Scottish Crop Research Institute

Annual Report 1992

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

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

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

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

Price £15

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Governing Body

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JANET (non UK) :	SCRI @ scri.sari.ac.uk

Editors :	D.A. Perry, T.D. Heilbronn
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Printed by :	Burns & Harris (Print) Ltd, Dundee

ISSN 0263 7200
ISBN 0 9058 7506 0

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Report of the Director

J.R. Hillman

Contrasting with 1991 when the Soviet State collapsed and there were prospects of a new world order, 1992 will be associated with schisms and perception of a downturn in international political and economic cooperation. Civil conflicts in eastern Europe, the Indian sub-continent and Africa were exacerbated in many other regions by political deadlocks, unleashing of nationalistic centrifugal fervour, migration, unemployment, economic recession and retrenchment. Scientific research, however, is becoming increasingly international as nations seek to attach funding priorities to their research effort and benefit from cooperation, and scientists share the excitement of discovery regardless of political boundaries. Agricultural science at major institutions such as SCRI is already strongly international, enhanced by collaboration and networking with Centers (Institutes) supported by the Consultative Group on International Agricultural Research, scientific societies, universities and institutes, the Commission of the European Communities (CEC) and private industry.

During the year there were three international cooperative events which are likely to affect the future direction of research in the life and environmental sciences. Firstly, the United Nations (UN) Conference on Environment and Development held in Rio de Janeiro, the 'Earth Summit', addressed for the first time major issues of global warming, biodiversity, over-consumption and environmental principles. Although the levels of commitment and compliance amongst the 178 participating countries were not adequate for many environmental scientists and activists, and the subject of population growth was not addressed directly, it was the first global summit devoted to the environment. The Treaty on Climate Change, as well as the Convention on Protecting Species and Habitats, was signed by more than 150 countries at the summit. There was also general agreement on the Declaration on Sustainable Development to be remitted to the UN Sustainable Development Commission. An increase in official development assistance as a percentage of gross domestic product of industrial nations was agreed but economic recession coupled to the underrated impact of demographic trends will undoubtedly override wholesale attainment of the target of 0.7%.

The second major international event was the International Conference on Nutrition (ICN) convened by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization. It was noted that more than 700 million people did not have adequate food to meet basic needs. Worldwide, it was thought there is sufficient food but the main problem is inequitable access. A Global Declaration on Nutrition pledged the participating countries to eliminate famine before the end of the century. In addition, the ICN called for each nation to develop by the end of 1994 a plan of action for human nutrition. Sustainability of present agricultural practices, impacts of donor aid on local economies and the environmental impacts of transport and storage were neglected issues at the Conference.

The third cooperative event was a study on soil degradation financed by the UN Environment Program and carried out by the World Resources Institute in cooperation with the International Soil Reference and Information Centre in the Netherlands. Since 1945, more than 10% of the world's vegetation-covered surface has been seriously damaged, much beyond restoration, by human activity, but this has been masked by improved agricultural productivity. Overgrazing, deforestation, inappropriate water management and urban growth were the main culprits.

Of particular importance to agriculture and food is the establishment of a world trade accord under the auspices of the General Agreement on Tariffs and Trade (GATT). Farming subsidies, together with indirect mechanisms to protect rural infrastructures and prevent vulnerability to food imports, appear to be primary targets for enabling free trade to operate. The protracted multilateral trade negotiations reported in previous SCRI Annual Reports were not concluded and bilateral agreements represented the normal interface between trading blocs. To accommodate GATT negotiation positions, the CEC made several changes to the Common Agricultural Policy (CAP). CAP reform was scheduled over a three-year period beginning 1993-1994, building on the oilseed-sector changes introduced in 1991 and extending to grains, livestock and dairy sectors. The package of measures is designed to reduce expenditure on price and income support for agricultural products thereby curtailing burgeoning surpluses held in intervention stores. Trade restrictions through importation barriers and export subsidies remain a controversial and complex issue. Most countries appreciate the need to protect domestic markets from cartels and unfair trading practices.

Preliminary indices of world agricultural and food production in 1992 published by the FAO pointed towards a levelling off of total agricultural production. There was a slight rise in total food production but a slight decline in *per capita* food production reflecting a global population increase in excess of 90 million for the year. Since 1950, the world's population has increased from 2.5 billion to 5.4 billion, subject to the veracity of data from the less-developed countries where nearly all the population growth took place.

Modest increases were expected in global grain output and stock holdings despite the inexorable rise in grain use. CAP reform would undoubtedly affect the position of the European Communities (EC) as the second largest grain exporter after the US. Intervention prices were reduced and appreciable proportions of farmland were to be 'set-aside' or fallowed. Prospects for revitalising natural flora and fauna depend on the management strategies allowed for set-aside land; many regard the failure to promote cultivation of non-food industrial crops on a portion of set-aside land a lost opportunity for economic development. Global oilseed production was expected to increase, especially in rapeseed, soybeans and cottonseed. A potential trade dispute triggered by the EC oilseed regime between the EC and US was seemingly averted towards the end of the year.

Food consumption patterns in the developed world continued the trend away from traditional meals to convenience products, dietary food and fresh fruit and vegetables. There was a remarkable rise in the UK of sales of vegetarian products. Although the effects of recession in the food industry were readily detected as declining profit margins, new opportunities arose with access to the markets of the former Soviet Union and the rapid-growth economies of the Pacific rim. Parallel with developments in engineering markets, retailers tried to refine further the concept of 'just-in-time' deliveries to reduce warehousing costs. Food technology attracted the attention of consumer groups although their responses to a range of technologies varied somewhat. Food irradiation and the introduction of genetically modified organisms tended to invoke opposition from certain groups on the grounds of potential safety risks to consumers and the environment. Such opposition was not overtly aimed at those processes developed to remove natural food constituents causing allergies, toxicity effects or potential dietary problems. So-called 'healthy' foods with reduced levels of fats, cholesterol, sugar, salt, or those products claimed to be 'environmentally friendly' or 'green' showed record growth. The additives market continues to expand, reflecting sales of convenience foods. Contaminants such as pesticide residues and food-poisoning organisms attracted considerable attention from the regulatory authorities, food producers and retailers alike. Developments in food packaging, most notably for fruit and vegetables, were based on long-established lines of research on controlled atmospheric compositions, edible films and coatings, and 'intelligent' marking.

Declining profitability from conventional agriculture and horticulture, as well as greater emphasis on the environment, sustainability and diversification, force those involved in the industry to reappraise low-over-

head options for farming. Foremost is the requirement for high-yielding cultivars that are naturally resistant to the ravages of pests and diseases. Prophylactic treatments of synthetic pesticides are no longer thought to be acceptable or affordable. That most pests and diseases show phenomenal powers of adaptation to circumvent control measures in the crop and resistance mechanisms in the host ensures that research and development allied to new cultivar production will remain the cornerstone for world agriculture and hence economic development. Of course, for much of the world, agrochemicals are the only option to maintain productivity, permit food storage and ensure predictable food supplies. Integrated pest management, combining both chemical and organic approaches, together with biological pest control, are rapidly developing areas of research, development and extension work. Most of the major advances are those involving modern biotechnology.

The Scottish Crop Research Institute.

Throughout the EC it is clear that the direct agricultural labour force has declined markedly, with an associated increase in average farm size and product specialisation. The UK has the highest average farm size - 68 hectares compared with 14 hectares in the rest of the EC - and has 14% of total area farmed. Technological advances have been rapidly incorporated and have enabled restructuring of the workforce alongside creation of new markets and specialised products. Sophisticated commercial agreements link together growers, processors and retailers. It is often forgotten the extent to which the economies of developed nations are dependent on efficient food supplies and agriculturally derived industrial commodities.

Agricultural research has now been recognised by funding agencies to cover all aspects of the life sciences and to draw heavily on engineering and a wide range of other sciences, especially mathematics, chemistry and physics. The interplay between fundamental, strategic, applied and extension studies has resulted in dramatic advances, raising in turn planning targets and expectations of politicians and governments.

SCRI occupies a crucial place in international and national agricultural and horticultural research. During a phase of major reorganisations, restructuring and waves of redundancies in most of the institutes of the UK Agricultural and Food Research Service, our Institute has grown steadily. The remit of SCRI is to conduct multidisciplinary research on agricultural, horticultural and industrial crops to advance knowledge of plant biology, and to improve agricultural sustainability and diversification, by providing an understanding of the complex interactions involving soils, water, microorganisms, pests and plants. The range of multidisciplinary skills, from fundamental studies on genetics and physiology, through agronomy, pathology, chemistry dynamics and biomathematics to glasshouse and field trials with exploitation of genetic resources, is unique within the UK research service. Close liaison with other research organisations, universities and colleges in the UK and abroad is an integral part of the scientific development of the Institute; such links are continually being developed and strengthened. Novel lines of research covering nearly all our activities have been introduced whilst retaining valuable traditional areas of study that link laboratory science with the field situation.

Building on the strength of plant virology at SCRI, Professor T.M.A.Wilson was appointed to the post of Head of Virology at the beginning of March 1992. A graduate of the University of Edinburgh, he completed his PhD at the University of Cambridge in

1976, before taking up posts at the University of Nottingham in 1976, the University of Liverpool in 1979 and the John Innes Institute in 1983. He has recently been on a secondment from the John Innes Institute as Professor at the Center for Agricultural Molecular Biology at Rutgers University in New Jersey, USA, leading a team working on the molecular biology of plant viruses.

Towards the end of 1992, Dr W.W. Christie was appointed Head of the Chemistry Department with effect from January 1993. A chemistry graduate of the University of St Andrews, he also gained his PhD and completed a post-doctoral fellowship at the University. After holding a Research Fellowship in Minnesota in 1964-1966 he worked at the Hannah Research Institute becoming internationally recognised for his contributions to lipid chemistry. In 1982 he was awarded the degree of DSc by the University of St Andrews.

The benefits of scientific research extend to all aspects of human progress, not just to those that carry out or finance the research. Harnessing scientific output to promote national prosperity and ultimately the quality of life in all its aspects is a particular challenge for all organisations supported by the public purse. SCRI is grant-aided by the Scottish Office Agriculture and Fisheries Department (SOAFD). In order to protect the charitable status of SCRI and to expand our funding base and the range of technology-transfer activities, Mylnefield Research Services (MRS) Ltd was incorporated as a wholly owned subsidiary company in November 1989. Trading commenced in April 1991 marketing the Institute's scientific expertise and products, and undertaking contract work and project management with commercial and other sponsors. The performance of MRS Ltd greatly exceeded our projections. Dr N.W. Kerby was appointed Commercial Manager of MRS in August 1992. He is a graduate of the University of Leeds, continuing his Doctoral and Post-Doctoral studies in the Department of Botany until 1982 when he joined the Department of Biological Sciences at the University of Dundee. Originally a member of the Cyanobacteria group led by Professor W.D.P. Stewart FRS, he became a Lecturer in Microbiology in 1990.

