can differ in their ability to transmit particular isolates of a virus which may be serologically indistinguishable. In contrast to this specificity, some nepoviruses are transmitted by more than one vector species, e.g. the serologically distinguishable Scottish and English strains of raspberry ringspot virus (RRSV) are each transmitted by *L. elongatus* and, even more surprisingly, some vectors are capable of transmitting more than one virus e.g. *X. diversicaudatum* transmits arabis mosaic (ArMV) and the serologically unrelated strawberry latent ringspot (SLRSV) viruses. However, with two exceptions, nepoviruses are not transmitted by nematodes in more than one genera.

Recent research has shown that the pattern of vector and virus specificity differs between *Longidorus* and *Xiphinema* species and between the *Xiphinema* virus-vector species occurring in Europe and those in North America. In Europe, the *Longidorus* transmitted nepoviruses occur as several serologically distinct strains with the viruses and their vector nematodes usually occurring in small, discrete geographical areas, frequently identified because they cause a disease in a particular crop. Recent examples were *L. arthensis* transmitting CRV to cherry trees in northern Switzerland, *P. maximus* transmitting raspberry ringspot to grapevines in the German Palatinate region, and *L. fasciatus* and *L. apulus* transmitting serologically distinct strains of artichoke Italian latent virus to artichoke in western Greece and south eastern Italy, respectively. In contrast, viruses transmitted by *Xiphinema* spp. in Europe usually show substantial serological homology and are widely distributed. *Xiphinema diversicaudatum* and its associated ArMV occur in most European countries, from Portugal in the west to the Moscow region in the east. Similarly, *X. index* and grapevine fanleaf nepovirus (GFLV), which occur together world-wide in the major viticulture areas, are widespread in European vineyards.

Recently, it has been shown that there is less specificity between nepoviruses and their vectors in North America. *Xiphinema americanum sensu stricto, X. californicum* and *X. rivesi*, all obtained from North America, were each shown to transmit cherry rasp leaf (CRSV), tobacco ringspot (TRSV) and tomato ringspot (ToRSV) viruses whilst *X. bricolensis* only transmitted ToRSV. Also, three populations of *X. americanum sensu stricto* differed in their ability to transmit CRLV and strains of ToRSV. These viruses and their vectors present a potential hazard if introduced into Europe. *Xiphinema rivesi* is already established in several European countries but has not been associated with a virus. However, ToRSV has been reported to be present and spreading in Yugoslavia. These viruses and their vectors are included in quarantine pests lists of the European Union and of the European and Mediterranean Plant Protection Organization.

The 41 putative species in the *X. americanum*-group are differentiated by minor morphological differences but molecular techniques were used to identify genetic discontinuities between the populations to support the morphological identification of the four North American species. The morphological studies showed that all of the populations had only three developmental juvenile stages rather than the four stages usually found in the Nematoda. Populations of European species belonging to the *X. americanum*-group that have not been associated with nepoviruses have four developmental stages. Consequently, the number of developmental stages may provide an objective method for distinguishing virus-vector populations within the *X. americanum*-group. *Longidorus martini*, the vector of mulberry ringspot virus in Japan, also has only three developmental juvenile stages which makes it unique amongst *Longidorus* virus-vector species.

In laboratory tests, European *Xiphinema* species transmitted their associated viruses with high frequencies. *Longidorus* spp. were more variable: only 5% to 10% of individual *L. elongatus* transmit RRSV and TBRV, whereas 50% to 70% of individual *L. attenuatus* and *L. fasciatus* transmit TBRV and AILV, respectively. Transmission frequency is a function of the ability of the nematode to retain and subsequently release virus particles from the specific sites of retention within the feeding apparatus. The sites of virus retention have been identified by electron microscopy of thin sections through the nematode's feeding apparatus. Virus particles are associated with the cuticle lining of the

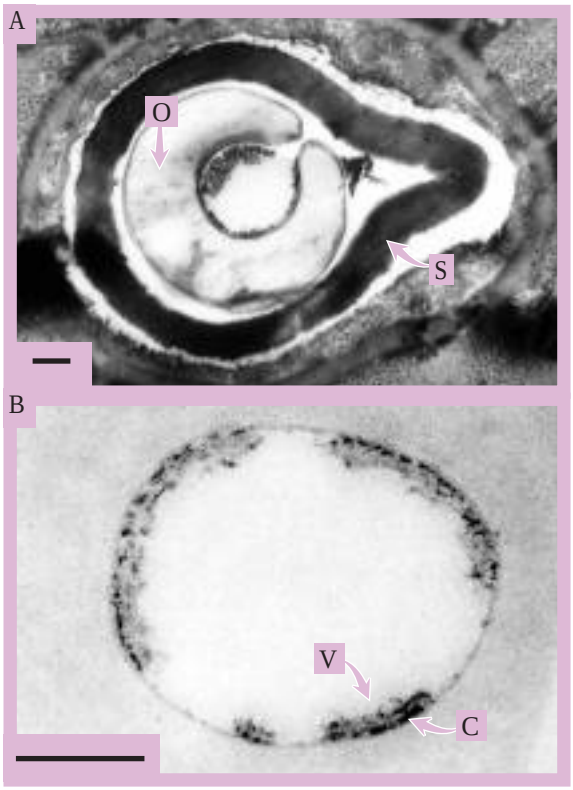


Figure 3 Electron micrographs of transverse sections through A) *Longidorus macrosoma* showing raspberry ringspot nepovirus particles in the lumen of the odontostyle (O) and trapped between the odontostyle and guide sheath (S); B) *Xiphinema diversicaudatum* stained for carbohydrate deposits (C) on the surface of the lumen of the odontophore some of which have "cloud" material indicating the presence of arabis mosaic nepovirus particles (V). Bars represent 200 nm.

odontophore and the oesophagus in *Xiphinmema*, and with the inner surface of the odontostyle and between the odontostyle and the guiding sheath in *Longidorus* species (Fig. 3). Inefficient transmission of RRSV by *L. macrosoma* is associated with a lack of dissociation of virus from the specific sites of retention within the vector.

Mechanisms of virus retention probably differ between *Longidorus* and *Xiphinema* spp., and between European and North American *Xiphinema* spp. Virus release must occur when the nematode injects saliva into the plant cells upon which it is feeding. The wall of the food canal in *Xiphinema* spp. stains for carbohydrate and particles of ArMV retained within the

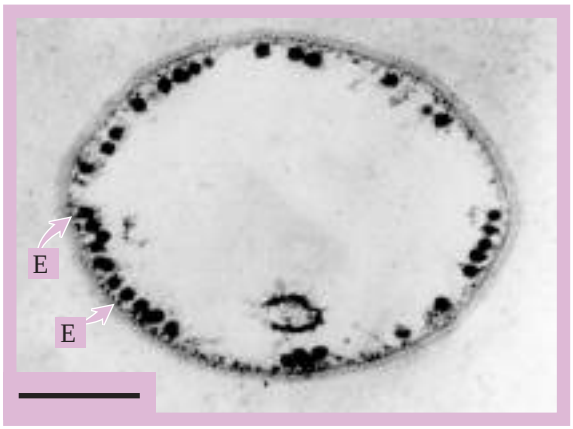


Figure 4 Electron micrograph of a transverse section through *Xiphinema diversicaudatum* showing strawberry latent ringspot nepovirus particles lining the lumen of the food canal with electron-dense structures (E) linking the body of particles to one another and to the surface of the lumen. Bar represents 200 nm.

odontophore of *X. diversicaudatum* have been observed surrounded with carbohydrate-containing material (Fig. 3). Therefore, the protein coat of the virus particles may have lectin-like properties, with carbohydrates being involved in both retention and release of particles in *Xiphinema*.

Vector specificity is correlated with serological characteristics, and they are both considered to be determined by the composition of the virus coat protein. Thin linking structures, assumed to be associated with "protruding" amino acid sequences on the C-terminal of tobacco rattle tobravirus have been identified in electron micrographs occurring between virus particles and the wall of the food canal of the vector trichodorid nematode. Recently, similar structures have been observed with a nepovirus and its vector (Fig. 4). We are currently investigating the role of these structures, and their possible involvement with a viral determined helper factor, in the specific retention of the viruses in their associated vectors. Also, the possible presence of nematode derived protein(s) which may bind with the viruses indicating their potential role in the specific recognition between the viruses and their vectors will be studied using several monoclonal antibodies raised to selected nepoviruses. Elucidation of the mechanisms involved in the specific transmission of viruses is integral to our research to develop novel virus disease control strategies.

The role of nematode exudates in plant pathogenesis

W.M. Robertson, J.T. Jones, L. Duncan[1] & J.R. Kusel[1]

Studies of plant nematode parasites have shown that their success is crucially dependent on their ability to manipulate their hosts. This can occur by both mechanical and chemical means and it is clear that the most economically important nematodes (e.g. potato cyst and root knot nematodes) use their secretions either to improve the suitability of the host or to induce plant cell modifications that are necessary to the development of the nematodes. Consequently, the susceptible reaction probably involves a range of interactions between nematode genes and their products, and plant genes. Also, it is likely that these secretions are important in resistant plants which contain gene products that are able to recognise some of the nematode secretions and switch on chemical defence mechanisms.

Recent studies have shown that nematodes produce several candidate exudates which may be involved in the process of invading host plants and inducing a susceptible or a resistant reaction. As populations of nematodes often belong to pathotypes or races which differ in their virulence, it is possible that differences in the virulence of particular populations may reflect differences in the activity of the exudates. We have identified a range of chemical treatments of nematodes which stimulate release of exudates that have been observed to emanate from the amphids, cuticle, excretory pore and the oesophageal glands. Where a nematode invades the plant with its whole body (endoparasites), it is possible that all the sources of secretion may be involved to a greater or lesser extent. In ectoparasitic species where the feeding nematode only penetrates the cells with its stylet and remains external to the root, observations of the feeding behaviour suggest that only secretions from the oesophageal gland are involved in inducing the plant cell modifications. A few of these ecto-parasitic nematodes transmit plant viruses and the oesophageal gland secretions are the only likely means of releasing and transporting the virus particles from sites of retention on the wall of the food canal into the plant.

Comparisons of the modifications induced in plants by both endo- and ectoparasitic species of nematodes suggests that the oesophageal secretions alone are responsible for the more elaborate changes in plant cell metabolism. The most profound changes are caused by root knot nematode (*Meloidogyne* spp.) which induces the cells it feeds upon to become enlarged, multi-nucleate, cytoplasm enriched and display synchronised nuclear division. Surrounding cells increase in number resulting in very large galls on the roots of host plants. Potato cyst nematodes (*Globodera* spp), in common with other cyst-forming genera, induce multi-nucleate cells by breaking down adjacent cell walls and causing the cell cytoplasm to fuse. Although no nuclear division takes place, the nuclei contain increased amounts of DNA suggesting that the nematode can induce DNA synthesis.

The production of relatively large volumes of amphidial and excretory pore exudates from nematodes can be induced by application of a mixture of solvents with Coomassie blue stain and a modified technique has been used to obtain exudates from large numbers of nematodes. At least part of the secretion from around the excretory pore was observed to spread across the surface of the nematode body (Fig. 1). This observation has suggested that the pair of longitudinal grooves known as alae, and which extend for almost the entire length of the nematode body, may be involved in the efficient transfer of material from the excretory pore along the length of the nematode.

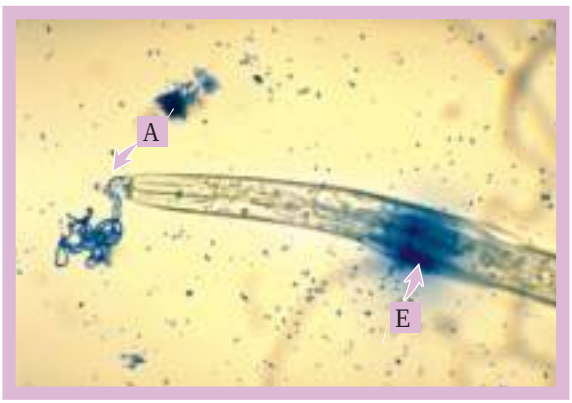


Figure 1 *Globodera pallida* showing exudates (stained blue) from amphids (A) and excretory pore (E) that have spread along the body. Many small blue particles which originate from the excretory pore surround the nematode.

[1] Department of Biochemistry, University of Glasgow

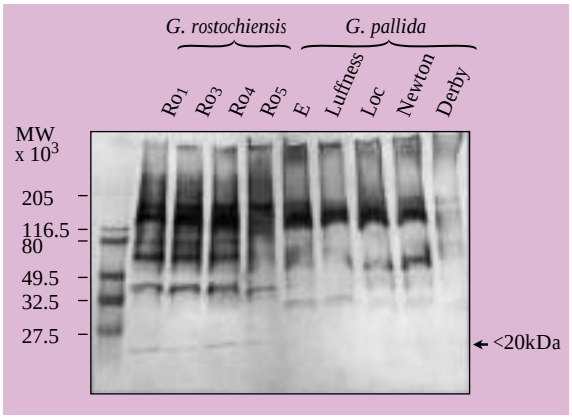


Figure 2 Western blot of whole body extracts of *G. pallida* and *G. rostochiensis* populations probed with antisera produced to exudates from the *G. pallida* Luffness population.

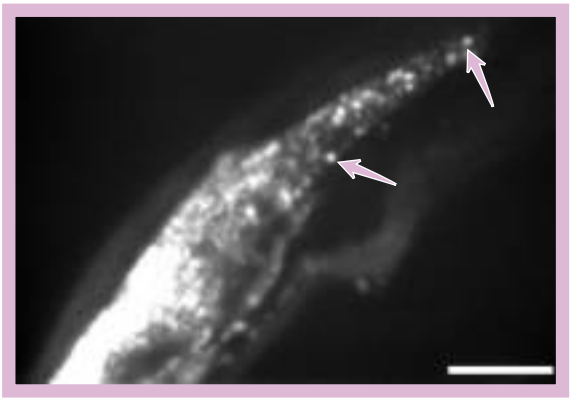


Figure 3 Confocal fluorescent image of granules in the subventral gland of *G. pallida* labelled with antisera raised to the subventral gland secretions (arrowed). Bar represents 10μm.

Exudates collected from a population of *Globodera pallida* were used to raise antibodies which were employed to probe and compare secretions from several populations of *G. pallida* and *G. rostochiensis* following separation by gel electrophoresis. A common band at <20 kDa and several bands between 40-60 kDa were revealed showing that differences occurred between the species, and between some populations. Western blots of whole body protein extracts using anti-exudate sera also showed differences between the two *Globodera* species as well as variation within each species (Fig. 2).

The serotonin agonist 5-methoxy DMT oxalate, was found to stimulate the production only of oesophageal gland secretions. However, the anti-exudate sera could be used on DMT stimulated nematodes to monitor changes in the secretions on the surface of the cuticle. Electron immunohistochemistry showed that granules in the oesophageal glands could be labelled with the antiserum using a gold conjugated secondary antibody.

A second antibody was raised using a protocol designed to suppress common sites of antigenicity. This antibody labelled the secretory granules of the subventral glands of both *G. pallida* and *G. rostochiensis* (Fig. 3). Western blots of total protein extracts revealed two bands of approximately 45 kDa. However, bioassays showed that this antibody could not detect surface secretions from nematodes treated with DMT. A series of bioassays were used to identify a plant component which caused the same effect as the DMT. This research represents significant progress towards isolating and identifying the nematode products involved in both resistant and susceptible reactions in plants. Further work will concentrate on using the antibodies to differentiate between components of the secretions, identifying their site of action in the plant and on characterising the molecules they recognise.

Molecular ecology of aphids

B. Fenton, A.N.E.Birch, G. Malloch, J.A.T.Woodford, A.T. Jones & C. Gonzalez[1].

Aphids are one of the most widespread and damaging group of plant pests. Their feeding is directly detrimental to crop plants and they also transmit many harmful plant viruses. Control using insecticidal sprays and resistant plants have been effective in the past. However, aphids have responded to these selection pressures in recent years by the development of insecticide resistance and virulent biotypes which overcome plant resistance genes. Biotypes are populations within species which are normally identical morphologically, but which differ in one or more important biological traits. In most instances, the underlying genetic mech-

[1]European Molecular Biological Laboratories, Heidelberg, Germany.

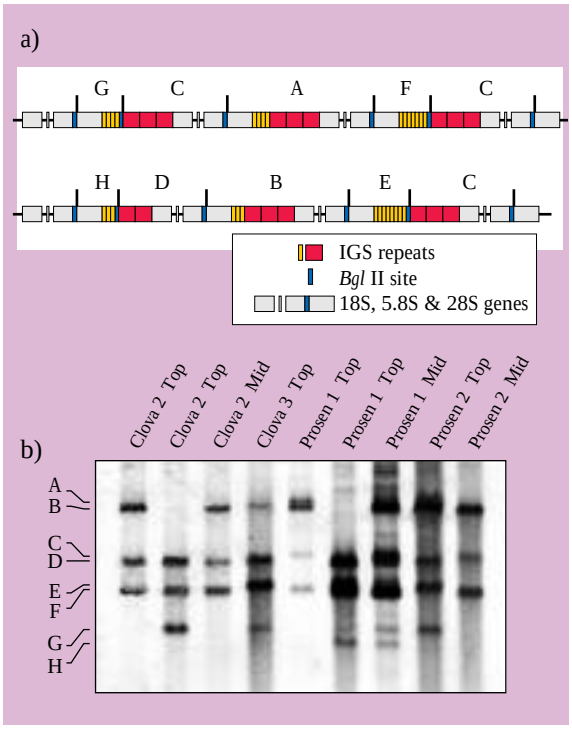


Figure 1 a) A graphic representation of variation found in the *A. idaei* IGS region. Repeat regions are shown by coloured blocks and *Bgl* II sites by a blue line. b) Analysis of single aphids sampled from a field. Different letters correspond to the fragments depicted in a).

anisms involved in biotype formation and the degree of gene flow between different biotypes is poorly understood.

The large raspberry aphid *Amphorophora idaei*, is host specific to raspberries and has a holocyclic life cycle (i.e. sexual reproduction occurs in autumn and the entire population overwinter as eggs). From inter-biotype mating a gene-for-gene hypothesis has been proposed to describe the interactions of *A. idaei* biotypes with major *A. idaei*-resistance genes in raspberry cultivars[1] and it was concluded that two pairs of alleles were involved in determining the ability of *A. idaei* biotypes to overcome resistance genes A_1 or $A_{3,4}$. These genes have been used widely in raspberry breeding programmes and currently more than 90% of the U.K. raspberry area is planted with cultivars containing one or more resistance genes to *A. idaei*. In response to this trend, resistant biotypes have increased until they now dominate the *A. idaei* population in the UK[2].

Recent progress in molecular genetics has increased the understanding of the genetics of insects such as the fruit-fly (*Drosophila melanogaster*). Studies of the ribosomal gene family have been crucial in understanding the population genetics and evolution of this organism. The current study reports an analysis of *A. idaei* biotypes using their ribosomal DNA region.

In animals and plants, the ribosomal genes are highly conserved and may be repeated 100-1000 times per cell in tandem arrays. Between the ribosomal DNA (rDNA) genes are the intergenic spacers (IGS; Fig. 1). The IGS regions evolve much more rapidly than the rDNA genes because they contain short tandem repeats which recombine at meiosis to form increased or decreased copy numbers in individual progeny. When *A. idaei* DNA is cut and hybridized with a rDNA probe a series of bands are seen on a blot. The different bands correspond to different sized IGS fragments (Fig. 1). The pattern of bands is highly variable and forms a DNA-fingerprint which allows the relationship of individual aphids to be assessed. Figure 1 shows an analysis of nine aphids taken from different positions on plants in an unsprayed raspberry plantation containing two cultivars. In this example, all but two aphids have a different pattern. On the plant

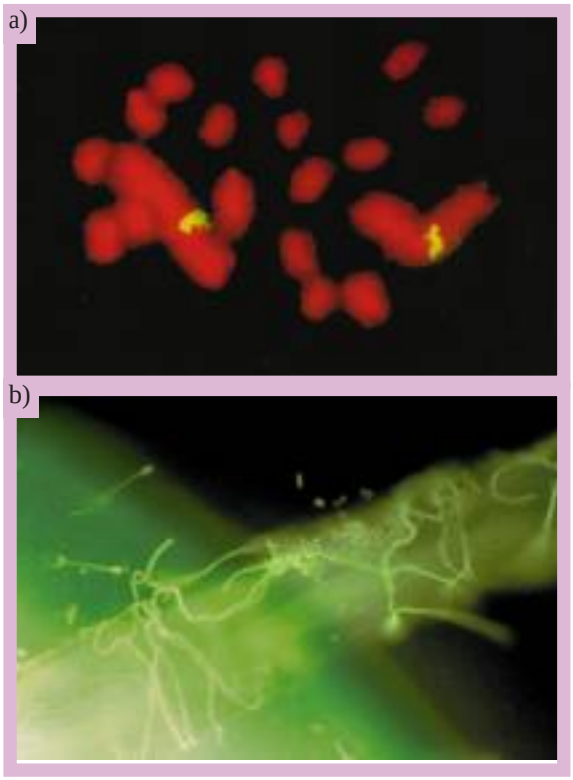


Figure 2 a) Dividing *A. idaei* cells prepared by *in-situ* hybridization with a ribosomal DNA probe. Chromosomes stain red (propidium iodide) and the ribosomal site yellow/green. b) The external surface of *A. idaei* covered with fungi stained with a fluorescent dye.

'Prosen 1', single aphids collected from the top sampling zone were genetically different from those sampled from the middle zone. Differences were also detected in aphids collected from adjacent rows. By analysing more samples from more locations throughout the UK, it was possible to conclude that the two virulent *A. idaei* biotypes, 2 and X, consist of many genetically distinct clones. There was also evidence of out-breeding between *A. idaei* populations under field conditions. However, there may be a more restricted gene flow between the more widely separated English and Scottish populations [2].

The rDNA genes were located on *A. idaei* mitotic chromosomes by *in situ* hybridization. In Figure 2 the full 18 chromosome complement of *A. idaei* is clearly distinguishable and two regions of staining, approximately 2/3 of the way along the two longest chromosomes (the Xs), show the location of the main rDNA arrays of this aphid. The *in-situ* analysis confirmed that *A. idaei* DNA was being analysed and not that of micro-organisms which cover the outside of these aphids [3] (see Fig. 2).

Implications for the control of *A. idaei* using resistance genes In the past 3 years, very large infestations of *A. idaei* on cultivars Glen Moy and Glen Prosen, both of which have *A. idaei* resistance gene A1, have occurred in many areas of England, Wales and Scotland. This indicates the widespread distribution of A_1 resistance-breaking biotypes 2 and X throughout the UK, and a large shift from avirulent biotype 1, which was dominant in the 1960s (97% of all *A. idaei* tested[1]) because of the replacement of susceptible with resistant raspberry cultivars. However, our data indicate that within the apparently simple phenotypic adaptation to specific *A. idaei*-resistance genes in raspberry, there is a great deal of heterogeneity. This suggests that the biotypes arose when virulence genes spread through the *A. idaei* population, rather than by clonal expansion of a small number of host-adapted genotypes through asexual reproduction and self-fertilisation within biotypes. The former process would ensure that the *A. idaei* population is genetically diverse and unlikely to suffer any adverse effects due to inbreeding.

The widespread breakdown of *A. idaei* resistance gene A_1 places increasing reliance in future on the alternative single major resistance gene A_{10}. The latter gene is already present in *c*. 30% raspberries grown in England and Wales, where it currently provides protection against all known *A. idaei* biotypes. However, there is now a real threat that new virulent biotypes will be selected before additional *A. idaei* resistance genes can be identified and transferred into commercial raspberries. This emphasises further the need to understand the genetic and phenotypic mechanisms leading to biotype development in *A. idaei* and other aphids, so that strategies which maximise the durability of resistance genes can be deployed in the future.

References

1 Briggs J.B. (1965). *Journal of Horticultural Science* 40, 109-117.

2 Birch, A.N.E., Fenton, B., Malloch, G., Jones, A.T., Phillips, M.S., Harrower, B.E., Woodford, J.A.T., Catley, M. A. (1994). *Insect Molecular Biology* 4, 239-245.

3 Fenton, B., Malloch, G., Birch, A.N.E., Woodford, J.A.T., Gonzalez C. (1994). *Insect Molecular Biology* 3,183-190.

Effects of aphid feeding behaviour on the transmission of potato leafroll virus

J.A.T. Woodford, C.A. Jolly & A.J. Nisbet

Potato leafroll virus (PLRV) is the most important virus affecting potato crops throughout the world. It causes particular problems for seed potato production and has been investigated extensively at SCRI in studies ranging from molecular and biochemical composition of the virus (*Ann Rep. 1989, 73; 1993, 97*), and plant resistance to infection (*Ann. Rep. 1992, 92; 1993, 103*) to epidemiology and control of spread (*Ann. Rep. 1990, 95)*. This report highlights the influence of aphid feeding behaviour on the specificity of PLRV transmission by different vector aphid species, and the effects of systemic antifeedant compounds on feeding behaviour and virus transmission.

PLRV, like other luteoviruses, is confined to the phloem tissues of infected plants, and is transmitted by only a few aphid species. Aphids that can feed and reproduce on potato are all potential vectors because

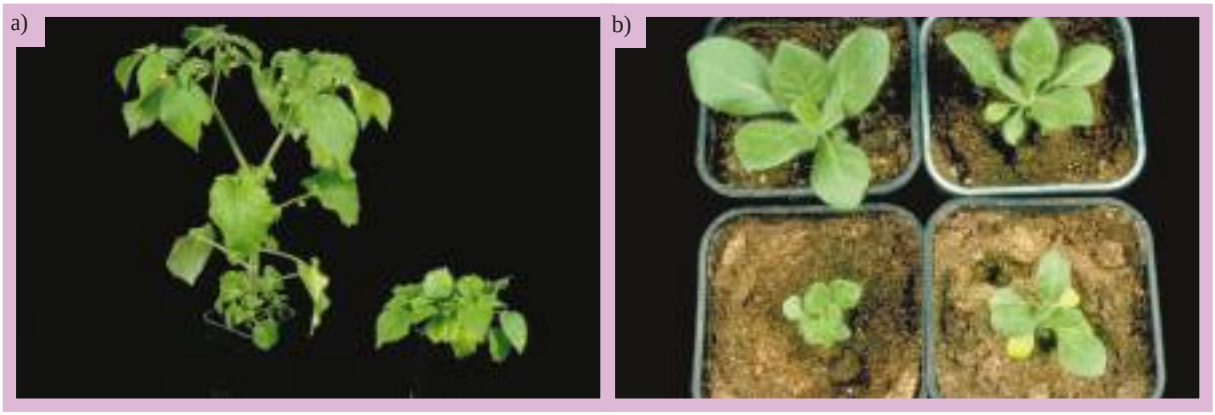


Figure 1 Stunted, clorotic plants infected with PLRV. a) *P. floridana* (right), healthy control plant left and b) *N. clevelandii* (front), healthy control plants at back.

their stylets penetrate phloem tissues. Three species commonly infest potato crops throughout the UK, but their importance as vectors of PLRV differs. In some areas, the potato aphid, *Macrosiphum euphorbiae*, and the glasshouse and potato aphid, *Aulacorthum solani*, are the most abundant species found on potato crops, but the peach-potato aphid, *Myzus persicae*, is usually considered to be the most efficient vector. This has focused attention on the recognition mechanisms responsible for the specificity of PLRV transmission in *M. persicae*. However, for epidemiological studies, it is necessary to consider the extent to which transmission efficiencies in laboratory experiments represent transmission rates to potato plants.

Virus transmission is a highly variable process because it involves interactions between virus, vector, and plant. PLRV is transmitted in a persistent, circulative manner. Successful transmission depends not only on the ability of the virus to circulate through the aphid and enter the saliva, but also on the feeding behaviour of the vector aphid during acquisition and inoculation. Most experimental transmissions of PLRV have been made using *Physalis floridana*, both as a virus source plant and test plant, because early work with *M. persicae* showed it to be a more reliable experimental plant than potato. Plants of *P. floridana* inoculated with PLRV at the seedling stage become stunted and the leaves show interveinal necrosis after 2-3 wk (Fig. 1). *P. floridana* is a poor host plant for other aphid species, particularly *M. euphorbiae*. However, since previous work had shown that PLRV could be detected by ELISA in *M. euphorbiae* that had fed on infected potato plants, or even *P. floridana*, the inability of this species to transmit the virus to *P. floridana* test plants has been attributed to a failure of PLRV particles to pass from the haemolymph to saliva. When apterous aphids were caged on PLRV-infected *P. floridana* or potato foliage and then transferred to young *P. floridana* or potato test plants, *M. persicae* transmitted PLRV efficiently to *P. floridana*, while *M. euphorbiae* did not transmit PLRV after feeding on *P. floridana*, but did so occasionally after feeding on potato (Table 1).

Potato-colonising aphid species have been shown recently to settle and reproduce on seedling *Nicotiana clevelandii*. This host plant also developed clear symptoms after infection with PLRV (Fig. 1), providing a means to determine if *M. euphorbiae* and *A. solani* transmitted PLRV more efficiently to a more accept-

Aphid species	PLRV source	Test plant	
		P. floridana	Potato
Myzus persicae	*P. floridana**	96 (195†)	57(30)
Macrosiphum euphorbiae	*P. floridana**	0(113)	Not tested
Myzus persicae	Potato*	44(243)	25 (44)
	Potato**	71(221)	42(12)
Macrosiphum euphorbiae	Potato*	4(182)	4(128)
	Potato**	2(175)	9(158)

† Number of test plants shown in parenthesis
* 3 aphids / test plant
** 5 aphids/ test plant

Table 1 Percentage of *Physalis floridana* or potato test plants infected with PLRV after inoculation by *Myzus persicae* or *Macrosiphum euphorbiae.*

Figure 2 *Myzus persicae* attached to thin gold wire.

able test plant species. When apterous aphids were caged on PLRV-infected potato plants and then transferred to *N. clevelandii* seedlings *M. euphorbiae* and *A. solani* transmitted PLRV just as efficiently as *M. persicae*, suggesting that the efficiency with which virus particles were transferred to saliva partly depended on aphid feeding behaviour on source plants and test plants.

The ability of an aphid to acquire PLRV from an infected plant depends on the length of time it feeds in the phloem, and caging *M. persicae* for acquisition access periods (AAP) of at least 3 days usually ensures high transmission rates. It is assumed that the aphids feed in the phloem sieve elements for long periods during the AAP. More direct evidence of the duration of phloem feeding has been obtained using the Electrical Penetration Graph (EPG) technique to monitor aphid feeding behaviour. An aphid is attached by a thin gold wire (Fig. 2) to a high impedance amplifier and the aphid and leaf form part of a DC circuit. The EPG is a record of the amplified signals produced when the aphid completes the circuit by puncturing the leaf with its stylets. Several EPG wave forms, characterised by amplitude, frequency, voltage level and electrical origin, have been described and, in some cases, associated with the location of the aphid stylet tips in plant tissues and specific feeding

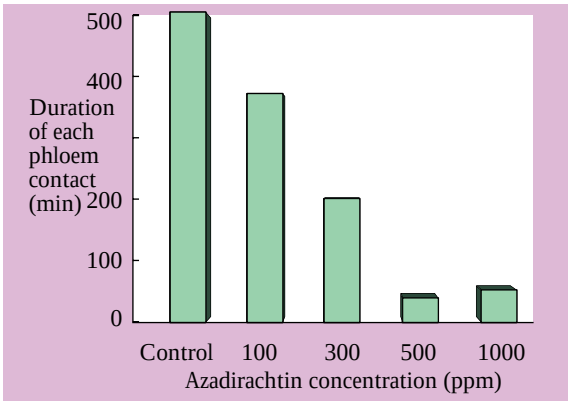


Figure 4 Effect of azadirachtin on the duration of each period of sustained phloem contact feeding behaviour.

activities[1]. One wave form, a lengthy series of small peaks and waves at a low potential level (Fig. 3), has been correlated with sieve element penetration and sap ingestion, and is particularly relevant to luteovirus acquisition.

In a search for natural products that might deter aphids from feeding and thus decrease the transmission of plant viruses, EPGs were recorded from *M. persicae* on *N. clevelandii* plants with their roots immersed in solutions of azadirachtin, a tetranortriterpenoid compound extracted and purified from the seeds of the neem tree (*Azadirachta indica*). The antifeedant effect of azadirachtin on many groups of insects has long been known, but evidence for its effect on aphids has been inconclusive. EPGs recorded for 9 h showed that as the concentration of azadirachtin in the solutions around the roots increased, aphids made more frequent, but briefer phloem contacts (Fig. 4), and the proportion of phloem contact activity during the total EPG was decreased at concentrations of 300ppm and above[2] (Fig. 5). Recent studies in The Netherlands[3] showed that non-viruliferous *Rhopalosiphum padi* placed onto wheat plants infected with barley yellow dwarf virus (BYDV), another luteovirus, acquired the virus most reliably if they produced prolonged sequences of the

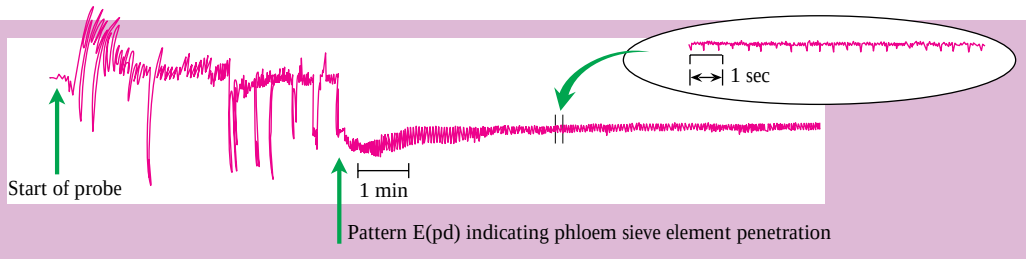


Figure 3 Part of an Electrical Penetration Graph of *M. persicae* on an untreated potato leaf.

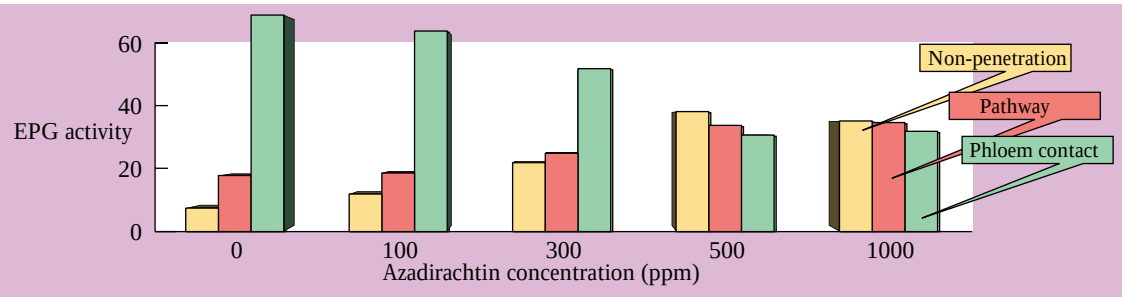


Figure 5 Effect of the antifeedant azadirachtin on the percentage of time aphids spent in stylet contact and non-penetration behaviour during 9h records of EPG activity.

wave form now called E2 which indicated phloem sap ingestion.

Antifeedants should decrease the acquisition of luteoviruses by decreasing phloem feeding but this has proved difficult to establish with virus-free aphids allowed to move freely on *N. clevelandii* seedlings infected with PLRV because many aphids walked off the plants and died from starvation during the 3 day AAP. Groups of *M. persicae* were placed on PLRV-infected *N. clevelandii* seedlings with their roots immersed in 0-150 ppm solutions of azadirachtin, and when surviving aphids were confined on virus-free *N. clevelandii* test plants, no differences in virus transmission were found between the concentrations of azadirachtin used, but overall, the treatment decreased virus acquisition by 72% (Table 2).