CAROS International Ltd (the Consortium of Agricultural Research Organisations in Scotland) is a new company formed in 1992 to promote and market the international research, educational and consultancy expertise of the Scottish science base in agriculture and related disciplines. The participating organisations are the Macaulay Land Use Research Institute, the Moredun Research Institute, (via Animal Diseases Research Association and Moredun Animal Health Ltd), the Rowett Research Institute (via Rowett Research Services Ltd), the Scottish Agricultural College (SAC) and SCRI (via MRS Ltd), with the backing of Scottish Enterprise. MRS provides accommodation and secretarial services for CAROS. Lord Sanderson of Bowden, businessman and former Minister of State in the Scottish Office, was appointed Chairman of CAROS with Dr D.J. Thomson the Chief Executive. Scheduled to take up his post in January 1993, Dr Thomson was formerly Director of Technical and Commercial Marketing at the University of Bath. He is a graduate of the University of Glasgow and gained his PhD from the University of Reading. After joining in 1961 the Agricultural and Food Research Council (AFRC) Institute of Grassland and Environmental Research he was seconded in 1986 to the CEC for a period of two years. Thereafter he returned to AFRC Central Office as Head of the International Marketing Office until he departed to Bath in 1989.

Staff at SCRI were deeply saddened by the untimely death in post of two highly dedicated and respected colleagues. Dr M.J. Allison died suddenly in January 1992. He graduated BSc in Forestry at the University of Edinburgh in 1959 and then completed National Service with the Black Watch as a commissioned officer. After returning to Edinburgh, he graduated BSc in Genetics in 1964 and PhD in Genetics in 1968. He then worked at the Medical Research Council Mutagenesis Unit in Edinburgh from 1967-68 under the supervision of Professor Charlotte Auerbach who was the first person to discover chemical mutagenesis. In 1969, he joined the Scottish Plant Breeding Station (SPBS) at Pentlandfield, Edinburgh, on a two-year appointment funded by the Home-Grown Cereals Authority to look at malting quality aspects of barley, an area in which he was to stamp his authority. He applied his knowledge of mutagenesis to study the potential for using beneficial mutations in the barley breeding programme to improve malting quality. His work proved so useful that in 1971 he was offered permanent employment in the Forage Division of SPBS, and became Head of Chemistry in 1973. Following the merger of SPBS and the Scottish Horticultural Research Institute in 1981, the Chemistry Department moved from Pentlandfield to Invergowrie in 1987. A gentle, kindly man noted for

his love of sport, he was a scientist of true originality and integrity.

Mr R MacDonald, Higher Professional and Technical Officer in the Engineering and Maintenance Department died suddenly in October 1992. Originally appointed to the Institute in 1960 as an Assistant Scientific Officer in the Laboratory Service Section, he was a time-served fitter with experience in the maintenance of scientific equipment. He subsequently received several promotions, and during his 32-year career at the Institute became one of its best-known staff members, noted for thorough knowledge of the buildings and services. He had particular experience in the design of growth cabinets, which was not only of considerable value to the Institute, but was also of benefit to a number of commercial manufacturers of such cabinets. He was also a popular member of the Civil Service Club and highly regarded as the 'skip' of the Institute curling team.

Research funding is a sensitive issue for scientists. In recent years substantial changes have been made to the process of commissioning research by Government departments. Coordination and monitoring mechanisms have become more complex; more effort is directed to *ex ante* and *ex post* assessments in an attempt to improve the returns to research and development spending. Government funding is now directed towards basic (fundamental or pure) research, and strategic research which underpins the improvement of competitiveness and efficiency by industry. Basic or fundamental research is defined as experimental work undertaken to acquire new knowledge of the underlying foundation of phenomena and observable facts without any particular application or use in view, whereas strategic research has practical aims and objectives although not sufficiently advanced to the stage where eventual applications can be clearly specified. Within the framework of basic and strategic research, the programme of SCRI is concerned with mechanisms, processes, relationships and systems. With the exception of policy-related topics, Government no longer funds research which it regards as being of direct application to industry ('near-market', for example plant breeding). There are, however, still differences in perception of the definition of 'near-market' between private industry and Government departments. Despite the formation of Levy Boards and similar bodies, technology transfer between many research organisations in the UK and private industry is generally constrained to the detriment of wealth creation. By way of contrast, at SCRI the main vehicles for technology transfer are MRS Ltd, CAROS International, the Scottish Society for Crop Research, publications, scientific meetings, patents, and the activities of the Scientific Liaison and Information Services Department. The remarkable productivity and efficiency of the Institute scientists demonstrate unequivocally that it is possible to foster wealth creation within the existing framework. Those changes brought about by the cuts in 'near-market' research and development undoubtedly stimulated the quality and quantity of our research and refocused the efforts to exploit the resource base.

The Governing Body of SCRI. Back (l. to r.) A.M. Jacobsen, L.M. Thomson, Prof. T.A. Mansfield, Prof. J.D. Hayes, J.B. Forrest, A. Logan, Prof. J.W. Parsons, Prof. J.A. Raven. Front (l. to r.) Prof. D.L. Lee, Prof. H.M. Dick, J.L. Millar (Chairman), J.A. Inverarity (Vice Chairman), T.P.M. Thomson.

Other than non-commissioned 'seed-corn' projects amounting to 4.15% of the SCRI funding, all other projects commissioned by SOAFD form part of the ROAME appraisal system (Rationale, Objective, Appraisal, Monitoring and Evaluation) implemented in full during 1991. A small part of the SOAFD funding is allocated through the 'Flexible Fund' which is used to support specific short-term projects under the ROAME system; some of which address policy requirements. Over 100 ROAME projects are funded at the Institute, presenting both project management opportunities and enormous bureaucratic responsibilities. During 1992, the 'seed-corn' funding was applied to the Stable Isotope and Woody Plant Initiatives, offering warmly welcomed freedom to explore conceptually new areas of scientific enquiry.

To ensure that commissioned work progresses satisfactorily and that the overall publicly funded programme responds adequately to the changing requirements of industry and science, the research programme is reviewed and amended by (i) four-yearly Visiting Group reviews and recommendations; (ii) various reviews of activity, commodity or topic sectors involving industry; (iii) continuing management and Governing Body assessment of the programme and its quality and productivity; (iv) decisions by SOAFD to commission new projects or cease existing work in the light of new research opportunities, emerging needs of industry or completion of projects; (v) advice of the AFRC Plants and Environment Research Committee and sub-groups; (vi) advice of SOAFD Scientific Advisor's Unit (SAU), Division C and Division D; and (vii) operation of the ROAME system.

Preparation of the Visiting Group documentation started in advance of the review scheduled to take place in April 1993. Changes to the arrangements for AFRS Visiting Groups include the submission of the documentation to independent referees to advise the Visiting Group, and substantially greater emphasis on performance indicators such as full economic costing per scientific paper, productivity, quality and technology transfer. Professor E.C.D. Cocking FRS who also chaired the 1989 Visiting Group was designated Chairman of the 1993 Visiting Group.

The Institute's management accounting systems were reviewed by a team from the Scottish Office Audit Unit from 7-18 December. Their report is expected in the first half of 1993. The Institute's accounts received their annual statutory audit in May/June by KPMG Peat Marwick, who also made separate special reports on our compliance with Income Tax, National Insurance and Value Added Tax regulations. These reviews should ensure that the Institute operates at a continuing high level of efficiency and propriety.

In addition to the AFRC organisation, the five Scottish Agricultural Research Institutes (SARIs) are part of the well-respected 'Scottish System' in which the SARIs are linked, under the administrative funding umbrella of SOAFD, to the Scottish Agricultural College (SAC) which has special development, advisory and educational functions as well as responsibility for specific areas in agricultural and related research. SOAFD also funds research by the Scottish Agricultural Science Agency in support of regulatory duties concerned mainly with the testing of potential cultivars and plant health, and provides grant-in-aid to the Royal Botanic Garden, Edinburgh. Close liaison between the Scottish organisations is maintained to our mutual advantage.

This Annual Report details a selection of the research achievements of the Institute, including the Scottish Agricultural Statistics Service (SASS), incorporating advances in fundamental and strategic science, contributions to the protection and understanding of the environment, and discoveries of both direct and indirect benefit to agriculture, horticulture and various biologically based industries. The outstanding performance of SCRI and SASS in respect of publications, reports, new cultivars, patents, contracts, supervision of students, and teaching reflects not only the range of facilities and resource capacity of the Institute but most notably the dedicated commitment of talented staff in every department and section.

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, operating at the highest professional standards of public service. Grants, contracts, donations and advice from the Scottish Society for Crop Research, governmental and non-governmental agencies, grower levy boards, local and regional authorities, commercial companies, farmers and other individuals are also warmly appreciated. In all respects, SCRI has a bright future. Our research output, grant income, research links, research student base, visiting scientists, requests for assistance from external bodies, international reputation and influence, appointment of new staff, the first-class facilities, and not least the quality of the staff and Governing Body, collectively point to success.

Notable Events

The Institute opened its doors to invited guests on 3 July and to the general public on 4 July. Among the distinguished guests who viewed displays of many aspects of the work of the Institute, toured the farm and glasshouse facilities, and enjoyed some of the fruits of the research projects, were the Lord Provost of Dundee, the Provost of Perth and Kinross,

The Lord Provost of Dundee (left) and the Provost of Perth and Kinross (right) welcomed by Professor Hillman, Director (centre) to the Open Day.

members of the Governing Body and representatives of MAFF and SOAFD and of the industry levy boards. Around 1500 members of the general public were entertained on 4 July to a similar programme with an in-house video production of the aims and objectives of the Institute, conducted tours around the laboratories and rides on a tractor/trailer round the farm proved to be very popular with the visitors. Many complimentary comments about the standard of the displays and the willingness and enthusiasm of the staff to explain their work were received after the event.

Plant molecular biology being explained to visitors by Dr. Amar Kumar.

During the year many individuals 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 and local club organisations. The Minister of Agriculture and Deputy Minister of Economic Affairs for the Moscow region of the CIS visited on 10 July and several groups from the former Eastern Bloc countries and four separate delegations from China were entertained.

A Russian delegation toured SCRI during their visit to Dundee to develop trade links.
Left to right : Dr. M.F.B. Dale; J. Korolev, Minister of Agriculture, Moscow Region; Ken MacDonald, Development Manager, Tayside Region Industrial Office; V. Ashitikov, Deputy Minister of Economic Affairs, Moscow Region.