Treatment		Pertcentage of infected test plants
Azadirachtin*		9
Control		34
Imidacloprid	2.50ppm	0
	1.25ppm	0
Control		52

* Mean of three concentrations (50, 100 and 150ppm); results pooled from three experiments

Table 2 Effects of azadirachtin and imidacloprid on the acquisition of PLRV by *M. persicae.*

Imidacloprid, a synthetic nitroguanidine, is a second compound with possible antifeedant activity which induces aphids to walk off treated leaves. Petioles of excised potato leaves were immersed in low concentrations of imidacloprid and EPGs from *M. persicae* on treated leaves showed that the total probing time was less than half that on untreated leaves, and the duration of phloem contact was decreased from almost 2 h to less than 11 min (Fig. 6). Many probes were terminated abruptly before sieve elements were penetrated. However, in contrast with the antifeedant effects of azadirachtin, aphids that made brief stylet contact with leaves treated with imidacloprid were killed by toxicant action, not starvation. Therefore, in subsequent tests aphids were given a 3 day AAP on PLRV-infected potato leaves placed in low concentrations of imidacloprid, 1.25 and 2.50 ppm. Surviving aphids from control leaves, given a 4 day IAP on *N. clevelandii*, infected 52% of the test plants, while those that had been confined on treated leaves were unable to transmit PLRV (Table 2).

Less is known about the specific feeding behaviour involved when virus-carrying aphids inoculate PLRV. Inoculation is thought to involve salivary secretion and non-vascular ('pathway') feeding activities rather than ingestion from phloem elements, but there is

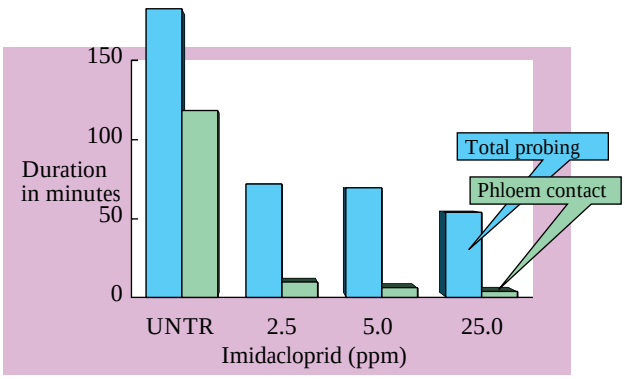


Figure 6 Effect of imidacloprin on total probe time and the duration of phloem contact feeding behaviour during 3 hour EPG records.

recent evidence[3] to show that the E1 wave form at the start of phloem contact is important for inoculation of BYDV. Some conventional insecticides decrease the proportion of aphids acquiring PLRV, but they are not very effective in preventing the inoculation of PLRV by aphids that are already viruliferous. Similarly, azadirachtin treatments that decreased the

Treatment		Pertcentage of infected test plants
Azadirachtin	500ppm	45
Control		30
Imidacloprid	25ppm	5
	2.50ppm	23
	1.25ppm	31
Control		50

Table 3 Effects of azadirachtin and imidacloprid on the inoculation of PLRV by *M. persicae.*

acquisition of PLRV did not prevent inoculation (Table 3). However, PLRV-infected *M. persicae* confined for a 2 day IAP on *N. clevelandii* seedlings with their roots immersed in imidacloprid infected fewer test plants than aphids confined on control seedlings (Table 3), and imidacloprid treatments have also prevented inoculation of PLRV to potato plants in the field[4]. Since it has also been reported that imidacloprid prevents *M. persicae* inoculating beet mild yellowing virus (another luteovirus) to sugar beet plants, this compound may interfere in a novel fashion with inoculation feeding. Further work is in progress to investigate the feeding behaviour of aphids, using EPG methods, during the inoculation phase of PLRV transmission.

References

1 Tjallingii, W.F. (1988). In: *Aphids, Their Biology, Natural Enemies and Control.* Vol 2B. A.K. Minks & P. Harrewijn (eds.) Elsevier, Amsterdam, 95-108.

2 Nisbet, A.J., Woodford, J.A.T., Strang, R.H.C. & Connolly, J.D. (1993). *Entomologia experimentalis et applicata* 68, 87-98.

3 Prado, E. & Tjallingii, W.F. (1994). *Entomologia experimentalis et applicata* 72, 157-165.

4 Woodford, J.A.T. & Mann, J.A. (1992). *Proceedings Brighton Crop Protection Conference - Pests and Diseases 1992,* 557-562.

Scottish Agricultural Statistics Service

Rob A. Kempton

The Scottish Agricultural Statistics Service (SASS) was established in 1987 to provide statistical and mathematical support, principally to the five SABRIs, SAC and the Scottish Agricultural Science Agency. Its broad aim is to contribute research, consultancy and training in statistics and mathematics in support of biological and related sciences, aimed at improving scientific quality and efficiency, and providing a better understanding of biological systems, particularly in agriculture, food and the environment. The broader definition of SASS activities will be reflected during 1995 in a change of name to Biomathematics and Statistics Scotland.

The implementation of Government strategy for science and technology, as set out in the White Paper 'Realising our Potential', and the wide-ranging debate generated by the scrutiny of public sector research establishments, have stimulated SASS to look at its own strategic development. Four features of the new policy directions will have particular impact:

1) The reduction in Government core-funding and opening up of 'flexible funding' to wider competition.

2) The clear recognition that SOAFD support of research and development within the Scottish System extends to all biological and related sciences, including agriculture, fisheries, food, environmental, economic and social science, together with relevant physical science and mathematics. There is also a move towards funding larger coordinated projects.

3) The greater recognition by Research Councils and SOAFD of the contribution that mathematics can make to understanding biological and environmental processes. For greatest effect, there needs to be integration of deterministic and stochastic (ie statistical) approaches.

4) The emphasis on exploitation of UK's research achievement, through better technology transfer.

In this changing research environment, SASS is well placed to exploit its strengths in statistics, mathematics and information science, and its experience of applications in the biological and related sciences, to access more diverse sources of funding. A possible bar to realising this aim is our name, Scottish Agricultural Statistics Service, which gives potential clients a misleadingly narrow impression of the work we do. It has therefore been agreed to change the name to Biomathematics and Statistics (BioSS) Scotland from April 1995. This report thus provides a suitable occasion to review the development of SASS over the last eight years and look ahead to the new opportunities for BioSS.

SASS was established in 1987 as a centre of statistical expertise, principally to support the SOAFD-funded research in SABRIs and SAC. Support has been provided at three levels: training; over the desk consultancy; and collaborative research. The training programme was aimed to give scientists more confidence in carrying out a preliminary analysis of their data and a greater awareness of statistical issues of design and analysis. However, as the requirements of scientists have become more sophisticated, SASS has met the demand for more specialist statistical courses, courses on mathematical modelling and on computational molecular biology. Twelve different courses have now been prepared and presented on over 200 occasions. They have proved to be a most efficient and effective means of improving the quantitative skills of scientists.

Advice and consultancy continues to be the backbone of the support SASS provides to scientists. The ready availability of a consultant with some familiarity with the scientific background of a problem is invaluable and 'routine' consultancy problems frequently develop into collaborative, research projects. Research has always been strongly motivated by applications. The range of topics is illustrated by projects currently supported by the SOAFD Flexible Fund: genetic structure of bacterial populations with particular reference to Listeria monocytogenes; vegetation dynamics in heterogeneous, species-rich vegetation; development of sensory methodology for soft fruit and dairy products; evaluation of new mathematical and statistical techniques in the analysis of personological, financial and physical data; and development of population assessment methods and management models for red deer in Scotland. Methodological research has been influenced by the advent of almost limitless, cheap computing power, which has made accessible a wide range of computer-intensive methods. These include image analysis, knowledge-based systems, artificial neural networks, geographic information systems and other data base methods.

Another development is the extension of research on modelling individual biological processes to understanding the behaviour of complex systems. This work has drawn on recent developments in non-linear mathematics applied to modelling physical systems. However, a key difference of biological systems is their essential variability, or stochasticity, which can have a major effect on model prediction, but which is ignored by much current modelling research. Recently, SASS has set about bridging the gap between the stochastic and deterministic schools of mathematics by appointing staff with broader mathematical interests and expertise to work alongside statisticians. The work has been strengthened through links with the Dundee Centre for Non-linear Systems in Biology and Department of Statistics and Modelling Science at Strathclyde. SASS is now involved in a number of multi-disciplinary projects in animal epidemiology, vegetation dynamics, and a far-reaching pilot study of the feasibility of a systems approach to modelling the Scottish rural economy.

SASS clients and collaborators now extend considerably wider than the SABRIs and SAC. We have long standing agreements with MAFF, Scottish Agricultural Science Agency, Institute of Terrestrial Ecology and British Society of Plant Breeders. Several contracts have been carried out for Scottish Natural Heritage, the Red Deer Commission and Torry Research Station. We work closely with many Scottish universities and co-supervise PhD students. Through involvement in EU programmes, strong links have also developed with sister organisations in Europe, particularly with the Department of Biometry and Artificial Intelligence, INRA, France, the Agricultural Mathematics Group, DLO, Holland and the Danish Informatics Network in the Agricultural Sciences. Further afield, we also undertake work for international agricultural research organisations in the developing world.

Our new name, Biomathematics and Statistics Scotland, has been chosen to better reflect the group's coverage of applications and disciplines. BioSS will be unique in UK in combining expertise in mathematics, statistics and information science, a broad experience of applications in the agricultural, biological and food sciences, close collaboration with scientists, and extensive links with universities and research organisations, throughout the world. It is thus well fitted to meet the needs of the bio-revolution in the new millennium.

Statistical analysis of molecular marker data

J. W. McNicol

Recent developments in molecular biotechnology have led to a dramatic increase in the number of marker data sets for which some statistical analysis is required. The role of SASS in this area is to develop statistical methods which are appropriate to these new technologies. This article describes both standard and new approaches to analysing molecular data.

Presenting the data Regardless of the biotechnology method, Isozymes, Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) or microsatellite simple sequence repeats (SSR), the data are presented as a series of 0's and 1's where a 1 denotes the presence of a band and zero its absence. Figure 1 shows part of a RAPD assay of 27 single egg mass lines of the root knot nematode Meloidogyne. Nine primers were used, generating 122 bands. From the picture of the full set of data, it is difficult to discern any patterns. The first step in analysing such a data set is to construct a suitable summary diagram or plot to help highlight dominant features and detect extreme results. Figure 2 shows part of the 'bandmap' for the same data set. The rows, which correspond to individual RAPD bands, have been sorted according to decreasing frequency. The columns, which correspond to the individual lines, have been re-ordered according to a clustering procedure which places lines with similar band patterns close to each other. The margins of the bandmap show the band row and column totals. Finally, an asterisk indicates that the band or line has an identical pattern to its preceding neighbour.

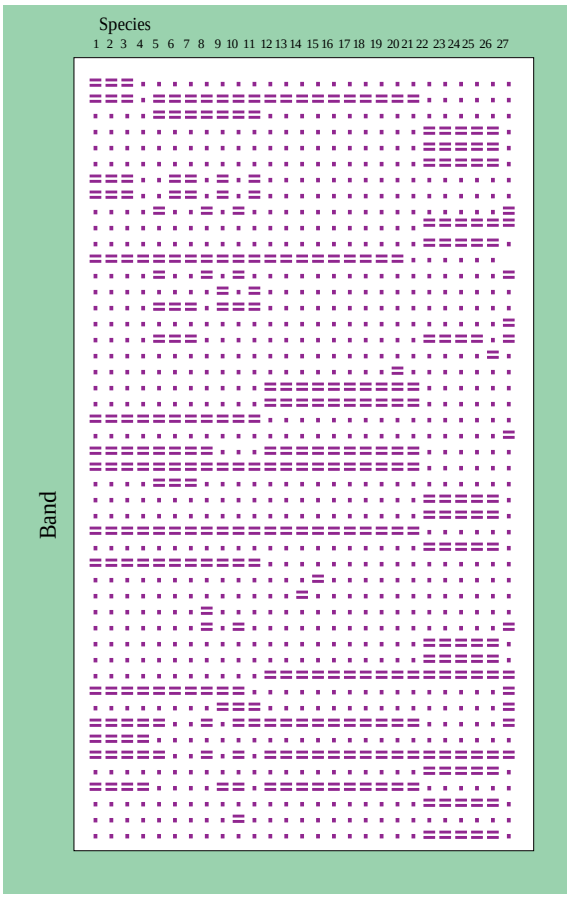


Figure 1 Part of RAPD assay of 27 *Meloidogyne* spp. egg mass lines. Each column represents a line, each row represents a band.

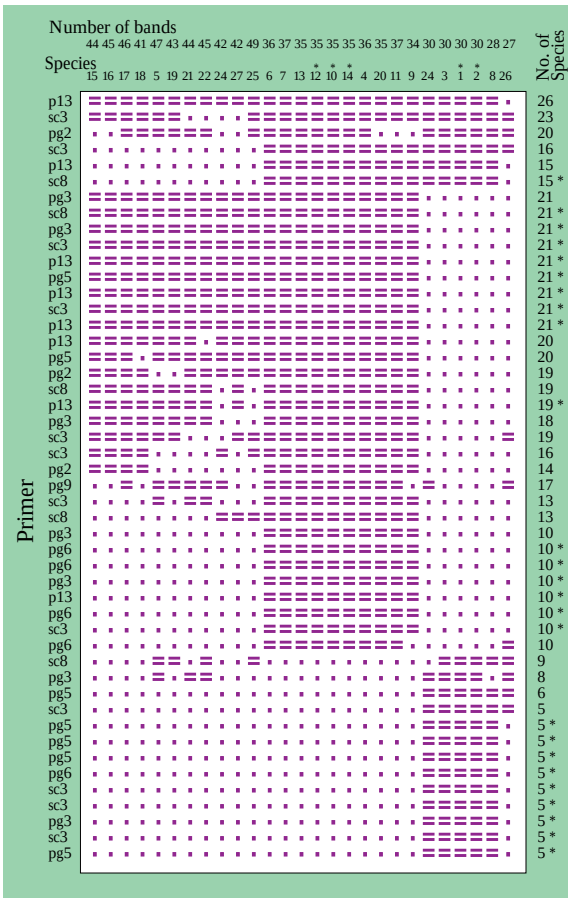


Figure 2 Part of RAPD assay of 27 *Meloidogyne* spp. bandmap.

This section of the Meloidogyne bandmap shows that there are distinct groups of lines and the common bands can be distinguished from the rare bands, information not obvious from the original presentation.

Similarity coefficients The second step is to quantify the similarities among the lines. Similarity measures are constrained to lie in the range zero to one; lines with identical band patterns will have a similarity of one, and lines which do not share any bands will have a similarity of zero. Within this framework there are many ways of measuring similarity and the three most commonly used with marker data, Simple Matching, Jaccard and Nei and Li, are illustrated using the data in Table 1. Suppose two lines A and B have been scored on 10 bands shown in Table 1a. The data can be re-arranged into a more convenient form as in Table 1b which shows the total number of bands which fall into each of the four presence/absence categories for the two lines. Simple Matching defines the similarity between A and B as the proportion of bands which are the same, namely (2+3)/10, or 50%. The Jaccard coefficient ignores bands for which both lines are not expressed ('double zeros') and uses the proportion of bands which are the same. Thus the Jaccard similarity in this case is 2/(2+4+1), or 29%. The Nei and Li coefficient also ignores the 'double zero' bands but uses the ratio of common bands to the average number of bands expressed by the two lines, namely 2/[{(2+1)+(2+4)}/2] or 44%.

a)

A	1	1	0	0	0	0	0	0	0	1
B	0	1	0	1	1	0	0	1	1	1

b)

		A	
		Present	Absent
B	Present	2	4
	Absent	1	3

Table 1 a) Artificial marker data for two genotypes assayed on 10 bands. b) The same marker data summarised in a presence/absence table.

There is considerable debate over which of these methods of estimating similarity is most appropriate for each of the common marker systems. For RAPDs and RFLPs both Jaccard and Nei and Li similarities are used. However, it can be shown that there is a simple relationship between these two coefficients, and Figure 3 shows that there is often very little difference between them. The relationship between the Simple Matching coefficient and both Jaccard and

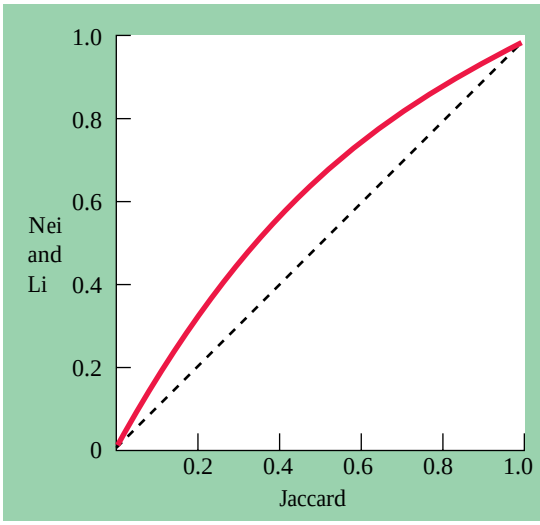


Figure 3 Relationship between the Nei and Li similarity coefficient and the Jaccard coefficient. The dotted line represents y=x.

Nei and Li coefficients is not so straightforward; the greater the percentage of double zeros in the data the greater the difference between the Simple Matching coefficient and the others. The difference can be as much as 0.4 in extreme cases.

Graphical representation Once a suitable similarity coefficient has been selected the next step is to calculate similarities between all pairs of samples. The matrix in Table 2 shows these similarities for the Meloidogyne species, rounded down to the nearest 10% so that the matrix is more easily read. For example the similarity between species 14 and 21 is 0.4. Asterisks denote cases for which no data were available. A graphical representation of the information in this matrix, however, is commonly sought, and the two popular methods are dendrograms and principal co-ordinate plots.

Dendrograms In the most common type of dendrogram each sample is considered as a group of size one. The first step is to join the two most similar groups to form a group of size 2. There are now n-1 groups instead of n groups, where n is the total number of samples. The second step is to join the two most similar among the n-1 groups. The third step joins the two most similar among the n-2 groups and so on. The key feature is the hierarchical approach to clustering of individual samples. The dendrogram displays this grouping process graphically and highlights groups of similar individuals There are several ways of defining similarity between two individuals as described above and the same is true for two groups.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1																											
2	9																										
3	9	9																									
4	9	9	9																								
5	7	7	7	7																							
6	7	7	8	7	8																						
7	7	7	7	7	8	9																					
8	7	7	7	7	9	7	7																				
9	6	6	6	6	6	7	7	6																			
10	6	6	6	6	7	6	6	7	8																		
11	6	7	6	6	6	7	7	6	9	8																	
12	5	4	5	5	4	4	4	4	4	4	4																
13	5	5	5	5	4	4	4	4	4	4	4	9															
14	4	4	5	4	4	4	4	4	4	3	4	9	9														
15	5	5	5	5	4	4	4	4	4	4	4	9	9	9													
16	5	5	5	5	4	4	4	4	4	4	4	9	*	9	9												
17	5	5	5	5	4	4	4	4	4	4	4	9	*	9	9	*											
18	5	5	5	5	4	4	4	4	4	4	4	9	*	9	9	*	*										
19	5	5	5	5	4	4	4	4	3	4	4	9	9	9	9	9	9	9									
20	4	4	5	5	4	4	4	4	4	4	4	9	9	9	9	9	9	9	9								
21	5	5	5	5	5	5	4	5	4	4	4	9	9	9	9	9	9	9	9	9							
22	0	0	1	0	1	1	1	1	0	0	0	2	2	1	1	2	2	2	1	2	2						
23	0	0	0	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	9					
24	0	0	0	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	9	*				
25	0	0	0	0	1	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	9	*	*			
26	0	0	0	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	8	9	9	9		
27	1	1	1	1	2	1	1	2	1	2	1	1	1	1	1	1	1	1	0	1	1	2	2	2	1	1	

Species

Table 2 The Nei and Li similarity matrix, rounded down to the nearest 10%, for all 27 *Meloidogyne* spp.

The three commonly used methods define the similarity between two groups as: the similarity between the individuals which are closest to each other, 'nearest neighbour'; the similarity between the individuals which are furthest from each other, 'furthest neighbour'; the average similarity between members of one group and members of the other group, 'average linkage'. The dendrogram displays this grouping process. Selection of the grouping method should be determined by the characteristics of the samples being studied.

Figure 4 shows the dendrogram for the Meloidogyne data using average linkage between groups, with individual similarities based on the Nei and Li coefficient. The lines have clustered into known species groups. This gives us confidence that the same methods, applied to samples of unknown species, are likely to identify species types correctly.

Principal co-ordinate plots Principal co-ordinate (PCO) plots are also based on the matrix of similarities, but avoid any emphasis on hierarchical clustering. The aim is to create a two-dimensional plot of the samples which represents as closely as possible the original distances, or similarities, among all the samples. A commonly used analogy is the creation of a map of the towns of Great Britain using only knowledge of inter-town distances. In this case the PCO plot can represent the original distances exactly but for data based on similarities and distances appropriate to

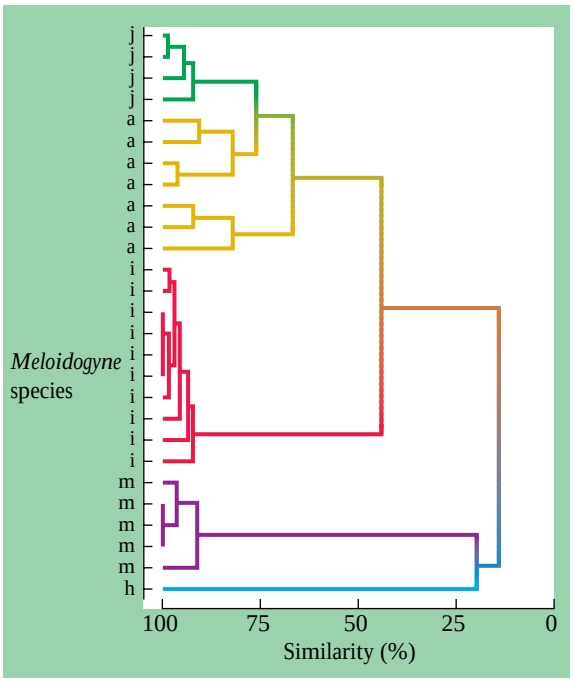


Figure 4 Dendrogram of the 27 *Meloidogyne* lines. Symbols a,h,i,j,m denote known species.

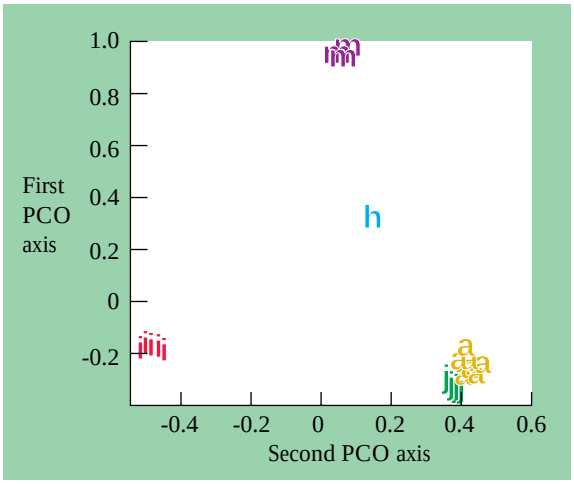


Figure 5 Principal co-ordinate plot of the 27 *Meloidogyne* lines. Symbols a,h,i,j,m denote known species.

marker data the PCO plot can only be an approximation to the original distances. Figure 5 shows the PCO plot for the Meloidogyne data. There is a little more information in this plot compared with the dendrogram. The single 'h' sample is shown to be approximately equidistant from all the other species and the three species groups, i, m and a+j are approximately equidistant. These conclusions, however, must be taken in light of how good the PCO approximation is to the original distances.

Simple sequence repeat data As new marker methods become available it is important to ensure that the standard statistical methods are still appropriate. Recently microsatellite simple sequence repeat (SSR) data have become another part of the biotechnologists' armoury. In effect the technique counts the number of times a specific short sequence of nucleotides occurs between two primers. Several such sequences, each with its own primer pair, are targeted in each investigation. The data again appear as bands and Figure 6 shows part of a microsatellite investigation of 49 genotypes of soybean in which the genotypes are represented by columns, and the rows have been partitioned into 3 groups, each group representing a targeted short nucleotide sequence. The position of a band indicates the number of times the sequence has been found; bands near the top of the group indicate greater frequency. Extra work is involved in deriving the exact number of repeats (sometimes referred to as 'allele size') for each band position, and it is tempting to proceed directly with familiar statistical procedures based on the presence or absence of bands.

Applying the Nei and Li coefficient to the bands in Figure 6 results in the similarity between genotype 1 and genotypes 2 and 3 both being 1/3, but between genotypes 2 and 3 being 0. For SSR data of this type the Nei and Li coefficient derives the proportion of target sequences which have the same frequency. No account is taken of the actual frequencies, or of the range of frequencies. Table 3a shows the frequencies for the first three genotypes.

It seems unreasonable to conclude that there is no similarity between genotypes 2 and 3 when their allele sizes for the third sequence differ by only 1, and yet for other genotypes the difference can be as large as 8. However until the causes of differences in repeat frequencies are better

a)

Genotype	Sequence 1	2	3
1	105	95	131
2	111	95	132
3	98	83	131
Range 49 genotypes :	38	30	8

b)

Genotype			
1			
2	0.91		
3	0.81	0.68	

Table 3 a) Allele sizes, based on three simple sequence repeats, for 3 soybean genotypes from a complete set of 49 genotypes. b) Similarities, based on allele sizes, for the same genotypes.

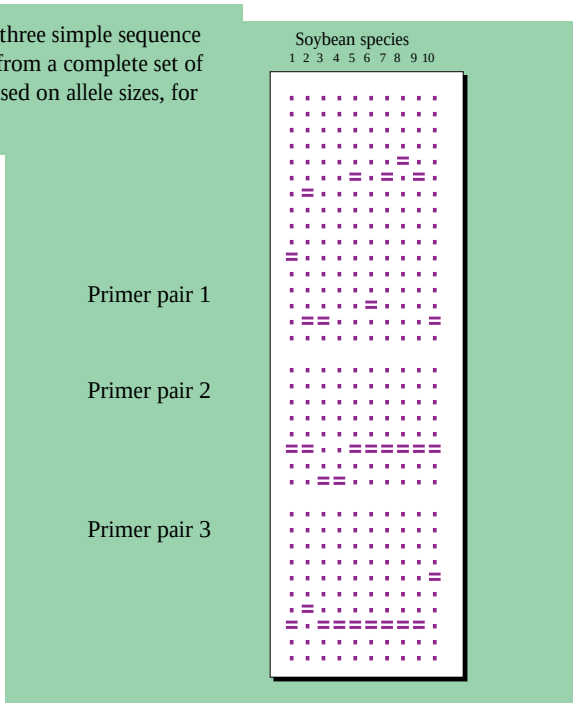


Figure 6 Microsatellite bands for 10 out of 49 soybean genotypes, separated into three primer pair groups.

understood some degree of arbitrariness in calculating similarities for microsatellite data is inevitable.

An alternative approach is to define the distance between two samples, based on a single target sequence, as the difference in frequency divided by the frequency range. The corresponding similarity is then defined as 1.0 minus the distance, and the similarity based on all the target sequences would then be the average of the individual similarities. Using only the data in Table 4a the resulting similarities shown in Table 3b.

These values reflect better the idea that genotypes 2 and 3 have some degree of similarity and that genotype 1 has more in common with genotype 2 than genotype 3.

Measurement of the sensory qualities of foods

E. A. Hunter, D. D. Muir[1] & R. M. Brennan

A partnership of SASS, the Hannah Research Institute (HRI) and SCRI is developing expertise in the sensory properties of soft fruits and their products. New funding from SOAFD will allow the expertise on the sensory properties of dairy foods at HRI and the statistical design and analysis of sensory experiments at SASS to be extended to soft fruit work at SCRI. The sensory information obtained will be used to increase understanding of the inheritance and genetic control of sensory properties, thereby allowing the efficient selection of high quality genotypes.

Unless food products are attractive to the consumer they will not be purchased and low price is no compensation for poor sensory qualities. Only very simple characteristics can be measured easily by instruments and food retailers and manufacturers are increasingly turning to sensory assessment to ensure the quality of food products. The development of new products, the improvement of existing products and production methods are areas where this methodology is proving to be effective and in many cases essential. For producers of the raw materials for the food industry, the sensory quality of the final product is an increasingly important factor in the selection of new methods of production. There is also a large and growing use of sensory testing for health care and cosmetic products.

Sensory assessment uses human beings as the measuring instrument. There are many kinds of sensory test of which difference tests and sensory profiles are the most widely used. Difference tests include triangular and other similar tests, in which the assessor picks out the "odd" samples, and also the direct estimation of the magnitude of sensory differences between samples. One deficiency of these tests is that the nature of the differences are not defined. Work at HRI, has concentrated on the sensory profile test which is interpretable and is very widely used for research, development and quality control.

A simple use of sensory profiling is when several people (assessors) rate samples for a number of sensory attributes. For example, bitterness is commonly rated on a five point scale,

1 = no bitterness,
2 = trace of bitterness,
3 = slightly bitter,
4 = bitter,
5 = very bitter.

External standards (such as solutions of varying concentrations of quinine for bitterness), may help to define attributes and standardise the scale for each assessor. Developing and refining a vocabulary is an essential part of sensory profile work and can be done in an objective manner[1].

Improving the objectivity and precision of the measurement process and analysing and interpreting the data pose many challenges. These include:

- the selection, training and calibration of assessors
- the provision of a suitable testing environment
- order of sample presentation and number of samples presented in a session
- data capture and management
- statistical analysis
- reporting results

[1]Hannah Research Institute, Ayr.

Over a number of years, SASS has contributed to the development of the sensory expertise at HRI. The testing environment was greatly improved by the conversion of a building into a customised sensory laboratory in 1991. Assessors are now isolated from each other during testing, so their assessments are completely independent; the temperature and lighting are closely controlled giving a standardised testing environment; and the sensory booths are supplied with tempered fresh air from above at a positive pressure to ensure that assessors are free of aromas from the sample preparation area. It is now possible to control the order of presentation, a factor shown to be very important in the control of variation[2]. A computerised data collection system has been developed and this has made a major contribution to the efficient use of technical staff and to good record keeping and storage. It allows the routine use of an undifferentiated line scale, rather than a 5, 7 or 9 point scale, without extra technical inputs. This has been shown to be a more precise method of capturing information.

Few statisticians work in food science and technology and, consequently, there is a poor appreciation of the contribution statistical methods can make to the design and analysis of experiments. Only simple designs, which ignore known sources of variability, are used in many sensory laboratories and sophisticated multivariate methods of analysis are rarely used. Unlike biological and medical science, food science has not yet generated intermediate level books which describe up-to-date statistical methods. The major SASS input to the HRI programme has been the development of a statistically based strategy for the design of sensory experiments, the analysis of data and the interpretation of results.

Solutions have been developed to many of the problems of sensory assessment and 11 papers have been published on sensory methodology and applications in the last 5 years. The facilities at HRI have attracted repeated funding from industrial sponsors thus aiding wealth creation directly.

SOAFD funding for the new joint project has allowed a panel of 14 assessors to be recruited and trained to supplement the existing laboratory panel at HRI and in addition a research assistant has been recruited for three years to work on the development of statistical methods for the design and analysis of sensory studies. Until recently, each experiment has been designed and analysed separately but the increased flow of experiments through the sensory laboratory has required

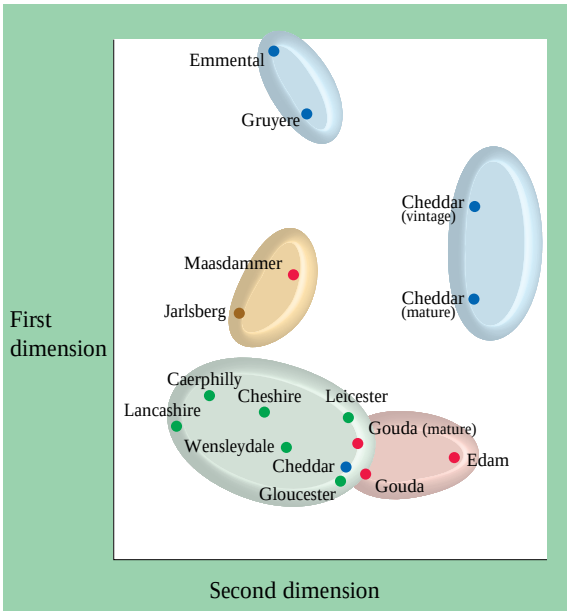


Figure 1 Sensory space map for flavour attributes of cheese constructed from scores on first and second dimensions of the consensus configuration derived by Generalised Procrustes Analysis of individual assessor matrices.

critical evaluation of the existing design and analysis process for profile experiments. We have committed ourselves to building a system which interfaces with the existing data collection system and which is designed to run on a powerful PC.

The system specification calls for the ability to routinely implement the best levels of design and analysis reported[3]. It will be run by food scientists at HRI and so must be user friendly. Good labelling of output and relevant publication quality graphics are regarded as essential (Figures 1 & 2). Already, sufficient progress has been made on a prototype system to ensure that the final product can be delivered. The system will be examined in the context of the on-going programmes of work on dairy products at HRI and on soft fruit genetic improvement at SCRI.

In order to illustrate the techniques that are being used to analyse data from the HRI sensory laboratory, a recent study[3] in which the sensory properties of 16 samples of hard cheese were evaluated by 16 assessors is considered. Each cheese was rated by each assessor for five aroma, ten flavour and five textural attributes. The aroma, flavour and textural data were analysed (separately) by Generalised Procrustes Analysis (GPA) of the data for each assessor to provide sensory maps. Figure 1, which is the sensory map for flavour, shows that the English regional cheeses form a sub-group

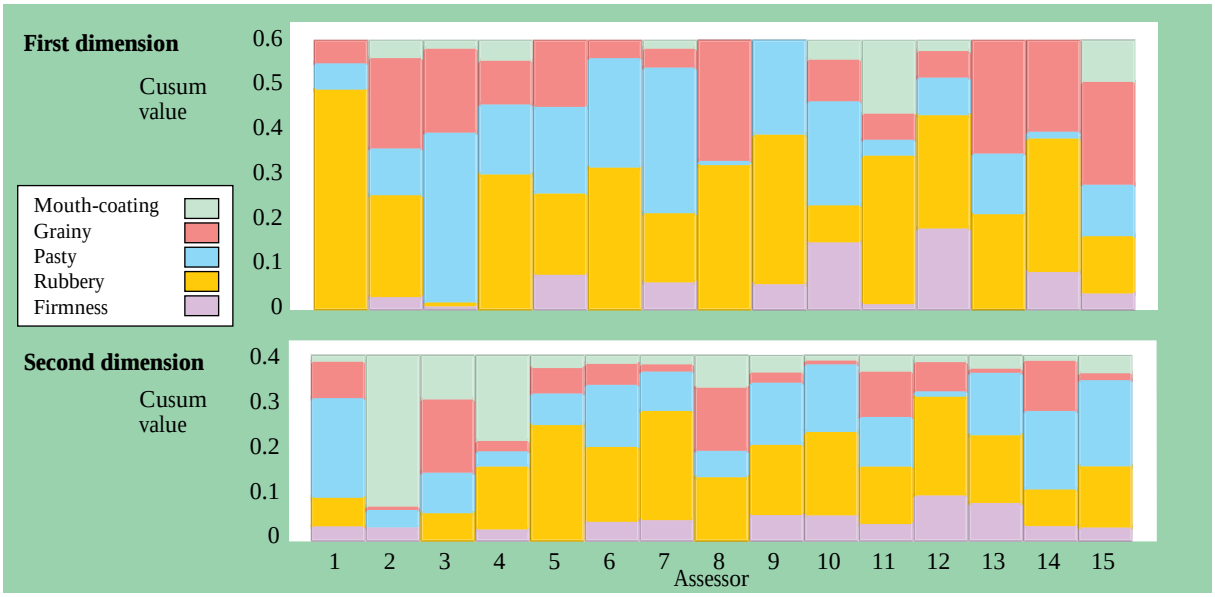


Figure 2 Single-value CUSUM diagrams showing the dependence of the first three textural dimensions on the original variables (textural attributes) for all assessors after Generalised Procrustes Analysis, using the fitted (smoothed) projections.

with Cheddar, which adjoins the Dutch cheeses of Gouda and Edam. The Swiss cheeses Emmental and Gruyere are shown to be different from the other cheeses as are the mature and vintage Cheddar. Interestingly the new Dutch cheese Maasdammer is perceived to be similar to the Norwegian cheese Jarlsberg. Compared to working on estimated treatment means, the advantage of using GPA is that it allows different assessors to use attributes terms such as "firmness", "rubbery", "pasty", "grainy" and "mouth-coating" in different ways. It also allows different assessors to use different proportions of the scale. Assessors exhibit surprising differences in their use of terms. Using a projection technique, Figure 2 shows the way each assessor's data was related to the consensus for texture. For the first dimension there was good agreement between assessors whilst for dimension 2 the agreement is poorer.