Plant genetics

G.R. Mackay & R.J. McNicol

The Vavilovian concept of centres of origin as the centres of diversity of agricultural crop species and their primitive or wild progenitors is now enshrined in evolutionary dogma. As most important European crop species have their origins in regions of the world outside Europe, the collection and evaluation of germplasm from these regions has been necessary for modern plant breeders to augment the genetic diversity of crops. This has been particularly true of potatoes where the narrow genetic base of the modern European form, Solanum tuberosum *ssp* tuberosum, *and lack of genetic variation for resistance to major problems such as late blight and potato cyst nematode continue to cause concern. Many of the pests and diseases are controlled by routine use of chemicals, some of which may be environmentally damaging.*

Centres of origin of crop species are also the centres of origin and diversity of many of the pests and diseases of those crops. Indeed, the co-existence and co-evolution of both hosts and pathogens has ensured the existence of heritable variation for resistance needed by breeders. Consequently, immediate access to potentially valuable germplasm can be complicated, not only by geography, but by strict quarantine procedures designed to ensure that non-indigenous or novel strains of pathogens are not introduced along with the germplasm. Thus genebanks such as the Commonwealth Potato Collection (CPC) and breeders' working collections are valuable resources, providing immediate access to genetic diversity for breeding and fundamental research. The H_1 gene that confers resistance to potato cyst nematode (*Globodera rostochiensis*) and which has been incorporated into many modern cultivars, provides one example of the value of the collection. Genebanks such as the CPC cannot replace the natural diversity of centres of origin, but evaluation and exploitation of genetic diversity for crop improvement would be more difficult and costly without them.

Most recent research involving wild species and primitive forms of potatoes is directed to the identification of specific traits, usually disease resistance, and their rapid incorporation into *S. tuberosum*. However, there are seven cultivated species of potato in South America and one, *S. phureja*, a diploid, has been widely used in the breeding of modern varieties, often as a "bridge" between *S. tuberosum* and other more distantly related wild species with which it will not hybridise easily or directly. *S. phureja*, in common with similar South American species, is adapted to short day growing conditions. Moreover, it has been

Figure 1 *Solanum phureja* "Gourmet potatoes".

selected by native farmers for almost continuous cropping and, consequently, its tubers have very short dormancy. A population of *S.phureja* has been developed at SCRI that is adapted to long days and whose tubers have improved keeping qualities. In collaboration with industrial partners, we have been examining the possibility of using these improved clones for consumption as novel vegetables as they possess unique culinary properties and flavours. Several independent experts in the food industry, to whom samples have been supplied, have reported enthusiastically on these "gourmet" potatoes.

The gene base in raspberry cultivars is also very narrow and recent analysis of the pedigrees of cultivars released worldwide during the past 30 years confirmed that it is narrowing further. Indeed, molecular fingerprinting techniques developed at SCRI have established that the genetic base is even more restricted in *Rubus* than previously suggested from the pedigree analysis.

An active breeding and genetics programme for a slow growing, woody perennial crop needs a diverse and healthy source of plant material from which genes are readily available. If the source material has to be imported, tested for health and grown on to maturity before use, a period of at least seven years will have elapsed before its progeny can be fully field evaluated. The availability of a wide range of germplasm is also an asset when new unexpected breeding objectives arise. The maintenance of collections is not commercially attractive and adequate resources must be provided from public funding.

Whilst phytosanitary barriers to free exchange of germplasm of small grain crops such as barley are less fraught than in potatoes and soft fruit, access to the diversity that exists in centres of origin is complicated by geography. However, collaboration has brought together the conventional skills of scientists with access to the natural habitats of wild barley and the expertise of SCRI geneticists to provide insights into the genetic architecture of barley as well as identifying potentially valuable germplasm.

Hybrid vigour has often been exploited but the genetic mechanism of heterosis remains unclear. The conclusion reached in an article by Bradshaw (p. 17) that inbred lines can be as high, or higher yielding than F_1 hybrids is fundamental to plant breeding. His research on swedes, designed to exploit heterosis by producing F_1 hybrids in an otherwise facultative inbreeder suggested that the most heterotic F_1 hybrids are probably a better starting point for an inbreeding programme than its end point. This project demonstrated the substantial benefits of a biometrical approach to genetic studies of complex traits such as yield.

The development and utilisation of modern genetic transfer techniques continues but, as the products approach commercial exploitation, they will require field trials and testing similar to the products of conventional breeding. The release of genetically modified organisms, including crop plants, into the environment is subject to statutory control and the plant breeding skills and experience at SCRI are now being harnessed to assess the potential risks of the release of genetically modified crops. A SOAFD-funded project on pollen movement and evidence of gene escape by surveying wild indigenous populations for phenotypically expressed marker genes will provide more accurate estimates of the possible distribution of genetically modified cultivars in the field. Contracts have been awarded by the Department of the Environment to examine the safety of selectable markers present in most gene constructs, that are additional to the gene of interest. The information obtained will be used to implement the regulatory aspects of release experiments. In a second contract, the population dynamics of feral oil seed rape is being investigated to determine the likelihood of oil seed rape becoming established in the natural weed flora, and what characteristics of genetically modified rape might enhance this possibility.

Conservation and utilization of germplasm collections of potato and faba bean

M.J. Wilkinson, K. Harding & G. Ramsay

The importance of germplasm conservation to plant breeding and related research cannot be overstated, and the launching of the Darwin initiative at the 1992 Earth Summit in Rio de Janeiro has served to increase public awareness of both biodiversity and genetic conservation.

The selection of elite lines has been a feature of farming since the birth of agriculture. Concurrent with enhancement of crop performance through genetic selection is an associated decline in genetic diversity. As the genetic base of a crop diminishes, there is an increasing risk of catastrophic losses following minor changes in pest or disease virulence leading to economic ruin and even famine. Indeed, changes in the pathogenicity of late blight (*Phytopthora infestans* (Mont.) de Bary) probably contributed to the famous Irish potato famine of the 1840's. It is not surprising, therefore, that breeders of all major crops have sought to insure against excessive genetic erosion through the repeated introduction of novel germplasm, primarily from sources that carry resistances to major pests and diseases.

Most plant breeding research is conducted in areas remote from the centres of biodiversity. Clearly, it is impractical for breeders and research workers to travel to these regions whenever they have need of new plant material. Genebanks containing a representative selection of genotypes have been established for all major and many minor crops in several countries. The function of these repositories is to provide breeders and research workers with easy access to plant material from which useful genes can be identified and transferred into commercial material. SCRI houses an international genebank containing relatives of the cultivated potato (*Solanum tuberosum* L.) and a substantial collection of related wild species, land races and experimental material of *Vicia faba* L.

Figure 1 Two species from the CPC found to contain resistance to potato cyst nematode: *S. tuberosum* ssp. *andigena* (left) and *S. toralapanum* (right).

POTATO COLLECTION. Potato is the fourth most important food crop after wheat, maize and rice, and there is a wide range of useable genetic material outside the crop. Hawkes [1] recognised 235 species belonging to the tuber-bearing section of the genus *Solanum* (Section *petota*). These plants have evolved an impressive battery of resistance genes to most of the pests and diseases that currently attack the cultivated potato.

The Commonwealth Potato Collection (CPC) is a genebank containing accessions of primitive cultivated species and wild relatives of the cultivated potato. The collection currently houses over 1400 accessions representing 73 species from 16 series, and 21 accessions of interspecific hybrids. Since its creation in 1938/9, it has been extensively used for breeding purposes. For example, in the 1940s the potato crop was severely affected in parts of the UK by potato cyst nematode (PCN). Accessions held in the collection were screened (Fig. 1) and a source of resistance was identified in *Solanum tuberosum* ssp. *andigena* Hawkes (accession ADG1673). The major resistance gene responsible, the H_1 gene, was identified and subsequently transferred into several commercial cultivars. These resistant cultivars were widely grown and have been commercially successful.

The CPC has been comprehensively screened and declared free of potato spindle tuber viroid (PSTV) on an individual plant basis. This provides the collection with a high health status and enables seed samples to be distributed freely throughout the UK. Computer assisted packages play an important role in stock-keeping, request information, and updating the inventory.

Figure 2 Somatic hybrids between *S.tuberosum* cv. Brodick and **a)** *S. megistacrolobum;* **b)** *S. sanctae-rosae* and **c)** *S. sparsipilum.*

MAINTENANCE In common with most other international collections of potato germplasm, the CPC is maintained primarily as botanical seed (true potato seed) which is divided into a rapid access collection used for seed requests in which sealed samples of 25 seeds are stored under controlled humidity at 6°C, and a long-term base collection (duplicated at Braunschweig) stored at -20°C under desiccated conditions. In recent years, collections of pollen, tubers, herbarium specimens, plants, and freeze-dried material have been established. A smaller number of interesting genotypes are also maintained in tissue culture. The CPC is in the process of expansion through the incorporation of the personal collection of Professor J.G.Hawkes held previously in the University of Birmingham. This collection contains over 1500 accessions representing 86 species of which 33 were not previously extant in the CPC. All plants from Hawkes collection that are being used to provide seed for the CPC are first screened for PSTV and other viruses known to be seed-transmissable, viz: Andean potato latent virus (APLV); tobacco ringspot nepovirus, Andean potato calico strain (TRSV-Ca); Arracacha B nepovirus, oca strain (AVB-O), and potato T capillovirus (PVT). In consequence, a significant portion of the CPC is unique in meeting the most stringent of European quarantine standards for plant health and may ultimately enable the distribution of seed samples throughout the European Community without the need to pass through quarantine.

DOCUMENTATION At a meeting of the International Collaboration of Potato Genebanks (1991) sponsored by the International Board for Plant Genetic Resources (IBPGR), a standardized format was agreed for the storage of passport and evaluation data. Information on the CPC has been assimilated in the new format onto a relational database system (ORACLE). Passport data includes the CPC code number, source locality, name of collector and number, type of material available, endosperm balance number (EBN), chromosome number, and notes on altitude, latitude, longitude, habitat etc. Evaluation data includes resistance to late blight, PCN (*Globodera rostochiensis* and *G. pallida*), potato viruses X, Y, A, B and C, potato leafroll virus, wart (*Synchytrium endobioticum*), aphids and blackleg (*Erwinia carotovora ssp. atroseptica*), and tolerance to frost and drought. A revised inventory for the collection is now available upon request.

UTILIZATION Since 1968, in excess of 3,600 accessions have been requested from 28 countries for a number of wide ranging activities [2]. Utilization of the CPC in the potato genetics programme at SCRI has concentrated on identifying new sources of resistance to PCN and late blight in species to be used as parents. Recently PCN resistance has been transferred from *S. vernei* Bitt. et Wittm. into cultivated material and genes from *S. demissum* Lindl. have been bred into cultivars to introduce field resistance to late blight (*Ann. Rep. 1991,16*).

A programme of protoplast fusion experiments has been initiated to transfer disease-resistant traits. For example, three species with resistance to PCN, *S. megistacrolobum* Bitt., *S. sanctae-rosae* Hawkes and *S. sparsipilum* (Bitt.) Juz. et Buk. were fused with *S. tuberosum* cv. Brodick to produce in excess of 800 putative somatic hybrids (Fig. 2). Hybrid status is

Figure 3 Electrofusion between gametic and somatic protoplasts.

being confirmed by chromosome number and conventional isozyme analysis, and resistance to PCN infection is being assessed. Limited genetic transfer is also being attempted in a series of gameto-somatic protoplast fusion experiments. Protoplasts isolated from meiotic tetrads of various wild species have been isolated and fused with mesophyll protoplasts of the cultivated potato to regenerate gameto-somatic hybrids (Fig. 3).