SASS organised the second SENSOMETRICS conference held in Edinburgh in September 1994 which was attended by 70 delegates from UK, continental Europe, USA, Canada and Australia. The UK delegates included several from local industry as well as food scientists from Queen Margaret College and Strathclyde University. SASS is coordinating an EU AAIR concerted action to bring together 10 laboratories to consider the problems of sensory assessment in different cultures, using internationally traded cheeses as a model product. This builds upon previous work with The Norwegian Food Research Institute[4]. Through participation in the EU Flair concerted action "SENS" (1991-94), SASS has made contacts with statisticians working in food science throughout Europe thus providing a comparison with the performance of other sensory laboratories.

References

[1] Hunter E. A. & Muir D. D. (1993). *Journal of Sensory Studies* 8, 213-227.

[2] Muir D. D. & Hunter E. A. (1991/2). *Food Quality & Preference* 3, 141-145.

[3] Muir D. D., Hunter E. A., Banks J. M. & Horne D. S. (1995). *International Dairy Journal* 5, 157-177.

[4] Hirst D., Muir D. D. & Næs T. (1994). *International Dairy Journal* 4, 743-761.

Research services

Analytical Facilities

W.W. Christie

Laboratory Accreditation

An essential requirement for further successful marketing of SCRI scientific expertise and facilities on a competitive basis will be the operation of an approved Quality Assurance (QA) System covering all aspects of our activities. This may also apply with regard to the award of research funding both from central government and external sources. In other scientific organisations, the introduction of quality systems has led to an improvement in the overall efficiency of activities at all levels. With this in mind, Dr T. Shepherd has been appointed quality assurance officer for the Institute, and a programme to implement a QA system within SCRI has been initiated under the auspices of the Chemistry Department. Stable Isotopes analysis is the first area to seek accreditation. The system is being extended to other areas within chemistry in a phased process, before it is applied to other selected and general activities within SCRI. With the support of Scottish Enterprise Tayside (SET) and MRS Ltd, consultants have been appointed to monitor and advise on the introduction of QA. Within the Chemistry Department we are working towards application for blanket EN ISO 9000 (BS 5750) registration for the full range of our activities, and EN 45000 (NAMAS) registration for selected areas. Such internationally recognised standards are also suitable for coverage of most other activities within SCRI.

Stable Isotope Facilities

Stable isotopes are now basic tools for the study of plant physiology, crop genetics, ecology and food webs. Valuable information comes both from studying natural variation in stable isotope composition and from following the fate of added isotopic tracers. SCRI is equipped with a comprehensive range of modern instrumentation for stable isotope analysis. With these, we can tackle most of the biologically important low atomic number elements, ^{13}C, ^{15}N, ^{18}O and ^{34}S, in a wide range of solid, liquid and gas samples. All the instrumentation is based on continuous-flow isotope-ratio-mass-spectrometers that are fully automated and operated through computer data systems. Automation allows a high through-put of samples, essential for many biological experiments where large data sets are required. For solid samples, the Europa Scientific Tracermass and 20-20 mass spectrometers are interfaced to Roboprep CN and ANCA-*NT* SL combustion sample converters. A Roboprep G+ gas purification unit is used for gas analysis. Plant samples of 1 to 5 mg are used, containing 25 to 100μg of the element of interest. Where possible, analytical protocols are devised to minimise sample preparation and fully exploit the automation.

Stable Isotope Facilities. Gas samples being prepared for automated analysis of ^{13}C and ^{18}O in CO_2.

SCRI also has expertise and resources for sample preparation from a wide range of sample types. This includes plant sample drying and grinding, freeze drying and weighing facilities. Research support is aimed at developing new methods to assist the Institute's commissioned programme.

Services to Molecular Biologists

DNA synthesis The DNA synthesis facility is based on an Applied Biosystems model 394 DNA/RNA synthesiser. The department also has a single column model 391 synthesiser which provides cover during servicing and repairs. The 394 instrument allows the

simultaneous synthesis of four oligonucleotides and is equipped with automated amidite dissolution, on line trityl monitoring and automatic cleavage facilities. Side chain deprotection and recovery of the DNA remain manual operations. Both instruments synthesise DNA by a step-wise solid phase method which allows over 100 nucleotides to be coupled in a single nucleic acid molecule. Oligonucleotides containing between 20 and 30 residues are normally required and cycle times are such that eight oligonucleotides can be made in a day.

Custom peptide synthesis Peptide synthesis is carried out using an Applied Biosystems model 431A peptide synthesiser, an instrument which automates the coupling of amino acids to form a peptide of any desired sequence. Peptides of up to 35 amino acids can be constructed easily, but with suitable care and alterations to the chemistry 50 to 60 residue peptides are possible. The cycle times for amino acid couplings are variable with a minimum of two hours and so the construction of an average peptide takes several days. Peptides can be made in a variety of formats; as single chain products fully deprotected and either bound to a resin bead or not; or as branched structures containing four copies of the desired peptide in a single, soluble, molecule. An Applied Biosystems 151a HPLC system is available for the analysis and purification of peptides.

DNA sequencing Automated DNA sequencing is performed on an Applied Biosystems model 373 Stretch Sequencer, with data handling and analysis sofware on an Apple MacIntosh Quadra 650. The sequencing reactions are performed using a Perkin Elmer 9600 thermal cycler in collaboration with the CMG Department. By using fluorescent dyes and laser scanning technology this system can generate large amounts of sequence data rapidly. Up to 35 clones can be loaded on a single gel and an accurate sequence of 450 bases called in each, resulting in nearly 16 kilobases of sequence generated in 24 hours.

DNA sequencing equipment.

Mass Spectrometry

The Institute is particularly well equipped in the field of mass spectrometry (MS) with three state-of-the-art instruments devoted to structural analysis of organic compounds. Housed in a new purpose-built laboratory suite, all systems have integrated computer control, library search capabilities and distributed data processing facilities. The core instrument is a high-performance double focusing magnetic sector mass spectrometer, a Kratos 8/90. With electron impact (EI) and chemical ionization (CI) and +ve/-ve ionization capability, high resolution (50,000 at 10% valley) and a mass range of up to 16000, this instrument can provide mass and structural data on a wide range of organic compounds.

Mass spectrometry equipment for the analysis of volatiles.

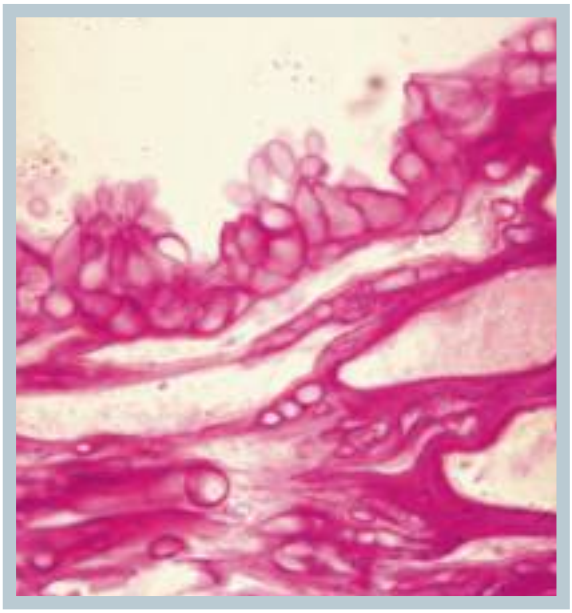
In addition, a bench top instrument is dedicated to the analysis of organic volatiles. This consists of a Perkin Elmer automated thermal desorption system (ATD) linked to a VG TRIO-1000 quadrupole gas chromatography (GC)-MS which permits detailed characterization of the profiles of organic volatiles generated by biological systems. The Institute's most recent acquisition, a Finnigan SSQ 710C dedicated liquid chromatography-MS instrument with atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) interfaces completes the facility. This has an ability to analyse samples whose high molecular weight, lack of volatility or polarity, precludes analysis on the other instruments. APCI and ESI are soft ionization techniques and generally only produce molecular ions, e.g. $[M\text{-}H]^+$ or MH^+, but the multicharge ionization mechanism of electrospray can extend the basic 2000 mass range of the instru-

ment by a factor of about 20 giving a mass range of >40,000 amu.

Mass spectrometric analysis at SCRI covers a broad spectrum of chemical investigations generated by the research programme of the Institute. A wide range of plant metabolites have been analysed, both in the native form and as derivatives, and include sterols, monoterpenes, sesquiterpenes, pentacyclic triterpenes, dimeric forms of phenolic acids, glucosinolates, long-chain wax esters, peptides, essential oils, carbohydrates, polychlorinated biphenyls and fatty acids. Finally, there is an experienced and expert staff ready to tackle and to solve most structural problems. They are actively seeking full laboratory accreditation status and working practices are commensurate with recognised standards.

Microscopy

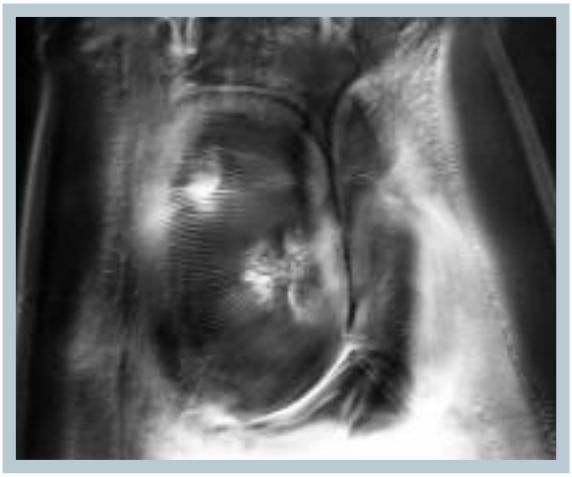
Light and fluorescence microscopy (B. Williamson) As the first step in any microscopic investigation of biological materials, the surface morphology of the specimen must be appreciated and recorded. Information of this type is best gained with stereoscopic dissecting microscopes with a large working distance between the objective lenses and the specimen and with zoom attachment for continuous enlargement. SCRI has a wide range of these microscopes suitable for studying sporulation of fungi, aphids feeding on leaves, or nematodes penetrating root apices *in vitro*. Many biological specimens are subject to vibration, are sensitive to intense light and prone to rapid desiccation. To provide photographic records of these specimens a range of photomicrographic equipment is available. An Olympus PM-10AD photomicrographic system is available which can be mounted on a variety of microscopes fitted with a trinocular head and used in conjunction with fibre-optic cold light sources or with an electronic flash attachment to provide shadow-free, sharp images with the correct colour balance.



The fungus *Elsinoe veneta* producing conidia and invading surface of raspberry stem affected by cane spot disease. (Sectioned in resin at 1.5µm thickness, stained for carbohydrate by periodic acid-Schiff reagent, observed by bright field microscopy (x 1000).

For the examination of small groups of single plant cells, microorganisms and other relatively transparent microscopic invertebrates, a wide range of microscopic techniques are available in several Departments. Over three decades, SCRI has invested in Zeiss Universal Research microscopes and we possess a wide choice of specialised lenses and imaging systems including bright field, phase contrast, differential interference (DIC) and polarising optics. For example, living plant and fungal protoplasts, epidermal cells of onion or pollen tubes grown *in vitro* can be observed best by DIC and the integrity of membranes, cytosol, nuclei and plastids revealed. Motile bacterial and fungal cells, however, can be seen and recorded with phase contrast microscopy, because the flagellae are shown in sharp relief, and high speed time-lapse video recording can be useful to record their behaviour.

The surface of largely opaque specimens can be studied with epifluorescence microscopy, providing the specimen is autofluorescent at the chosen wavelength or if a fluorochrome (fluorescent dye) is introduced. For example, aniline blue in phosphate buffer solutions stains cell walls of fungal hyphae on the surface of leaves and flowers and can be examined with no further preparation after a few minutes. Fluorochromes introduced into tissues also provide highly versatile and specific tools for analysis when



Groove patterns in the buccal capsule of predatory nematode *Anatonchus tridentatus* viewed by DIC (x1000).

combined in a stable complex (conjugate) with specific polyclonal or monoclonal antibodies which recognise particular epitopes, or complexed to plant-derived proteins called lectins which combine with particular sugar residues.

In some cases, the epitopes or sugar groups of interest are exposed on the surface of the specimen, but in most cases the specimen must be sectioned to expose them. To preserve the chemical specificity of the antibody or lectin the live specimen must then be frozen rapidly before freeze-sectioning (3-15 μm thick), or chemically 'fixed' with aldehydes, infiltrated with resin, polymerised and sectioned for light microscopy in the thickness range 0.5-4.0 μm. Sections can be produced at SCRI from frozen specimens with a Bright Cryostat fitted with a heavy-duty rotary microtome; prepared from small resin-embedded specimens using a range of ultramicrotomes, or for large format specimens the Reichert-Jung 1140 Autocut Universal microtome with 12 mm glass knives would be more appropriate. Resin-embedded sections of plant or invertebrate specimens, sometimes freeze-substituted before embedding to preserve translocated fluorochromes and other labile molecules (e.g. lucifer yellow transported in tissues), can then be examined by fluorescence microscopy after sectioning to localise the fluorochome-antibody conjugate, or stained with other dyes to reveal key features (e.g. secondary cell wall layers in flax fibres, or fungal hyphae growing in plant cell walls).

To record such detail at the highest magnification the quality and choice of lenses is of paramount importance. SCRI has a full range of Zeiss, Olympus and Nikon objective lenses suitable for fluorescence, flat field optics with colour correction and DIC optics. To provide a permanent record for measurement of chromosomes from *Solanum* spp. the recent acquisition of a Sony CCD-IRIS black and white digital camera mounted on a Nikon Optiphot-2 microscope and video printer has proved invaluable for data capture.

For some work there is a need for inverted microscopy, particularly for inspection of protoplasts, cell suspension cultures or hybridoma cells in liquid media. Low power objective lenses with a long working distance are needed to cope with the thickness of culture dishes and media. For example, a Nikon Diaphot-TMD inverted microscope fitted with epi-fluorescence system and x2 and x4 objective lenses of long working distance are used for identification of antibody-labelled bacterial colonies (*Erwinia carotovora* ssp. *atroseptica*) grown in plastic 24-microwell dishes.

Confocal laser scanning microscopy (K.J. Oparka) In August 1993 SCRI acquired a Bio-Rad MRC 1000 confocal laser scanning microscope (CLSM), the first of its generation in the world to be installed (see page 73). In the CLSM laser light is focused onto the specimen through a small aperture located at a conjugal focal plane to the specimen. The illuminated spot of light is then scanned rapidly across the specimen to produce a confocal image, a clear optical 'slice' of the tissue at a fixed depth within it. Confocal microscopes are exceptionally good at giving clean optical sections of biological material and for producing three-dimensional reconstructions at the cellular and subcellular levels. In practice, light scattering and absorption by plant tissues limits confocal 'sectioning' to a depth of about 100 μm below the tissue surface. However,

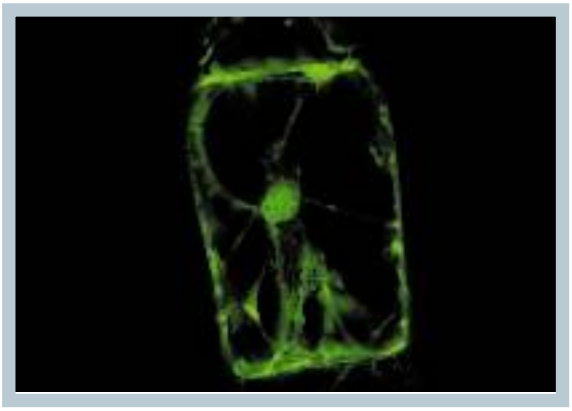


Figure 1 Leaf trichomes of *Nicotiana clevelandii* systemically infected with PVX carrying the GFP gene.

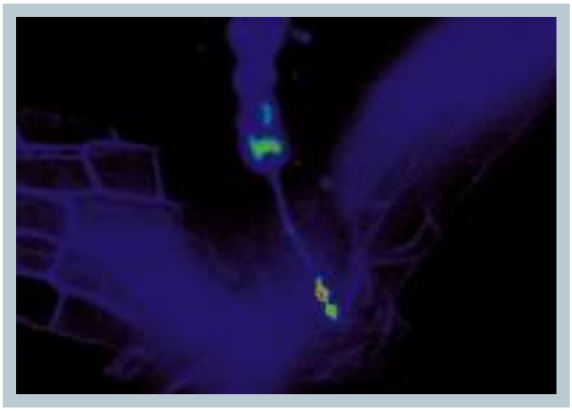


Figure 2 Confocal optical section of the head of a feeding nematode withdrawing a fluorescent probe from a parasatised *Arabidopsis* root.

CLSM is a very powerful technique and has begun to revolutionise several areas of plant cell biology.

Within SCRI, CLSM imaging is now being used in a range of diverse projects and applications. Physiologists have used the microscope extensively in the non-invasive imaging of plant transport processes, such as the phloem transport and unloading of a range of fluorescent probes Using this approach, together with thin biological specimens such as *Arabidopsis* roots, it has proved possible to study the cell-cell movement of small molecules within intact, functioning biological systems (see Annual Report 1993). Coupled to recent advances in microinjection techniques being made at SCRI (see page 75), CLSM is also proving invaluable in tracing the movement of macromolecules such as viruses as they move systemically throughout plants. A recent breakthrough, involving collaborative research between virologists and physiologists, is utilising plant viruses genetically modified to express the green fluorescent protein (GFP) from the jellyfish *Aqeuorea victoria*. The gene for GFP has recently been cloned[1] and the ability to generate fluorescence *in situ* (without additional cofactors) has opened up tremendous possibilities for continuously monitoring, by CLSM, gene expression, cell development and protein trafficking in unperturbed living plant cells and tissues[2]. In viral constructs of potato virus X (PVX) and tobacco mosaic virus (TMV) which carry the GFP gene, the GFP is released into the plant-cell cytoplasm using the virus coat protein promoter. Thus, each new cell which becomes infected during cell-cell spread of the virus accumulates high levels of GFP. Such cells are easily identified using CLSM and the level of resolution which can be achieved on single living cells is striking (Fig. 1).

In addition, CLSM has found uses in quantitative measurements of ions and pH using ratio imaging techniques and has also been used to image nematode-host interactions (Fig. 2). In Crop Genetics CLSM has been used to examine chromosomes within intact potato embryos and has also begun to be used with *in situ* hybridisation techniques.

At present, the CLSM is equipped with a 25 mW Krypton/Argon laser and a 100 mW Argon laser. The latter is used predominantly in studies of fluorescence redistribution after photobleaching (FRAP). The microscope also posseses a fast-photon counting facility to detect and enhance extremely weak fluorescence signals and is equipped with extensive software packages for image analysis and 3-dimensional image reconstruction. For photography, a Kodak XLS 8300 digital printer provides colour images of high resolution directly from the microscope.

[1] Prasher, D.C., Eckenrode, V.K., Ward, W.W., Prendergast, F.G. & Cormier, M.J. (1992). Gene 111, 229-233.
[2] Baulcombe, D., Chapman, S. & Santa Cruz, S. (1995). The Plant Journal 7, 1045-1053.

Electron Microscopy Facilities (I.M. Roberts)

Electron microscopy at SCRI has a long history dating back over 30 years to the early sixties when the then Scottish Horticultural Research Institute (SHRI) purchased its first transmission electron microscope (TEM), an elderly second-hand Metropolitan Vickers EM3A. Since those early days the Institute has had the foresight to continually upgrade and update its electron microscope facilities so that we are now very well equipped to tackle a wide range of electron microscope studies. At the present time we have three transmission electron microscopes *viz.* a Philips CM 10, a Jeol 1200 EX and a Jeol 100 S. Two of these have fully eucentric goniometer stages which facilitate 3-D reconstruction of images and we also have a high resolution Scanning Transmission module (STEM)

Philips CM10 transmission electron microscope.

for the study of surface features on small specimens. Ancillary equipment for TEM includes a specialised vacuum coating unit with facilities for freeze-drying and high resolution shadowing, and contamination-free carbon evaporation for thin support films, together with modern ultramicrotomes (and Knifemakers) with conventional glass knives and diamond knives for ultrathin section studies. In addition to the three TEMs we also have a Jeol T 200 scanning electron microscope (SEM). For SEM, most specimen preparation utilises an Emscope SP 2000 Sputter-Cryo unit interfaced with a low temperature SEM stage thus allowing examination of unfixed, fully hydrated samples. This is supported by standard sputter coating units and Critical Point Drying modules.

EM scope SP2000 sputter-cryo system for specimen preparation for scanning electron microscopy.

Originally, electron microscopy at SCRI was used exclusively for studies on plant viruses and the Institute maintains a deserved reputation for its considerable expertise on techniques for the detection and identification of viruses in plants and in their insect and nematode vectors. The principal techniques involved in these studies include negative staining, immunoelectron microscopy, ultramicrotomy (fixation, embedding etc), and specialised extraction methods. More recently we have developed immunogold labelling (IGL) techniques for the study of epitope sites on the surfaces of virus particles and for viral and other proteins in thin sections of plants and disease vectors. SEM studies have been greatly helped by the cryo-preparation and transfer module. This has made it possible to examine fully hydrated fungal or bacterial infections of highly vacuolate fruits such as black currant, raspberry and others and, together with freeze fracturing, critical point drying and sputter coating, offers a wide range of specialist techniques and equipment for surface studies

Fourier-Transform Infrared Microspectroscopy (D. Stewart) An FT-IR spectrometer with a microspectrometer attachment is a recent addition to the microscopical and spectroscopic facilities of the institute. FT-IR spectroscopy involves directing a laser beam, with frequencies normally in the range 4000-600 cm^{-1}, at a sample and analysing the unabsorbed radiation. This type of radiation, when absorbed causes chemical bonds to vibrate and the frequency of the absorbed radiation is characteristic of a specific type of bond. For example, aromatic compounds absorb at 1595 and 1510 cm^{-1}, whereas protein amide bonds exhibit two absorbances at 1650 and 1550 cm^{-1}. FT-IR spectroscopy can therefore be used to look at the chemical composition and structure of a wide range of materials and tissues.

The microspectrometer functions in the same manner as a normal FT-IR spectrometer. However, by using redundant apertures the incident laser beam can be narrowed down to a beam of 8 μm diameter. This allows FT-IR spectroscopy to be performed on very small samples, e.g. starch granules, or localised regions of large tissues e.g. cell walls, nucleus etc. The microspectrometer also has a camera attachment so that the area under analysis can be photographed.

The sample under analysis can be scanned in two modes; reflectance and transmission. In reflectance mode, the incident laser is directed at the area of interest, then the reflected radiation is detected and compared to the incident beam. This gives information on the absorbed IR frequencies and hence chemical and structural information. In this mode the reflected radiation gives information about the first few microns of the sample surface. Examples of its

FT-IR spectrometer.

possible use are the study of fungal microspore surfaces, changes in seed coats during dormancy break and the effects of processing on fibre surfaces.

Transmission microspectroscopy involves detecting the radiation after its passage through the material. This has been used successfully to study the effect of bacterial infection on the starch granules and cell walls of potato, and to follow the changes in structure and composition of anatomically different flax cells during development. Examples of other potential applications are the effect of high salt concentration on cell wall and membrane structure and the expression of antisense genes on intra- and extra-cellular composition and structure.

NMR Microscopy (B.A. Goodman) The Spectroscopy Group has a Bruker AMX300/SWB Fourier Transform NMR spectrometer with a micro-imaging accessory. The arrangement of the system is such that there is convenient access for the investigation of small trees. The 7.05 Tesla magnet has a 150 mm bore and two separate probeheads, one for variable temperature operation in the range -100 to +100°C and exchangeable RF inserts for samples with 5, 10, 15, 20 and 25 mm diameters, the other a 'birdcage' design for large sample access (up to 65 mm diameter). In addition, a surface coil modification to the "birdcage probe" is being developed and should be available towards the end of 1995. The system contains a Bruker X32 host data system and the Bruker library of imaging pulse sequences; the CPU has sufficient memory for the analysis of 3-dimensional image datasets containing 256^3 voxels (volume elements).

NMR microscopy is able to produce images of the internal structure of small biological specimens (from a few millimetres to several centimetres diameter, with typical spatial resolution of a few tens of micrometres), based on the physical and chemical properties of protons in the main fluid-state molecules (usually water, but occasionally lipids or sugars). Because of the non-invasive nature of the technique it is particularly suited to the investigation of developmental processes *in vivo*. Such measurements are not limited to the observation of structural changes in the specimen, but can produce separate images of the distributions of the major fluid components (e.g. of water and lipids in plant seeds or insect pupae). The extent of tissue damage caused by either biotic or abiotic processes can also be readily visualised as a result of changes induced in the physical characteristics of tissue water (examples of successful applications include the study of bruising and fungal damage in soft fruits and of frost damage to buds of woody perennials). In addition, it is expected that facilities will be available by early 1996 to perform image directed spectroscopy (ie the generation of conventional ^{1}H NMR spectra from selected regions of a specimen defined from its microscopic image). Finally NMR microscopy is a powerful technique for the observation of fluid transport phenomena *in vivo*, and is able to envisage directly rates of water movement in different tissues (subject to the overriding limitation of resolution).

NMR facility.

Data Processing Unit

R.J. Clark

The DP Unit provides the computing service to scientists. Information Technology has an impact on all science and the aim is to provide the benefits of IT at least cost in time and effort. Scientists working from their desktop personal computers (PCs) can access SCRINet, the Local Area Network (LAN) for centrally mounted software services, and the Internet. A Unix system is available

running on SUN workstations and a Novell service via a PC server. Planning, configuring and running this expanding network, with all the software mounted and devices linked, is the major responsibility of the Unit.

Advice is given on the selection of appropriate computer equipment and software for particular tasks and user support for fault finding and software problems are provided on a day to day basis. Standardising on particular hardware and software options aids the training and support of users, and the production of guides to the use of SCRINet and software applications, and the solving of software problems is an ongoing task. Maintenance contracts and licence agreements are negotiated on a site basis to minimise costs.

Hardware

SCRINET The Local Area Network (LAN), installed in 1991, has Thin Ethernet cabling within buildings, with optical fibre connections in a star topology connecting to a central hub in the Data Processing suite. There are currently eleven Sun SPARCstations of various models attached to SCRINet.

Four Sun SPARCstations running the Solaris (Unix) operating system provide a central processing service to PCs acting as terminals. In addition, another six Sun SPARCstations are connected to SCRINet for various purposes. Some are used by specialist groups, e.g. modellers; others are devoted to specific tasks, e.g. image analysis.

A variety of peripheral devices are attached including hard disks, optical disk, CD-Rom and digital tape drives, line-printer and laser-printers and a plotter. Data files can be archived on DAT tape. A server for Novell Netware provides a similar service for Microsoft DOS (MS-DOS) and Windows software over SCRINet. Novell Netware also enables easy installation and management of departmental laser printers.

IBM PCS AND COMPATIBLES Nearly 250 IBM PCs and compatibles are in use, ranging from IBM Model 30s (with the 8086 processor) to the most recent Pentium based micros. Seventy per cent of PCs are fitted with the 80386 chip or higher and have MS Windows installed. Most are connected to SCRINet, with network software that allows access to the Sun hosts both as a terminal and as a network disk and printer server. The Novell network server acts similarly for sharing MS-DOS files and software

The DP suite has a user area with ten PCs available to users and visiting workers. These are attached to SCRINet with the full range of software and printing devices set up ready to use.

Portable PCs are used principally for data collection, demonstrations and conferences. They are also available to all staff for work at home. Portable computers have been used at SCRI since 1983 for recording data in the field or glasshouse. Palm top portables have recently been purchased to replace the older Epson HX20 for similar tasks.

EXTERNAL CONNECTIONS The network is connected to Dundee University, JIPS and the Internet by a 2Mb/s BT Megastream line on a Chernikeef router.

Eview	- display of structured information (Edinburgh University).
Genstat	- a powerful and versatile statistical package written by Rothamsted Experimental Station.
SPlus	- statistical package with programming interface.
Khoros	- a large image processing programming library (public-domain).
Maple	- for mathematical operations and interactive graphics.
Minitab	- a user-friendly interactive statistical program.
NAG Fortran library	- mathematical and statistical procedures.
Oracle	- a powerful relational database capable of working efficiently with large databases and many simultaneous users.
Uniras	- a versatile interactive graphics package.
BioImage	- molecular biology gel scanning software (Millipore).
Latex	- Document processor.
xv	- an image viewer and manipulator.

Table 1 Sun Application Software - A representative sample of the software.

Software

SUN SOFTWARE Application software is installed in addition to the basic set of Unix tools and compilers. Selections of them are described in Table 1.

PC SOFTWARE POLICY AND SUPPORT The operating system for PCs is MS-DOS Version 5, and MS-Windows Version 3.1 is installed on most recently-purchased machines. The network software is PC-NFS Version 4 and Novell IPX.

Standardisation offers cost benefits in the staff time needed for training and support. A comprehensive range of software is available to cover most applications and full use is made of shareware and academic site license agreements. Supported software can be mounted on the Novell server with sufficient licenses to cover demand, with obvious cost benefits to departmental budgets.

SOFTWARE APPLICATIONS DEVELOPED BY THE UNIT

CHIP CHIP was originally written by a member of the Crop Genetics Department and is used by crop geneticists for managing genetic databases, generating experiment plans and labels, performing statistical analyses and selecting and comparing progenies.

Data Collection from Scientific Instruments Occasionally there is a need for software to be written to collect data from a scientific instrument or data-logger. The principal applications in use at SCRI are data capture from, and control of, balances, the Squirrel data-logger and digitising tablets. Other interesting examples are described below.

ELISA program Originally written for the BBC micro and rewritten for the PC. It takes optical density measurements from a Titertek plate reader to measure the amount of pathogen (fungus, virus) in a plant extract detected with an antibody. Samples are randomised on plastic plates, and Trakwell Markers show the application sequence. A hyperbolic curve is fitted to a series of standards, and used to calculate the concentration in each sample.

Image Analysis Measurements from biological images, ranging from the microscopic (e.g. protozoa and bacteria) to the macroscopic (e.g. ground cover), are automated in one of two ways:

Semi-automatic, where an operator traces lines on a photograph or in a microscope image with the cursor of a digitising tablet connected to a microcomputer.

Automatic, where a video camera is used to record a digital high resolution image, from which features are extracted and measurements are made. A Millipore Bioimage System was installed in 1992, with a solid-state 1024 x 1024 element camera linked to a Sun IPC workstation. The BioImage software is principally used for analysis of bands from electrophoresis gels (radiographs, photographs, ethidium bromide fluorescence) with molecular weight determination from internal standards.

Nematode Measurement Program A versatile program capable of a variety of measurements with a digitising tablet, either directly from photographs or by superimposing an LED cursor in the field of view of a microscope fitted with a drawing arm. Used initially and principally for nematodes but anything of a similar shape may be measured, e.g. RNA virus particles.

Oracle Relational Database Management system

CONTROL OF SUBSTANCES HAZARDOUS TO HEALTH (COSHH) Detailed information, e.g. suppliers, hazards, handling, storage and physical data, on all the chemicals in the institute is held on a database. In addition, site specific information on room numbers, first aid and safe disposal is stored. This uniformity of data format covering information from various chemical suppliers, coupled with the detailed site specific information combine to make it an important tool for research, safety, and stock ordering. Information on any specific chemical is available to staff both on-line over SCRINet and as Hazard Data Sheets, printed and bound for each laboratory.

COMMONWEALTH POTATO COLLECTION Designed for the Crop Genetics Department, to hold records for the Commonwealth Potato Collection of wild and cultivated clones. It includes provenance, disease resistance, appearance, location, and quantity held. Stocks are regularly supplied to other organisations world-wide. This database is a prototype for proposed future work on genebanks for soft fruit, viruses, etc, that other users in the UK could access.

FIELD EXPERIMENTS APPLICATION RECORDS (FEAR) A database maintained by the Field Experiments Officer in collaboration with the Unit, containing information on preparation and management (fertilizer, pesticides) of field experiments, is available to all users.

Training

Training is an important part of the Unit's work. As scientific staff become more computer literate, they increase their productivity and less on-going support is needed from Unit staff. However, software is con-

Training in the use of the Internet.

stantly being upgraded making training a constant requirement for DP staff as well as users.

Seminars and workshops are arranged as required and 'User Notes' are written to guide the user through the basics with pointers to the full documentation. Some users have been trained in the use of graphics software and spreadsheet graphics so that they can prepare their own displays for seminars, conferences and publications.

External Services

WORLD WIDE WEB A browser for World Wide Web pages is installed on most PCs running Windows. Plans are in hand for the Institute to become a Web server.

Electronic mail Scientists use electronic mail and file transfer facilities for fast communication with many UK and overseas universities and research stations. The Unit provides support for scientists in making contact with colleagues across the world. A service is available through Unix and also on Windows using Pegasus mail, a popular package among academic users which allows attachments to email for copying directly into word processors.

BBSRC COMPUTING CENTRE Meteorological data for the UK are available from Harpenden.

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

Part of the library at SCRI.

Video editing facilities in the Visual Aids section.

publicity purposes. Graphics are produced on an Apple Macintosh system and are used to illustrate scientific results in publications and lectures and, during 1994, to create quality displays in exhibitions including Scotgrow, PMB Potato Harvesting & Handling Demonstration and the World Potato Congress. 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.

SCRI is committed to the improvement of the public understanding of science and has, since 1993, contributed to the National Week of Science, Engineering & Technology.

Estate, Glasshouse & Field Experiments Department

G. Wood

During 1994 there were a number of significant changes for the Estate, Glasshouse and Field Experiments Department. W.I.A. Jack, the Head of the Department since 1975, retired after 41 years' contribution to horticultural and agricultural research in Scotland (see People and Events), and E.A.M. Gardiner retired after 25 years valued service in the glasshouse area.

Since 1977 the area of field trials and numbers of plants produced in glasshouses have almost doubled to current levels of 56 ha and 300,000 plants, respectively, despite a reduction in staff numbers. Major efficiency gains have been made in order to continue to provide an uninterrupted high-quality service to the Institute's field and glasshouse research programmes. Significant steps have been taken to reduce costs and manpower inputs and to mechanise certain aspects of work wherever possible.

To maximise available space, we have continued to install new mobile aluminium benching (Fordingbridge) wherever possible in glasshouses. These benches lend themselves to auto- irrigation, -feeding and control systems (Sarnia) which have also been installed. In 1995 we shall be looking at auto-internal blinds (Fordingbridge) for shading and energy conservation plus auto-sensing daylength/intensity control of lighting systems in glasshouses. Refurbishment and updating of our growth cabinet/controlled environment facilities are now almost complete. The latest batch of 12 cabinets (Sanyo Fitotron) included 5 specifically for tissue culture.

Growth cabinet facilities.

On the field trial side several new machinery/equipment items were purchased. An 18m air-assisted tractor-mounted sprayer (Hardi) gives better cover of target and allows reduced water volumes to be used resulting in a bigger area covered with one fill. A machine harvester for potato plots (Bray Valley Gymnast) will allow trials to be harvested individually (or bulked) with a much reduced labour input. We shall be looking at the natural partner to this machine in 1995: a machine planter for potato plots (Edwards Farm Machinery F300L/2). Similarly, a machine planter for cane fruit and blackcurrant trials (MJF Amplanter) was purchased and proved very successful. In 1995 we shall be looking at the advantages to be gained from use of a new Wintersteiger plot combine in cereal trials. This should allow plot weighing, selection and data-logging on the move thereby improving throughput times and reducing manhandling problems.

Potato plot harvester.