Future storage of material

SEED The maintenance of the core collection through long-term storage of seed samples has low labour requirement and minimal risk of accession extinction through disease. However, although seed samples of most species retain some viability for up to 25 years, regeneration of fresh seed is generally required at intervals of 8-15 years. When the viability of a seed stock falls below 75-80%, fresh seed is generated. Limitations on space and manpower resources dictate that, in general, the new seed is derived from a parental population of about 40 plants. This represents only a small fraction of the original seed sample which may contain over 20,000 seeds and is a source of severe genetic erosion. Genetic erosion between successive generations of seed also leads to a reduction in the number of incompatibility groupings (S-alleles) and leads eventually to a completely self-incompatible accession. Crosses within such an accession fail to set seed and new stocks can only be generated by inter-accession hybridizations. Several hundred of the original CPC accessions have been saved as inter-accession crosses, usually as intraspecific hybrids.

A reassortment of genes is obligately associated with the sexual process and so individual clones (eg cultivars) cannot be maintained in seed form. Therefore, alternative methods of storage need to be employed for individuals with a desirable assortment of genes.

TUBERS Storage of tubers has the major advantage of allowing individual genotypes to be maintained in clonal form, although the annual regeneration of tuber progenies is a labour-intensive exercise. Plants grown in the field or glasshouse are prone to infection by pests and diseases, and may fail to produce tubers under certain environmental conditions. In some years this can lead to heavy losses of material and to the extinction of accessions. The CPC was originally maintained as tubers, but excessive losses and running costs forced a change to seed storage in the 1960s. However, a small collection of tubers has been created to allow storage of some desirable genotypes, and to provide a ready supply of material for research workers interested in physiology and epidemiology of tuber diseases.

IN VITRO CONSERVATION The conservation of germplasm *in vitro* may be achieved by introducing explants into tissue culture or by the use of medium-term (slow growth) and long-term (cryopreservation) storage procedures. An important aspect in the application of *in vitro* methods for the conservation of genetic resources is the stability of plants regenerated from these procedures.

Potato shoots and plantlets can be regenerated from a variety of explants and techniques have been developed to micropropagate shoot-tips, where adventitious shoots have been used in breeding and genetic manipulation programmes. Culture techniques were restricted initially to a few genotypes, but increasing refinements to methodology has extended the range of responsive cultivars and species. The *in vitro* induction of tuberisation in some species is an alternative to continuous micropropagation and provides a practical means of transplantation.

USE OF TISSUE CULTURE IN CONSERVATION Micropropagation procedures are invaluable for germplasm storage studies and shoot-tip cultures can be held in culture for extended time periods under minimal growth conditions or with growth retardants. Propagation by shoot-tip cultures ensures genetic stability in the cultured progeny and the stability of regenerated plants. However, time-related genomic changes associated with prolonged culture and use of growth retardants for *in vitro* conservation needs to be assessed, especially as mannitol supplemented media causes vitrification and stunting in cultured potato

shoots. Genomic analysis of slow growing plants in mannitol medium demonstrated that changes related to stress culture had taken place within the ribosomal DNA [3]. Clearly, tissue culture has a role to play in germplasm conservation, although the benefits must be balanced against the risks of genetic instability and the costs of routine maintenance.

USE OF CRYOPRESERVATION IN CONSERVATION The possibility of storing material in a cryopreserved state from which whole plants can be regenerated is another approach to conserving potato genetic resources. A wide range of techniques are described in the literature and include recent advances in cryoprotective dehydration, vitrification and encapsulation. The feasibility of cryopreserving *Solanum phureja* Juz. et Buk. by encapsulation of meristems in alginate beads has been demonstrated [4]. The potential use of this technique on a wider range of *Solanum* species is presently being examined at SCRI in collaboration with Dr E.E.Benson at Dundee Institute of Technology.

The SCRI faba bean collection

The faba bean (*Vicia faba* L.), also known as the field, tic, horse and broad bean, is the most important grain legume crop in the UK. Research on faba bean genetics requires ready access to a diverse range of germplasm and a collection of 754 accessions, consisting predominantly of *V. faba* but also containing other *Vicia* species, other grain legumes and their wild relatives is maintained. The *V. faba* accessions have diverse geographical origins, covering 34 countries in five continents (Fig. 4). Intraspecific variation is well represented in the collection which ranges from the smallest seeded *V. faba* var. *paucijuga* to the largest seeded European broad bean cultivars. The *V. faba* accessions are grouped into modern or old cultivars, inbred lines, landraces, cytogenetic stocks, miscellaneous genetic stocks and induced mutants.

MAINTENANCE The collection supplies material for studies on variation for anti-nutritional factors, tissue culture response, genetic markers, molecular taxonomy and a range of other studies. Many of the accessions held are not available elsewhere in the UK, consequently care is taken to rejuvenate, multiply, store and catalogue material to a high standard. Faba bean plants are attractive to bees which, in open pollinated conditions, bring about outcrossing at rates of between 0 and 90% according to genotype and conditions. Accordingly, stocks are multiplied in insect-screened polythene tunnels, giving good control over pollination. Accessions are maintained by self-pollination but as few lines set seed in any quantity without manually opening and closing each flower, multiplication is a time-consuming and labour-intensive process. Accessions which began as mixed populations, includ-

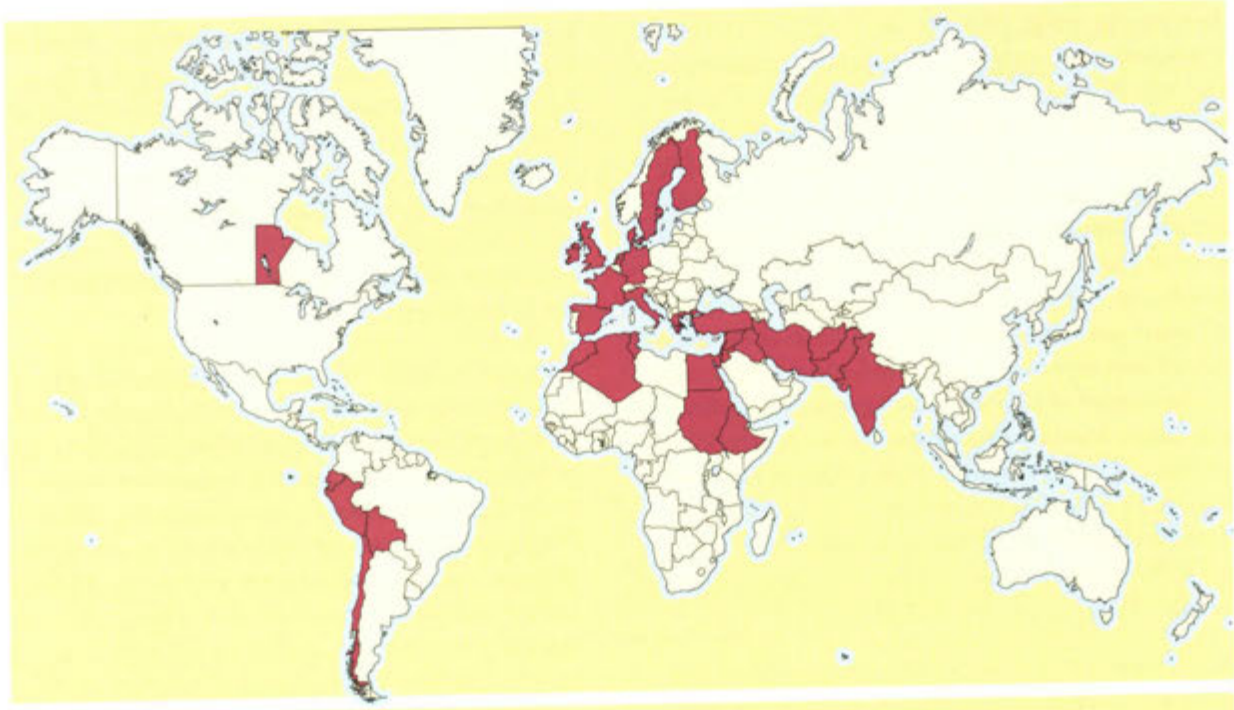

Figure 4 Geographical origins of *Vicia faba* accessions.

ing most landraces and many cultivars, are maintained as self-pollinated bulk samples and representative inbred lines have been derived from some of these populations.

EXPERIMENTAL MATERIAL One unique feature of the collection is a growing number of induced mutants created as a by-product of work on anti-nutritional factors. Sodium azide mutagenesis yielded mutants of pre-existing types (including dwarf, white flower and terminal inflorescence) and a range of novel types worthy of conservation. A total of 144 mutants in 95 classes have been identified. Several are likely to be useful in studies of faba bean physiology including wilting, cold sensitivity and etiolation mutants. A number of different types of mutation may give rise to a wilting phenotype but one of particular interest causes reduced levels of abscisic acid which leads to poor stomatal regulation. Mutants which fail to de-etiolate following seedling emergence may have a defective gene for phytochrome, or its chromophore, and can help determine the plants response to its environment. Among the new flowering mutants there are several with new flower colours and some flower morphology mutants. Seeds with wrinkled phenotypes or with yellow or red testas were also found. Most of these mutants are in the process of being grown under self pollinating conditions and will be included in the collection when a sufficient bulk of homozygous seed has been produced.

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Breeding to exploit heterosis in swedes

J.E. Bradshaw

Swedes (*Brassica napus* ssp. *rapifera* L.) have been grown in the UK to provide winter forage for cattle and sheep since the early 1800s. During the 19th century many new strains and varieties were produced by visual selection of attractive looking roots which were then seeded together in isolation from other multiplications. They were, however, very variable as a result of insects making cross-pollinations between the plants in an isolation site. Between 1900 and 1930 the chemical composition of swedes was determined with a view to improving their feeding value, but opinions differed over the relationship between chemical composition and feeding value. Research workers in Scotland concluded that the swede with the highest dry-matter yield per unit area was the most profitable one to grow, and from 1930 this became the main selection criterion in the breeding programme at the Scottish Plant Breeding Station (SPBS) in Edinburgh[1]. Furthermore, the desire to produce high yielding, uniform cultivars became the major influence on the choice of breeding method and type of cultivar.

Pure-line breeding Although swedes are insect-pollinated, Davey at SPBS showed that they are usually self-fertile and that inbreeding depression is mild. He produced uniform lines from variable commercial cultivars by a number of generations of natural self-pollination in insect-proof bags (bag-selfing, Fig.1), but did not consider the best ones to be sufficiently superior or distinct from their parent cultivars for release

Figure 1 Natural self-pollination in insect-proof bag.

as new cultivars. Interestingly, however, two such lines which were recently produced primarily for genetical research have been released. A line from cultivar Criffel is being marketed in New Zealand as an improved and distinct cultivar, Highlander, and a superior line from the shopping swede Acme is being marketed in the UK as an improved stock of Acme.

Pedigree inbreeding During the 1930s, Davey changed his approach to combining desirable characteristics from different cultivars, strains and pure lines through hybridizations (Fig. 2), followed by bag-selfing and selection for a number of generations to produce true breeding lines with the required attributes. This pedigree method which he developed has now been in use for over 50 years. The cultivars Angus and Melfort came from crosses made in 1967 and have been on the NIAB Recommended List of Swedes since 1982. Airlie was provisionally recommended for general use in 1992 and Brora and SS5 have completed their second and first year, respectively, in National List Trials.

Figure 2 Hybridization in the glasshouse.

F_1 hybrid breeding The vigour of the first hybrid generation (F_1) from crosses between inbred line cultivars was appreciated as early as 1930 by Davey, but at that time there was no way of producing large quantities of F_1 seed to exploit this heterosis in hybrid cultivars. It was not until 1957 that Davey had the idea of using self-incompatibility, following his discovery in 1954 of a self-incompatible plant in the cultivar backcross (Western Perfection x Champion) x Champion[2]. Gowers developed methods of using sporophytic self-incompatibility in preference to cytoplasmic male sterility to produce hybrid swedes on a commercial scale from 1971 onwards[3]. During this period, McNaughton and Munro[4] and Gowers[2] confirmed the superiority of the F_1 generation over the better parent of the cross (better parent heterosis), with dry

Figure 3 Testing plant for self-incompatibility.

matter yield increases in the ranges 6-26% and 12-29%, respectively. Self-incompatible *B. napus* lines for use in hybrid breeding were obtained by detection of naturally occurring self-incompatible plants, by artificial syntheses of *B. napus* from its self-incompatible progenitors, *B. campestris* and *B. oleracea*, and by introgression from *B. campestris* which readily crosses with *B. napus*[5].

During the 1980s, F_1 hybrid breeding consisted of backcrossing self-incompatibility into agronomically desirable inbred lines with good combining ability. These were built up over a number of years for use in genetical research and for parents in the breeding programmes, and were tested from time to time for their combining ability in a diallel set of crosses in all combinations. As self-incompatibility (SI) is dominant to self-compatibility (SC) the backcrossing was straightforward but laborious. Plants of each backcross were tested for self-incompatibility by physically self-pollinating 10 newly opened flowers on an inflorescence (Fig. 3). Self-compatible plants were discarded along with any that were partially self-compatible (Fig. 4). The self-incompatible individuals were then crossed with the agronomically desirable inbred line to provide seed of the next backcross generation. Backcrosses with a poor seed set were discarded to avoid problems from poor fertility. After six backcrosses true breeding SI lines were extracted by two

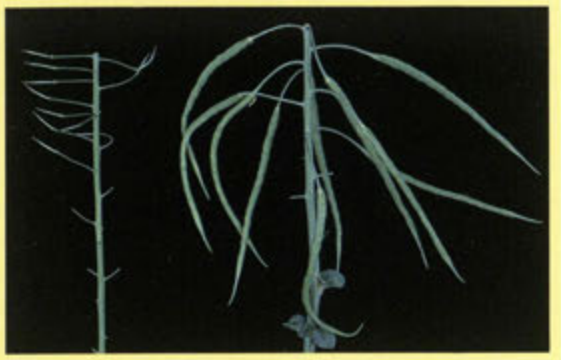

Figure 4 No seed set in self-incompatible plant compared with full set on self-compatible one.

generations of selfing at the bud stage, i.e. before self-incompatibility started to function.

The first SI lines to emerge from the programme were SIRE, which was produced from a line from cv. Ruta Øtofte (BR) and SI line E over the period 1979 to 1988, and SICF which was produced from a line from cv. Criffel (CR) and SI line F over the period 1980 to 1989. It was concluded from the two generations of bud-selfing required to isolate SIRE and SICF that self-incompatibility was conferred by a single dominant gene in both lines.

For F_1 hybrid production on a commercial scale, Gowers' modified double-cross method could be used[3] but for research purposes, a simpler method successfully produced hybrids between SIRE and seven other inbred lines. Seed of each hybrid was obtained from 10 plants of SIRE, arranged in two central rows with five plants of the male parent on either side in a polythene tunnel (Fig. 5). Blowflies were introduced as pollinators and the self-compatible parent was physically removed when the plants had finished flowering.

The percentage of selfs in six of the seven hybrids was readily determined because the roots of SIRE had a green skin colour which is recessive to purple. For all six, around 98% of the seed produced was of the desired hybrid.

The seven hybrids were assessed in a trial in which dry-matter yields were based on approximately 300 plants (Fig. 6). The hybrids outyielded their better parents by between 1.9% and 19.2%, but only the hybrid between SIRE and CR was significantly better than cv. Angela which was the best of the three controls. SICF was therefore used subsequently to produce hybrids between CR and lines from Bangholm

Figure 5 F_1 hybrid production: SI row (nearest) with SC pollinator behind.

Magres (BM), Bangholm Wilby (BW) and Marian (MN) which were compared in field trials, together with SIRE x CR. The most heterotic hybrid, SICF x BM which outyielded its better parent by 14.9% was also the highest yielding at 11.28 t/ha. However, whilst it outyielded Airlie, Angela and Brora, its yield was less than SS5 at 12.54 t/ha, a particularly significant result because SS5 was an F_6 line from the cross BM by CR. The superiority of SS5 was confirmed in the following year when it again outyielded SICF x BM, which in turn outyielded all of the other commercial cultivars. The production of a recombinant inbred line which outyielded the F_1 from which it came suggests that the genetical basis of the heterosis was a dispersion of partially dominant genes between the parents, rather than overdominance.

Biometrical genetical studies The hybrids CR x MN and CR x BW had already been used for a joint project with Dr M J Kearsey of Birmingham University in which early generation biometrical analyses were used to determine the genetical basis of heterosis, and to predict the yield distribution of the recombinant inbred lines which could be derived from the crosses.

Figure 6 Yield trial.

Figure 7 CR (white flesh), MN (yellow) and F_1 hybrid in centre (white).

Two augmented triple test crosses (TTC) were produced by Dr L D Ramsay (whilst a SERC-RCCA postgraduate student) and assessed in field trials. The F_1's of CR x MN (Fig. 7) and CR x BW outyielded their better parent by 14.5% and 11.9% and the average dominance ratios were less than unity, namely 0.50 and 0.94 respectively. The genetical basis of heterosis in the first cross was therefore a dispersion of partially dominant genes between the parents, and in the second a dispersion of almost completely dominant genes. There was no evidence of overdominance and it was predicted that 12.0% and 1.6% respectively, of the recombinant inbred lines from the two crosses should outyield the F_1. There was evidence of epistasis (non-allelic interactions) affecting the generation means in both crosses, and the variances in the second cross, but there was no consistent pattern and epistasis was not considered a major feature of the genetic architecture of dry matter yield. There were however large and unexpected reciprocal differences, particularly in all TTC and F_3 generations of the first cross, and these require further investigation.

Conclusions Throughout the history of swede breeding at SPBS and SCRI there was a strong desire to produce hybrids, based on the belief that this was the only way to fully exploit the heterosis known to exist for dry matter yield. However, the main conclusion from our recent work is that it is possible to produce inbred cultivars of swede in a modest sized breeding programme that will outyield F_1 hybrids. Hence high yielding heterotic F_1's should be the starting point rather than the finale of swede breeding programmes, and research into breeding methods should concentrate on improving the efficiency of Davey's pedigree inbreeding method, rather than on ways of producing F_1 hybrids.

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The use of *Hordeum spontaneum* Koch in barley improvement

R.P. Ellis, W.T.B. Thomas, B.P. Forster, M. Macaulay & E. Nevo[1]

The evolution of the modern barley (*Hordeum vulgare*) crop coincided with the migration of human populations from the Middle East into Europe. During this process, farmers made selections from wild populations of barley, resulting in locally adapted landraces. Selection reduced the genetic base of the crop and much potentially useful variation in the wild populations was lost during adaptation to cultivation. One major adaptation was the selection of a non-shattering ear which made harvesting much easier. Wild barley ears naturally shatter to ensure effective seed dispersal. The narrowing of the crop's genetic base was accelerated further by selection for malting and brewing quality and, in the early years of the 20th century, by the development of commercial plant breeding. Breeders and geneticists have recognised this problem and have made collections of wild barley in an attempt to preserve some of the diversity. The closest wild relative of cultivated barley, *Hordeum spontaneum* Koch, shows a wide range of variation in

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Figure 1 Collection expedition for *H. spontaneum* in Israel.

isozymes, storage proteins and tolerance of biotic and abiotic stresses. This variability makes *H. spontaneum* a very useful source of genes for the improvement of the cultivated species. Many expeditions have been made to collect (Fig. 1) *H. spontaneum* from the wild. In 1977, the AFRC and the Hebrew University of Jerusalem sponsored a collection which sampled 213 sites in Israel. UK Research Institutes in Cambridge, Aberyswyth and Edinburgh grew over 10,000 of the accessions collected and this material has been used in breeding programmes and research studies.

Cultivars produced by breeding programmes are adapted to their environments. In Europe, suitable environments are well supplied with nitrogenous fertilisers and, ideally, free from weeds and pests or pathogens. Breeders have exploited some of the diversity available in wild barleys, including *H. spontaneum*, to identify and deploy new disease resistance genes. However, the wild species possesses considerable variation for other characters of economic importance and we are assessing its value as a source of variation for components of malting quality and salt tolerance as well as disease resistance. One problem encountered in previous attempts to use *H. spontaneum* as a source of new variation was the introduction of deleterious, weedy characteristics along with the desired one. More efficient exploitation of *H. spontaneum* in the production of new cultivars would follow from a better knowledge of the variation within the species. Modern genetic techniques allow the identification and precise introgression of new genes affecting important characters from the wild species thus avoiding problems caused by linkage with deleterious characters.

Malting quality Malting quality in barley is a complex trait associated with grain composition, germination and the production of hydrolytic enzymes.

Figure 2 Geographic localities of 10 populations of *Hordeum spontaneum*.
Collection sites:

10	Maalot	26	Caesarea
15	Eyzariya	27	Herzlujya
22	Mehola	28	Ashgelon
24	Akhzir	31	Ha Machtesh-Ha Gadol
25	Atlit	32	Ein-Zukim

Milling energy, a simple screening test for malting quality, is determined by a number of aspects of grain composition and thus, while not directly related to hot water extract, can give useful information on malting potential. The Comparamill measures milling energy by monitoring the reduction in the speed of rotation of a flywheel attached to a mill when grinding a grain sample.