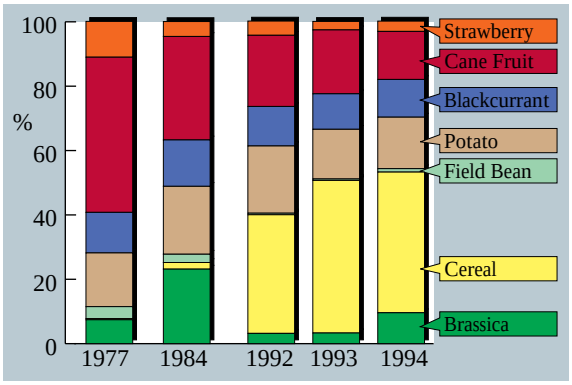
Rotation cropping operations and quality of ground preparation for trials were also improved by a number of new items. Wide, extremely low-pressure Trelleborg tyres were fitted to a Massey Ferguson 3095 tractor. These give better grip, allow tractor usage under marginal soil conditions, alleviate tracking problems and reduce soil structure damage. We have changed from a labour-intensive, multiple-handling small bale to a minimal-handling large bale system for dealing with hay and straw. This was very successful and yielded immediate and dramatic reductions in labour inputs and time usage. We have moved to a 3m Väderstad 300C combine drill for cereals, and tests on w. barley and w. oats showed that this machine, coupled with supply of seed and fertilizer in $^1/_2$ -tonne bags, will improve throughput, uniformity and accuracy of drilling operations. A one-pass Kverneland Packomat four furrow 16" reversible plough with press (pulled by the MF3095 tractor complete with Trelleborg tyres) has improved finish as well as covering a larger area in less time.

The vast majority of the Department's staff are now fully trained and NPTC-certificated in safe use of pesticides and agrochemical application (knapsack/tractor-mounted/misting/fogging/smokes) and fork-lift truck operation (rough-terrain/industrial-masted). These are vital skills in both glasshouse and field trial operations. Further aspects of a training development programme are being pursued and include biological and integrated pest control methods, supervisory and first-line management skills, as well as the specialist practical skills involved with field trialling machinery and operations.

The range of crops covered by the Department, in both glasshouse and field trials, continues to increase. Included are cereals (winter and spring barley/oats), field bean, grass, forage brassica (swede/turnip, kale, rape), potato, blackcurrant, cane fruit (raspberry, black- and hybrid-berry), strawberry, *Lilium*, *Narcissus*, novel fruits (blueberry, *Rosa spp*, sea buckthorn, *Sambucus*), fibres and other industrial crops (hemp, reed canary grass, nettles, lupins, willows), reserve collection of top fruit stocks (apple, pear, plum, damson), mixed tree species windbreaks and woody perennials, *Rubus* and *Ribes* nuclear stocks of germplasm, cassava, coffee, groundnuts, maize, and a miscellany of virus-indicator/screening species test plants.

The Estate, Glasshouse and Field Experiments Department fulfils the fundamental and crucial rôles of producing and maintaining plant material for the Institute's scientific research and contractual undertakings. A wide variety and large number of plants are made available throughout the year for work both in contained/controlled environments and in the field. The landscaping of amenity areas within the various sites and the maintenance of all estate and field boundaries are additional responsibilities.

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



Share of total trials area.

Control of raspberry root rot.

land and/or controlled environment régimes for inputs to be undertaken in varying degrees by scientific clients.

The work undertaken spans sub-cellular to whole-plant aspects within the range from high-technology research on the genetic modification of plants, virus manipulation and testing; vegetation dynamics, bioremediation, woody perennials, fibres and biomass; defining data parameters for deriving mathematical models of crops; through studies on the effects of nutrient, pest, disease, weed environment on crops; to "traditional" variety trials and maintenance of germplasm and nuclear stocks.

Within the controlled environment and glasshouse facilities, more and more accent is being placed on GMO/transgenic work and, as a consequence, on the specialist protocols and facilities required to undertake this type of work.

On the field trial side, cereals now occupy the largest proportion of the total trials area for all crops. In some part this has been due to increased activity in studies of disease progression in mixtures and resistance elicitor spray trials. New developments taken on board in 1994 included a regional trial of autumn-sown determinate white lupins (in conjunction with IACR Rothamsted); a comparison of yield and quality of fibres from two cultivars of hemp; assessing integrated control of raspberry root rot involving fungicide, mulch and ridge treatments; and the setting up of sites for new initiatives in vegetation dynamics which will examine the environmental and genetical causes of variability in plants and plant populations.

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 farm workshop section providing maintenance facilities for a substantial fleet of tractors and agricultural machinery. The Department provides a general stores facility and a cleaning and security service. The

workshops are generally well equipped to deal with the maintenance tasks assigned to them.

The 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.

Electrician in the Engineering and Maintenance Department.

Major works completed during the year included: relocation of the Institute's Mass Spectrometry section to a completely refurbished area within the Spectroscopy Building; upgrading of the Soft Fruits glasshouse laboratories; refurbishment of the Cereals glasshouse laboratories; the division of one of the large seedlings glasshouses to allow for accommodation of transgenic plant material; replacement fume cupboards within the Virology and Zoology Departments; upgrading of two laboratories within the Virology glasshouse to facilitate transgenic work; and the conversion of two small laboratories and an office area into a large laboratory within the Virology Department. These works have been carried out using a combination of outside contractors and in-house staff.

The rapidly changing and wide ranging scientific aims of the Institute ensures that laboratory alterations will always be a part of the Engineering Department's work. With this in mind services to laboratories must be as flexible and adaptable as possible. Over the last few years systems have been introduced which allow the Department to respond quickly and efficiently when changes are necessary thus reducing laboratory down times to a minimum. Scientists can now confidently bring new and diverse projects to the Institute knowing that a team is on hand to ensure their facilities will meet whatever requirement they may have.

An additional twelve replacement controlled environment growth cabinets were purchased this year to replace the oldest of the Institute's 46 cabinets. As a reflection of the changing work within the Institute, five of the new cabinets are specifically designed to accommodate tissue culture jars or plates.

The controlled environment cabinet area is situated in the same building as the engineering workshops. As an energy saving exercise a heat recovery system was installed between the two areas which uses the excess heat from the cabinet area to provide 'free-heating' within the workshops. The system has proved itself to be so successful that it is proposed to extend it to cover the electronic laboratory area and joiners' workshop in the coming year. Health and Safety considerations are foremost in any work undertaken by the Department and with this in mind safety guard rails have been fitted around the perimeter of all flat roofed buildings within the Institute, ensuring safer conditions for maintenance staff working on roof mounted equipment.

Mylnefield Research Services

N.W. Kerby

Mylnefield Research Services (MRS) Ltd was established in 1989 as the commercial arm of the Scottish Crop Research Institute (SCRI). Primarily set up to market products, intellectual property and expertise, MRS Ltd places particular emphasis on developing partnerships, forging stronger relationships with customers and technology transfer.

MRS Ltd acts as the gateway to a variety of skills unique within the UK biological, agricultural and horticultural research service, ranging from fundamental studies on genetics, molecular biology and physiology, through agronomy and pathology to glasshouse and field trials. MRS Ltd is in a position to offer a wide variety of services which frequently utilise the unique range of modern facilities and resources resident at SCRI.

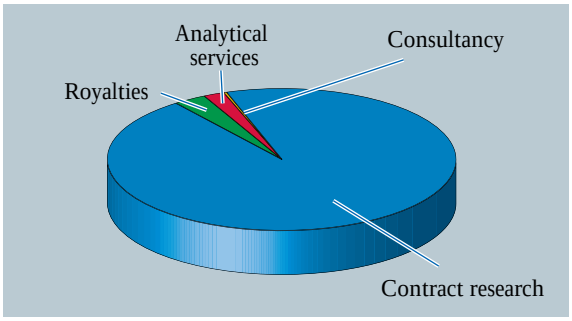


Figure 1 Summary of MRS income 1993-94.

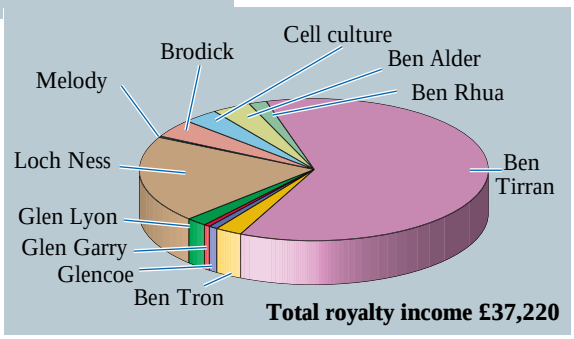


Figure 2 Royalty income 1993-94.

Promoting SCRI as a centre of scientific excellence, MRS Ltd recognises the necessity of marketing science and technology in wealth creation, improving quality of life and industrial competitiveness. Additionally, we place the utmost importance on achievement and meeting both the demands and expectations of customers and sponsors.

Both MRS Ltd and SCRI are aware of the need to effectively manage intellectual property (IP). In particular, MRS Ltd has a key role in identifying, protecting and exploiting the intellectual property rights of SCRI.

During 1994, MRS Ltd played a key role in financially supporting scientific research at the Institute. MRS Ltd gratefully acknowledges the valuable and essential contributions made by SCRI staff. The year was the most successful for MRS Ltd.

Financial

31 March 1994 saw the close of our third financial year. Turnover was in excess of £1.3 million representing a 20% increase on the previous financial year. The financial contribution to SCRI, in addition to intercompany transfers for services provided by the Institute, included a gift aid of £150,000 (an increase of 20% on the previous year), management fees of £101,000 and a payment of £90,000 towards the costs of the unique Nuclear Magnetic Resonance (NMR) facility.

MRS Ltd predicts that royalty income from products developed at SCRI, in particular new plant cultivars, will dramatically increase in the future. We see this revenue especially important for the growth of the Company and the Institute. Consequently we place importance on all forms of IP as it is at the heart of any technology transfer process. Figure 2 shows the breakdown of royalty income which is dominated by blackcurrant royalties, largely due to a back payment for the cultivar Ben Tirran. The blackberry Loch Ness continues to perform well.

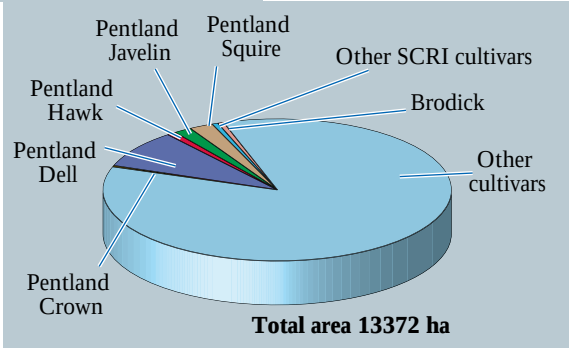


Figure 3 1994 area of Scottish certified seed potato attributable to SCRI.

Royalty income from potatoes is currently low, reflecting the brief period of establishing the breeding and trialling agreements, but will rise significantly due to the increase in certified area of Brodick seed sown in 1994. The importance of SCRI's potato breeding in the UK potato industry is clearly shown by Figure 3. The Pentland series occupy a significant sector of the market; however, MRS Ltd and SCRI do not receive royalties on these cultivars which pre-dated the formation of MRS Ltd.

External Contracts

During 1994 external contracts with a value of £3,335,926 were signed demonstrating the high level of activity and achievement by the SCRI scientific staff. MRS Ltd was awarded two additional SMART (Small Firms Merit Awards for Research and Technology) awards by the Scottish Office Industry Department for innovative research.

The SMART Stage I Award is for the development of a novel microinjection system in collaboration with Karl Oparka and Denton Prior of SCRI. The SMART Stage II Award is for the continuing development of novel soft fruit confectionery in collaboration with Ronnie McNicol of SCRI. MRS Ltd was the only company in Scotland to receive two 1994 SMART awards.

Major Agreements Concluded During 1994

Sharpes International Seeds Ltd A major agreement was negotiated with Sharpes International Seeds Ltd in May 1994 for the commercialisation of certain brassica cultivars and for a brassica breeding programme focused primarily on swedes. This agreement is particularly welcome in the light of the withdrawal of public funds for brassica research and development at SCRI.

Zenith Potatoes Ltd September 1994 saw an important and valuable agreement being negotiated with Zenith Potatoes Ltd (Aberdeen Seed Potato Organisation Ltd, H and JM Bennett International Ltd and Grampian Growers (Services) Ltd), for the commercialisation of potato clones that are no longer of interest to the consortium of Nickerson Seeds Ltd and Dalgety Agriculture Ltd.

Bruker Spectrospin Ltd A joint venture purchase agreement for a wide-bore imaging NMR (Model AMX 300 with a superconducting 7.05 Tesla, 150 mm bore magnet) (see photo page 176) was negotiated and the NMR was installed during December 1994. This agreement is for 4 years and will provide the Institute with an unique facility. It is the intention of MRS Ltd to specifically target the provision of particular services and facilities rather than non-specifically supporting the Institute through the Gift Aid Scheme.

Staff

We welcome the following scientific staff who were appointed to MRS Ltd during 1994 and are employed on various external contracts: Jan van den Berg, Alison Dolan, Rick Harrison, Ewen Simpson, Joao Pontes, Nathan Harris and Peter Hedley.

Scientific Research

The scientific research efforts of MRS Ltd have largely concentrated on a collaborative research programme with Scottish Soft Fruit Growers Ltd. This programme is funded from a variety of sources including the Scottish Office, the European Union and members of the growers association. Research areas include:-

1. Raspberry variety development with specific objectives of developing finished varieties with improved fruit quality, machine and hand harvest ability and extended harvest periods. In excess of 100 crosses were made and 12,000 seedlings were raised for screening for spinelessness and aphid resistance. All other stages of selections were progressed and several advanced selections were identified for further trialling and testing.

2. Research into the major loss of raspberry fruit quality established the role of ethene in raspberry fruit ripening. Ethene evolution from detached fruit increases concurrently with the degree of softening and anthocyanin production. Various cell wall hydrolases have been identified and a loss of cell wall material together with a decrease in cell wall methylation accompanies the ripening process. An objective of this programme is to establish a screening aid to assist breeders to provide future cultivars with significantly improved quality and/or shelf life.

3. Trials to investigate the control of raspberry root rot (*Phytophthora fragariae* var *rubi*) through cultural practices that require reduced chemical inputs were established on infected ground (see photo page 182). The trials incorporate mulching vs non-mulching and ridging vs non ridging, all at a range of fungicide doses with two different commercial cultivars with varying susceptibility.

4. The control of fungal pathogens through the incorporation and/or manipulation of genes naturally occurring in raspberries. A polygalacturonase inhibitor protein (PGIP) has previously been shown by staff of SCRI to markedly inhibit the growth of the predominant fruit rotting fungus (*Botrytis cinerea*). The raspberry gene coding for PGIP is being isolated and sequenced as a prelude to cloning and the transformation of raspberry cultivars.

CAROS International Ltd

J. Duffin

CAROS International Ltd was established in 1992 by the Scottish Agricultural and Biological Research Institutes (SABRIs) and the Scottish Agricultural College (SAC) to generate extra income for consortium members through bidding for, and winning, external consultancy contracts. CAROS has targeted the major international development funding agencies, concentrating in particular upon European Union technical assistance programmes.

In 1994 CAROS experienced great success in Poland, winning contracts with the European Commission's PHARE programme and the UK's Know How Fund (KHF).

Scottish expertise in the cultivation, storage and marketing of potatoes was responsible for CAROS winning two of these projects. Potatoes are a major crop in Poland; cultivation taking up 13% of arable land, but production is characterised by primitive cultivation technology, high production costs and high storage losses due to the absence of modern storage facilities. Production is not market-led and consumers are faced with low-quality produce.

A PHARE study and the KHF project sought to help reorientate Polish potato production; making the industry more consumer and market-driven. The PHARE programme funded a strategy proposal to develop the potato sector. This was complemented by the KHF project, an integrated, market-led pilot project incorporating a storage and grading facility, a training centre and a producers' group. SAC is responsible for providing the project management and technical assistance in both projects.

A third project in Poland, "Commercialisation and restructuring of agricultural research institutes", funded by PHARE, showed it is possible to gain through pain. Dr Jeff Wilson (MLURI; Team Leader) and Ronnie McNicol (SCRI) brought first-hand experience of research institutes' response to intense government scrutiny and increasing economic pressures to this project.

Working in co-operation with the Foundation for the Development of Polish Agriculture (FDPA), a private

Joan Duffin and Anne Pack - CAROS

Polish consultancy, they audited the resources of two research institutes, making recommendations on future strategy to the Polish Ministry of Agriculture and Food Economy. The joint report was highly commended by the Polish authorities and CAROS looks forward to further co-operation with FDPA in 1995.

There were disappointments as well as successes in 1994. CAROS was invited to tender for a major European Commission project in a remote province of China, Qinghai. The project's main objective was to reduce poverty amongst the rural population through increasing farm level potato production, improving cultivation and storage techniques and strengthening the scientific capability of Qinghai Academy of Agricultural Sciences. Chen Jianping, an exchange scientist working on advanced virology techniques at SCRI, and Mike Potts, former Director of the International Potato Centre for SE Asia and the Pacific, made a site visit to Qinghai. The technical proposal they produced was rated highly by the Commission but was insufficient to win the contract.

1994 was also a year of change for CAROS. In November Dr David Thomson resigned his position as Chief Executive. Joan Duffin took over the day-to-day running of the company, advised by a Management Committee composed of representatives from each member organisation and a non-executive director of CAROS, Robin Forrest. A sharp refocusing of CAROS' remit and highly selective targeting of funding agencies should bring even greater rewards in 1995.

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 312 on 31 December 1994.

The AGM of the Society was held on 4 May when John Heading, Chairman of the PMB addressed the members on the funding of potato research in the United Kingdom.

The committee of Management met on two occasions (4 May and 15 November).

Travel grants were awarded to:-

Dr D K L MacKerron, Cellular and Environmental Physiology Department, to Wageningen.

Dr A C Newton, Mycology and Bacteriology Department, to Poland.

Dr J Duncan, Mycology and Bacteriology Department, to Mexico.

Grants were also awarded to the Society for Experimental Biology and the Association of Applied Biologists.

A Cereal Walk was held on Thursday 7 July where the recurrent theme for the evening was the emphasis that is placed by the Institute on varieties specifically suited for the Scottish Environment. Topics covered included future varieties, work on lupins and field beans.

A Soft Fruit Walk was held on Thursday 28 July when topics covered included raspberry bushy dwarf virus, new cultivars, *phytophthora* root rot-trial, downy mildew and novel confectionary.

A Potato Walk was held on Thursday 4 August when new varieties, drought tolerance, breeding for resistance to potato cyst nematodes and new anti-viral technologies were exhibited.

The SSCR Autumn Meeting l. to r. A. Jacobsen, T. Guthrie, J. Vipond, J. Taylor, F. Rexen, P. Cook, W. Macfarlane Smith, J. Seed, K. Walker, J. Bradshaw, I. Morrison, M. Sutton, J. Hillman.

The Society Autumn Meeting was entitled Brassicas and Alternative Crops for Scotland and was held in conjunction with Sharpes International Seeds Ltd on the 24 November in Dundee. Delegates heard from Dr J Vipond, Mr M Sutton, Mr P Cook and Dr K Walker, SAC; A S Guthrie, Sharpes International Seeds Ltd; Professor J R Hillman, Dr I M Morrison, Dr. J E Bradshaw and Dr W H Macfarlane Smith, SCRI; Dr F Rexen, Ministry of Agriculture, Denmark; Mr J Seed, Border Biofuels; Mr J Taylor, PMB. A Bulletin has been issued of the proceedings of this Conference.

Officers of the Society

Trustees:
G B R Gray, OBE, Smeaton, East Linton EH40 3DT
A Patullo, MC, 6 Castle Way, St Madoes, Glencarse, Perthshire PH2 7NH
A G M Forbes, Omachie, Kingennie, Nr Dundee DD5 3RE
I E Ivory, Ruthven House, Meigle, Blairgowrie, Perthshire PH12 8RF
Chairman: A M Jacobsen, Grange of Kinneff, Inverbervie, Montrose DD10 0TL
Vice Chairman: T P M Thomson, Bramblebank Works, Blairgowrie PH10 7HY

Members of Committee of Management

J Arbuckle, East Flisk, Newburgh, Fife KY14 6HP
A C Bain, Flocklones, Invergowrie, Dundee DD2 5LE
Dr D A S Cranstoun, Corehouse, Lanark ML11 9TN
R G Galloway, East Balmirmer, Arbroath DD11 2PN
W P Laird, Cairnie Lodge, Cupar, Fife KY15 4QD
G D Morrison,OBE, Slatefield, Forfar DD8 1XD
G Rennie, Stenton, St Monans, Fife KY10 2DF
Secretary and Treasurer: D L Hood, 25 North Balmossie Street, Monifieth, Dundee DD5 4QL
Registered Office:
c/o Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA

Publications

Ahmad, A., Dunbar, L.J., Green, I.G., Harvey, I.W., Shepherd, T., Smith, D.M. & Wong, R.K.C. 1994. Polyaza heterocycles. Part 2. Nucleophilic substitution of halogens in halogenoquinoxalino[2,3-*c*]cinnolines. *Journal of The Chemical Society Perkin Transactions 1*, 2751-2758.

Alphey, T.J.W., Birch, A.N.E., Fellows, L.E. & Robertson, W.M. 1994. Control of parasitic nematodes. *UK Patent Application No. 9401331.5.*

Alphey, T.J.W., Birch, A.N.E., Fellows, L.E. & Robertson, W.M. 1994. Control of parasitic nematodes. *UK Patent Application No. 9401344.8.*

Amrine, J.W., Duncan, G.H., Jones, A.T., Gordon, S.C. & Roberts, I.M. 1994. *Cecidophyopsis* mites (Acari:Eriophyidae) on *Ribes* spp. (Grossulariaceae). *International Journal of Acarology* 20, 139-168.

Andreeva, L., Järvekülg, L., Rabenstein, F., Torrance, L., Harrison, B.D. & Saarma, M. 1994. Antigenic analysis of potato virus A particles and coat protein. *Annals of Applied Biology* 125, 337-348.

Anwar, S.A., Trudgill, D.L. & Phillips, M.S. 1994. The contribution of variation in invasion and development rates of *Meloidogyne incognita* to host status differences. *Nematologica* 40, 579-586.

Arif, M., Torrance, L. & Reavy, B. 1994. Improved efficiency of detection of potato mop-top furovirus in potato tubers and in the roots and leaves of soil-bait plants. *Potato Research* 37, 373-381.

Asmar, G., Clark, G.P., Vaux, P., Simpson, G.G. & Brown, J.W.S. 1994. Molecular characterisation of a family of potato RNA helicases. *Journal of Experimental Botany* 45, 11.

Bailey, E., Deighton, N., Clulow, S.A., Goodman, B.A. & Benson, E.E. 1994. Changes in free radical profiles during the callogenesis of responsive and recalcitrant potato genotypes. *Proceedings of the Royal Society of Edinburgh, B*, 102, 243-246.

Banks, J.M., Brechany, E.Y., Hunter, E.A., Muir, D.D. & Christie, W.W. 1993. Cheddar cheese flavor and chemical indexes - changes during maturation. *Abstracts of Papers of the American Chemical Society*, 206 Pt1, 28-AGFD.

Barker, H., Reavy, B., Arif, M., Webster, K.D. & Mayo, M.A. 1994. Towards immunity to potato leafroll virus and potato mop-top virus by using transgenic and host gene-mediated forms of resistance. *Aspects of Applied Biology* 39, 189-194.

Barker, H., Solomon-Blackburn, R.M., McNicol, J.W. & Bradshaw, J.E. 1994. Resistance to potato leafroll virus multiplication in potato is under major gene control. *Theoretical and Applied Genetics* 88, 754-758.

Barker, H., Webster, K.D., Jolly, C.A., Reavy, B., Kumar, A. & Mayo, M.A. 1994. Enhancement of resistance to potato leafroll virus multiplication in potato by combining the effects of host genes and transgenes. *Molecular Plant-Microbe Interactions* 7, 528-530.

Barton, J.S., Hindmarsh, P.C., Scrimgeour, C.M., Rennie, M.J. & Preece, M.A. 1994. Energy-expenditure in congenital heart-disease. *Archives of Disease in Childhood* 70, 5-9.

Barua, U.M., Chalmers, K.J., Thomas, W.B.T., Hackett, C.A., Lea, V., Jack, P., Forster, B.P., Waugh, R. & Powell, W. 1994. Molecular mapping of genes determining height, time to heading and growth habit in barley *(Hordeum vulgare* L.). *Genome* 36, 1080-1087.

Baty, A. & McDougall, G.J. 1994. Deposition of glucomannan in flax fibre walls. *Proceedings of the Fourth Scottish Cell Wall Group Meeting*, Glasgow. Paper 8.

Baty, A., McDougall, G.J., Stewart, D., Weyers, J.D.B. & Morrison, I.M. 1994. Control of plant fibre quality: Identification and characterization of key enzymes in cell wall synthesis. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 73-78.

Bedrock, C.N., Cheshire, M.V., Chudek, J.A., Goodman, B.A & Shand, C.A. 1994. ^{31}P NMR studies of humic acid derived from a blanket peat. In: Senesi, N. & Miano, T.M. (eds.). *Humic Substances in the Global Environment and Implications on Human Health.* Elsevier, 227-232.

Bedrock, C.N., Cheshire, M.V., Chudek, J.A., Goodman, B.A & Shand, C.A. 1994. Use of ^{31}P NMR to study the forms of phosphorus in peat soils. *Science of the Total Environment* 152, 1-8.

Bengough, A.G. 1994. Problems associated with modelling plant root systems. *Proceedings of the International Conference on the Dynamics of Roots and Nitrogen in Cropping Systems of the Semi-Arid Tropics*, Hyderabad, November 1994.

Bengough, A.G. & Mackenzie, C.J. 1994. Simultaneous measurement of root force and elongation for seedling pea roots. *Journal of Experimental Botany* 45, 95-102.

Bengough, A.G., Mackenzie, C.J. & Elangwe, H.E. 1994. Biophysics of the growth responses of pea roots to changes in penetration resistance. *Plant and Soil* 167, 135-141.

Beven, A., Simpson, G.G., Brown, J.W.S. & Shaw, P. 1994. The organisation of spliceosomal components in the nuclei of higher plants. *Journal of Cell Biology* 108, 509-518.

Birch, A.N.E., Fellows, L.E., Geoghegan, I.E., Robertson, W.M. & Watson, A.A. 1994. Control of parasitic nematodes. *UK Patent Application No. 9401345.5.*

Birch, A.N.E., Fenton, B., Malloch, G., Jones, A.T., Phillips, M.S., Harrower, B., Woodford, J.A.T. & Catley, M.A. 1994. Ribosomal spacer length variability in the large raspberry aphid, *Amphorophora idaei* (Aphidinae:Macrosiphini). *Insect Molecular Biology* 3, 239-245.

Birch, A.N.E., Gatehouse, A., Geoghegan, I.E. & Robertson, W.M. 1994. Nematicidal proteins. *UK Patent Application No. 9406371.6.*

Blok, V.C., Fargette, M., Phillips, M.S. & Trudgill, D.L. 1994. Approaches to describing the genetic variation in root-knot nematodes using molecular markers. *Abstracts of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 37.

Blok, V.C., Fargette, M., Phillips, M.S. & Trudgill, D.L. 1994. Inter- and intraspecies relationships in root-knot nematodes, *Meloidogyne* spp. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 114-116.

Blok, V.C., Hamilton, A., Phillips, M.S. & Brown, D.J.F. 1994. Genetic variation in *Xiphinema index*. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 37-38.

Blok, V.C., Ziegler, A., Robinson, D.J. & Murant, A.F. 1994. Sequences of 10 variants of the satellite-like RNA-3 of groundnut rosette virus. *Virology* 202, 25-32.

Boag, B. & Neilson, R. 1994. Nematode aggregation and its effect on sampling strategies. *Aspects of Applied Biology* 37, 103-111.

Boag, B. & Neilson, R. 1994. The Rotylenchimae of Great Britain. *Proceedings of the 22nd International Symposium of the European Society of Nematologists*, Gent, Belgium, 38.

Boag, B., Lawson, H.M., Neilson, R. & Wright, G.McN. 1994. Observations on the diversity of soil nematode fauna and weed seedbanks under different set-aside management regimes. *Aspects of Applied Biology* 40, 443-452.

Boag, B., Legg, R.K., Neilson, R.L, Palmer, L.F. & Hackett, C.A. 1994. The use of Taylors Power Law to describe the aggregated distribution of earthworms in permanent pasture and arable soil in Scotland. *Pediobiologia* 38, 303-306.

Boag, B., Yeates, G.W., Johns, P.M., Neilson, R., Palmer, L.F. & Legg, R.K. 1994. Distribution of the New Zealand flatworm (*Artioposthia triangulata*) in New Zealand and Scotland. *Acta Zoologica Fennica* 196, 212-214.

Bradshaw, J.E. 1994. Assessment of five cultivars of potato (*Solanum tuberosum* L.) in a competition diallel. *Annals of Applied Biology* 125, 533-540.

Bradshaw, J.E. 1994. Quantitative genetics theory for tetrasomic inheritance. In: Bradshaw, J.E. & Mackay, G.R. (eds.). *Potato Genetics*. CAB International, Wallingford, 71-100.

Bradshaw, J.E. 1994. Theory for locating quantitative trait loci. In: Bradshaw, J.E. & Mackay, G.R. (eds.). *Potato Genetics*. CAB International, Wallingford, 101-108.

Bradshaw, J.E. & Mackay, G.R. 1994. Breeding strategies for clonally propagated potatoes. In: Bradshaw, J.E. & Mackay, G.R. (eds.). *Potato Genetics*. CAB International, Wallingford, 467-498.

Bradshaw, J.E. & Mackay, G.R. (eds.). 1994. *Potato Genetics*. CAB International, Wallingford, 552 pp.

Bradshaw, J.E., Dale, M.F.B., Mackay, G.R., Phillips, M.S., Stewart, H.E. & Wastie, R.L. 1994. Potato breeding viewed as multitrait genotypic recurrent selection. *Potato Research* 37, 334.

Brechany, E.Y. & Christie, W.W. 1994. Identification of the unsaturated oxo fatty acids in cheese. *Journal of Dairy Research* 61, 111-115.

Breese, W.A., Shattock, R.C., Williamson, B. & Hackett, C.A. 1994. *In vitro* spore germination and infection of cultivars of *Rubus* and *Rosa* by downy mildews from both hosts. *Annals of Applied Biology* 125, 73-85.

Breese, W.A., Shattock, R.C., Williamson, B. & Hackett, C.A. 1994. Studies of spore germination and infection with downy mildew isolates from *Rubus* and *Rosa*. *Annals of Applied Biology* 125, 73-85.

Brennan, R.M. 1994. Currants. In: Dale, A. & Hanson, E.J. (eds.). *Mechanical Harvesting of Berry Crops*. *Horticultural Review* 16, 312-328.

Brennan, R.M. 1994. *Ribes* research at SCRI. *HDC Soft Fruit Subject Day*, HRI, East Malling, March 1994.

Brown, D.J.F., Halbrendt, J.M., Jones, A.T., Taylor, C.E. & Lamberti, F. 1994. An appraisal of some aspects of the ecology of nematode vectors of plant viruses. *Nematologia Mediterranea* 22, 253-263.

Brown, D.J.F., Halbrendt, J.M., Jones, A.T., Vrain, T.C. & Robbins, R.T. 1994. Transmission of three North American nepoviruses by populations of four distinct *Xiphinema americanum*-group species (Nematoda: Longidoridae). *Phytopathology* 84, 646-649.

Brown, D.J.F., MacFarlane, S.A., Legorburu, F.J., Robinson, D.J. & Bol, J. 1994. The role of tobravirus genes in vector transmission by trichodorid nematodes. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 42.

Brown, J.W.S., Simpson, C.G., Clark, G.P. & Waugh, R. 1994. Complementary deletions in expressed potato U2snRNA gene variants support the hypothesis that stem-loop IIb is dispensible for splicing. *The Plant Journal* 6, 921-925.

Brown, J.W.S., Simpson, G.G., Hamilton, J.I., Clark, G.P., Beven, A., Shaw, P., Hedges, P. & Beggs, J.D. 1994. Plant snRNP proteins and nuclear organisation of snRNP components. *Messenger RNA Processing and Nuclear Organisation*, Strasbourg, France, September 1994.

Brown, S.M., Oparka, K.J., Sprent, J.I. & Walsh, K.B. 1995. Symplastic transport in soybean root nodules. *Soil Biology and Biochemistry* 27, 387-399.

Buckland, S.T. & Elston, D.A. 1994. Use of groundtruth data to correct land cover area estimates from remotely sensed data. *International Journal of Remote Sensing* 15, 1273-1282.

Burch, L.R., Davies, H.V., Ross, H.A., Machray, G.C., Hedley, P.E. & Waugh, R. 1994. Processing of a 58 x 10^3 M_r invertase from potato tubers. *Phytochemistry* 35, 579-582.

Butcher R.D., Macfarlane Smith W.H., Robertson, G.W. & Griffiths, D.W. 1994. The identification of potential aeroallergen/irritant(s) from oilseed rape (*Brassica napus* spp. *oleifera*): volatile organic compounds emitted during flowering progression. *Clinical and Experimental Allergy* 24, 1105-1114.

Carter, C.G., Owen, S.F., He, Z.Y., Watt, P.W., Scrimgeour, C.M., Houlihan, D.F. & Rennie, M.J. 1994. Determination of protein-synthesis in rainbow-trout, *Oncorhynchus mykiss*, using a stable-isotope. *Journal of Experimental Biology* 189, 279-284.

Chalmers, K.J., Newton, A.C., Waugh, R., Wilson, J. & Powell, W. 1994. Evaluation of the extent of genetic variation in mahoganies (Meliaceae) using RAPD markers. *Theoretical and Applied Genetics* 89, 504-508.

Chen, J. & Wilson, T.M.A. 1994. Genetic plasticity in rod-shaped plant RNA viruses - Bad news for cereals, good

news for biotechnology. In: Li Debao (ed.). *Proceedings of the 2nd Hangzhou International Symposium on Plant Pathology*, Hangzhou, China, 95.

Chen, J., MacFarlane, S.A. & Wilson, T.M.A. 1994. Detection and sequence analysis of a spontaneous deletion mutant of soil-borne wheat mosaic virus RNA2 associated with increased symptom severity. *Virology* 202, 921-929.

Chen, J., MacFarlane, S.A. & Wilson, T.M.A. 1994. Spontaneous gene deletion events associated with increased symptom severity in a fungus-transmitted virus of cereals. *Annual Report of the Scottish Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 106-109.

Chen, J., MacFarlane, S.A. & Wilson, T.M.A. 1995. An analysis of spontaneous deletion sites in soil-borne wheat mosaic virus RNA2. *Virology* 209, 213-217.

Chen, J., MacFarlane, S.A. & Wilson, T.M.A. 1995. Effect of cultivation temperature on the spontaneous development of deletions in soil-borne wheat mosaic furovirus RNA2. *Phytopathology* 85, 299-306.

Chong, P.K.K., Jung, R.T., Scrimgeour, C.M. & Rennie, M.J. 1994. The effect of pharmacological dosages of glucocorticoids on free-living total-energy expenditure in man. *Clinical Endocrinology* 40, 577-581.

Chong, P.K.K., Jung, R.T., Scrimgeour, C.M., Rennie, M.J. & Paterson, C.R. 1994. Energy-expenditure and body-composition in growth-hormone deficient adults on exogenous growth-hormone. *Clinical Endocrinology* 40, 103-110.

Christie, W.W. 1994. High-performance liquid chromatography of chiral lipids. In: Tyman, J.H.P. & Gordon, M.H. (eds.). *Developments in the Analysis of Lipids*. Royal Society of Chemistry, Cambridge, 59-72.

Christie, W.W. 1994. High-performance liquid chromatography of plant phospholipids. *INFORM*, 5, 462A.

Christie, W.W. 1994. Separation of phospholipids by high-performance liquid chromatography. 2. The column. *Lipid Technology* 6, 17-19.

Christie, W.W. 1994. Silver ion and chiral chromatography in the analysis of triacylglycerols. *Progress in Lipid Research* 33, 9-18.

Christie, W.W. 1994. Why I dislike boron trifluoride-methanol. *Lipid Technology* 5, 66-68.

Christie, W.W. 1994. Why doesn't your method work when I try it? *Lipid Technology* 6, 121-123.

Christie, W.W., Brechany, E.Y., Marekov, I.N., Stefanov, K.L. & Andreev, S.N. 1994. The fatty acids of the sponge *Hymeniacidon sanguinea* from the Black Sea. *Comparative Biochemistry and Physiology* 109B, 245-252.

Clulow, S.A. 1994. The potential of the True Potato Seed. *Scottish Agricultural Colleges Newsletter, March 1994*, 2-3.

Clulow, S.A. & Bradshaw, J.E. 1994. Glasshouse-based selection to identify agronomically adapted, uniform true potato seed (TPS) progenies. *Annals of Applied Biology* 124, 519-529.

Clulow, S.A., McNicoll, J. & Bradshaw, J.E. 1995. Producing commercially attractive, uniform True Potato Seed progenies: the influence of breeding scheme and parental genotype. *Theoretical and Applied Genetics* 90, 519-525.

Commandeur, U., Koenig, R., Manteuffel, R., Torrance, L., Lüddecke, P. & Frank, R. 1994. Location, Size, and Complexity of Epitopes on the Coat Protein of Beet Necrotic Yellow Vein Virus Studied by Means of Synthetic Overlapping Peptides. *Virology* 198, 282-287.

Connelly, S., Leader, D.J., Brown, J.W.S. & Filipowicz, W. 1994. Small nuclear RNA genes transcribed by either RNA polymerase II or III in monocot. plants share three promoter elements and use a different strategy to regulate gene expression than their dicot. counterparts. *Molecular and Cellular Biology* 14, 5910-5919.

Cooper-Bland, S., De,Maine, M.J., Fleming, M.L.M.H., Phillips, M.S., Powell, W. & Kumar, A. 1994. Synthesis of intraspecific somatic hybrids of *Solanum tuberosum*: assessments of morphological, biochemical and nematode (*Globodera pallida*) resistance characteristics. *Journal of Experimental Botany* 45, 1319-1325.

Craig, A.L., Morrison, I., Baird, E., Waugh, R., Coleman, M., Davie, P. & Powell, W. 1994. Expression of reducing sugar accumulation in interspecific somatic hybrids of potato. *Plant Cell Reports* 14, 401-405.

Crawford, J.W. 1994. Linking knowledge in a systems approach to modelling. SOAFD Feasibility Study on a Systems Approach to Modelling Agriculture and Land Use in Scotland.

Crawford, J.W. 1994. The relationship between structure and the hydraulic properties of soil. *European Journal of Soil Science* 45, 493-502.

Croser, C., Pritchard, J., Tomos, D. & Bengough, A.G. 1994. The response of expanding root cells to soil compaction: the biophysics of growth recovery in hydroponics. *Journal of Experimental Botany* 45 (Suppl.), 40.

Dale, M.F.B. 1994. Potatoes - from South America to modern day cultivars. *National Vegetable Society Conference*, Stirling, November 1994.

Dale, M.F.B. 1994. Seed-borne diseases - Problems, control and current research and development. *West Midland Potato Group Conference*, Staffordshire College of Agriculture, Stafford, November 1994.

Dale, M.F.B. & Mackay, G.R. 1994. Inheritance of table and processing quality. In: Bradshaw, J.E. & Mackay, G.R. (eds.). *Potato Genetics*. CAB International, Wallingford, 285-315.

Dale, M.F.B. & Phillips, M.S. 1994. The effect of physiological age of potato tubers on female production of potato cyst nematodes (*Globodera pallida* Pa 2/3). *European Journal of Plant Pathology* 101, 51-56.

Davies, D.H.K., Fisher, H.M., Proven, M.J., Courtney, A.D., Lawson, H.M. & Wright, G.McN. 1994. Towards reducing cereal herbicide costs as gross margins fall. *Aspects of Applied Biology* 40, 307-316.

Davies, H.V. 1994. Developmental changes in carbohydrate metabolism in potato. *Abstracts of XVIIth Congress of the Scandinavian Society for Plant Physiology*, Elsinore, Denmark, August, Abstract 3.12.

Davies, H.V. & Mackay, G.R. 1994. Exploitation of genetic variation to improve potato quality. *Aspects of Applied Biology* 39, 45-49.

Davies, H.V. & Viola, R. 1994. Control of sugar balance in potato tubers. In: Belknap, W.R., Vayda, M., Park, W.D. (eds.). *The Molecular and Cellular Biology of the Potato*. CAB International, Wallingford, 67-81.

Dawson, I.K., Simons, A.J., Waugh, R. & Powell, W. 1994. Diversity and genetic differentiation among subpopulations of *Gliricidia sepium* revealed by PCR-based assays. *Heredity* 74, 10-18.

Deighton, N. & Goodman, B.A. 1994. The speciation of metals in biological systems. In: Davidson, C.M. & Ure, A.M. (eds.). *Chemical Speciation in Natural Systems*. Chapman & Hall, Glasgow, 307-334.

Deighton, N., Glidewell, S.M. & Goodman, B.A. 1994. Spin-trapping methods for investigating unstable biological free radicals. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 77-79.

Deighton, N., Glidewell, S.M. & Goodman, B.A. 1994. The nature and origin of the EPR spectra from irradiated bone and hydroxyapatite. *Abstracts of Analytical Detection Methods for Irradiation Treatment of Food*, Belfast.

Deighton, N., Deans, S.G., Glidewell, S.M. & Goodman, B.A. 1994. The chemical fate of the endogenous plant antioxidants carvacrol and thymol during oxidative stress. *Proceedings of the Royal Society of Edinburgh, B*, 102, 247-252.

Deighton, N., Glidewell, S.M., Goodman, B.A. & Pérombelon, M.C.M. 1994. Identification of irradiated fruit from the pectin-derived EPR signal. *Abstracts of Analytical Detection Methods for Irradiation Treatment of Food*, Belfast.

Deighton, N., Lyon, G.D., Johnston, D., Glidewell, S.M. & Goodman, B.A. 1994. Are free radical generation and phytoalexin biosynthesis coupled? *Proceedings of the Royal Society of Edinburgh* 102B, 253-255.

Deighton, N., Vicente, L., Glidewell, S.M., Empis, J.A. & Goodman, B.A. 1994. A spin-trapping procedure for the direct detection of free radical generation in heated cooking oils. *Abstracts of the European ESR Meeting on Recent Advances and Applications to Organic and Bioorganic Materials*, Paris, 135.

De,Maine, M.J. 1994. Comparisons of tetraploid progenies of potato dihaploids, their chromosome-doubled derivatives and second generation dihaploids. *Potato Research* 37, 173-181.

De,Maine, M.J. 1994. The ploidy composition of offspring from *Solanum tuberosum* (4x) and *S. phureja* (2x) crosses with special reference to triploid frequencies. *Annals of Applied Biology* 125, 361-366.

Duncan, J.M. & Kennedy, D.M. 1994. Effect of temperature and host genotype on production of secondary inoculum by *Phytophthora fragariae* var. *fragariae*. *Plant Pathology* 44, 10-21.

Duncan, J.M. 1994. Powdery mildew and crown rot. *Report of SAC Protected Soft Fruit Seminar*, SAC Craibstone, Aberdeen, 11 October 1994, 39-34.

Duncan, L.H., Robertson, W.M. & Kusel, J.R. 1994. An analysis of amphidial/excretory exudates of the plant parasitic nematodes *Globodera pallida* and *G. rostochiensis*. *Proceedings of the 22nd International Nematology Symposium*, Gent, Belgium 7-12 August, 1994, 54.

Evans, K., Trudgill, D.L. & Webster, J.M. 1994. Plant parasitic nematodes in temperate agriculture. CAB International, Wallingford, 648 pp.

Fargette, M., Blok, V.C., Phillips, M.S. & Trudgill, D.L. 1994. Genetic variation in tropical *Meloidogyne* spp. *Advances in Molecular Plant Nematology*. Plenum Press, New York, 91-96.

Fargette, M., Davies, K.G., Robinson, M.P. & Trudgill, D.L. 1994. Characterisation of resistance breaking *Meloidogyne incognita* - like populations using lectins, monoclonal antibodies and spores of *Pasteuria penetrans*. *Fundamental and Applied Nematology* 17, 537-542.

Fargette, M., Davies, K.G., Blok, V., Phillips, M.S. & Trudgill, D.L. 1994. Differential attachment of *Pasteuria penetrans* to *Meloidogyne* species. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 57.

Fawcett, T., Simon, W., Swinhoe, J.R., Shanklin, J., Christie, W.W. & Slabas, A.R. 1994. Expression of mRNA and steady-state levels of protein isoforms of enoyl-ACP reductase from *Brassica napus*. *Plant Molecular Biology* 26, 155-163.

Fenton, B., Birch, A.N.E., Malloch, G., Woodford, J.A.T. & Gonzalez, C. 1994. Molecular analysis of ribosomal DNA from the aphid *Amphorophora idaei* and an associated fungal organism. *Insect Molecular Biology* 3, 183-189.

Fenton, B., Malloch, G., Birch, A.N.E. & Woodford, J.A.T. 1994. Use of ribosomal DNA intergenic spacer variation to study aphid populations. *Abstracts of the 5th European Congress of Entomology*, University of York, 62.

Fenton, B., Malloch, G., Jones, A.T., Birch, A.N.E., Gordon, S.C., A'Hara, S., McGavin, W.J. & Amrine, J.W. 1995. Species identification of *Cecidophyopsis* mites (Acari:Eriophyidae) from different *Ribes* species and countries using molecular genetics. *Molecular Ecology* 4, 383-387.

Fenton, B., Woodford, J.A.T., Malloch, G. & Birch, A.N.E. 1994. Phylogenetic relationships within the *Aphididae* using sequence analysis of their ribosomal RNA genes. Poster presentation at *The impact of genetic variation on sustainable agriculture*. Association of Applied Biologists, Presidential Conference, Dundee, 14 September.

Flavell, A.J., Pearce, S.R. & Kumar, A. 1994. Plant transposable elements and the genome. *Current Opinion in Genetics and Development* 4, 838-844.

Forrest, J.M.S. 1994. Stress response in plants. In: Müller, W.E.G. (ed.). *Use of aquatic invertebrates as tools for monitoring environmental hazards*. Gustav Fischer Verlag, Stuttgart, 119-128.

Forrest, J.M.S. & Kerby, N.W. 1994. A licensing opportunity for the further development of an HIV diagnostic and therapeutic agent. Technical report, MRS Ltd.

Forster, B.P., Handley, L.L., Pakniyat, H., Scrimgeour, C.M., Nevo, E. & Raven, J.A. 1994. Genetic and ecological factors controlling carbon isotope discrimination in barley. *Aspects of Applied Biology* 38, 139-143.

Forster, B.P., Pakniyat, H., Macaulay, M., Matheson, W., Thomas, W.T.B., Phillips, M.S. & Powell, W. 1994. Variation in the leaf sodium content of the *Hordeum vulgare* (barley) cultivar Maythorpe and its derived mutant cv. Golden Promise. *Heredity* 73, 249-253.

Foster, G.N., Pallet, D. & Woodford, J.A.T. 1994. Suppression of the spread of potato leafroll virus and potato virus Y by aphicide sprays. *Proceedings Brighton Crop Protection Conference - Pests and Diseases - 1994*, 223-228.

Gibson, G.J., Glasbey, C.A. & Elston, D.A. 1994. Monte-Carlo evaluation of multivariate normal integrals and sensitivity to variate ordering. *Proceedings of the Third International Conference in Numerical Methods and Applications*, Sofia, Bulgaria, 120-126.

Glasbey, C.A. 1994. Contribution to discussion on 'Regression using fractional polynomials of continuous covariates' by P. Royston and D.G. Altman. *Applied Statistics* 43, 457-459.

Glasbey, C.A. & Gibson, G.J. 1994. Contribution to discussion on 'Assessment and propagation of model uncertainty' by D. Draper. *Journal of the Royal Statistical Society B* 57, 83-84.

Glasbey, C.A. & Wright, F. 1994. An algorithm for unwarping multitrack electrophoretic gels. *Electrophoresis* 15, 143-148.

Glasbey, C.A., Horgan, G.W. & Hitchcock, D. 1994. A note on the greyscale response and sampling properties of a desktop scanner. *Pattern Recognition Letters* 15, 705-711.

Glidewell, S.M. & Goodman, B.A. 1994. Direct methods of metal speciation. In: Davidson, C.M. & Ure, A.M. (eds.). *Chemical Speciation in Natural Systems.* Chapman & Hall, Glasgow, 33-64.

Glidewell, S.M. & Goodman, B.A. 1994. Irradiation and free radicals. *Food and Beverage Europe*, 171-173.

Glidewell, S.M., Goodman, B.A. & Skilling, J. 1994. Quantified maximum entropy and biological EPR spectra. *Abstracts of the 14th International Maximum Entropy Workshop*, Cambridge, 57.

Glidewell, S.M., Goodman, B.A. & Skilling, J. 1994. Quantitative maximum entropy applied to the analysis of isotropic EPR spectra. *Abstracts of the XVIth International Conference on Magnetic Resonance in Biological Systems*, Veldhoven, Netherlands, 27.

Glidewell, S.M., Skilling, J. & Goodman, B.A. 1994. Quantified maximum entropy methodology for the production of quantitative information from EPR spectra of solutions of free radicals in low concentrations. *Abstracts of the European ESR Meeting on Recent Advances and Applications to Organic and Bioorganic Materials*, Paris, 151.

Glidewell, S.M., Deighton, N., Morrice, A.E. & Goodman, B.A. 1994. Time course study of the EPR spectra of seeds of soft fruit irradiated in wet and dry states. *Abstracts of Analytical Detection Methods for Irradiation Treatment of Food*, Belfast.

Goldringer, I., Brabant, P. & Kempton, R.A. 1994. Adjustment for competition between genotypes in single-row plot trials of winter wheat *(Triticum aestivum). Plant Breeding* 112, 294-300.

Goleniewski, G. & Newton, A.C. 1994. Modelling mildew spread in cereal mixtures using a nearest neighbour approach: the effect of geometrical arrangement. *Plant Pathology* 43, 631-643.

Goodman, B.A. 1994. Molecular spectroscopy - introduction and general principles. In: Wilson, M.J. (ed.). *Clay Mineralogy: Physical Determinative Methods*. Chapman & Hall, Glasgow, 1-10.

Goodman, B.A. 1994. Mössbauer spectroscopy. In: Wilson, M.J. (ed.). *Clay Mineralogy: Physical Determinative Methods.* Chapman & Hall, Glasgow, 68-119.

Goodman, B.A. 1994. The involvement of free radicals in plant-pathogen interactions. *Proceedings of the Royal Society of Edinburgh* 102B, 479-493.

Goodman, B.A. & Chudek, J.A. 1994. Nuclear magnetic resonance spectroscopy. In: Wilson, M.J. (ed.). *Clay Mineralogy: Physical Determinative Methods*. Chapman & Hall, Glasgow, 120-172.

Goodman, B.A. & Deighton, N. 1994. Can EPR spectroscopy be used to measure specifically free radicals produced by natural senescence processes in plants? *European ESR Meeting on Recent Advances and Applications to Organic and Bioorganic Materials*, Paris, 19.

Goodman, B.A. & Hall, P.L. 1994. Electron paramagnetic resonance spectroscopy. In: Wilson, M.J. (ed.). *Clay Mineralogy: Physical Determinative Methods*. Chapman & Hall, Glasgow, 173-225.

Goodman, B.A., Deighton, N. & Glidewell, S.M. 1994. Detection of irradiated foods of plant origin by electron paramagnetic resonance spectroscopy. *Food Science and Technology Today* 8, 110-111.

Goodman, B.A., Deighton, N. & Glidewell, S.M. 1994. Optimisation of experimental parameters for the EPR detection of the "cellulosic" radical in irradiated foodstuffs. *International Journal of Food Science and Technology* 29, 23-28.

Goodman, B.A., Deighton, N. & Glidewell, S.M. 1994. Studies on the origins and stabilities of radiation-induced free radicals in plant-derived food products. *European ESR Meeting on Recent Advances and Applications to Organic and Bioorganic Materials*, Paris, 130.

Goodman, B.A., Cheshire, M.V., Berrow, M.L. & Deighton, N. 1994. Identification of paramagnetic species and determination of the copper coordination environments in organic matter from soils used for the disposal of distillery wastes. In: Senesi, N. & Miano, T.M. (eds.). *Humic Substances in the Global Environment and Implications on Human Health.* Elsevier, 1025-1030.

Goodman, B.A., Glidewell, S.M., Deighton, N. & Morrice, A.E. 1994. Free radical reactions involving coffee. *Food Chemistry* 51, 399-403.

Goodman, B.A., Simpson, E., Williamson, B. & Brennan, R.M. 1994. 3-Dimensional nuclear magnetic resonance (NMR) microscopy of soft fruit crops. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 80-82.

Goodman, B.A., Williamson, B., Simpson, E.J., Chudek, J.A. & Hunter, G. 1994. High field NMR microscopy of strawberry fruits. *Abstracts of the XVIth International Conference on Magnetic Resonance in Biological Systems*, Veldhoven, Netherlands, 78.

Gordon, S.C., Brennan, R.M., Jones, A.T., Fenton, B. & Birch, A.N.E. 1994. The blackcurrant gall mite (*Cecidophyopsis ribis*): its biology and strategies for control. *Proceedings of British Crop Protection Council Conference-Pests and Diseases*, Brighton, 1994.

Graham, J. 1994. Genetic modification in food production. *Report to SmithKline Beecham.*

Graham, J. & McNicol, R.J. 1995. An examination of the ability of RAPD markers to determine the relationships within and between *Rubus* species. *Theoretical and Applied Genetics* 90, 1128-1132.

Graham, J., McNicol, R.J. & Kumar, A. 1995. *Agrobacterium*-mediated transformation of soft fruit *Rubus*, *Ribes* and *Fragaria*. *Methods in Molecular Biology* 44, 129-133.

Graham, J., McNicol, R.J. & Lanham, P.G. 1994. The use of RAPD markers in the analysis of *Rubus* species. In: Van Ooijen, J.W. & Jansen, J. (eds.). *Biometrics in plant breeding: Applications of molecular markers*, 226-227.

Graham, J., McNicol, R.J., Greig, K. & Van de Ven, W.T.G. 1994. Identification of red raspberry cultivars and an assessment of their relatedness using fingerprints produced by random primers. *Journal of Horticultural Science* 69, 123-130.

Griffiths, B.S. 1994. Microbial-feeding nematodes and protozoa in soil: their effects on microbial activity and nitrogen mineralization in decomposition hotspots and the rhizosphere. *Plant and Soil* 164, 25-33.

Griffiths, B.S. 1994. Soil Nutrient Flows. In: Darbyshire, J.F. (ed.). *Soil Protozoa*. Commonwealth Agriculture Bureau International, Wallingford, 65-91.

Griffiths, B.S. & Young, I.M. 1994. The effects of soil structure and moisture on protozoa in a clay-loam soil. *European Journal of Soil Science* 45, 285-292.

Griffiths, B.S., Ritz, K. & Wheatley, R.E. 1994. Nematodes as indicators of enhanced microbiological activity in a Scottish organic farming system. *Soil Use and Management* 10, 20-24.

Griffiths, B.S., Van Vuuren, M.M.I & Robinson, D. 1994. Microbial grazer populations in a ^{15}N labelled organic residue and the uptake of residue-N by wheat. *European Journal of Agronomy* 3, 321-325.

Griffiths, B.S., Ritz, J., Hendriksen, N. & Torsvik, V. 1994. Broad-scale approaches to the analysis of community DNA. *Abstract of New Approaches in Microbial Ecology, International Workshop*, Elsinore, Denmark.

Griffiths D.W., Birch A.N.E. & Macfarlane Smith, W.H. 1994. Induced changes in the indole glucosinolate content of oilseed and forage rapes (*Brassica napus*) plants in response to turnip root fly (*Delia floralis*) larvae and artificial damage. *Journal of the Science of Food and Agriculture* 65, 171-178.

Griffiths D.W., Dale, M.F.B. & Bain, H. 1994. The effect of cultivar, maturity and storage on photoinduced changes in the total glycoalkaloid and chlorophyll contents of potatoes. *Plant Science*, 98, 103-109.

Griffiths D.W., Macfarlane Smith W.H. & Boag, B. 1994. The effect of cultivar, harvest date and grazing on the concentration of S-methyl cysteine sulphoxide in oilseed and forage rapes (*Brassica napus*). *Journal of the Science of Food and Agriculture* 64, 283-288.

Gu, Y., Crawford, J.W., Peiris, D.R. & Jefferies, R.A. 1994. An approach for treating the uncertainties in the impact of climate change. *Environmental Pollution* 83, 87-93.

Gu, Y.Q., Peiris, D.R., Crawford, J.W., McNicol, J.W., Marshall, B. & Jefferies, R.A. 1994. An application of belief networks to future crop production. *Proceedings of 10th Conference on Artificial Intelligence for Applications*, IEEE Computer Press, Los Alamos, 305-309.

Guerineau, F., Waugh, R. & Brown, J.W.S. 1994. Parameters affecting the activity of antisense RNA sequences in tobacco protoplasts. *Plant Cell Reports* 13, 703-708.

Hackett, C.A. 1994. Selection of markers linked to quantitative trait loci by regression techniques. *Ninth meeting of the Eucarpia section Biometrics in Plant Breeding*, Wageningen, *Euphytica*, 99-106.

Halbrendt, J.M. & Brown, D.J.F. 1994. Inter- and intraspecific variation in wild-type and single female-derived populations of *Xiphinema americanum*-group nematodes. *Journal of Nematology* 26, 212-221.

Handley, L.L., Odee, D. & Scrimgeour, C.M. 1994. $\delta^{15}N$ and $\delta^{13}C$ patterns in savanna vegetation: dependence on water availability and disturbance. *Functional Ecology* 8, 306-314.

Handley, L.L., Nevo, E., Raven, J.A., Martinez-Carrasco, R., Scrimgeour, C.M., Pakniyat, H. & Forster, B.P. 1994. Chromosome-4 controls potential water-use efficiency ($\delta^{13}C$) in barley. *Journal of Experimental Botany* 45, 1661-1663.

Harris, N, Forster, J.M., Kumar, A., Wray, J. & Davies, H.V. 1995. Nitrogen economy of the potato. *Abstracts of the Society of Experimental Biology Annual Meeting*, St Andrews, April 1995, P7.13.

Harrison J.G. 1995. Factors involved in the development of potato late blight disease (*Phytophthora infestans*). In: Haverkort, A. & MacKerron, D.K.L. (eds.). *Potato Ecology and Modelling of Potato Crops Under Conditions Limiting Growth*. Current Issues in Production Ecology, Kluwer Academic Publishers, Dordrecht, Netherlands, 215-236.

Haverkort, A.J. & MacKerron, D.K.L. (eds.). 1995 *Potato Ecology and Modelling of Potato Crops Under Conditions Limiting Growth.* Current Issues in Production Ecology, Kluwer Academic Publishers, Dordrecht, Netherlands, 379pp.

Haverkort, A.J. & Trudgill, D.L. 1995. Crop physiological responses to infection by potato cyst nematode (*Globodera* spp.) In: Haverkort, A. & MacKerron, D.K.L. (eds.). *Potato Ecology and Modelling of Potato Crops Under Conditions Limiting Growth.* Current Issues in Production Ecology, Kluwer Academic Publishers, Dordrecht, Netherlands, 167-183.

Hedley, P.E., Machray, G.C., Davies, H.V., Burch, L. & Waugh, R. 1994. Potato (*Solanum tuberosum*) invertase-encoding cDNAs and their differential expression. *Gene* 145, 211-214.

Hillman, J.R., Glidewell, S.M. & Deighton, N. 1994. The senescence syndrome in plants: an overview of phytogerontology. *Proceedings of the Royal Society of Edinburgh*, 102B, 447-458.

Hirst, D.J. 1994. An improved removal method for estimating animal abundance. *Biometrics* 50, 501-505.

Hirst, D.J. & Naes, T. 1994. A graphical technique for assessing differences among a set of rankings. *Chemometrics* 8, 81-93.

Hirst, D.J., Muir, D.D. & Naes, T. 1994. Definition of the organoleptic properties of hard cheese: a collaborative study between Scottish and Norwegian panels. *International Dairy Journal* 4, 743-761.

Hong, Y., Fargette, D., Swanson, M.M., McGrath, P.F. & Harrison, B.D. 1993. Sequence and epitope variation among coat proteins of tomato leaf curl geminiviruses from different regions. *Abstracts of the IXth International Congress of Virology*, Glasgow, 105.

Horgan, G.W. 1994. Optimal linear filters for SAR. *International Journal of Remote Sensing* 15, 1053-1064.

Horgan, G.W. & Ball, B.C. 1994. Simulating diffusion in a Boolean model of soil pores. *European Journal of Soil Science* 45, 483-491.

Horgan, G.W. & Glasbey, C.A. 1994. Digital ways to unwarp gels. *Image Processing* 6, 26-28.

Hunter, E.A. 1994 (ed.). Method of analysis of data from consumer studies. *SENS* 3, 36-77.

Hwang, D-J., Roberts, I.M. & Wilson, T.M.A. 1994. Assembly of tobacco mosaic virus and TMV-like pseudovirus particles in *Escherichia coli*. *Archives of Virology* 9, 543-558.

Hwang, D-J., Roberts, I.M. & Wilson, T.M.A. 1994. Expression of tobacco mosaic virus coat protein and assembly of pseudovirus particles in *Escherichia coli*. *Proceedings of the National Academy of Sciences USA* 91, 9067-9071.

Hyman, L.J., Wallace, H., Lopez, M.M., Cambra, M., Gorris, M.J. & Pérombelon, M.C.M. 1995. Characterization of monoclonal antibodies against *Erwinia carotovora* subsp. *atroseptica* serogroup I: specificity and epitope analysis. *Journal of Applied Bacteriology* 78, 437-444.

Ingram, B., Leslie, M., Cipriani, G., Golmirzaie, A. & Clulow, S.A. 1994. Somatic embryoid formation in potato: the effects of hormonal concentration, explant source and genotype. *Proceedings of the VII International Congress of Plant Tissue and Cell Culture*, Kluwer, Dordrecht, 186.

Jefferies, R.A. 1995. Physiology of crop responses to drought. In: Haverkort, A. & MacKerron, D.K.L. (eds.). *Potato Ecology and Modelling of Potato Crops Under Conditions Limiting Growth*. Current Issues in Production Ecology, Kluwer Academic Publishers, Dordrecht, Netherlands, 61-74.

Jefferies, R.A. & Brennan, R.M. 1994. Effects of low temperature on the growth of blackcurrant cultivars. *Scientia Horticulturae* 57, 293-301.

Jefferies, R.A. & MacKerron, D.K.L. 1994. Genotypic differences in water use efficiency in potato. *Aspects of Applied Biology* 38, 63-70.

Jensen, J.L., Glasbey, C.A. & Corbett, P.W.M. 1994. On the interaction of geology, measurement, and statistical analysis of small-scale permeability measurements. *Terra Nova* 6, 397-403.

Johnston, D.J., Williamson, B. & McMillan, G.P. 1994. The interaction *in planta* of polygalacturonases from *Botrytis cinerea* with a cell wall-bound polygalacturonase-inhibiting protein (PGIP) in raspberry fruits. *Journal of Experimental Botany* 45, 1837-1843.

Jolly, C.A. & Mayo, M.A. 1994. Changes in the amino acid sequence of the coat protein readthrough domain of potato leafroll luteovirus affect the formation of an epitope and aphid transmission. *Virology* 201, 182-185.

Jones, A.T. 1994. Black currant reversion and its eriophyid mite vector. *Proceedings of the International Rosette Symposium*, Iowa State University.

Jones, A.T. 1994. *Technical Guidelines for the Safe Movement of Small Fruit Germplasm.* In: Diekmann, M., Frison, E.A. & Putter, C.A.J. (eds.). FAO/IPGRI, Rome, 1-124.

Jones, A.T. & Mitchell, M.J. 1994. Properties of a host range and coat protein variant of broad bean true mosaic comovirus from *Vicia faba*. *Annals of Applied Biology* 125, 123-131.

Jones, A.T., Amrine, J.W., Roberts, I.M., Duncan, G.H., Fenton, B., Malloch, G., McGavin, W.J. & Birch, A.N.E. 1994. The ultrastructure and taxonomic evaluation of eriophyid mites of *Ribes*. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 116-119.

Jones, A.T., Brown, D.J.F., McGavin, W.J., Rüdel, M. & Altmayer, B. 1994. Properties of an unusual isolate of raspberry ringspot virus from grapevine in Germany and evidence for its possible transmission by *Paralongidorus maximus*. *Annals of Applied Biology* 124, 283-300.

Jones, D.A.C., Hyman, L.J., Tumeseit, M., Smith, P., & Pérombelon, M.C.M. 1994. Blackleg, potential of potato seed: determination of tuber contamination by *Erwinia carotovora* subsp. *atroseptica* by immunofluorescence colony staining and stock and tuber sampling. *Annals of Applied Biology* 124, 557-568.

Jones, E.R.L., Newton, A.C. & Clifford, B.C. 1994. *Rhynchosporium* of barley. *United Kingdom Cereal Pathogen Virulence Survey Annual Report for 1993*, 58-64.

Jones, J.T. & Perry, R.N. 1994. Analysis of gene expression during the hatching process in the potato cyst nematode, *Globodera rostochiensis* using the differential display technique. *Proceedings of the 22nd International Symposium of the European Society of Nematologists*, Gent, Belgium.

Jones, J.T., Perry, R.N. & Johnston, M.R.L. 1994. Changes in the ultrastructure of the aphids of the potato cyst nematode, *Globodera rostochiensis*, during development and infection. *Fundamental and Applied Nematology* 17, 369-382.

Jones, J.T., Robertson, W.M., Forrest, J.M.S. & Smith, A. 1994. Preliminary characterisation of a protein produced in the subventral gland of the potato cyst nematode, *Globodera pallida*. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 78-79.

Juanéda, P., Sebedio, J.L. & Christie, W.W. 1994. Complete separation of the geometrical isomers of linolenic acid by high performance liquid chromatography with a silver ion column. *Journal of High Resolution Chromatography* 17, 321-324.

Kempton, R.A., Seraphin, J.C. & Sword, A.M. 1994. Statistical analysis of two-dimensional variation in variety yield trials. *Journal of Agricultural Science* 122, 335-342.

Kennedy, D.M. & Duncan, J.M. 1994. A papillate *Phytophthora* species with specificity to *Rubus*. *Mycological Research* 99, 57-68.

Kumar, A., Mad Arif, S., Taylor, M.A., George, L., Burch, L., Davies, H.V., Butler, A. & Stark, M. 1994. Molecular and functional analysis of the cDNA clone of S-adenosylmethionine decarboxylase (SAMDC) of potato. *Journal of Experimental Botany* 45 (Suppl.), 43.

Lanham, P.G., Brennan, R.M., Hackett, C. & McNicol, R.J. 1994. Genetic variation in blackcurrant (*Ribes nigrum* L.) detected by molecular markers. *Biometrics in plant breeding: Applications of molecular markers*, 241-242.

Lanham, P.G., Brennan, R.M., Hackett, C. & McNicol, R.J. 1995. RAPD fingerprinting of blackcurrant (*Rubus nigrum* L.) cultivars. *Theoretical and Applied Genetics* 90, 166-172.

Lanham, P.G., Foster, B.P., McNicol, P., Moss, J.P. & Powell, W. 1994. Storage protein initiation in *Arachis* species. *Genome* 37, 487-496.

Lawson, H.M., Wright, G.McN., Bacen, E.T.G. & Yeoman, D. 1994. Impact of set-aside management strategies on the soil seedbank and weed flora at Woburn. *Aspects of Applied Biology* 40, 453-460.

Leader, D.J., Sanders, J.F. & Brown, J.W.S. 1994. Novel genomic organisation of plant U14 small nucleolar RNAs. *Journal of Experimental Botany* 45, 11.

Leader, D.J., Connelly, S., Filipowicz, W. & Brown, J.W.S. 1994. Characterisation and expression of a maize U3snRNA gene. *Biochemica et Biophysica Acta* 1219, 145-147.

Leader, D.J., Sanders, J.F., Turnbull-Ross, A., Waugh, R. & Brown, J.W.S. 1994. Genomic organisation of plant U14snoRNA genes. *RNA Processing Meeting*, Madison, USA, May 1994, 239.

Leader, D.J., Sanders, J.F., Turnbull-Ross, A., Waugh, R. & Brown, J.W.S. 1995. Genomic organisation of plant U14snoRNAs. *Biochemical Society Transactions* 314S, 23.

Leader, D.J., Sanders, J.F., Waugh, R., Shaw, P. & Brown, J.W.S. 1994. Molecular characterisation of plant U14 small nucleolar RNA genes: closely linked genes are transcribed as polycistronic U14 transcripts. *Nucleic Acids Research* 22, 5196-5203.

Leese, G.P., Nicoll, A.E., Varnier, M., Thompson, J., Scrimgeour, C.M. & Rennie, M.J. 1994. Kinetics of $^{13}CO_2$ elimination after ingestion of ^{13}C bicarbonate: the effects of exercise and acid base balance. *European Journal of Clinical Investigation* 24, 818-823.

Liskova, M. & Brown, D.J.F. 1994. The occurrence and distribution of Longidoridae and Trichodoridae in the Slovak Republic. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 84-85.

Liu, J., Crawford, J.W. & Viola, R. 1995. Complex oscillations, chaos and coexistence of attractors in a model system coupling activation and inhibition in parallel. *Journal of Theoretical Biology* 10, 311-325.

Luby, J.J. & McNicol, R.J. 1994. The potential for exploitation of genetic resources in *Fragaria, Rubus* and *Ribes*. *Aspects of Applied Biology* 39, 7-14.

MacFarlane, S.A., Mathis, A. & Bol, J.F. 1994. Heterologous encapsidation of recombinant pea early browning virus. *Journal of General Virology* 75, 1423-1429.

Machray, G.C., Burch, L., Hedley, P.E., Davies, H.V. & Waugh, R. 1994. Characterisation of a complementary DNA encoding a novel plant enzyme with sucrolytic activity. *FEBS Letters* 354, 123-127.

Machray, G.C., Burch, L., Hedley, P.E., Davies, H.V. & Waugh, R. 1994. Genes of sucrose metabolism in potato and manipulation of their expression. *Proceedings of the International Colloquium 'Impact of Plant Biotechnology on Agriculture'*. Planprint, Ljubljana, 201-210.

Machray, G.C., Hedley, P.E., Simpson, C.G., Waugh, R. & Brown, J.W.S. 1994. PCR methods for the analysis of expression from plant multigene families. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 42-44.

Mackay, G.R., Wastie, R.L. & Stewart, Helen E. 1994. Breeding potatoes for warm climates. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 20-23.

MacKerron, D.K.L. & Jefferies, R.A. 1994. Seasonal and ontogenetic changes in water use efficiency in potato as indicated by isotope discrimination. *Aspects of Applied Biology* 38, 101-111.

MacKerron, D.K.L. & Lewis, G.J. 1995. Modelling to optimize the use of both water and nitrogen. In: Haverkort, A. & MacKerron, D.K.L. (eds.). *Potato Ecology and Modelling of Potato Crops Under Conditions Limiting Growth*. Current Issues in Production Ecology, Kluwer Academic Publishers, Dordrecht, Netherlands, 129-146.

Madulu, J.D., Trudgill, D.L. & Phillips, M.S. 1993. Rotational management of *Meloidogyne javanica* and effects on *Pasteuria penetrans* and tomato and tobacco yields. *Nematologica* 40, 438-455.

Magill, W., Deighton, N., Pritchard, H.W., Benson, E.E. & Goodman, B.A. 1994. Physiological and biochemical studies of seed storage parameters in *Carica papaya*. *Proceedings of the Royal Society of Edinburgh*, 102B, 439-442.

Marshall, B. & Wallace, J.W. 1994. Water and agriculture - scaling from soil grains to regions. *5th International Congress for Computer Technology in Agriculture: Enabling Technologies for Land Use and Resource Management*, Royal Agricultural Society for England, NAC, Stoneleigh, Warwickshire, UK, 65-68.