At SCRI we have used the Comparamill to screen the seed of wild barley populations collected in Israel (Fig. 2) and multiplied at Haifa. Many populations had higher milling energies than some commercial barley cultivars (Fig. 3) but some had low milling energies,

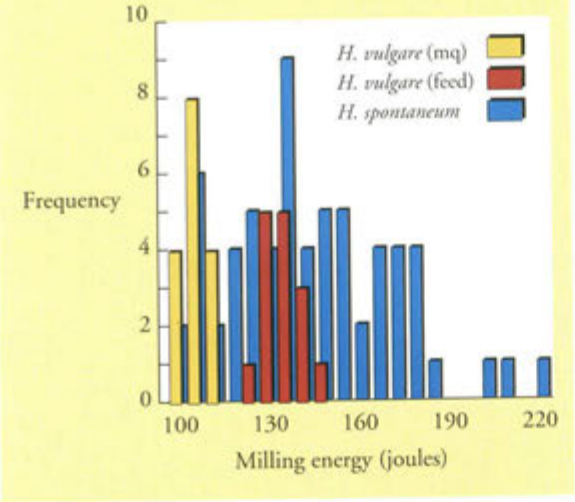

Figure 3 Milling energy of *H. spontaneum*.

particularly those collected from desert sites. As the seed that we analysed was the product of multiplication at one site, our results must reflect the effects of natural selection in the wild populations of *H. spontaneum*.

We found lines in some populations that had similar milling energies to malting cultivars, suggesting that pre-screening for this trait could be carried out before using *H. spontaneum* lines in a breeding programme to improve other characters. This is especially true when the high levels of milling energy in *H. spontaneum* are related to the toughness of the husk and lemma awn. Indeed, we have used cross prediction techniques to reveal that it is possible to derive low milling energy lines from crosses with *H. spontaneum*.

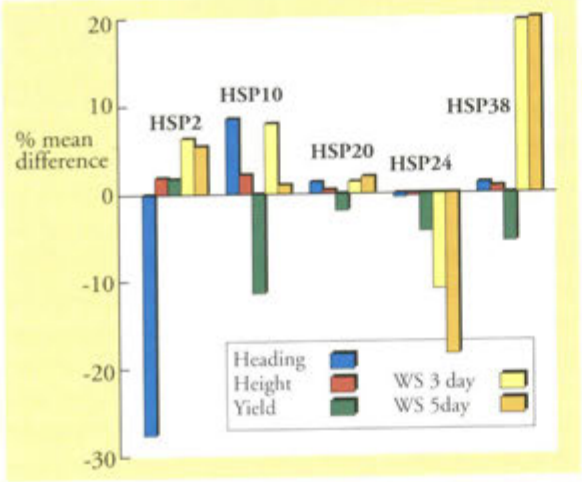

Figure 4 Effects of introducing resistance to mildew from *H. spontaneum* (HSP) into *H. vulgare* cv. Prisma.

Disease resistance. *H. spontaneum* is recognized as a rich source of disease resistance and one estimate indicated that 75% of wild collections contained resistance to powdery mildew, the most prevalent disease of barley in the UK. In addition, new gene loci for resistance to brown rust and *Rhynchosporium* have been found in *H. spontaneum*. At SCRI, we selected mildew resistant lines from a bulk population of *H. spontaneum* and crossed 38 of them to cv. Golden Promise. When segregation of their resistance genes was monitored, we found that resistance in 24 lines behaved as a single gene character. The single gene resistances were used in a backcrossing scheme with cv. Prisma as the recurrent parent and four backcrosses were made for 14 of the resistant genotypes. The F_3 progenies were tested to identify homozygous resistant and susceptible lines and then grown in a fungicide treated field trial at the F_4 generation. The estimated effects of each of the resistance genes from the differences in the means of the resistant and susceptible groups are shown in Figure 4.

We found eight of the resistance genes were associated with large effects upon water sensitivity, which hinders malting by slowing germination after steeping. Six of the resistant groups showed a poorer germination than the corresponding susceptible group, e.g. HSP38. In contrast, HSP24 showed a large, although not quite significant, increase in germination rate. We also found that some of the resistance genes affected heading date. In the HSP2 group, resistance was associated with a significant increase in heading date but in the HSP10 group it was associated with a reduction. The resistance genes had slight effects on the other characters, especially if the yield increases were discounted because they probably resulted from incomplete disease control.

We are still assessing the backcross lines for further characters but the results so far indicate the value of the approach. Sources of resistance have been found with little effect on other characters, e.g. HSP20, or with possible beneficial effects, e.g. HSP24, on water sensitivity. In general, however, our study has highlighted some deleterious associations which could be broken by suitable selection procedures during the production of commercial cultivars.

Salt tolerance The genetic diversity of *H. spontaneum* has also been exploited in research on salt tolerance. Chromosome 4 of *Hordeum* species is known to carry genes for salt tolerance, it also carries the gene *Vrn1* which controls spring or winter habit. *Vrn* genes

Figure 5 β-amylase phenotypes found at SCRI in *H. spontaneum.*

are of interest in salt tolerance as they are associated with effects on sodium uptake. We therefore examined the region of the barley genome around the *Vrn1* locus in an attempt to locate genes for salt tolerance. We required genetic markers close to *Vrn1* and chose the β-amylase gene (*Bmy1*) as it is located a short distance, 4 cM, from *Vrn1*. *H. spontaneum* shows wide variation for *Bmy1* and to date we have detected nine forms of this gene in accessions collected from Israel (Fig. 5). European cultivars in contrast have only two forms of the gene. *H. spontaneum* therefore shows greater variation than *H. vulgare* in this part of the genome which we hope to exploit in improving the salt tolerance of the crop. We have crossed *H. spontaneum* accessions which differ in this region of the genome with a salt sensitive barley cultivar as an initial investigation into its effects on salt tolerance. The crosses include three *H. spontaneum* lines which grow naturally in saline sites.

Our current studies of *H. spontaneum* when completed will provide a better picture of genome organisation in this species. Given the successful development of gene marker technology in other parts of the SCRI programme, we will be in a good position to characterise genes and identify those which would be beneficial to cultivated barley. We could then manipulate genes more effectively in germplasm enhancement and transfer just the target character without any deleterious characters.

Applications of biotechnology to soft fruit breeding

Julie Graham

Breeders of fruit crops have to overcome a number of obstacles during the production of improved cultivars. These include long generation times associated with extensive periods of juvenility, and highly heterozygous germplasm which requires the evaluation of large seedling populations.

Biotechnological strategies should significantly ease the production of improved new cultivars and should permit some developments that are currently impossible by conventional techniques. Tissue culture can be used to promote the rapid clonal multiplication of new cultivars, for the production of secondary metabolites in cell culture, and for *in vitro* screening of useful somaclonal variation; while transformation systems can be employed to incorporate horticulturally desirable genes into established cultivars, and to understand basic gene structure and function. Furthermore, the polymerase chain reaction (PCR) can be used for direct examination of the plant genome and has applications in variety identification, location of economically important genes and analysis of complex polygenic characters such as low temperature tolerance.

The application of these biotechnological techniques to soft fruit genetics at SCRI is described in this article.

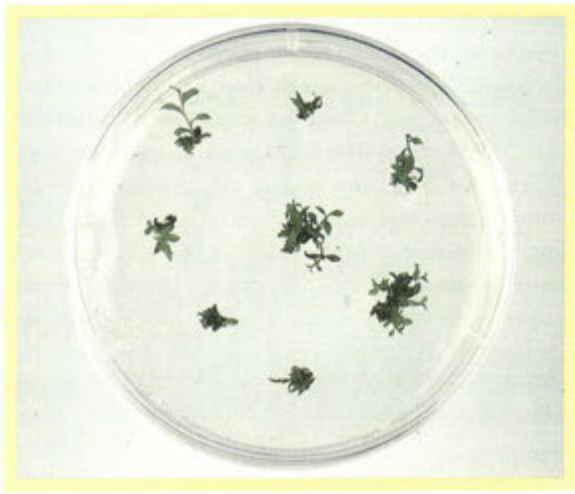

Figure 1 Regeneration of blueberry from leaf explants.

Figure 2 Inoculation of strawberry with CpTi and regeneration of whole plants on kanamycin medium.

Plant regeneration The development of efficient whole plant regeneration systems from small explants is a prerequisite for the application of a number of biotechnological approaches to crop improvement, including genetic transformation and *in vitro* selection. Regeneration can be achieved either through organogenesis or somatic embryogenesis and, in the case of soft fruit, the former pathway has been successful. Leaf and stem explants of *Rubus* and *Fragaria*, stem explants of *Ribes* and leaf explants of *Vaccinium* have all yielded regenerants and suitable media have been developed for the purpose (Fig. 1).

Transformation of potentially useful genes Conventional plant breeding techniques have been highly successful in horticulture and agriculture, but they are constrained by the extended timescale required, the limited ability to incorporate a specific character and the co-transfer of undesirable characteristics in a crossing programme. The techniques for transforming foreign genes into plants provides a means of introducing potentially useful genes from any closely or distantly related organism into improved germplasm without disturbing any other characteristic of the material. In soft fruit, we have successfully used the soil bacterium *Agrobacterium tumefaciens* to introduce marker genes into *Rubus, Ribes, Fragaria* and *Vaccinium* species (Figs. 2-4)

There are a number of potentially useful genes that we are attempting to introduce into soft fruit. The cowpea trypsin inhibitor gene(CpTi), isolated from *Vigna unguiculata*, encodes an insect digestive enzyme inhibitor which may control of a number of soft fruit pests including the raspberry beetle, strawberry weevil

Figure 3 Histochemical analysis of strawberry transformed with the GUS marker gene.

Figure 4 Trypsin assay on strawberry transformed with the cowpea protease trypsin inhibitor. The yellow colour which develops in the control reactions (1 and 4) is inhibited by transgenic plants (2 and 3).

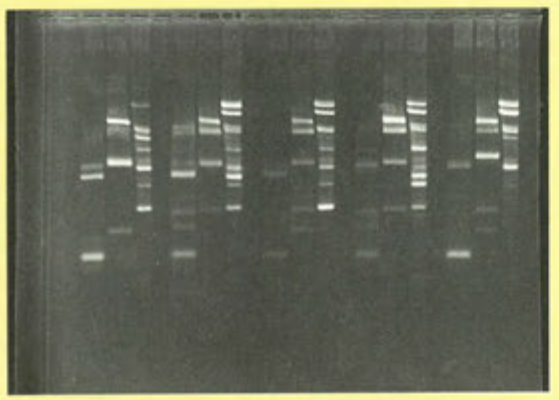

Figure 5 Fingerprints produced on 5 different red raspberry cultivars by 3 random primers.

and blackcurrant gall mite. These pests can cause significant crop loss and their control often requires high volumes of chemical sprays, some of which are applied close to harvest. By providing the plant with a genetic-based, self defence mechanism, the use of pesticides may be greatly reduced. Other useful genes include coat protein genes of arabis mosaic virus, a major problem in some common raspberry and strawberry cultivars, and raspberry bushy dwarf virus, a seriously debilitating disease, spread by pollen and seed. At present, virus diseases can be controlled by the use of resistant cultivars providing genetic sources of resistance are available or by eliminating the vector with pesticides. Virus coat protein genes inserted into model plants such as tobacco protect against the virus by a process analagous to the phenomenon of cross-protection whereby infection with a mild strain reduces severity of a later infection with a virulent strain.