Marshall, B., Crawford, J.W. & McNicol, J.W. 1993. Flexible modelling accessing knowledge and uncertainty. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 62-65.

Marshall, B., Crawford, J.W. & McNicol, J. 1994. Handling qualitative and uncertain information. In: Haverkort, A. & MacKerron, D.K.L. (eds.). *Potato Ecology and Modelling of Potato Crops Under Conditions Limiting Growth*. Current Issues in Production Ecology, Kluwer Academic Publishers, Dordrecht, Netherlands. 323-340.

Mayo, M.A. 1994. Nepoviruses. In: Webster, R.G. & Granoff, A. (eds.). *Encyclopedia of Virology* London, Academic Press, 901-907.

Mayo, M.A. 1994. Satellites. In: Murphy, F.A., Fauquet, C.M., Bishop, G.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A. & Summers, M.D. (eds.). *Virus Taxonomy - The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses*. Springer-Verlag, Vienna, 487-497.

Mayo, M.A. 1994. Unassigned Viruses. In: Murphy, F.A., Fauquet, C.M., Bishop, G.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A. & Summers, M.D. (eds). *Virus Taxonomy - The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses*. Springer-Verlag, Vienna, 504-507.

Mayo, M.A. & Fritsch, C. 1994. A possible concensus sequence for VPg of viruses in the family Comoviridae. *FEBS Letters* 354, 129-130.

Mayo, M.A. & Murphy, F.A. 1994. Modifications to the rules for virus nomenclature. *Archives of Virology* 134, 213-215.

Mayo, M.A., Jolly, C.A., Duncan, G.H., Lamb, J.W. & Hay, R.T. 1994. Experimental approaches to determining roles for the proteins in the particles of PLRV. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 97-100.

Mayo, M.A., Lamb, J.W., Duncan, G.H. & Hay, R.T. 1994. Formation of virus-like particles in insect cells infected with a recombinant baculovirus containing the gene for the coat protein of potato leafroll luteovirus. *Abstracts of the XIIIth Annual Meeting of the American Society of Virology*, Madison, 184.

Mayo, M.A., Miller, J.S., Wesley, S.V., Naidu, R.A. & Reddy, D.V.R. 1993. Current research on Indian peanut clump virus at SCRI. In: Reddy, D.V.R., McDonald, D. & Moss, J.P. (eds.). *Working together on groundnut virus disease*, ICRISAT, Patancheru, India, 46.

Mayo, M.A., Murant, A.F., Turnbull-Ross, A.D., Reavy, B., Hamilton, R.I. & Gingery, R.E. 1994. Family Sequiviridae. In: Murphy, F.A., Fauquet, C.M., Bishop, G.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A. & Summers, M.D. (eds.). *Virus Taxonomy - The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses*. Springer-Verlag, Vienna, 337-340.

Mayo, M.A., Robertson, W.M., Legorburu, F.J. & Brierley, K.M. 1994. Molecular approaches to an understanding of the transmission of plant viruses by nematodes. In: Lamberti, F., De Giorgi, C. & Bird, D. McK. (eds.). *Advances in Molecular Nematology*. Plenum Press, New York, 277-293.

Mayo, M.A., Wesley, S.V., Miller, J.S. & Reddy, D.V.R. 1994. Nucleotide sequence studies with Indian peanut clump virus. In: Witcombe, J.R. & Wright, D. (eds.). *ODA PSRP Compendium of Research on Plant Sciences 1993*. Overseas Development Administration, London, 55-57.

McDougall, G.J. 1993. Accumulation of wall-associated peroxidases during wound-induced suberization of flax. *Journal of Plant Physiology* 142, 651-656.

McDougall, G.J. & Fry, S.C. 1994. Fucosylated xyloglucan in suspension-cultured cells of the graminaceous monocotyledon, *Festuca arundinacea*. *Journal of plant Physiology* 143, 591-595.

McDougall, G.J. & Morrison, I.M. 1995. Ionically-bound and covalently-bound wall peroxidases differ in their substrate specificity. *Biochemical Society Transactions* 23, 150.

McDougall, G.J., Stewart, D. & Morrison, I.M. 1994. Cell-wall-bound oxidases from tobacco (*Nicotiana tabacum*) xylem participate in lignin formation. *Planta* 194, 9-14.

McDougall, G.J., Stewart, D. & Morrison, I.M. 1994. Cell-wall-bound oxidases participate in lignin formation. *Proceedings of the Fourth Scottish Cell Wall Group Meeting*, Glasgow, 15.

McDougall, G.J., Stewart, D. & Morrison, I.M. 1994. Oxidation of coniferyl alcohol to lignin-like products by cell walls of tobacco xylem. *Phytochemistry* 37, 683-688.

McMillan, G.P., Barratt, A.M. & Pérombelon, M.C.M. 1994. An isoelectric focusing study of the effect of methyl-esterified pectic substances on the production of extracellular pectic isoenzymes by soft rot *Erwinia* spp. *Journal of Applied Bacteriology* 77, 175-184.

McNicol, R.J. 1994. Brambles. In: Dale, A. & Hanson, E.J. (eds.). *Mechanical Harvesting of Berry Crops*. *Horticultural Review* 16, 282-298.

McNicol, R.J. 1994. New cultivars from SCRI. *HDC Soft Fruit Subject Day*, HRI, East Malling, March 1994.

McSorley, R. & Phillips, M.S. 1993. Modelling populations dynamics and yield losses and their use in nematode management. In: Evans, K., Trudgill, D.L. & Webster, J. (eds.). *Plant Parasitic Nematodes in Temperate Agriculture*. CAB International, Oxford.

Meyer, R., Machray, G.C., Hedley, P.E., Davies, H.V. & Waugh, R. 1994. Promoter deletion analysis of invertase gene expression. *Journal of Experimental Botany* 45 (suppl.), 6.

Millam, S., Craig, A. & Christie, W.W. 1994. Case studies in the investigation of potential industrial oil crops. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 26-29.

Millam, S., Craig, A., Harvey, C., Mackay, G.R. & Morrison, I.M. 1994. Modification of oilseed rape by applied plant tissue culture methodology. In: Hennink, S., van Soest, L.J.M., Pitham, K. & Hof, L. (eds.). *Alternative oilseed and fibre crops for cool and wet regions of Europe*, Wageningen, The Netherlands, 198-202.

Millam, S., Craig, A., Robertson, G., Griffiths, D.W., Christie, W.W. & McNicoll, J. 1994. The generation of modified fatty acid lines of *Brassica napus* by somaclonal variation techniques. *Abstracts of the VIII International Congress of Plant Tissue and Cell Culture*, Florence, 119.

Modha, J., Robertson, W.M., Kennedy, M.W. & Kusel, J.R. 1994. Characterisation of a major surface associated excretory-secretory antigen of *Trichinella spiralis* larvae with antibodies to keyhole limpet haemocyanin. *Parasitology* 109, 487-495.

Modha, J., Robertson, W.M., Kusel, J.R. & Kennedy, M.W. 1994. *Trichinella spiralis*: second messengers and surface transformation of infective larvae. *Proceedings of the British Society for Parasitology* 6-8 April, 1994, 37-38.

Molina-Cano, J-L., Ramo, T., Ellis, R.P., Swanston, J.S., Bain, H., Uribe-Echeverria, T. & Perez-Vendrell, A. 1995. Effect of grain composition on water uptake by malting barley: a genetic and environmental study. *Journal of the Institute of Brewing* 101, 79-83.

Morrison, I.M. 1994. Glycolipids. In: Chaplin, M.F. & Kennedy, J.F. (eds.). *Carbohydrate Analysis*: *A Practical Approach-2nd Edition.* I.R.L. Press, Oxford. 295-317.

Morrison, I.M. 1994. Industrial fibres from Scottish crops. In: Perry, D.A. (ed.). *Brassicas and Alternative Crops for Scotland*. Proceedings of the Scottish Society for Crop Research. Bulletin No. 10. Scottish Crop Research Institute, Dundee, 32-35.

Morrison, I.M. 1994. Plant Fibres. *New Directions in Agriculture*, Grampian Regional Council Conference, Aberdeen.

Morrison, I.M. 1994. Range, provision and processing of non-wood fibres from temperate crops. *Non-wood fibres for industry*, PIRA/SRI Conference. Paper 2, 1-11.

Morrison, I.M. & Burrows, S.E. 1994. The fibre composition of *Brassica* species and cellulase degradability of chemically treated brassicas. *Industrial Crops and Products* 2, 171-177.

Morrison, I.M. & Mulder, M.M. 1995. Pyrolysis mass spectrometry for the characterisation of straw and chemically treated straw. *Phytochemical Analysis* 6, 74-84.

Morrison, I.M. & Stewart, D. 1994. Brassica cultivars as sources of plant fibre. *Crucifer Newsletter*. No. 16, 101-102.

Morrison, I.M. & Stewart, D. 1994. The potential of *Brassica* species and cultivars as novel sources of plant fibres. In: Hennink, S., van Soest, L.J.M., Pitham, K. & Hof, L. (eds.). *Alternative oilseed and fibre crops for cool and wet regions of Europe*, Wageningen, The Netherlands, 113-121.

Murant, A.F. 1994. Parsnip yellow fleck virus group. In: Webster, R.G. & Granoff, A. (eds.). *Encyclopedia of Virology*. London, Academic Press, 1042-1046.

Murant, A.F. & Mayo, M.A. 1994. Genus *Idaeovirus*. In: Murphy, F.A., Fauquet, C.M., Bishop, G.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A. & Summers, M.D. (eds.). *Virus Taxonomy - The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses*. Springer-Verlag, Vienna, 458-460.

Murant, A.F., Robinson, D.J., Torrance, L., Blok, V.C., Farmer, M.J., Scott, K. & Zeigler, A. 1994. Current research on groundnut rosette disease at SCRI. In: Reddy, D.V.R., McDonald, D. & Moss, J.P. (eds). *Working together on groundnut virus diseases: Summary and recommendations of a meeting of international working groups on groundnut virus diseases*, 15-19 August, 1993. ICRISAT, International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India, 25-26.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A. & Summers, M.D. (eds.). 1994. *Virus Taxonomy - The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses*. Springer-Verlag, Vienna, 586pp.

Naes, T., Hirst, D.J. & Baardseth, P. 1994. Using cumulative ranks to detect individual differences in sensory profiling. *Journal of Sensory Studies* 9, 87-99.

Neilson, R. & Boag, B. 1994. Environmental impact assessment - sampling for nematodes, a family decision. *Aspects of Applied Biology* 37, 251-254.

Newton, A.C. 1994. Mildew of barley in Scotland. *United Kingdom Cereal Pathogen Virulence Survey Annual Report for 1993*, 49-50.

Newton, A.C. & Hackett, C.A. 1994. Subjective components of mildew assessment on spring barley. *European Journal of Plant Pathology* 100, 395-412.

Newton, A.C. & Reglinski, T. 1995. Environmental Biotic Factors in Integrated Plant Disease Control. *BSPP News*, Poznan, Poland.

Newton, A.C. & Thomas, W.T.B. 1994. Detection of tolerance of barley cultivars to infection by powdery mildew (*Erysiphe graminis* f.sp. *hordei*). *Euphytica* 75, 179-187.

Newton, A.C. & Thomas, W.T.B. 1994. Disease tolerance in spring barley. *Aspects of Applied Biology* 39, 201-206.

Newton, A.C., Harrison, J.G. & Lowe, R. 1994. Interaction of the expression of partial plant disease resistance and the environment. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 89-91.

Newton, A.C., Reglinski, T. & Lyon, G.D. 1995. Resistance elicitors from fungi as crop protectants. In: *Environmental Biotic Factors in Integrated Plant Disease Control. Proceedings of the Third European Foundation for Plant Pathology Meeting*, Poznan, Poland, 74.

Newton, A.C., Thomas, W.T.B. & Goleniewski, G. 1995. Effects of nitrogen on disease levels and yield in major gene and partial resistance spring barley cultivar mixtures. *Proceedings of the Third Workshop on Integrated Control of Cereal Mildew Across Europe*, Kappel a. Albis, Switzerland, 1994.

Newton, A.C., Leakey, R.R.B., Powell, W., Chalmers, K.J. & Waugh, R. 1994. Domestication of Mahoganies. In: Newton, A.C. & Leakey, R.R.B. (eds.). *Tropical Trees: The Potential for Domestication and Rebuilding of Forest Resources*, 256-266.

Nikolova-Damyanova, B., Christie, W.W. & Herslöf, B.G. 1994. Improved separation of some positionally isomeric monounsaturated fatty acids as phenacyl derivatives by silver ion thin-layer chromatography. *Journal of Planar Chromatogr*aphy 7, 382-385.

Nisbet, A.J., Woodford, J.A.T. & Strang, R.H.C. 1994. Quantifying aphid feeding on non-radioactive food sources. *Entomologia Experimentalis et Applicata* 72, 85-89.

Nisbet, A.J., Woodford, J.A.T. & Strang, R.H.C. 1994. The effects of azadirachtin-treated diets on the feeding behaviour and fecundity of the peach-potato aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata* 71, 65-72.

Nyange, N.E., McNicol, R.J., Williamson, B. & Lyon, G.D. 1994. *In vitro* selection of coffee plants resistant to coffee bean disease. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 93-94.

Olawoye, T.L., Minnikin, D. & Christie, W.W. 1993. HPLC separation of tertbutyldimethylsilyl (TBDMS) ether homologs of mycolic acids using a silver ion column. *Biokemistri* 3, 103-108.

Oparka, K.J. 1994. *The Living Plant Cell*. Educational video and booklet for schools and colleges. Scottish Crop Research Institute, Dundee.

Oparka, K.J. & Prior, D.A.M. 1993. Plasmodesmata: an open and shut case. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 50-53.

Oparka, K.J., Prior, D.A.M. & Wright, K.M. 1995. Non-invasive imaging of phloem transport processes. *Abstracts of 3rd International Conference on Botanical Microscopy*, Oxford.

Oparka, K.J., Prior, D.A.M. & Wright, K.M. 1995. Non-invasive imaging of phloem unloading in *Arabidopsis* roots. *Abstracts of Society of Experimental Biology Meeting*, St Andrews.

Oparka, K.J., Prior, D.A.M. & Wright, K.M. 1995. Symplastic communication between primary and developing lateral roots of *Arabidopsis thaliana*. *Journal of Experimental Botany* 46, 187-197.

Oparka, K.J., Duckett, C.M., Prior, D.A.M. & Fisher, D.J. 1994. Real-time imaging of phloem unloading in the root tip of *Arabidopsis*. *The Plant Journal* 6, 759-766.

Palivan, C.G., Cristescu, C. & Goodman, B.A. 1994. Characterisation by EPR spectroscopy of the coordination environment of copper in superoxide dismutase (SOD). *Proceedings of the Royal Society of Edinburgh, B*, 102, 273-277.

Palivan, C.G., Goodman, B.A. & Palivan, H. 1994. ^{14}N Superhyperfine structure in the spectra of frozen solutions of copper/zinc superoxide dismutases (SOD). *Abstracts of the European ESR Meeting on Recent Advances and Applications to Organic and Bioorganic Materials*, Paris, 109.

Pearce, S.R., Kumar, A. & Flavell, A.J. 1994. *Ty-Copia* group retrotransposons in *Solanum tuberosum*. *Abstracts of PMB II-BBSRC Meeting*, Norwich.

Pereira, L.G., Torrance, L., Roberts, I.M. & Harrison, B.D. 1994. Antigenic structure of the coat protein of potato mop-top furovirus. *Virology* 203, 277-285.

Pérombelon, M.C.M. 1994. Diversity in erwinias as plant pathogens. *Colloques, No. 66, Plant Pathogenic Bacteria*, Versailles, France, June 9-12, 1992, 113-128.

Pérombelon, M.C.M. & McMillan, G.P. 1995. Purification and characterization of a high pl pectin methyl esterase isoenzyme and its inhibitor from tubers of *Solanum tuberosum* subsp. *tuberosum* cv. Katahdin. *Physiological and Molecular Plant Pathology* 46, 413-427.

Pérombelon, M.C.M. & Salmond, G.P.C. 1995. Bacterial soft rots. In: Singh, V.S., Kohmoto, K. & Singh, R.P. (eds.). *Pathogenesis and Host specificity in Plant Diseases.* Pergamon Elsevier, Oxford, Vol. 1, Chapter 1, 1-20.

Phillips, M.S. 1994. Inheritance of resistance to nematodes. In: Bradshaw, J.E. & Mackay, G.R. (eds.). *Potato Genetics*. CAB International, Wallingford, Oxford.

Phillips, M.S., Blok, V.C., Dale, M.F.B. & Trudgill, D.L. 1994. Virulence to plant resistance in potato cyst nematodes. *Aspects of Applied Biology* 39, 139-143.

Phillips, M.S., Blok, V.C., Harrower, B. & Malloch, G. 1994. The use of PCR to examine intraspecific variation in potato cyst nematodes. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematology*, Gent, Belgium.

Phillips, M.S., Hackett, C.A., Trudgill, D.L., Spaull, A.M. & Hancock, M. 1994. Modelling the population dynamics of *Globodera pallida* and yield losses to potatoes differing in tolerance and resistance. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 102.

Ramel, M.E., Brown, D.J.F., Vallotton, R., McGavin, W. & Gugerli, P. 1994. Occurrence of infection by a strain of arabis mosaic nepovirus of barley (cv. Express) and viruliferous *Xiphinema diversicaudatum* in fields near Fribourg in Switzerland. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 106-107.

Ramsay, L.D., Bradshaw, J.E. & Kearsey, M.J. 1994. The inheritance of quantitative traits in *Brassica napus* ssp. *rapifera* (swedes): augmented triple test cross analysis of yield. *Heredity* 73, 84-91.

Ramsay, L.D., Bradshaw, J.E. & Kearsey, M.J. 1994. The inheritance of quantitative traits in swedes (*Brassica napus* L. spp. *rapifera*). Diallel analysis of dry matter yield. *Journal of Genetics and Breeding* 49, 253-258.

Raven, J.A., Johnston, A.M., Newman, J.R. & Scrimgeour, C.M. 1994. Inorganic carbon acquisition by aquatic photolithotrophs of the Dighty Burn, Angus, U.K.: uses and limitations of natural abundance measurements of carbon isotopes. *New Phytologist* 127, 271-286.

Reaich, D., Graham, K.A., Cooper, B.G., Scrimgeour, C.M. & Goodship, T.H.J. 1994. Recovery of ^{13}C in breath from infused $NaH^{13}CO_3$ increases during euglycemic hyperinsulinemia. *Clinical Science* 87, 415-419.

Reavy, B., Barker, H., Webster, K.D., Arif, M., Kashiwazaki, S., Jolly, C.A. & Mayo, M.A. 1994. Exploiting coat protein transgenesis to create improved forms of resistance. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee 103-105.

Reglinski, T., Lyon, G.D. & Newton, A.C. 1994. Assessment of elicitor activity of scotiatec extracts and their ability to induce resistance to plant pathogens. *Report to Scotia Pharmaceuticals Ltd.*

Reglinski, T., Lyon, G.D. & Newton, A.C. 1994. Induction of resistance mechanisms in barley by yeast-derived elicitors. *Annals of Applied Biology* 124, 509-517.

Ritz, K. 1994. Fungal growth in heterogeneous environments. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 54-57.

Ritz, K. 1995. Growth responses of some soil fungi to spatially heterogeneous nutrients. *FEMS Microbiology Ecology* 16, 269-280.

Ritz, K. & Griffiths, B.S. 1994. Potential application of a community hybridization technique for assessing changes in the population structure of soil microbial communities. *Soil Biology and Biochemistry* 26, 963-971.

Ritz, K., Griffiths, B.S., Ebblewhite, N. & Caul, S. 1995. Assessment of the impact of plants upon microbial communities by broad-scale analysis of community DNA. Abstract of Soil Ecology Society National Conference, *Influence of Belowground Biodiversity on Aboveground Community Structure*, Fort Collins, USA.

Ritz, K., Griffiths, B.S., Ebblewhite, N. & Caul, S. 1995. Broad-scale approaches to the analysis of microbial community DNA extracted from the rhizosphere. *Abstract in Plant and Soil Interactions*, Society of Experimental Biology Annual Meeting, St Andrews, UK.

Robbins, R.T. & Brown, D.J.F. 1994. A computer program "LONG-SORT" to assist with the diagnosis and identification of *Longidorus* species. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 110-111.

Robbins, R.T. & Brown, D.J.F. 1994. Diagnoses and relationships of Thorne's *Longidorus sulphus*, *L. crassus* and *L. fragilis*. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 111-112.

Robbins, R.T. & Brown, D.J.F. 1995. Amended descriptions of *Longidorus sylphus* Thorne, 1939, *L. crassus* Thorne, 1974 and *L. fragilis* Thorne, 1974 (Nematoda:Longidoridae). *Journal of Nematology* 27, 94-102.

Roberts, I.M. 1994. Factors affecting the efficiency of immunogold labelling of plant viruses in thin sections. *Journal of Virological Methods* 50, 155-166.

Roberts, I.M., Jones, A.T. & Amrine, J.W. 1994. Ultrastructure of the black currant gall mite, *Cecidophyopsis ribis* (Acari:Eriophyidae), the vector of the agent of reversion disease. *Annals of Applied Biology* 125, 447-455.

Robertson, G.W., Griffiths, D.W., Woodford, J.A.T. & Birch, A.N.E. 1994. Changes in the chemical composition of volatiles released by the flowers and fruits of the raspberry cv. Glen Prosen. *Phytochemistry* 38, 1175-1179.

Robertson, W.M. & Mayo, M.A. 1994. Observations and speculations on the retention of plant virus particles within plants. *Proceedings of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 112-113.

Robertson, W.M., Forrest, J.M.S., Jones, J.T. & Smith, A.M. 1994. Preliminary characterisation of a protein produced in the subventral oesophageal gland of the potato cyst nematode, *Globodera pallida*. *Abstracts of the BSPP spring meeting 1994*.

Robinson, D. 1993. The efficiency of plant root systems in nutrient capture: black box, grey area or red herring? In: Clarkson, D.T. (ed.). *Genetic Techniques for Adapting Crop Plants to Lowered Fertiliser Inputs and Nutrition Stresses*. CEC, Brussels, 53-62.

Robinson, D. 1994. The responses of plants to non-uniform supplies of nutrients. Tansley Review No. 73. *New Phytologist* 127, 635-674.

Robinson, D., Linehan, D.J. & Gordon, D.C. 1994. Nitrate recovery from soil by wheat in relation to root length in flow and nitrogen availability. *New Phytologist* 128, 297-305.

Robinson, D.J. 1994. Sequences at the ends of RNA-2 of I6, a recombinant tobravirus. *Archives of Virology* 9, 245-251.

Robinson, D.J. 1994. The role of recombination in generating the diversity of tobraviruses. *Abstracts of the Juan March Institute Workshop on Genetic Recombination and Defective Interfering Particles in RNA Viruses*, Madrid, 1994, 44-45.

Robinson, D.J. & Dale, M.F.B. 1994. Susceptibility, resistance and tolerance of potato cultivars to tobacco rattle virus infection and spraing disease. *Aspects of Applied Biology* 39, 61-66.

Ross, H.A., Davies, H.V., Burch, L.R., Viola, R. & MacRae, D. 1994. Developmental changes in carbohydrate content and sucrose degrading enzymes in tuberising stolons of potato (*Solanum tuberosum*). *Physiologia Plantarum* 90, 748-756.

Rouselle, P., Wastie, R. & Waugh, R. 1994. Revue des methodes pour l'amélioration génétique de la resistance aux parasites et aux maladies. I. Les approaches traditionelles. *La Pomme de Terre Française*, No. 480, 13-19.

Rouselle, P., Wastie, R. & Waugh, R. 1994. Revue des methodes pour L'amélioration génétique de la resistance aux parasites et aux maladies. II. Les approaches Nouvelles. *La Pomme de Terre Française*, No. 481, 69-75.

Ruxton, G.D. 1994. Local and ensemble dynamics of linked populations. *Journal of Animal Ecology* 63, 1002.

Ruxton, G.D. 1994. Low levels of immigration between chaotic populations can reduce system extinctions by inducing asynchronous regular cycles. *Proceedings of the Royal Society B* 256, 189-193.

Ruxton, G.D. 1994. Mathematical modelling of ammonia volatilization from slurry stores and its effect on cryptosporidium oocyst viability. *Journal of Agricultural Science* 123, 152-153.

Ruxton, G.D. & Gibson, G.J. 1994. Aerobic deterioration of big-bale silage and its implications for development of *Listeria monocytogenes*. *Journal of Agricultural Science* 122, 164.

Ruxton, G.D. & Gibson, G.J. 1994. Effect of deterioration of spraying the open face of a silage bunker with propionic acid. *Journal of Agricultural Engineering Research* 58, 159-168.

Ruxton, G.D. & Gibson, G.J. 1994. Self-compaction and aerobic deterioration of bunker silage: a simulation study. *Journal of Agricultural Engineering Research* 59, 189-193.

Ruxton, G.D. & Gurney, W.S.C. 1994. Deriving the functional response without assuming homogeneity. *American Naturalist* 144, 537-541.

Schröder, H.C., Scheffer, U., Forrest, J.M.S., Séve, A-P., Rytik, P.G. & Müller, W.E.G. 1994. Association of scrapie prion proteins and prion RNA stem loop with nuclear carbohydrate-binding protein 35 and other RNA-binding proteins. *Neurogeneration* 3, 177-189.

Scott, K.P., Kashiwazaki, S., Reavy, B. & Harrison, B.D. 1994. The nucleotide sequence of potato mop-top virus RNA-2: a novel type of gene organization for a furovirus. *Journal of General Virology* 75, 3561-3568.

Shepherd, T. 1994. The microchemistry of plant/microorganism interactions. In: Blakeman, J.P. & Williamson, B. (eds.). *Ecology of Plant Pathogens*. CAB International, Oxford, 39-62.

Shirako, Y. & Wilson, T.M.A. 1994. Furoviruses. In: Webster, R.G. & Granoff, A. (eds.). *Encyclopedia of Virology*, Saunders Scientific Publications, London, 508-516.

Simpson, C.G. & Brown, J.W.S. 1994. Selection of 3' splice sites in plant pre-mRNA processing. *Journal of Experimental Botany* 45, 11.

Simpson, G.G., Clark, G. & Brown, J.W.S. 1994. Isolation of a maize cDNA encoding a protein with extensive similarity to an inhibitor of protein kinase C and an agrobacterial open reading frame. *Biochimica et Biophysica Acta* 1222, 306-308.

Simpson, C.G., Clark, G., Davidson, D. & Brown, J.W.S. 1994. Branchpoint sequences are required for plant pre-mRNA splicing. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 44-45.

Simpson, C.G., Clark, G., Davidson, D. & Brown, J.W.S. 1994. Conserved branchpoint sequences are required for plant intron splicing. *RNA Processing Meeting*, Madison, USA, May 1994, 403.

Smith, K., Downie, S., Barua, J.M., Watt, P.W., Scrimgeour, C.M. & Rennie, M.J. 1994. Effect of a flooding dose of leucine in stimulating incorporation of constantly infused valine into albumin. *American Journal of Physiology* 266, 640-644.

Solomon-Blackburn, R.M., Mackay, G.R. & Brown, J. 1994. An evaluation of seedling progeny tests for resistance to potato leafroll virus in potato, *Solanum tuberosum*. *Journal of Agricultural Science* 122, 231-239.

Stewart, D. 1994. A physical and chemical study of plant fibres. *Ph.D. Thesis*, University of Dundee.

Stewart, D. 1994. Phenolic acid dimers on barley straw cell walls. *Abstracts of the Tenth Cellucon Conference: The Chemistry and Processing of Wood and Plant Fibrous Materials*, 43.

Stewart, D. & Brennan, R.M. 1994. Blackcurrant stems - an agri-waste with potential as a diluent for existing tree-based fibres. In: *The Chemistry and Processing of Wood and Plant Fibres Material*. Ellis Horwood, Chichester, 44.

Stewart, D. & Morrison, I.M. 1994. Delignification/bleaching of non-wood fibres with peroxy compounds. *Abstracts of the Tenth Cellucon Conference: The Chemistry and Processing of Wood and Plant Fibrous Materials*, 13.

Stewart, D., Baty, A. & McDougall, G.J. 1994. Fourier transform infra-red (FT-IR) microscopy of flax cell walls. *Proceedings of the Fourth Scottish Cell Wall Group Meeting*, Glasgow. Paper 7.

Stewart, D., Brennan, R.M. & Provan, G.J. 1994. Characterisation of blackcurrant stem lignin. *Phytochemistry* 37, 1703-1706.

Stewart, D., Brennan, R.M. & Provan, G.J. 1994. Characterisation of blackcurrant stem lignin. *Proceedings of 4th Scottish Cell Wall Group Meeting.*

Stewart, D., Forrest, J.M.S. & Müller, W.E.G. 1994. Anti-viral material patent. Application No. 08/143,500, 50522413.14, 90915787.7, OAP1 9652.

Stewart, D., Lyon, G.D. & Tucker, E.J.B. 1994. A Fourier-transform infrared spectroscopic and microscopic study of the infection of potato tubers by *Erwinia carotovora* ssp. *carotovora* in aerobic and anaerobic conditions. *Journal of the Science of Food and Agriculture* 66, 145-154.

Stewart, Helen E., Bradshaw, J.E. & Wastie, R.L. 1994. Correlation between resistance to late blight in foliage and tubers in potato clones from parents of contrasting resistance. *Potato Research* 37, 429-434.

Stewart, Helen E., Bradshaw, J.E., Wastie, R.L., Mackay, G.R., Erlich, O., Livescu, L. & Nachmias, A. 1994. Assessing progenies of potato for resistance to early blight. *Potato Research* 37, 257-269.

Stewart, G.R., Schmidt, S., Handley, L.L., Turnbull, M.H., Erskine, P.D. & Joly, C.A. 1994. ^{15}N natural abundance of vascular rainforest epiphytes: implications for nitrogen source and acquisition. *Plant, Cell and Environment* 18, 85-90.

Swanson, M.M. & Harrison, B.D. 1993. Differently conserved epitopes on the coat protein of whitefly-transmitted geminiviruses. *Abstracts of the IXth International Congress of Virology*, Glasgow, 1993, 357.

Swanson, M.M. & Harrison, B.D. 1993. Serological relationships and epitope profiles of isolates of okra leaf curl geminivirus from Africa and the Middle East. *Biochimie* 75, 707-711.

Swanson, M.M. & Harrison, B.D. 1994. Properties, relationships and distribution of cassava mosaic geminiviruses. *Tropical Science* 34, 15-25.

Swanston, J.S. 1994. Malting performance of barleys with altered starch composition. *Ph.D. Thesis*, Heriot-Watt University.

Swanston, J.S. & Ellis, R. 1994. Breeding barley varieties for improved malting quality. *Food & Beverage Europe 1995*, 105-106.

Swanston, J.S., Ellis, R.P. & Molina-Cano, J-L. 1994. Endosperm cell walls - barriers to malting quality. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 24-26.

Symons, M.C.R., Rowland, I.J., Deighton, N., Shorrock, K. & West, K.P. 1994. Electron spin resonance studies of nitrosyl haemoglobin in human liver, colon and stomach tumour tissues. *Free Radical Research Communications* 21, 197-202.

Talbot, M. 1994. The design of variety trials. *Proceedings of the Ninth Meeting of the EUCARPIA Section Biometrics in Plant Breeding*, Wageningen, The Netherlands, 43-56.

Talbot, M. & Leonard, S. 1994. Introducing statistics to the general public. *Teaching Statistics at its Best*, 147-149.

Taylor, C.E., Brown, D.J.F., Neilson, R. & Jones, A.T. 1994. The persistence and spread of *Xiphinema diversicaudatum* (Nematoda:Dorylaimida) in cultivated and uncultivated biotopes. *Annals of Applied Biology* 124, 469-477.

Taylor, M.A. & Davies, H.V. 1994. Nucleotide sequence of a cDNA clone for a 60S ribosomal protein L27 gene from potato (*Solanum tuberosum* L.). *Plant Physiology* 105, 1025-1026.

Taylor, M.A., Wright, F. & Davies, H.V. 1994. Characterisation of the cDNA clones of two β-tubulin genes and their expression in the potato plant. *Plant Molecular Biology* 26, 1013-1018.

Taylor, M.A., Ross, H.A., Gardner, A. & Davies, H.V. 1995. Characterisation of a cDNA encoding fructokinase from potato (*Solanum tuberosum* L.). *Journal of Plant Physiology* 145, 253-256.

Torrance, L. 1994. New developments in techniques for virus detection. In: Reddy, D.V.R., McDonald, D. & Moss, J.P. (eds.). *Working together on groundnut virus diseases*. ICRISAT, Patancheru, India 43-45.

Torrance, L., Jones, A.T. & Duncan, G.H. 1994. Properties of cocksfoot streak and cocksfoot cryptic, two viruses infecting cocksfoot (*Dactylis glomerata*) in Scotland. *Annals of Applied Biology* 124, 267-281.

Trudgill, D.L. 1994. An examination of host, temperature and other factors affecting nematode development rates and ecology. *Abstracts of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 127.

Trudgill, D.L. 1995. Why do tropical poikilothermic organisms tend to have higher threshold temperatures for development than temperate ones? *Functional Ecology* 9, 136-137.

Trudgill, D.L. & Perry, J.N. (1995). Thermal time and ecological strategies - a unifying hypothesis. *Annals of Applied Biology* 125, 521-532.

Trudgill, D.L. & Phillips, M.S. 1994. Mechanisms of tolerance differences of potato to damage by potato cyst nematode (*Globodera pallida*). *Nematologica* 40, 564-578.

Trudgill, D.L., Blok, V., Fargette, M. & Phillips, M.S. 1994. Variations in nematode heterogeneity - a discussion. *Abstracts of the 22nd International Nematology Symposium of the European Society of Nematologists*, Gent, Belgium, 128.

Trudgill, D.L, Blok, V.C., Phillips, M.S., Gowen, S. & Hahn, M. 1994. The importance of understanding the centres of origins of nematode pests. *Aspects of Applied Biology* 39, 133-138.

Turnbull-Ross, A.D., Simpson, G.G., Lyon, J., Watters, J., Clark, G., Shaw, P. & Brown, J.W.S. 1994. Transgenic plants in the analysis of plant splicesomal proteins. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 46.

van Bel, A.J. & Oparka, K.J. 1995. On the validity of plasmodesmograms. *Botanica Acta* 108, 174-182.

Van de Ven, W.T.G. & McNicol, R.J. 1994. Genetic 'fingerprinting' of selected Sitka spruce clones using RAPD markers. *Annual Meeting of the Tree Biotechnology Liaison Group, East Malling, UK*, 28-29 March.

Vicente, M.L., Deighton, N., Glidewell, S.M., Goodman, B.A. & Empis, J.A. 1994. An EPR investigation of free radical generation in heated lipids. *Abstracts of the XVIth International Conference on Magnetic Resonance in Biological Systems*, Veldhoven, Netherlands, 26.

Viola, R. & Davies, H.V. 1994. Effect of temperature on pathways of carbohydrate metabolism in tubers of potato (*Solanum tuberosum* L.). *Plant Science* 103, 135-143.

Wastie, R.L. 1994. Inheritance of resistance to fungal diseases. In: Bradshaw, J.E. & Mackay, G.R. (eds.). *Potato Genetics*. CAB International, Wallingford, Oxon, 411-427.

Wastie, R.L., Mackay, G.R. & Carbonell, J. 1994. Effect of *Erwinia carotovora* ssp. *atroseptica* on yield of potatoes from cut and uncut seed tubers in Valencia. *Potato Research* 37, 21-24.

Wastie, R.L., Mackay, G.R. & Nachmias, A. 1994. Effect of *Erwinia carotovora* ssp. *atroseptica* alone and with *Alternaria solani* or *Verticillium dahliae* on disease development and yield of potatoes in Israel. *Potato Research* 37, 113-120.

Wastie, R.L., Rousselle, P. & Waugh, R. 1994. A review of techniques for acquiring pest and disease resistance in potatoes. *Proceedings of the 12th Triennial Conference of the European Association for Potato Research*, Paris 1993, 57-74.

Waugh, R., Provan, J., Chalmers, K.J. & Powell, W. 1994. Intermicrosatellite amplification with 5' anchored simple sequence repeat containing primers generate highly variable and genotype specific genetic fingerprints. *Plant Genome II*, San Diego, 68.

Wesley, S.V., Mayo, M.A., Jolly, C.A., Naidu, R.A., Reddy, D.V.R., Jana, M.K. & Parnaik, V.K. 1994. The coat protein of Indian peanut clump virus: relationships with other furoviruses and with barley stripe mosaic virus. *Archives of Virology* 134, 271-278.

Wheatley, R.E. & Ritz, K. 1995. Elucidating the factors which regulate nitrification in arable soils. *Proceedings of the 8th Nitrogen Workshop*, Gent, Belgium, 20.

Wheatley, R.E. & Ritz, K. 1995. Dynamics of mineral nitrogen in soils supporting potato crops. *Biology and Fertility in Soils* 19, 36-40.

Wheatley, R.E., Millar, S. & Griffiths, D.W. 1995. Volatile organic compounds and nitrogen transformations in soils. *Proceedings of the 8th Nitrogen Workshop*, Gent, Belgium, 19.

Williams, C.M.J., Vitosh, M.L., Maier, N.A. & MacKerron, D.K.L. 1995. Nutrient management strategies for sustainable potato (*Solanum tuberosum*) production systems in the southern and northern hemispheres. *Proceedings of the Fourth International Solanaceae Conference*, Adelaide, 4-9 September 1994.

Williamson, B., Breese, W.A. & Shattock, R.C. 1994. Downy mildew in blackberries and raspberries: a problem in micropropagated plants. *Proceedings of Conference on New Developments in the Soft Fruit Industry*, Robinson College, Cambridge, 22 February 1994, 41-43.

Williamson, B., Duncan, G.H., Harrison, J.G., Harding, L.A. & Zimand, G. 1994. Grey mould (*Botrytis cinerea*) of roses. *Annual Report of the Scottish Crop Research Institute for 1993*. Scottish Crop Research Institute, Dundee, 91-93.

Williamson, B., Goodman, B.A., Chudek, J.A., Hunter, G. & Lohman, J.A.B. 1994. The vascular architecture of the fruit receptacle of red raspberry determined by 3D NMR microscopy and surface-rendering techniques. *New Phytologist* 128, 39-44.

Wilson, T.M.A. 1995. Disassembly mechanisms of plant RNA viruses: fine-tuning and possible sites for transgenic protection. *Fifth International Symposium on Biotechnology and Plant Protection*. University of Maryland, World Publishing Company, 1-34.

Wilson, T.M.A. 1994 Peptide antibiotics. *UK Patent Application No* 9420989.7.

Wilson, T.M.A. & Davies, J.W. 1994. New roads to crop protection against viruses. In: Atkin, R.K. & Lister, D. (eds.). *Outlook on Agriculture*. CAB International, Oxford, 33-39.

Wright, G. & Boag, B. 1994. The application of satellite remote sensing and spatial proximity analysis techniques to observations on the grazing of oilseed rape by roe deer. *International Journal of Remote Sensing* 15, 2087-2097.

Wright, K.M. & Oparka, K.J. 1994. Observations on the accumulation of five xenobiotic chemicals in phloem versus parenchyma tissues of celery. *Pesticide Science* 42, 17-24.

Yanishlieva, N.V., Marekov, I.N. & Christie, W.W. 1994. Comparison of the antioxidative effectiveness of *alpha*-tocopherol and caffeic acid during bulk phase and liposome oxidation of acylglycerols with a high linoleic acid content. *Seifenöle-Fette-Wache Journal* 120, 662-665.

Yeates, G.W. & Boag, B. 1994. Relation between macrofauna populations and soil physical properties in two agroforestry trials. *Acta Zoologica Fennica* 196, 212-214.

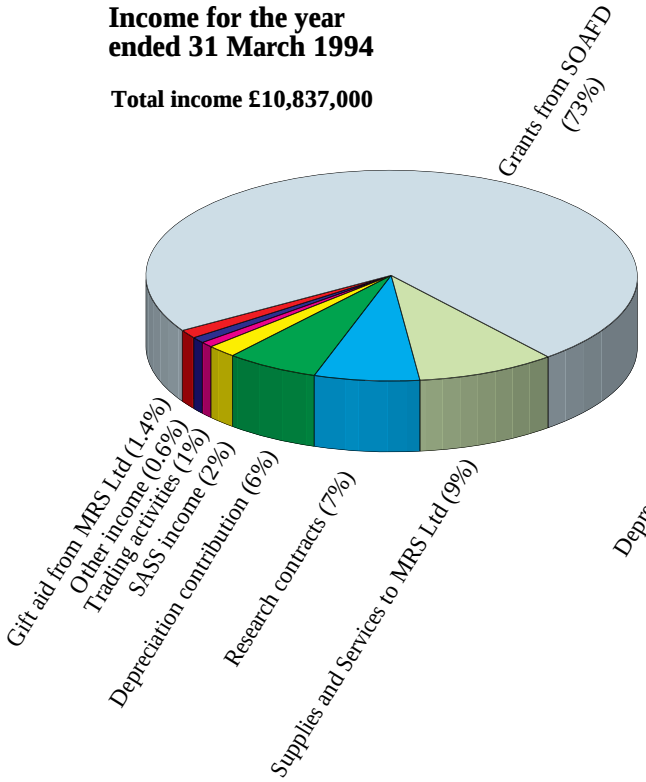
Yeates, G.W., Boag, B. & Small, R.W. 1994. Species diversity and biogeography of Mononchoidea (Nematoda). *Russian Journal of Nematology* 2, 45-54.

Young, I.M., Roberts, A., Griffiths, B.S. & Caul, S. 1994. Growth of a ciliate protozoan in model Ballotini systems of different particle sizes. *Soil Biology and Biochemistry* 26, 1173-117.

Summary of the Accounts

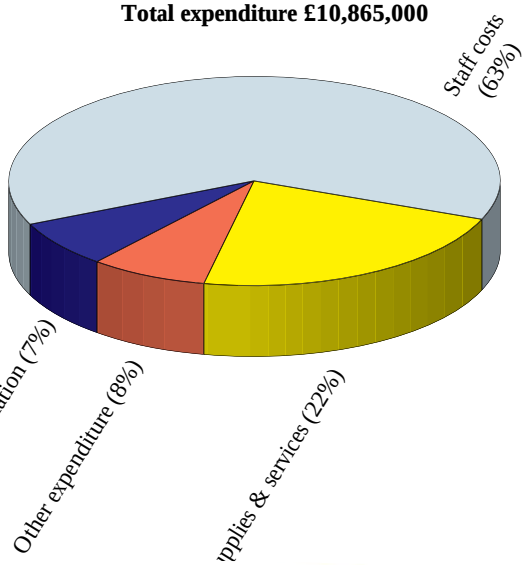
Income for the year ended 31 March 1994

Total income £10,837,000



Expenditure for the year ended 31 March 1994

Total expenditure £10,865,000



Balance sheet at 31 March 1994

Total value £12,134,000

Assets		Liabilities	
Fixed assets	94 %	Capital reserve	89 %
Stocks	1 %	Income & expenditure account	3 %
Debtors	5 %	Current liabilities	8 %

Staff list

as at 31 December 1994

Director	Professor J.R. Hillman, B.Sc., Ph.D., DSc., F.L.S., C.Biol., F.I.Biol., F.R.S.E.[1,2,3]	Band 1
Secretary	R.J. Killick, B.Sc., M.B.A., Ph.D., C.Biol., M.I.Biol.	Band 4
Assistant to Director	T.J.W. Alphey, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 4

Crop Genetics Department (CG)

Head : G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol.[4,5]	Band 3
J.E. Bradshaw, M.A., M.Sc., Ph.D.[6]	Band 4
M.F.B. Dale, B.Sc., Ph.D.[6]	Band 4
W.H. Macfarlane Smith, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 4
R.L. Wastie, M.A., Ph.D., F.I.S.P.[6]	Band 4
I. Chapman, B.Sc.	Band 5
M.J. De,Maine, B.Sc., M.Phil.	Band 5
S. Millam, B.Sc., Ph.D.	Band 5
G. Ramsay, B.Sc., Ph.D.	Band 5
Ruth M. Solomon-Blackburn, B.A., M.Sc.	Band 6
S.A.. Clulow, B.Sc., Ph.D.	Band 6
K. Harding, B.Sc., Ph.D.	Band 6
Helen E. Stewart, C.Biol., M.I.Biol.	Band 6
M.J. Wilkinson, B.Sc., Ph.D.	Band 6
Jill Middlefell-Williams, H.N.C.	Band 7
G.E.L. Swan	Band 7
D. Todd, B.Sc.	Band 7
R.N. Wilson, N.C.H.	Band 7
Eva Bennett	Band 8
P. Davie, O.N.C.	Band 8
Norma Dow	Band 8
Michelle L.M.H. Fleming, H.N.D., B.Sc.	Band 8
Frances Gourlay, H.N.C, B.Sc.	Band 8
Jane McNicoll, H.N.C., B.Sc.	Band 8
M.P.L. Campbell	Band 8
A. Margaret McInroy	Band 10
R. Milligan	Band 10
Moira Myles	Band 10
Gail Simpson	Band 10

Soft Fruit Genetics Department (SFG)

Head : R.J. McNicol, B.Sc. [6]	Band 4
R.M. Brennan, B.Sc., Ph.D.	Band 5
Julie Graham, B.Sc., Ph.D.	Band 6
W.T.G. van de Ven, Ir., Ph.D.	Band 6
Sandra L. Gordon, H.N.C.	Band 8
Kay Greig, Dip. H.E.	Band 8
Amanda J. Thomson, H.N.D.	Band 8
Linzi M. Ross	Band 10
Departmental Administrator: Maureen Murray	Band 8

Cell & Molecular Genetics Department (CMG)

Head : W. Powell, B.Sc., M.Sc., Ph.D.[4,6]	Band 3
J.W.S. Brown, B.Sc., Ph.D.[6]	Band 4
R. Ellis, B.Sc., Ph.D.[6]	Band 4 (Tr. from CG Nov)
B.P. Forster, B.Sc., Ph.D.[6]	Band 4
W.T.B. Thomas, B.Sc., Ph.D.	Band 4 (Tr. from CG Nov)
R. Waugh, B.Sc., Ph.D.[6]	Band 4
A. Kumar, B.Sc., Ph.D.	Band 5
G.C. Machray, B.Sc., Ph.D.	Band 5
J.S. Swanston, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 5 (Tr. from CG Nov)
C.G. Simpson, B.Sc.	Band 6
A. Young	Band 6 (Tr. from CG Nov)
E. Baird, H.N.C., B.Sc.	Band 7
Gillian Clark, H.N.C	Band 7
Jackie Lyon	Band 7
G. R. Young, H.N.C.	Band 7 (Tr. from CG Nov)
Nicky Bonar, H.N.C.	Band 8
A. Booth, H.N.C.	Band 8 (Tr. from CG Nov)
Diane Davidson	Band 8
R. Keith	Band 8 (Tr. from CG Nov)
M. Macaulay, H.N.C., B.Sc.	Band 8
A. Wilson	Band 8 (Tr. from CG Nov)
Alice Bertie	Band 10 (Tr. from CG Nov)
J.D. Fuller	Band 10 (Tr. from CG Nov)
Patricia E. Lawrence	Band 10 (Tr. from CG Nov)
Joyce I. Young	Band 10 (Tr. from CG Nov)

Cellular & Environmental Physiology Department (CEP)

Head : H.V. Davies, B.Sc., Ph.D.[5]	Band 3
K.J. Oparka, B.Sc., Ph.D.[5]	Band 3
B.S. Griffiths, B.Sc., Ph.D.	Band 4 (Prom. Apr)
D.K.L. MacKerron, B.Sc., Ph.D.	Band 4
B. Marshall, B.Sc., A.R.C.S., Ph.D.[7]	Band 4
D. Robinson, B.Sc., Ph.D.[6]	Band 4
G.R. Squire, B.A., Ph.D.	Band 4
A.G. Bengough, B.Sc., Ph.D.	Band 5
J.W. Crawford, B.Sc., Ph.D.[7]	Band 5
R.A. Jefferies, B.Sc., Ph.D.	Band 5
K. Ritz, B.Sc., Ph.D.	Band 5
M. Taylor, B.Sc., Ph.D.	Band 5
R. Viola, B.Sc., Ph.D.	Band 5 (Prom. Apr)
R.E. Wheatley, B.Sc.	Band 5
I. Young, B.Sc., Ph.D.	Band 5
G. Goleniewski, B.Sc., Ph.D.	Band 6
D.C. Gordon, H.N.C.	Band 6
Heather A. Ross, H.N.C., Ph.D., C.Biol., M.I. Biol.	Band 6
Kathryn M. Wright, M.A., Ph.D.	Band 6
Sandra Caul, H.N.C.	Band 7
Alison Cooper, H.N.D.	Band 7
Sandra E. Millar, O.N.C., H.N.C.	Band 7
D.A.M. Prior, H.N.C.	Band 7
Susan Verrall, H.N.C.	Band 7
Gladys Wright, H.N.C.	Band 7
D. Crabb	Band 8
G. Dunlop, O.N.C.	Band 8
Margaret Garland	Band 8
Lesley George	Band 8
Diane McRae	Band 8

[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. Band 4
D.W. Griffiths, M.A., Ph.D, C. Chem., M.R.S.C.. Band 5
G.W. Robertson, B.Sc., C.Chem., M.R.S.C. Band 5
H. Bain, H.N.C., L.R.S.C. Band 6
C.M. Scrimgeour, B.Sc., Ph.D.[6] Band 6
Winifred M. Stein, H.N.C. Band 6
K. Taylor, H.N.C. Band 7
Fiona Falconer, H.N.C. Band 8
Jean Wilkie Band 10
Quality Assurance Officer : T. Shepherd, B.Sc., Ph.D. Band 6

Director's Group (DG)

Unit for Industrial Crops (UIC)
Head : I.M. Morrison, B.Sc., Ph.D.[6] Band 4
J.M.S. Forrest, B.Sc., Ph.D. Band 4
G.J. McDougall, B.Sc., Ph.D. Band 5
D. Stewart, B.Sc. Band 6
Sandra A. Brocklebank, O.N.C. Band 10 (P/T)(Appt. Mar)
Julie A. Duncan Band 10 (P/T)

Spectroscopy (Spec)
Head : B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C.[6] Band 4

Mycology and Bacteriology Department (M & B)

Head : J.M. Duncan, B.Sc., Ph.D. Band 3
J.G. Harrison, B.Sc., Ph.D. Band 4
G.D. Lyon, B.Sc., M.Sc., Ph.D., D.I.C.[6] Band 4
B. Williamson, B.Sc., M.Sc., Ph.D.[6] Band 4
A.C. Newton, B.Sc., Ph.D. Band 5
Lizbeth J. Hyman, B.A. Band 6
R. Lowe Band 6
G. McMillan Band 7
Jacqueline Heilbronn, H.N.C. Band 7
P.P.M. Ianneta, B.Sc., Ph.D. Band 7 (Appt. Mar)
D.J. Johnston, B.Sc., PhD. Band 7 (On sabbatical)
Naomi A. Williams, H.N.C. Band 7
D.C. Guy, H.N.D. Band 8
Evelyn Warden Band 10

Virology Department (Vir)

Head : T.M.A. Wilson, B.Sc., Ph.D.[2] Band 3
A.T. Jones, B.Sc., Ph.D. Band 3
M.A. Mayo, B.Sc., Ph.D., C.Biol., M.I.Biol.[5] Band 3 (IMP)
H. Barker, B.Sc., Ph.D. Band 4
I.M. Roberts, H.N.C., Dip.R.M.S. Band 4
D.J. Robinson, M.A., Ph.D.[6] Band 4
Lesley Torrance, B.Sc., Ph.D.[6] Band 4
G.H. Duncan, H.N.C. Band 5
B. Reavy, B.Sc., D.Phil. Band 5
S.A. MacFarlane, B.Sc., D.Phil. Band 6
S. Santa Cruz, B.Sc., Ph.D. Band 6 (Appt. May)
Maud M. Swanson, B.Sc., Ph.D. Band 6
G.H. Cowan, H.N.D. Band 7
Sheila M.S. Dawson, H.C. Band 7
Anne C. Jolly, H.N.C., M.Sc. Band 7
Wendy J. McGavin, B.Sc. Band 7
Kara D. Webster, H.N.C. Band 7
Fiona Carr Band 8 (P/T)
Gillian L. Fraser Band 8
Ann Grant Band 8
Wendy Ridley Band 8

Zoology Department (Zoo)

Head : D.L. Trudgill, B.Sc., Ph.D., C.Biol., F.I.Biol.[5,6] Band 3
B. Boag, B.Sc., Ph.D. Band 4
D.J.F. Brown, B.A., Ph.D., C.Biol., M.I. Biol. Band 4
M.S. Phillips, B.Sc. Band 4
W.M. Robertson, N.H.C., F.L.S. Band 4
J.A.T. Woodford, M.A., Ph.D.[6] Band 4
A.N.E. Birch, B.Sc., Ph.D., C.Biol., M.I.Biol. Band 5
S.C. Gordon, H.N.C. Band 5
Vivian Blok, B.Sc., M.Sc., Ph.D. Band 6
B. Fenton, B.Sc., Ph.D. Band 6
J.T. Jones, B.Sc., Ph.D. Band 6
B. Harrower, H.N.D., B.Sc. Band 7
Gaynor Malloch, D.C.R., B.Sc. Band 7
R. Neilson, H.N.C., M.Sc. Band 7
Ailsa Smith, B.Sc. Band 7
Anne M. Holt Band 8
Sheena S. Lamond Band 8

Data Processing Unit (DP)

Head : R.J. Clark, B.A., M.B.C.S. Band 5
R. Kidger, B.Sc. Band 5(Prom. Nov)
P. Smith, B.Sc. Band 6
I. Black, H.N.C. Band 7
S. Clark, H.N.C. Band 7

Scientific Liaison & Information Services Department (SLIS)

Head : D.A. Perry, B.Sc., Ph.D. Band 4
T. G. Geoghegan, A.B.I.P.P., A.M.P.A. Band 5
T.D. Heilbronn, B.Sc., M.Sc. Band 6
I.R. Pitkethly, H.N.D. Band 6
Sarah E. Stephens, B.Sc., M.A., A.L.A. Band 6
S.F. Malecki, A.B.I.P.P. Band 7
Ursula M. McKean, M.A., Dip. Lib. Band 7
G. Menzies Band 7
Kristy L. Grant, B.A. Band 9
Barbara V. Gunn Band 10
Janette Keith Band 11 (P/T) (Appt. Dec)

Administration Department (Admin)

Secretary : R.J. Killick, B.Sc., M.B.A., Ph.D., C.Biol., M.I.Biol.	Band 4
Accountant : S.L. Howie, C.A.	Band 5
Assistant Secretary : D.L. Hood, B.Admin., Dip. Ed., L.T.I., A.I.I.M.	Band 6
Personnel Officer : I. Paxton, H.N.C., M.I.P.D.	Band 6
Freida F. Soutar	Band 6
Loraine Galloway	Band 7
Catherine Skelly	Band 7
Elizabeth L. Stewart	Band 8
Margaret Barnes	Band 9
Dianne L. Beharrie, Dip. Ed.	Band 9
Maureen E. Campbell	Band 9
Rhona G. Davidson	Band 9
Pam Duncan	Band 9
Wendy A. Patterson, H.N.D.	Band 9
Sarah-Jane Simms, H.N.D.	Band 9
Joyce Davidson	Band 10
Sheena Forsyth	Band 10
Elizabeth J. Fyffe	Band 10
Myra Purves	Band 10

Engineering & Maintenance Department (EM)

Institute Engineer : S. Petrie, B.Sc.	Band 5
D. Gray, H.N.C.	Band 6
A. Low	Band 7
K. Low	Band 7
I.C. McNaughton, H.N.C.	Band 8
G.C. Roberts	Band 8
I.M. Scrimgeour	Band 8
R. White	Band 8
J. Anderson	Band 9
D. Byrne	Band 9
W. J. Downes	Band 9
K. Henry	Band 9
F. Howie	Band 9
E. Lawrence	Band 9
R.D. McLean	Band 9
C.G. Milne	Band 9
R. Pugh	Band 9
J. Flight	Band 10
N. McInroy	Band 10
D.L.K.. Robertson	Band 10
T. Purves	Band 11
J. Rowe	Band 11
J. Oldershaw	Band 11
C. Conejo	Band 11
Departmental Administrator: Hazel Duncan	Band 9 (P/T)

Estate, Glasshouse & Field Experiments Department (EGF)

Acting Head : G. Wood, B.Sc., Ph.D., F.E.T.C.	Band 5
P.A. Gill, H.N.D.	Band 6
J.R.K. Bennett	Band 7
W.D.J. Jack, B.Sc.	Band 7
B.D. Robertson, N.E.B.S.M., H.N.C., Dip. Mgt., M.B.A.	Band 7
D.S. Petrie	Band 7
A. Grant	Band 8
A.W. Mills	Band 8
R. Ogg	Band 8
D.G. Pugh	Band 8
J.T. Bennett	Band 9 (Prom. May)
C.R. Dalrymple	Band 9 (Prom. May)
L.A. McNicoll	Band 9 (Prom. May)
J. Mason	Band 10
J.K. Wilde	Band 10
A.J. Adams	Band 10
G. Dow	Band 10
B. Fleming	Band 10
I. Fleming	Band 10
A.C. Fuller	Band 10
G.S. Lacey	Band 10
C. McCreadie	Band 10
T.A. Mason	Band 10
R. Murray	Band 10
Gillian Pugh	Band 10
M.J. Soutar	Band 10
Angela M. Thain	Band 10 (P/T)
Departmental Administrator: Lorna Doig	Band 9(P/T)

Scottish Agricultural Statistics Service (SASS)

King's Buildings, University of Edinburgh

Director : R.A. Kempton, M.A., B.Phil. [9]	Band 3
G.J. Gibson, B.Sc., Ph.D.	Band 4 (Prom. Apr)
C.A. Glasbey, M.A., Dip. Math. Stats., Ph.D.[9]	Band 4
E.A. Hunter, B.Sc., M.Phil.[9]	Band 4
Janet M. Dickson, B.Sc.	Band 5
G.W. Horgan, B.A., M.Sc.	Band 5
M. Talbot, F.I.S., M.Phil. [9]	Band 5
F.G. Wright, B.Sc., M.Sc., Ph.D.	Band 5
A.D. Mann, B.Sc.	Band 6
I.M. Nevison, M.A.	Band 6
G.D. Ruxton, B.Sc., Ph.D.	Band 6
Muriel A.M. Kirkwood, D.A.	Band 8
Diane Glancy	Band 10 (P/T)
Karyn Linton	Band 9 (P/T)
Amy G. Stewart	Band 10 (P/T)
Secretary : Elizabeth M. Heyburn, M.A.	Band 7

Ayr Unit

A. Sword, B.Sc., M.Sc.	Band 6
D.A. McNulty, B.Sc., Ph.D.	Band 6

Aberdeen Unit, RRI

Head : M.F. Franklin, B.Sc., M.Sc., Ph.D. [8]	Band 4
D.J. Hirst, B.Sc., Ph.D.	Band 5
C. J. Harbron, B.Sc.	Band 6
Karen A. Robertson, B.Sc.	Band 7

Aberdeen Unit, MLURI

Head : J.W. Kay, B.Sc., B.D., Ph.D.	Band 4 (Appt. Apr)
D.A. Elston, B.A., M.Sc.	Band 5
Elizabeth I. Duff, B.Sc.	Band 7
G. Jayasinghe, Grad. Dip. Inst. Stat., M.Sc.	Band 6

Dundee Unit

Head : J.W. McNicol, B.Sc., M.Sc.	Band 4
S.D. Chasalow, B.A., M.A., Ph.D.	Band 6
Christine Hackett, B.A., Dip. Math. Stats., Ph.D.	Band 6
T. Connolly, B.Sc., Ph.D.	Band 6

Short Term Contracts

SOAFD Flexible Funding

Cellular and Environmental Physiology

J. Liu, B.Sc., M.Sc., Ph.D.	Band 6
A. Anderson, H.N.D., B.Sc., M.Sc.	Band 7
K. Baldwin, B.Sc.	Band 7 (Appt. Nov)
D. Burn, M.Sc.	Band 7 (Appt. Mar)
N. Ebblewhite, B.Sc.	Band 7
J. Wishart, B.A.	Band 7
Alexandra Holmes, H.N.D., P.G.Dip. Biotech.	Band 10

Crop Genetics / Soft Fruit Genetics

Aileen Timmons, B.Sc., Ph.D.	Band 6
Sharon Dubbels	Band 8
Carol Taylor	Band 10

Director's Group (Spec)

N. Deighton, B.Sc., Ph.D.	Band 6
Sheila Glidewell, M.A., M.Sc.,Ph.D.	Band 6

Mycology and Bacteriology

D. Cox, B.A., M.Phil.	Band 7
S. Main	Band 10

SASS

Elizabeth J. Austin, M.A., D.Phil.	Band 6 (Appt. Nov)
Verena M. Trenkel, Dipl. Biol., M.Sc.	Band 6
S.A.R. Williams, B.Sc.	Band 7 (Appt. Nov)

Virology

S. Chapman, B.A., Ph.D.	Band 6 (Appt. Nov)
D.A.C. Jones, B.Sc., Ph.D.	Band 6
A. Ziegler, B.Sc., Ph.D.	Band 6

CIP/USDA

Cell and Molecular Genetics

Rhonda Meyer, Ph.D.	Band 6 (Appt. Apr)

Department of the Environment

Crop Genetics

Yvonne M. Charters, B.Sc.	Band 6
A. Robertson	Band 7 (Appt. Dec)

Virology

Livia Dyckhoff, B.Sc.	Band 7 (Appt. Nov)

CEC

Cellular and Environmental Physiology

Susan Jarvis, B.Sc., Ph.D.	Band 6
M.R. MacLeod, B.Sc., Ph.D.	Band 6 (Appt. Feb)
Sigrun Holdhus, Cand. mag.	Band 7

Chemistry

G. Dobson, B.Sc., Ph.D.	Band 6
I.S. Begley, B.Sc., Ph.D.	Band 7 (Appt. Dec)

Cell and Molecular Genetics

Joanne Russell, B.Sc., Ph.D.	Band 6 (P/T)

Director's Group (UIC)

Nadine Henderson, B.Sc., M.A.	Band 7(P/T) (Appt. Dec)

Virology

Sybil M. Macintosh, B.Sc.	Band 7

Zoology

M. Armstrong, B.Sc., M.Sc.	Band 7
Paula M. Hebden, B.Sc.	Band 8
A. Paterson	Band 10
Jane Roberts, S.N.C.	Band 10

CEC / ECSA

Cell and Molecular Genetics

F.E. McMahon, B.Sc.	Band 10 (Appt. Jan)

DTI/Dalgety Spillers

Crop Genetics

D. Matthews, B.Sc., Ph.D.	Band 6

Gene Shears

Cell and Molecular Genetics

D.J. Leader, B.Sc.	Band 6
A.D. Turnbull-Ross, B.Sc., Ph.D.	Band 6
Jennifer Watters, H.N.D.	Band 8

LINK/HDC

Mycology and Bacteriology

D.E.L. Cooke, B.Sc.	Band 6 (Appt. Feb)

MAFF

Cellular and Environmental Physiology

Sheena J. Rodger O.N.C.	Band 8

Soft Fruit Genetics

Emily Cobb, H.N.C.	Band 10

McCains PLC

Crop Genetics

Marjorie J. Grant, H.N.D.	Band10

Mylnefield Research Services Ltd

Cell and Molecular Genetics

P. Hedley, B.Sc.	n/a

Cellular and Environmental Physiology

J. van den Berg, M.Sc., Ph.D.	n/a
D.N. Harris, B.Sc., M.Sc.	n/a

Soft Fruit Genetics

Alison Dolan, H.N.C.	n/a
Jane E. Fairlie, O.N.C.	n/a
R.E. Harrison, B.Sc., M.Sc., Ph.D.	n/a
J. Pontes, B.Sc.	n/a
E. Simpson, B.Sc.	n/a

Mycology and Bacteriology

Vasantha Ramanathan, B.Sc., M.Phil., Dip. Biotech., Ph.D.	n/a

ODA

Cell and Molecular Genetics

I.K. Dawson, B.A., M.Sc.	Band 6

Virology

J.S. Miller, B.Sc.	Band 6
M. Taliansky, Ph.D., D.Sc.	Band 6 (Appt. Nov)
Bridget Jones, B.Sc.	Band 10

ODA/CIP

Crop Genetics

Beverly Ingram, B.Sc., M.Sc.	Band 6
Michele S. Leslie	Band 8

OECD

Mycology and Bacteriology

Isabelle Lacourt, Ph.D.	Band 6 (Appt. Jul)

PMB

Cellular and Environmental Physiology

G.J. Lewis, B.Sc., M.Sc.	Band 6
M. Young, H.N.D.	Band 7

Scotia

Chemistry

R.A. Unwin, B.Sc.	

Mycology and Bacteriology

A. Reglinski, B.Sc., Ph.D.	Band 6

Miscellaneous funding

Soft Fruit Genetics

P. Lanham, B.Sc., Ph.D.	Band 6

Resignations

Name	Dept.	Band	Month
E.J. Christie	EGF	10	September
Ann Donnelly	CG	8	August
Diana Kennedy	M&B	6	January
Karen McIlravey	CG	8	January
Amanda Thomson	SFG	8	August
D.A. Thomson	EGF	10	July
Sheila Unkles	M&B	6	November

Staff Retirements

Name	Dept.	Band	Month
E.A.M. Gardiner	EGF	10	August
W.I.A. Jack	EGF	5	February
H.M. Lawson	CEP	4	August
M.C.M. Pérombelon	M&B	4	June

Redundancies, Voluntary and Flexible Retirements

Name	Dept.	Band	Month
D. Linehan	CEP	4	March
J.S. Wiseman	CEP	6	March

Editorial Duties

Name	Position	Journal Title
H. Barker	Editorial Board	*Annals of Applied Biology*
G. Bengough	Joint Editor	*British Society of Soil Science 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*
B.P. Forster	Co-ordinator	*Barley Genetics Newsletter* (Chromosome 4)
M.F. Franklin	Editorial Board	*Journal of Agricultural Science*
	Editorial Board	*British Journal of Nutrition*
C.A. Glasbey	Associate Editor	*Biometrics*
	Editorial Panel	*Applied Statistics*
J.R. Hillman	Publication Committee	*Journal of Horticultural Science*
	Editorial Board	*Agricultural Systems*
	Editorial Board	*Journal of Agricultural Science*
R.A. Kempton	Associate Editor	*Journal of Agricultural Science*
	Associate Editor	*Journal of Agricultural, Biological and Environmental Statistics*
D.K.L. MacKerron	Assistant Editor	*Journal of Horticultural Science*
	Editorial Board	*Euphytica*
	Editor	Special issue of *Agricultural and Forest Meteorology* on 'Climate Change - from Impact to Interaction'
M.A. Mayo	Editorial Board	*Journal of General Virology*
J.W. McNicol	Statistical Editor	*Annals of Applied Biology*
I.M. Morrison	Management Committee	*Journal of the Science of Food and Agriculture*
K.J. Oparka	International Advisory Board	*Journal of Experimental Botany*
	Subject Editor	*Plant Physiology*
D.A. Perry	Editor	*SSCR Bulletin*
M.S. Phillips	Associate Editor	*Journal of Nematology*
D. Robinson	Associate Editor	*Journal of Horticultural Science*
D.J. Robinson	Editorial Board	*Journal of Virological Methods*
G.R. Squire	Editorial Board	*Experimental Agriculture, CUP*
	Advisory Board	*Crop Physiology Abstracts, CABI*
D.L. Trudgill	Editorial Board	*Nematologica*
	Editorial Board	*Fundamental and Applied Nematology*
R.L. Wastie	Editorial Board	*Annals of Applied Biology*
	Editor	*Potato Research*
R. Waugh	Board of Editors	*Molecular Biotechnology*
B. Williamson	Associate Editor	*Annals of Applied Biology*
T.M.A. Wilson	Associate Editor	*Molecular Plant-Microbe Interactions*
I.M. Young	Joint Editor	*British Soil Science Society Newsletter*

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.
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.
Joanne Badge	Vir	Narcissus latent virus.
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	UIC	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.
F. Chaubron	CEP	Cloning of sugar metabolising genes from sugarbeet.
J. Chen	Vir	Molecular biology of fungus-transmitted cereal viruses.
K. Cheung	CMG	Genetic transformation in Groundnut.
F.A. Comerford	CMG	Lamins in the plant nuclear membrane.
Clare Croser	CEP	Aspects of root growth through compacted soil.
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.
J. Forster	CEP/CMG	Genetic manipulation of nitrate reductase activity in potato.
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.
S. Hendy	Vir	Development of scFv antibodies for transgenic resistance to potato viruses.
Lizbeth Hyman*	M&B	Characterisation of pectolytic bacteria by monoclonal antibodies.
D.J. Leader	CMG	U5snRNA genes from potato and maize.
G.J. Lewis	CEP	Methods for simulation of water and nitrogen use in potato.
A. Mackie	CEP	Volatile organic compounds as signals between micro-organisms in the rhizosphere.
Anne Maddison	CMG	Molecular dissection of invertase gene expression in potato.
Pauline McConway	M&B/CMG	Molecular biology of potato resistance to erwinias derived from *Solanum brevidens.*
R.J. McNicol*	SFG	Investigations into running off in blackcurrants.
D. Milbourne	CMG	Molecular marker-assisted targeted breeding for potato cyst nematode and late blight.
Sarah Miller	M&B	Assessment of the potential to control potato diseases by resistance elicitors.
Adele Mooney	Vir	Replication of pea early browning virus.
A. Munir	Zoo	Management of potato cyst nematodes in Pakistan.
Shi Nongnong	Vir	Population genetics of barley yellow mosaic virus-resistance breaking isolates.
F. Nabugoomu	SASS	REML estimation in a series of varietal trials.
Martha Namfua	Zoo	Management of nematodes associated with coffee.
C. Orozco-Castillo	CMG	Molecular diversity and genetic linkage mapping of *Coffea* spp.
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.
C. Regalado	CEP	Spatio-temporal dynamics of microorganisms in a heterogeneous environment.
G. Rhandawa	CMG	Manipulation of potato genes.
W.Q. Ribeiro	CMG	Genetic variation in *Phaseolus vulgaris*.
A. Roberts	CEP	Plasmodesmata and virus transport.
Jessica Searle	M&B	Population genetics of *Rhynchosporium secalis.*
J. Shaw	SASS	Techniques for discrimination of seed types using imaging measurements.
F.N. Wachira	CMG	Molecular variation in tea.
R.E. Wheatley*	CEP	Nitrogen transformation in cultivated soils.
G. White	CMG	Development and application of molecular assays for tree populations.
A. Wilson*	CG	Gene position in a synthetic *Brassica napus* .
Stella Xenophontos	CG	The epidemiology of spraing disease (tobacco rattle virus) of potato.