Improved cultivars produced through the use of a transformation system may not be immediately acceptable because of the real or imagined risk associated with the co-transfer of antibiotic resistant selectable marker genes that are included to allow selection of regenerants. However, initial studies have shown that gene transfer can be accomplished in soft fruit without antibiotic selection suggesting that vectors can be used that do not contain antibiotic resistant sequences. Also, research into plant regeneration from tissue culture indicates that the majority of regenerants originate from single cells rather than from a few cells, making transformation in the absence of selectable markers possible in a wide range of plant species. It may even increase the transformation frequency as individual transformed cells may be killed off initially by the selective agent and, although large numbers of plants need to be screened, it can be accomplished with suitable marker genes.

The use of molecular markers in the genetic improvement of perennial fruit crops Inherited mutational changes that occur in DNA differ between individuals in a population and can be detected using RFLP or RAPD markers. The markers can be used to follow segregation of allelic forms of genes through a series of crosses, and linkage maps can be constructed of the markers and specific phenotypes with which they are correlated. Markers can tag desirable genes and can be positively selected for the trait of interest rather than relying on the phenotype.

Initially in *Rubus* the relatedness of ten red raspberry cultivars has been investigated using RAPD markers produced from ten random primers (Fig. 5). Similarity coefficients based on the primer bands were calculated based on the number of shared fragments and by using three or more primers with PCR, all cultivar patterns could be resolved by at least two bands.

Somaclonal variation When plants are regenerated from cultured cells, new phenotypes arise, sometimes at high frequencies, through somaclonal variation due to changes in chromosome number, chromosome rearrangements, alteration of gene copy number and DNA methylation. If the changes are heritable and produce desirable agronomic traits, somaclonal variation can be included in breeding programmes. Applying a stress *in vitro* can lead to the selection of somaclonal variation for a particular trait, e.g. to develop genotypes with improved disease resistance, toxins, the pathogen or its toxins can be included in the culture medium.

Biotechnology offers exciting opportunities for improving breeding efficiency in soft fruit and tree breeding programmes where the generation times are long.

Molecular biology

W. Powell

Transgenic biology, genome analysis, gene cloning and sequencing have become standard tools for experimental biologists. These technological methods have increased the range and efficiency of methods used to locate, identify and eventually exploit new plant genes. The accompanying articles provide examples of how these experimental tools are being deployed in our research to advance scientific knowledge in the areas of cell and molecular biology.

The main focus of the RNA processing group is to understand the mechanisms involved in intron removal. An analysis of RNA processing and pre-mRNA splicing is vital for a full understanding of plant gene expression. Research has concentrated on intron recognition and on the characterisation of the components of the plant spliceosome. A novel means of analysing gene expression based on reverse transcriptase-PCR (RT-PCR) has been developed and is being used to study the accuracy and efficiency of splicing in plants. Over 40 snRNA genes have been isolated and characterised from potato and maize and novel promoter elements have been identified in maize UsnRNAs and in potato U6snRNAs. The isolation of the potato U2B" gene represents the only plant spliceosomal protein gene to be isolated at present. The function of this RNA-binding protein is being analysed by the use of antisense technology and the creation of transgenic potato and tobacco plants. Fundamental knowledge emerging from this research is also relevant to applied biotechnology programmes. To this end, cloned snRNA genes are being modified for use as vectors to deliver gene inactivating molecules.

Research on the molecular basis of carbohydrate metabolism in potato is aimed at modulating levels of invertase activity in tubers in order to reduce hexose sugar accumulation during cold storage. Major findings include the cloning and characterisation of a fam-

ily of genes encoding apoplastic invertases (ß-fructofuranosidases). Transgenic plants are being created to examine the function of individual family members in sucrose metabolism and to determine their importance in low temperature sweetening. In addition, potato genes with homology to ß-glucanases are being examined and promoter analysis of these genes is being pursued. The genomic organisation of these genes is also being examined and several genes have been located to specific chromosomes on the potato linkage map.

Within the genome research group, considerable emphasis has been placed on PCR based, polymorphic assay procedures. Linkage maps of barley, faba bean and potato are at an advanced stage. Already molecular markers have been identified which are linked to traits of biological importance and these can be used in marker based selection programmes. RAPD products linked to genes controlling resistance to *Rhynchosporium secalis* in barley are being cloned and sequenced. Using this information, RAPD markers are being converted to Sequence Characterised Amplified Regions (SCARs) and may provide robust PCR based markers for plant breeding.

In theory, map based gene cloning approaches provide a route to clone any allele which is genetically mapped. However, the amount of effort (and luck!) required to implement map based gene cloning in agriculturally important crops is considerable. One of the requirements of such a procedure is to identify markers tightly linked to the trait of interest. Recently, population based methods have been devised to rapidly locate DNA markers to a specific region of the genome. Bulked segregant analysis and pooled DNA analysis have therefore been used to saturate specific regions of the potato and barley genome. Future research will focus on alternative PCR based detection methods such as simple sequence repeats (microsatellites) to maximise the number of informative loci for high resolution mapping and to facilitate fundamental studies of genome organisation. In addition to trait based mapping, genetic markers are providing new experimental tools for molecular taxonomic studies and a scientific framework for the characterisation, maintenance and cost-effective management of plant genetic resources. Studies of the molecular ecology of plant parasitic nematodes are being pursued. Two fundamental questions are being addressed, namely, how much variation and potential variation exists in various populations and what are the mechanisms by which changes in virulence occur? Several approaches are being used to assess the level of genetic diversity and the genomic organisation of particular gene families are being compared. For example, the intergenic regions of the tandemly repeated ribosomal genes (18S, 5.8S and 26S) of *Globodera pallida* are being studied. Restriction enzyme digestion of the PCR amplified product from this region have shown that different digestion patterns are produced from different pathotypes and populations from one of the pathotypes have mixtures of these different patterns. The results suggest that populations of this latter pathotype are mixtures of individuals from different genotypes or that the mixtures of repeat units of the ribosomal genes observed are the result of hybridisation of different genotypes. However, within the root-knot nematode, *Meloidogyne*, very little variation has been detected in the ribosomal genes. Mitochondrial DNA (mtDNA), which is inherited maternally, is being examined to determine if different lineages of PCN occur in Europe. Variation in the RFLP patterns of populations of *G. pallida* using a partially purified mitochondrial DNA probe has been observed. The presence of different lineages suggest that PCN has been introduced to Europe from South America on more than one occasion.

Amongst the repertoire of generic technologies available to plant scientists, the production of transgenic plants is now indispensable. For example, having cloned a gene, most methods of establishing gene function depend upon the creation of transgenic plants. In this capacity the role of the cell biology group is fundamental to much of our basic and strategic research. In addition to developing regenerative cell and tissue culture systems for temperate crops, emphasis has been placed on establishing protocols for tropical species. This aspect of our research is illustrated by the accompanying article on tissue culture response of groundnut (*Arachis hypogea* L.).

Research in the Cell and Molecular Genetics Department embraces many scientific disciplines. In order to continue to be successful, future research programmes will need to be focused and interactive. However, we are determined to ensure that our research is conducted within an international context and that our scientific links with leading laboratories in mainland Europe and the rest of the world are strengthened. Collaboration with institutes and scientists from developing countries will continue to be a priority providing a rational framework for training of scientists and technology transfer.

Genetic approaches to mapping genes conferring resistance to plant pathogens and pests

R. Waugh, N. Duncan, E. Baird, K. Chalmers, U. Barua, L. Hakim, B. Harrower, W.T.B. Thomas, M.S. Phillips & W. Powell

A significant portion of crop yield is lost each year through the actions of plant pests and diseases. A major challenge for plant breeders is therefore the identification of genes which increase levels of disease resistance within a crop species and the incorporation of these genes into adapted germplasm. Two types of plant disease resistance mechanisms are generally recognised - those conferred by single genes (major gene resistance) and those conferred by groups of genes or gene complexes (polygenic or partial resistance). While major gene resistances are generally effective against specific races of a pathogen, they are often easily overcome. Polygenic disease resistance on the other hand is more durable because a number of gene products interact to confer resistance. However, combining and manipulating different sources of resistance to a single pathogen is problematic and compounded by the fact that critical evaluation of disease resistance is generally quantitative. Furthermore, the precise contribution of specific resistance genes is only possible after the lengthy process of infection with different races of the pathogen. The identification of easily scorable genetic markers linked to individual disease resistance genes would be an invaluable tool for plant breeders for the development of marker based selection strategies and would provide the potential of combining different resistance genes in a single progeny (a concept known as gene pyramiding). In addition, markers linked to important disease resistance genes are the starting point for more strategic studies such as map-based cloning. In this report, different but complementary approaches used to locate both qualitative (major) and quantitative forms of resistance to plant pathogens and pests are described.

Leaf blotch of barley Leaf blotch, caused by the facultative fungus *Rhynchosporium secalis*, is an important disease of barley, particularly winter barley. A number of resistance genes have been identified and classified based on crossing and resistance testing of the progenies with different races of *Rhynchosporium*. By these criteria, a complex of major dominant resistance genes (*Rh*) were located to barley chromosome 3H. Other *Rh* resistance genes have been putatively located on other barley chromosomes. To identify markers linked to the major gene complex on chromosome 3, we initially screened a collection of four *Rh* resistant near isogenic lines (NILs) of barley, their donor and recurrent parents with 300 decamer oligonucleotide primers which detected 1500 RAPD loci. Near isogenic lines are the products of repeated cycles of backcrossing and selection, and theoretically contain only the selected genes from the donor parent and a limited amount of flanking DNA which is carried along through the backcrossing process by a phenomenon known as 'linkage drag'. Three of the four NILs were previously classified as possessing different *Rh* alleles on chromosome 3H while the fourth contained an *Rh* locus putatively located on chromosome 4H. One RAPD marker (SC10-65-H400) was consistently generated from the three NILs with *Rh* genes located on chromosome 3H but not from the line with an *Rh* gene on chromosome 4H (Fig. 1a). Formal proof that this marker was linked to the *Rh* locus was obtained by demonstrating its inheritance and co-segregation in a doubled haploid population generated from the F_1 of a cross between a *Rhynchosporium* resistant (E224/3) and a susceptible (Blenheim) barley genotype. This analysis indicated that the genetic map distance between SC10-65-H400 and the *Rh* locus was approximately 30 cM.