* Permanent members of staff

Service on External Committees or Organisations

Name	Position	Committee or Organisation
T.J.W. Alphey	Secretariat	CHABOS & SMAC
H. Barker	Member	Virology Group Committee, Association of Applied Biologists
G. Bengough	Committee Member	Scottish Soils Discussion Group
A.N.E. Birch	Session Organiser	AAB Entomology Group Committee for Presidential Meeting
	Member	IOBC Working Group for Resistance to Insects and Mites
R.M. Brennan	Member	AAB Plant Breeding Committee
	Adviser	SmithKline Beecham R&D Committee
D.J.F. Brown	Secretary & Treasurer	European Society of Nematologists
	Co-Chairman	Russian Society of Nematologists International Meeting
	Member	Society of Nematologists *Ad Hoc* Committee "International Federation of Nematology Societies"
	Member	European Plant Protection Organisation *Ad Hoc* Committee "*Xiphinema americanum*-group nematodes"
	Member	Institute of Biology "IOBS Link Group"
J.W. Crawford	Panel Member	BBSRC/EPSRC Biomathematics Panel
	Committee Member	Industrial Liaison Committee, University of Abertay, Dundee
	Working Group Member	SOAFD Feasibility Study of a Systems Approach to Modelling Agriculture and Land Use in Scotland
H.V. Davies	Member	Kluwer International Scientific Advisory Board
	Member	Organising Committee of International Symposium on Molecular Biology of Potato
J.M. Duncan	Committee Member	ISPP Phytophthora Committee
B.P. Forster	Co-ordinator	International Committee on Barley Chromosome Genetic Mapping: Chromosome 4
T.D. Heilbronn	Publicity Officer	Association for Crop Protection in Northern Britain
J.R. Hillman	Chairman	Agriculture, Natural Resources & Environment Sector Panel of the UK Technology Foresight Programme
	Chairman	SCRI/SASA/COSAC Liaison Group
	Chairman	Tayside Biocentre Group
	Chairman	SMAC
	Deputy Chairman	Board of Directors, Mylnefield Research Services Ltd
	Member	Board of Directors, CAROS International
	Member	Board of Directors, CHABOS
	Member	SOAFD Joint Consultative Committee for 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
	Adviser	International Foundation for Science, Stockholm
E. A. Hunter	Member	Management Committee of FLAIR Concerted Action No 2 "SENS"
J.W. Kay	Chairman	Royal Statistical Society: Highlands Group
	Committee Member	Royal Statistical Society: Research Section
R.A. Kempton	President	International Biometric Society (British Region)
G.D. Lyon	Committee Member	Steering group organising a workshop on molecular aspects of plant disease
W.H. Macfarlane Smith	Member	BBSRC Joint Committee on Health & Safety
	Member	BSPB Oilseed & Industrial Crop Group
	Member	AFRS Safety Officers Group
	Member	SABRI Safety Officers Group
	Member	NPTC Plant Variety Development Panel
G.R. Mackay	Chairman	EUCARPIA, Potato section
	Member	Biological Sciences Advisory Committee, Coventry University
D.K.L. MacKerron	Secretary	Potato Crop Sub-Committee, SSCR
	Secretary	Physiology Section, EAPR
	Co-organiser	AAB Meeting on 'Efficiency of Water Use in Crop Systems'
	Convenor	Conference: 'Climate Change - from Impact to Interaction'
	Co-organiser	Second International Potato Modelling Conference, Wageningen
B. Marshall	Member	NERC, Terrestrial Sciences, Higher Education Grants and Training Awards Committee
	Member	Soil Science Steering Group, BBSRC
	UK Co-ordinator	Soil-Plant-Microbial Interactions reporting to BBSRC
	Member	Advisory Board of 5th International Congress for Computer Technology in Agriculture
	Member	IND77E (DoE) Sub-Group, Soils
M.A. Mayo	Member	Executive Committee of the International Committee for the Taxonomy of Viruses (ICTV)
	Chairman	Plant Virus Sub-committee of ICTV
	Member	Virus data sub-committee of ICTV

Name	Position	Committee or Organisation
U.M. McKean	Member	Scottish Agricultural Librarians' Group
R.J. McNicol	Member	HDC Soft Fruit Trialling Sub-Committee
	Member	Soft Fruit Sub-committee, SSCR
	Adviser	SNSA Committee
	Adviser	SSFG Ltd Board
	Adviser	Soft Fruit Committee of Horticulture Research International
S. Millam	UK representative	COST 822: Mechanisms and monitors of regeneration and genetic stability
I.M. Morrison	Member	Agriculture and Environment Committee, Society of Chemical Industry
	Member	Energy and Industrial Cropping Group, National Farmers' Union of Scotland
A.C. Newton	Committee Member	UK Cereal Pathogen Virulence Survey
	Membership Secretary	British Society for Plant Pathology
K.J. Oparka	Member	International Organising Committee, Phloem Transport Conference, Canterbury, UK (1995)
	Member	International Organising Committee, Plasmodesmata Conference, Zichron Yakov, Israel (1996)
	Local Organiser	SEB Annual Meeting, St Andrews (1995)
I. Paxton	Member	Institute of Personnel and Development Committee, Tayside and North of Scotland Branch
D.A. Perry	Treasurer	British Society for Plant Pathology
B. Reavy	Member	BBSRC Protein Engineering Liaison Committee
K. Ritz	Member	BBSRC Soil Plant Microbe Interactions Working Party
	Member	SOAFD Soil/Plant/Microbe Group
	Member	Management Group, Centre for Non-Linear Systems in Biology, Dundee
I.M. Roberts	Safety Officer	Royal Microscopical Society
	Chairman	BBSRC EM Advisory Group
D. Robinson	Member	AAB Plant Physiology Committee
D.J. Robinson	Member	Advisory Committee on Releases to the Environment, DoE
G.R. Squire	Member	SOAFD Working Group on Vegetation Dynamics
S.E. Stephens	Member	Tayside Chief Librarians' Group
	Member	Information Services Group - Scottish Library Association
	Member	Scottish Agricultural Librarians' Group
M. Talbot	Member	Statistics Group of UK Plant Variety 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	Plant Breeding & Genetics Group of AAB
L. Torrance	Member	Management Committee, COST 88
	Member	Plant virus sub-committee, ICTV
	Chairperson	Study group on Furo- and Hordeiviruses, ICTV
B. Williamson	Secretary	Soft Fruit Sub-committee, SSCR
	Member	7th International Congress of Plant Pathology 1998 Finance Sub-committee
	Treasurer	Association for Crop Protection in Northern Britain
	Member	Scientific Advisory Committee of XIth International Botrytis Symposium 1996, Wageningen, The Netherlands
M.J. Wilkinson	SCRI representative	UK Plant Genetic Resources Group
T.M.A. Wilson	Member	Program Committee VIIth International Conference of Comparative Virology
	Member	Program/Advisory Committee Xth International Congress of Plant Virology, Jerusalem
	Member	Advisory Committee IVth Positive-Strand RNA Virus Meeting, Utrecht
	Member	Programme Committee VIIth International Congress of Plant Pathology, Edinburgh
	Member	Church of Scotland, Science Religion and Technology Project "Ethics of Genetic Engineering of Non-Human Life Forms"
J.A.T. Woodford	Regional Hon. Sec.	Royal Entomological Society
F. Wright	Member	SEQNET, CCP11 User Documentation Group (Daresbury Laboratory, UK)
	Member	BBSRC Protein Engineering Liaison Group
I.M. Young	Council Member	British Society of Soil Science
	Member	BBSRC Soil Physics Working Party

Short term workers and visitors

Name	Country of origin	Dept.	Month/yr of arrival	Length of stay
I.M. de O. Abrantes	Portugal	Zoo	Feb 94	1 week
S. A'Hara	UK	Zoo	Apr 94	6 months
R. Ahiabu	Ghana	Vir	May 94	6 months
I. Ahmad	Malaysia	Vir	Nov 94	9 months
M.T. Martins de Almeida	Portugal	Zoo	Feb 94	1 week
M. Alphey	UK	CG	Apr 94	1 week
Rachel Beacham	UK	Zoo	Sep 94	1 year
F. Bem	Greece	Vir	Sep 94	4 months
P. Bonanti	The Netherlands	M&B	Oct 94	1 week
N. Bouchireb	Algeria	CMG	Apr 94	4 months
J.S. Bran	India	Zoo	Apr 94	6 months
A. Brown	UK	Vir	Jul 994	2 months
M. Burke	UK	CMG	Oct 94	6 months
U. Butikofer	Switzerland	SASS	May 94	1 week
Giselle Cipriani	Peru	CG	Aug 94	1 month
M. Dannowski	Germany	CEP	Sep 94	2 weeks
O. David	France	SASS	Nov 94	2 weeks
M. Davidson	UK	CG	Jan 94	6 months
Catherine A. Davies	UK	SASS	Sep 94	1 year
M. Folling	Denmark	CMG	Nov 94	5 months
Laura Forsyth	UK	CG	Aug 94	1 month
L. Hiller	USA	CEP	Jul 93	1 year
F. Hussain	UK	Vir	Jul 94	2 months
V. Ivandic	Germany	CMG	Mar 94	3 months
B. Javornik	Slovenia	CMG	Mar 94	1 month
G. Jigjidin	Mongolia	CMG	Sep 94	6 months
Y-G. Joh	Korea	Chem	Mar 94	6 months
Rachel Jones	UK	CG	Apr 94	1 week
Rachel Jones	UK	CG	Sep 94	1 year
D. Kuklev	Russia	Chem	May 94	6 months
Janie Lyon	USA	CG	Sep 94	1 year
N. Malone	UK	CG	Aug 94	2 months
Mary McGregor	UK	CG	May 94	1 year
S. McKeown	UK	Zoo	Oct 94	6 months
K.B. McRae	Canada	SASS	Sep 94	1 year
Karen McWilliam	UK	CG	Jul 94	2 months
Susan Mitchell	UK	CG	Nov 94	6 weeks
J.M. Monier	France	CG	Jul 94	3 months
A. Mortazavi	Iran	CG	Jun 94	4 months
Elisabetta Moscheni	Italy	CG	Aug 94	1 month
Emma Moxey	UK	Zoo	Aug 93	10 months
G. Mrimi	Tanzania	CG	Sep 94	2 weeks
R.A. Naidu	India	Vir	Jul 94	4 months
C. Nieser	Germany	Zoo	Jun 94	3 weeks
B. Nikolova-Damyanova	Bulgaria	Chem	Aug 94	3 months
C. Olsson	Sweden	M&B	Oct 94	1 week
P. Parikka	Finland	M&B	Oct 94	1 week
G. Pember	UK	Zoo	Sep 94	1 year
A.T. Ploeg	The Netherlands	Zoo	Nov 94	3 weeks
S. Pluta	Poland	SFG/Zoo	Apr 94	6 Weeks
V. Rappordt	The Netherlands	CEP	Aug 94	3 months
N. Rauffer	France	Vir	Mar 94	1 month
A. Roberts	UK	Vir	Jun 94	2 months
A. Robertson	UK	CG	Sep 94	3 months
R.T. Robbins	USA	Zoo	Jul 94	2 weeks
Natalie Rodrigue	Canada	SASS	May 94	1 week
H. Rogasik	Germany	CEP	Sep 94	2 weeks
R. Rønn	Denmark	CEP	Oct 94	4 months

Name	Country of Origin	Dept.	Month/yr of Arrival	Length of stay
I Sandholt	Denmark	SASS	Jun 94	1 week
T. Sato	Japan	Vir	Jun 94	10 months
Benedicte Shek	UK	CG	Jan 94	5 months
L. Shepherd	UK	CMG	Oct 94	1 year
W. Sledz	Poland	M&B	Dec 94	1 week
A. Stensvand	Norway	M&B	Oct 94	1 week
J. Stewart	Canada	CEP	Aug 93	10 months
Irene Tierney	UK	CG	Apr 94	6 months
D. Tilak	India	CEP	Apr 94	1 year
C. Tosh	UK	CG	Aug 94	2 months
A. Vallette	France	CG	Jul 94	3 months
T.C. Vrain	Canada	Zoo	Jul 94	1 week
Marie Wade	UK	CG	Apr 94	6 months
Wang Lin Ping	China	CG/Vir	Jun 94	6 weeks
J. van der Wolf	The Netherlands	M&B	Nov 94	1 month
E. Woodford	UK	CEP	Oct 93	11 months
T. Yoneyama	Japan	CEP	Jun 94	3 months

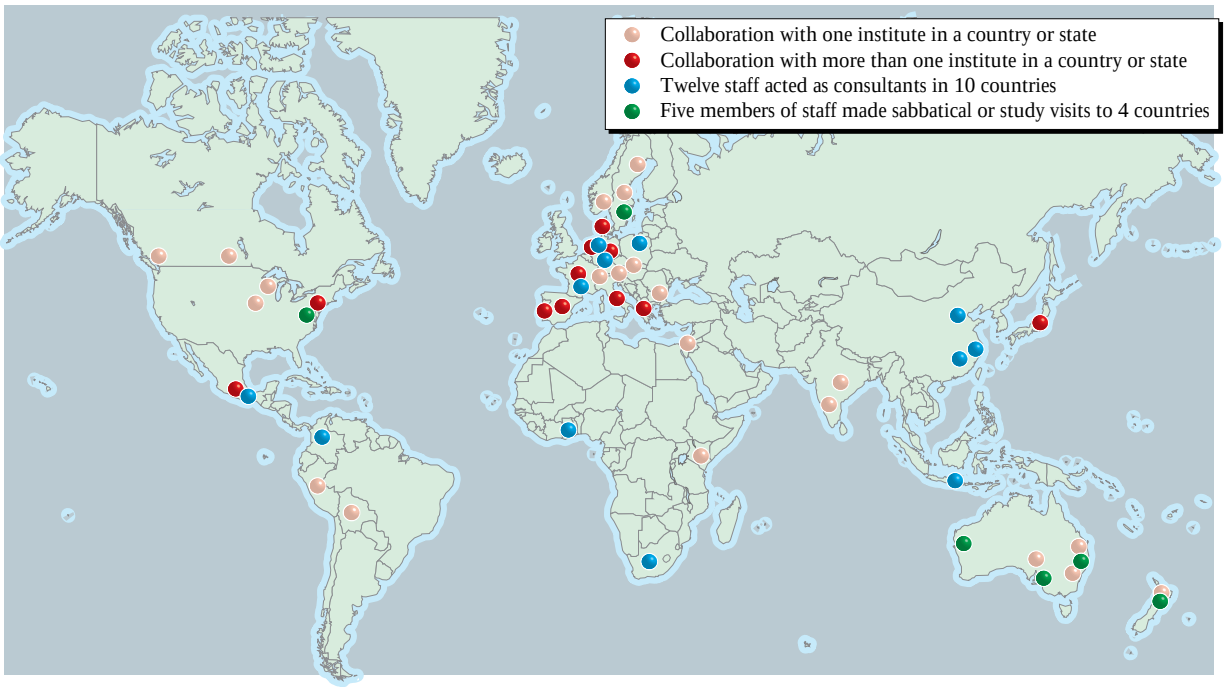
Longer-term visitors and Research Fellows

Name	Country of origin	Dept.	Month/yr of arrival	Length of stay
C. Barrera	Peru	Virology	Apr 93	15 months
Anne-Sophie Bournay	France	CMG	Oct 93	2 years
Linda L. Handley	UK/USA	CEP	Jun 91	5 years
Margret M.I. van Vuuren	The Netherlands	CEP	May 93	3 years
M. White	UK	CEP	Mar 93	16 months
P. Whitty	UK	CMG	May 92	3 years

Awards and Distinctions

Name	Dept.	Degree/Award/Distinction/Appointment
N.L. Innes	Admin	O.B.E
M.C.M. Pérombelon	M&B	M.B.E.
R.J. McNicol	SFG	Royal Horticultural Society Jones Bateman Cup
D.L. Trudgill	Zoo	Fellow, Society of Nematologists
L.L. Handley	CEP	Honorary Professor, University of Florida at Miami (FIU)
G.R. Mackay	CG	Honorary Senior Lecturer, University of Dundee
M.A. Mayo	Vir	Honorary Senior Lecturer, University of Dundee
J.E. Bradshaw	CG	Honorary Lecturer, University of Dundee
M.F.B. Dale	CG	Honorary Lecturer, University of Dundee
I.M. Morrison	DG (UIC)	Honorary Lecturer, University of Dundee
L. Torrance	Vir	Honorary Lecturer, University of Dundee
R.L. Wastie	CG	Honorary Lecturer, University of Dundee
J.R. Hillman	Admin	D.Sc. Honoris, University of Strathclyde
S. Fennell	CMG	Ph.D., University of Dundee
S.A. Mad Arif	CMG	Ph.D., University of Dundee
H.A. Ross	CEP	Ph.D., University of St Andrews
J. Russell	CMG	Ph.D., University of Reading
J.S. Swanston	CG	Ph.D., Heriot-Watt University
B.A. Robertson	EGF	M.B.A., University of Abertay, Dundee
C.A. Jolly	Vir	M.Sc., University of Dundee
Irene Tierney	CG	M.Sc. Biotechnology (Distinction), University of Abertay, Dundee
M. Young	CEP	M.Sc., University of Dundee
Frances Gourlay	CG	B.Sc. Biotechnology, University of Abertay, Dundee
M. Macaulay	CMG	B.Sc. Biotechnology, University of Abertay, Dundee
Jane McNicoll	CG	B.Sc. Biotechnology, University of Abertay, Dundee
D. Todd	CG	B.Sc. Biotechnology, University of Abertay, Dundee
C. Simpson	CMG	Waitangi Fellowship
K.M. Wright	CEP	NEBOSH National General Certificate

International Collaboration and Consultancies



Research is executed within an international framework that encourages information transfer. The extent of SCRI's international commitment during 1994 is reflected in the collaborative research that was undertaken with 68 institutions in 25 countries.

SCRI Research Programme
1994-1995

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.

40	SCR/006/91	ROA Genetic architecture of tetraploid potatoes and production of enhanced germplasm	Bradshaw J E
47	SCR/017/91	ROA Maintenance, improvement and evaluation of the Commonwealth Potato Collection	Wilkinson M J
152	SCR/042/91	ROA Identification and exploitation of genetic markers in crop improvement	Forster B
181	SCR/063/91	ROA Mechanisms determining specificity and efficiency of nepovirus transmission by longidorid nematodes	Brown D J F
215	SCR/067/91	ROA Biology and ecology of pests and beneficial arthropods associated with cane and bush fruits	Woodford J A T
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
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
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
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
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 of material for germplasm enhancement and genetical studies at the diploid level	De,Maine M J
303	SCR/015/91	ROA Inheritance of resistance to potato virus diseases and production of resistant enhanced potato germplasm	Solomon-Blackburn R 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
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
322	SCR/081/91	ROA Molecular analysis of species and virulence group relationships in *Meloidogyne* spp.	Trudgill D L
326	SCR/050/91	ROA Physical and physiological constraints on the growth and activity of plant root systems	Robinson D
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
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
340	SCR/004/91	FF Computation of safe isolation distances for field-grown genetically modified crops	Mackay G R
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 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
364	SCR/364/92	ROA Epidemiological and etiological studies of bacterial and fungal pathogens of potatoes, cereal crops and raspberries	Pérombelon M C 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
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, morphogenesis 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 M
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
393	SCR/393/92	FF Combined NMR and mathematical study of major metabolic pathways in higher plant cells	Viola R
394	SCR/394/93	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/395/93	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, xyloglucans 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 of brassicas	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
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 repertoire 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
418	SCR/418/94	ROA Free radicals, antioxidants and metalloenzymes; their identification and behaviour in plants and plant derived foods	Goodman B A

419	SCR/419/94	ROA Non-invasive approaches to the study of structure, composition and developmental processes in plants and plant parasites using magnetic resonance technologies	Goodman B A
420	SCR/420/94	ROA Production, isolation and characterisation of plant fibres for industrial applications	Morrison I M
421	SCR/421/94	ROA Biosynthetic control of fibre constituents during development and differentiation of fibre cells and genetic modification of these processes	Morrison I M
422	SCR/422/94	ROA Processing of plant fibres by novel and environmentally acceptable methods	Morrison I M
423	SCR/423/94	ROA Physiological and developmental regulation of plasmodesmata	Oparka K J
424	SCR/424/94	ROA Relating soil structure to biological function	Young I M
425	SCR/425/94	ROA Influence of the host on gene expression of plant parasitic nematodes	Jones J T
426	SCR/426/94	ROA Fundamental studies on longidorid and trichodorid nematode vectors in relation to the aetiology of nepo- and tobraviruses which are transmitted to a range of arable and fruit crops	Brown D J F
427	SCR/427/94	ROA Characterisation of nematode cuticular surfaces of *Globodera*, *Heterodera* and *Meloidogyne* involved in pathogenesis	Robertson W M
428	SCR/428/94	ROA Investigate inheritance of low temperature sugar stability and develop effective selection strategies to produce superior potato germplasm for processing	Mackay G R
429	SCR/429/94	ROA Genetic architecture of diploid potatoes and production of enhanced germplasm	Bradshaw J E
430	SCR/430/94	ROA Production, maintenance, distribution and associated management of facilities to produce disease-free tubers of genetic stocks of potato clones	Chapman I M
431	SCR/431/94	ROA Devise and operate methods for detecting and quantifying genetic resistance to pathogens of the potato causing late blight, early blight, blackleg, stem canker, skinspot, dry rot, silver scurf, gangrene, common scab and powdery scab	Wastie R L
432	SCR/432/94	ROA Integrated approaches for rapid and efficient gene transfer and characterisation in potato	Millam S
433	SCR/433/94	ROA Development of Polymerase Chain Reaction (PCR)-based sequence tagged site markers for potato and barley	Waugh R
434	SCR/434/94	ROA Dissection of regulatory mechanisms governing invertase gene expression in potato	Machray G C
435	SCR/435/94	ROA To clone the Hero gene of tomato which confers resistance to potato cyst nematode by transposon tagging	Kumar A
436	SCR/436/94	ROA Molecular approach to study the functions of polyamines in plant cell proliferation and morphogenesis	Kumar A
437	SCR/437/94	ROA Introducing physiological attributes and phenotypic plasticity to models of spatial and temporal dynamics in vegetation	Squire G R
438	SCR/438/94	ROA Role in pathogenesis of extracellular enzymes of *Phytophthora*, *Botrytis* and *Erwinia*	Williamson B

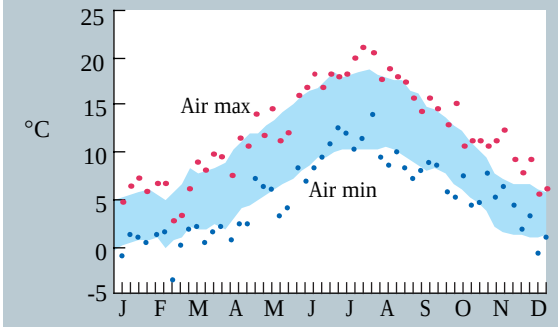
441	SCR/441/94	FF Studies of phloem transport using an artificial 'aphid'	Oparka K J
803	SCR/803/94	FF Fundamental studies to develop plant virus-like particles expressed in *Escherichia coli* as vaccine or therapeutic agents	Wilson T M A
805	SCR/805/94	FF Control of certain invertebrate pests of agricultural importance using gut membrane proteins as targets for antibodies	Fenton B
808	SCR/808/94	FF Development of molecular biological and physiological techniques in studies of the interaction between microbes, nutrient cycling and vegetation among a range of agriculturally important pastures, to enable scaling from microcosm to field	Ritz K
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

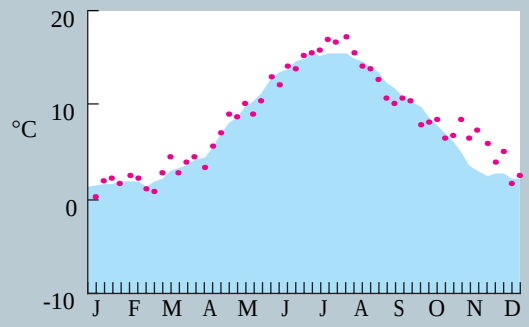
D.K.L. MacKerron

Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 1994 and the long term average for 1961-1990( ).

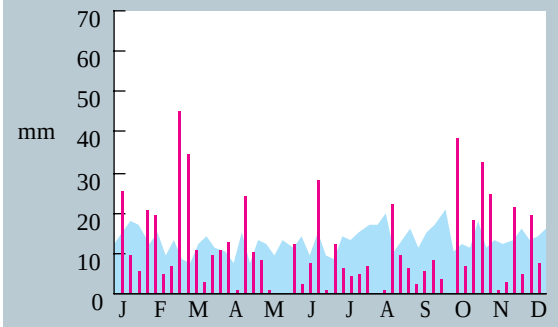
Air temperature



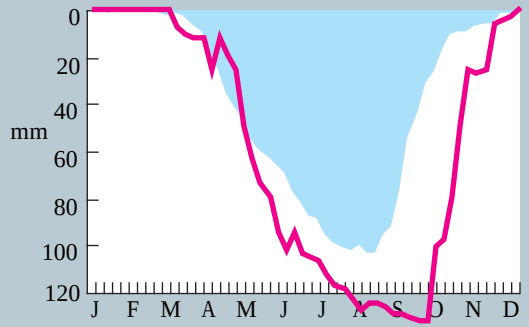
10cm soil temperature



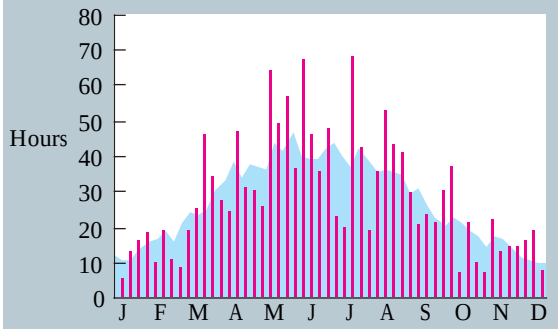
Rainfall



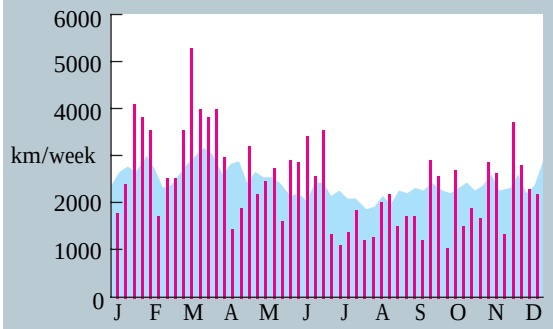
Soil moisture deficit



Sunshine



Run of wind



Institutes supported by the Biotechnology and Biological Sciences Research Council

BBSRC Office	Polaris House, North Star Avenue, Swindon, Wilts SN2 1UH	01793-413200
BBSRC Computing Division	West Common, Harpenden, Herts AL5 2JE	01582-762271
Babraham Institute	Babraham Hall, Babraham, Cambridge CB2 4AT	01223-832312
Laboratory of Molecular Signalling	Dept of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ	01223-336600
Institute for Animal Health	Compton, Near Newbury, Berkshire RG20 7NN	01635-578411
Compton Laboratory	Compton, Near Newbury, Berkshire RG20 7NN	01635-578411
Pirbright Laboratory	Ash Road, Pirbright, Woking, Surrey GU24 0NF	01483-232441
BBSRC & MRC Neuropathogenesis Unit	Ogston Building, West Mains Road, Edinburgh EH9 3JF	0131-667-5204
Institute of Arable Crops Research	Harpenden, Herts AL5 2JQ	01582-763133
Long Ashton Research Station	Long Ashton, Bristol BS18 9AF	01275-392181
Rothamsted Experimental Station	Harpenden, Herts AL5 2JQ	01582-763133
Broom's Barn Experimental Station	Highham, Bury St. Edmunds, Suffolk IP28 6NP	01284-810363
Institute of Food Research	Earley Gate, Whiteknights Rd, Reading RG6 6BZ	01734-357055
Norwich Laboratory	Norwich Research Park, Colney, Norwich NR4 7UA	01603-56122
Reading Laboratory	Earley Gate, Whiteknights Rd, Reading RG6 6BZ	01734-357000
Institute of Grassland and Environmental Research	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	01970-828255
Aberystwyth Research Centre	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	01970-828255
North Wyke Research Station	Okehampton, Devon EX20 2SB	01837-82558
Bronydd Mawr Research Station	Trecastle, Brecon, Powys LD3 8RD	01874-636480
Trawsgoed Research Farm	Trawsgoed, Aberystwyth, Dyfed SY23 4LL	01974-261615
John Innes Centre	Norwich Research Park, Colney, Norwich NR4 7UH	01603-452571
Roslin Institute	Roslin, Midlothian EH25 9PS	0131-440-2726
Silsoe Research Institute	Wrest Park, Silsoe, Bedford MK45 4HS	01525-860000
Horticultural Research International	Wellesbourne, Warwick CV35 9EF	01789-470382
HRI, East Malling	West Malling, Maidstone, Kent ME19 6BJ	01732-843833
HRI, Littlehampton	Worthing Road, Littlehampton, West Sussex BN17 6LP	01903-716123
HRI, Wellesbourne	Wellesbourne, Warwick CV35 9EF	01789-470382

Scottish Agricultural and Biological Research Institutes

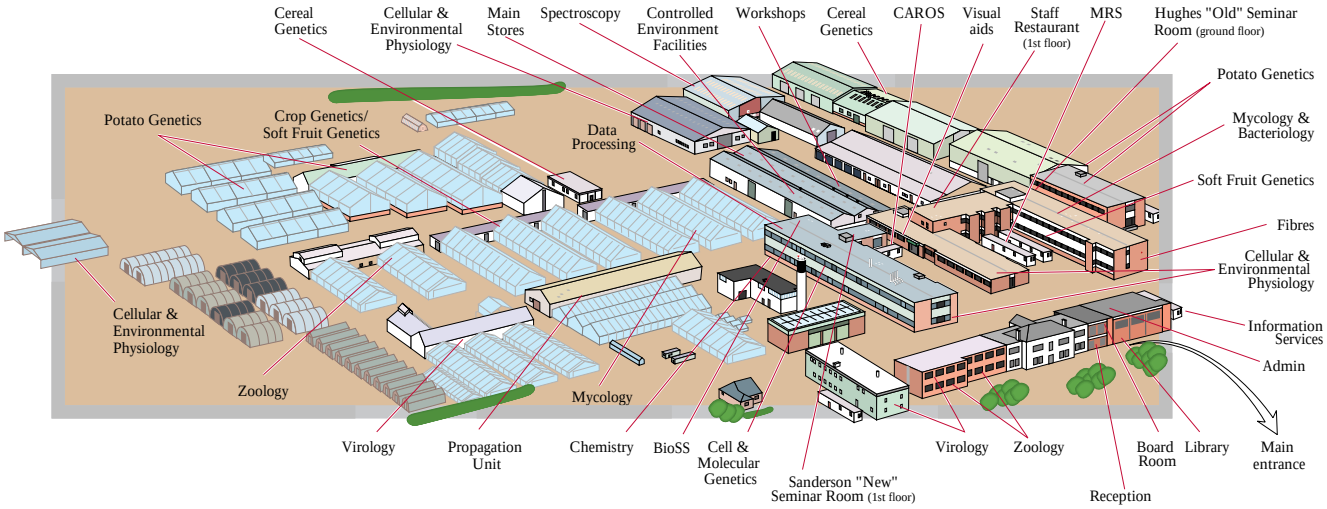
Hannah Research Institute	Ayr, Scotland KA6 5HL	01292-476013
Macaulay Land Use Research Institute	Craigiebuckler, Aberdeen AB9 2QJ	01224-318611
Moredun Research Institute	408 Gilmerton Road, Edinburgh EH17 7JH	0131-664-3262
Rowett Research Institute	Greenburn Road, Bucksburn, Aberdeen AB2 9SB	01224-712751
Scottish Crop Research Institute	Invergowrie, Dundee DD2 5DA	01382-562731
Biomathematics and Statistics Scotland (Administered by SCRI)	University of Edinburgh, James Clerk Maxwell Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JZ	0131-650-4900

List of Abbreviations

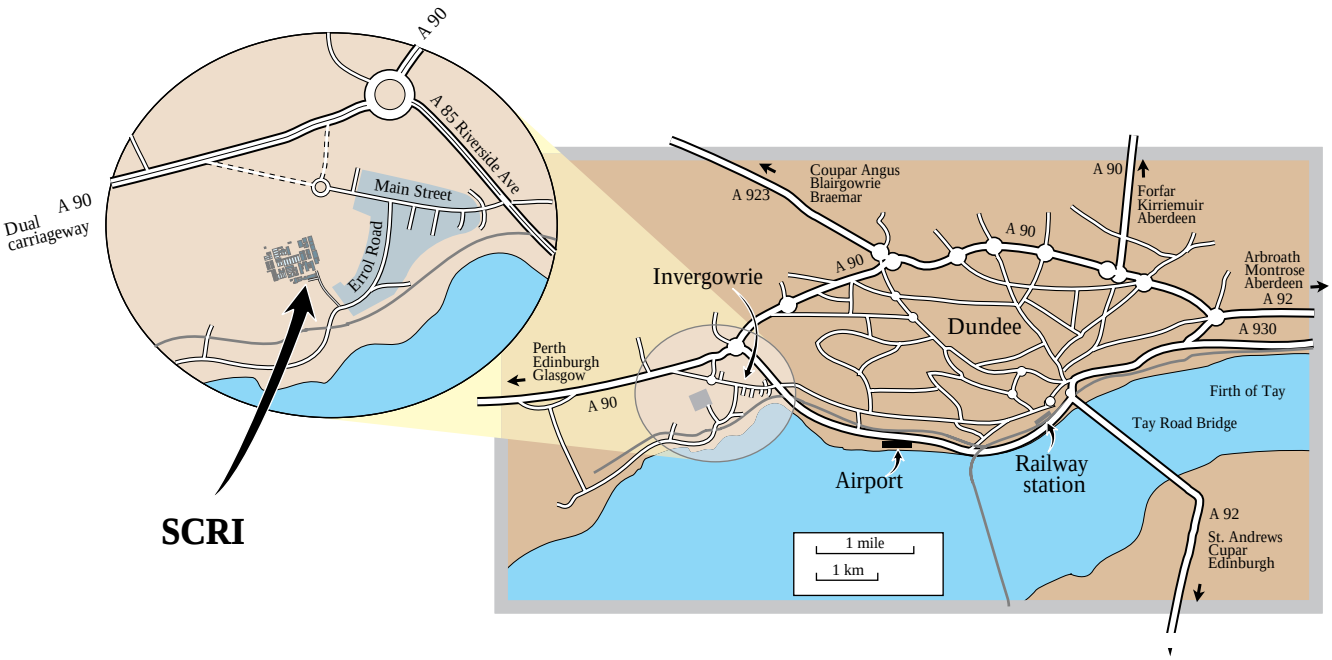
AAB	Association of Applied Biologists
ADAS	Agricultural Development and Advisory Service
BBSRC	Biotechnology & Biological Sciences Research Council
BCPC	British Crop Protection Council
BioSS	Biomathematics and Statistics Scotland
BSPB	British Society of Plant Breeders
BTG	British Technology Group
CAPS	Cleaved Amplified Polymorphic Sequence
CEC	Commission of the European Communities
CIP	International Potato Centre - Peru
COST	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
ICTV	International Committee for the Taxonomy of Viruses
IOBC	International Organisation for Biological Control
ISHS	International Society for Horticultural Science
ISPP	International Society for Plant Pathology
IVEM	Institute of Virology and Environmental Microbiology
MAFF	Ministry of Agriculture Fisheries and Food
MLURI	Macaulay Land Use Research Institute
MRI	Moredun Research Institute
NERC	National Environmental Research Council
NFT	National Fruit Trials
NIR	Near Infra-Red
NMR	Nuclear Magnetic Resonance
NPTC	National Proficiency Test Council
ODA	Overseas Development Administration
ORSTOM	Organisation for research in science and technology overseas
PCR	Polymerase Chain Reaction
PMB	Potato Marketing Board
PVRO	Plant Variety Rights Office
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RRI	Rowett Research Institute
SABRI	Scottish Agricultural and Biological Research Institutes
SAC	Scottish Agricultural College
SARI	Scottish Agricultural Research Institutes
SASA	Scottish Agricultural Science Agency
SASS	Scottish Agricultural Statistics Service
SCRI	Scottish Crop Research Institute
SEB	Society for Experimental Biology
SET	Scottish Enterprise Tayside
SNSA	Scottish Nuclear Stocks Association
SOAFD	Scottish Office Agriculture and Fisheries Department
SSCR	Scottish Society for Crop Research
SSFG	Scottish Soft Fruit Growers Ltd.
SSPDC	Scottish Seed Potato Development Council
STS	Sequence Tagged Sites
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 6km west of the centre of Dundee. Access is via the A85, Main Street and Errol Road.

British Rail has direct InterCity services between Dundee and London, Edinburgh and Glasgow and other UK cities.

Flights are available to Dundee Airport from Manchester and Aberdeen, and scheduled services operate from many domestic and international destinations to Edinburgh and Glasgow