Because only one marker was identified in the NILs, to increase the number of markers flanking this *Rh* locus a second approach known as 'bulked segregant analysis' was employed using selected individuals from the Blenheim x E224/3 doubled haploid population. In this procedure, equal quantities of DNA from five resistant individuals which possessed the SC10-65-H400 marker and from five susceptible plants which did not possess the marker allele were combined to form a 'resistant' and a 'susceptible' bulk. The two bulked DNA samples were screened with 700 decamer primers. Seven products were identified which were present in one bulk but not in the other (Fig. 1b). Segregation analysis confirmed that all of these markers were linked to the *Rh* resistance locus segregating in the doubled haploid population (Fig. 1c).

To investigate the chromosomal location of the RAPD markers and *Rh*, a set of previously mapped

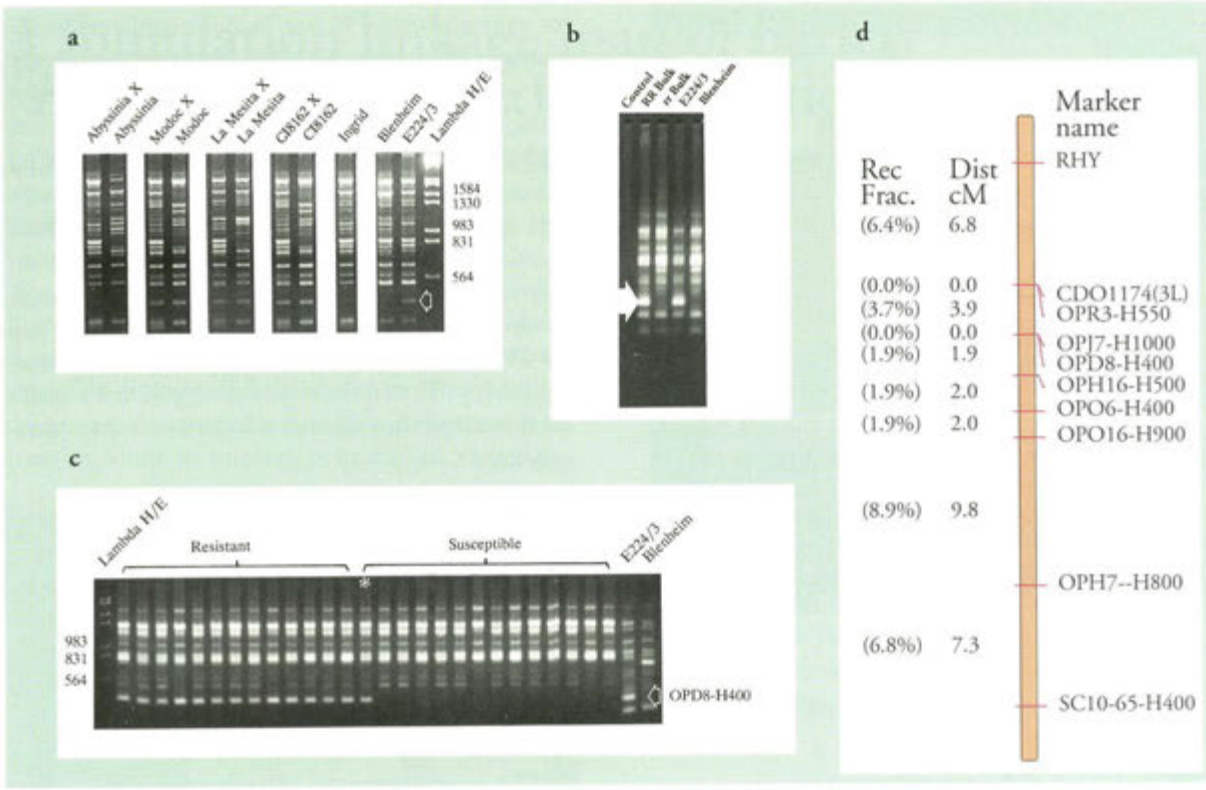

Figure 1 Markers linked to Rh genes were identified by a combination of approaches. Fig. 1a shows the amplification products generated when the 10-mer primer SC10-65 was used to generate RAPD markers from four different sets of near isogenic lines and the parents of a doubled haploid population which segregates for resistance to *Rhynchosporium secalis*. The arrowed band is absent from the recurrent parent (Ingrid) but present in three of the NILs, their resistant donors and the resistant (E223/4) parent of the doubled haploid population. Fig. 1b shows the products obtained by amplifying bulked DNA from five resistant and five susceptible doubled haploid individuals and the parental lines E224/3 and Blenheim with primer OPD8. The arrowed band amplified in the 'resistant' bulk is also amplified from the resistant parent. Segregation of this band in a selection of resistant and susceptible lines is shown in Fig. 1c. The starred track represents an individual in which recombination has occurred between the OPD8 derived marker and the Rh locus. In this example the presence or absence of the 400 bp amplification product (OPD8-H400) would lead to a correct classification of resistant or susceptible progeny from this cross 26 out of 27 times. Fig. 1d shows the linear order of all of the markers linked to Rh which we have identified by examining both the NILs and the 'bulks'. The closest marker, OPR3-H550 could be used to successfully identify resistant individuals approximately 94% of the time.

chromosome arm specific RFLP probes were used to screen the doubled haploid population. Using the MAPMAKER computer software, the RAPD markers identified by both approaches were linked to probes specific for the long arm of barley chromosome 3H. A local genetic linkage map of the region surrounding *Rh* is shown in Figure 1d. The markers which are genetically linked to *Rh* on chromosome 3H could now be used in marker assisted resistance selection. Using a similar approach we have also identified markers linked to a different *Rh* locus on chromosome 4H. Having markers linked to two distinct loci conferring resistance to *Rhynchosporium* will facilitate the combination of different *Rh* genes in a single genetic background by marker based selection. Isolation of markers linked to *Rh* genes in other regions of the genome will further enhance the possibility of gene pyramiding for *Rh* resistance in barley.

Nematode resistance in potato In potato, two major genes, H1 and H2 confer resistance to the potato cyst nematodes *Globodera rostochiensis* pathotypes Ro1 and Ro4 and *Globodera pallida* pathotype Pa1 (PCN). However they are ineffective against a number of other pathotypes (e.g. Ro2, Ro3, Ro5, Pa2/3) which are becoming increasingly important in European potato growing regions. These latter pathotypes are populations of individuals with quantitative rather than qualitative virulence characteristics and are differentiated by the number of cysts which they produce on a series of potato tester clones which exhibit differ-

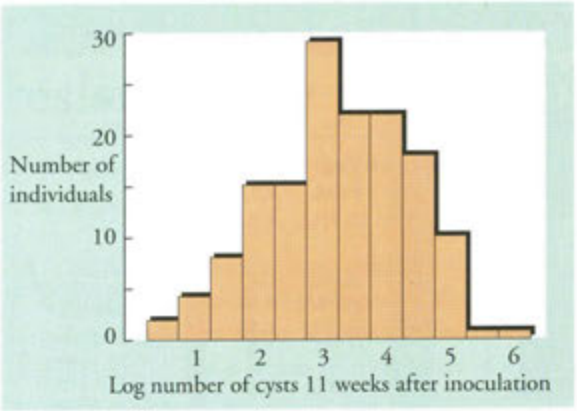

Figure 2 Frequency distribution of multiplication of *G. pallida* PA 2/3 on progeny from a cross between PDH7 & *S. vernei* a resistance class and the horizontal axis, the resistance classes. The overall shape of the graph illustrates the quantitative nature of resistance to PCN.

ent levels of resistance to PCN. We have initiated experiments to identify regions of the potato genome which contribute towards partial (polygenic) resistance to PCN.

Two different approaches have been pursued. Firstly, progeny from a cross between a susceptible diploid potato clone (PDH7) and a resistant diploid wild species (*S. vernei*) have been examined for resistance to PCN. A histogram of the average resistance scores is shown in Figure 2 and illustrates the quantitative inheritance pattern of PCN resistance in this progeny population. DNA isolated from a subset of the individuals from this population were screened with a series of potato chromosome arm specific markers (provided by Dr C. Gebhardt, MPI, Köln and Dr S. Tanksley, Cornell), previously unmapped RFLP markers and RAPDs. Segregation data was obtained for approximately 150 loci in this population and a number of chromosomal segments have been identified which contribute towards the expression of polygenic forms of resistance to PCN. To test these associations we have adopted a technique known as 'selective genotyping' in which only the 20 most resistant and 20 most susceptible individuals have been examined. The hypothesis is that the most resistant individuals will contain most or all of the positive *S. vernei* alleles which contribute towards resistance and the 20 most susceptible, a majority or all of the negative alleles. The results obtained suggest that a major component of the resistance is located on potato chromosome IX.

The second approach is based on the detection of 'rare' or 'exotic' alleles. The polygenic form of resistance to PCN is derived from *S. vernei* and has been introgressed into a restricted number of potato cultivars. The frequency with which alleles associated with PCN resistance will be detected in potato cultivars is therefore expected to be low. In order to test this hypothesis we have evaluated 54 potato cultivars with RAPDs in an attempt to identify exotic alleles. The RAPD profiles obtained with primer SC10-73 is shown in Figure 3. Three cultivars (Glenna, Morag and Nadine) possess an exotic allele and all have partial (polygenic) resistance to PCN. These cultivars are known to have *S. vernei* in their pedigrees. Further studies are required to conclusively demonstrate co-segregation of the informative allele with the expression of resistance to PCN. However, this experimental approach has the potential to expedite the identification of molecular markers linked to disease resistance gene(s) and is particularly relevant to alien gene introgression programmes.

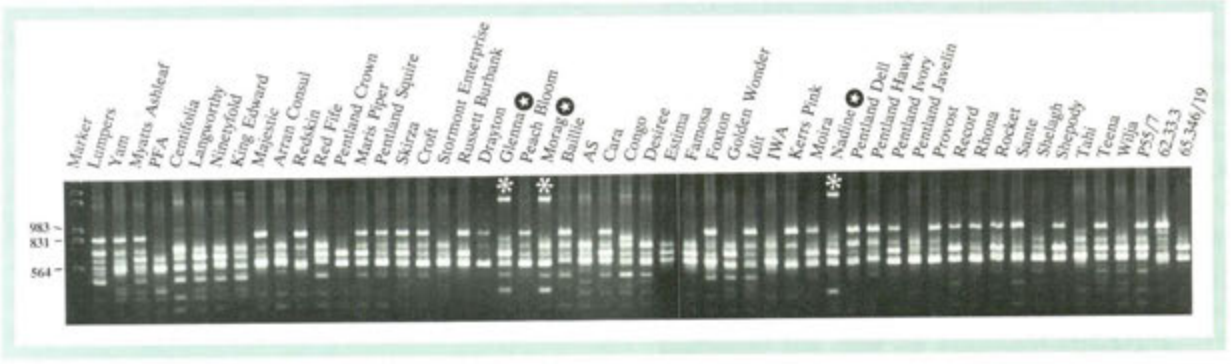

Figure 3 DNA from 54 tetraploid potato cultivars was isolated and surveyed at approximately 200 genetic loci with RAPD markers. In this example the starred tracks contain an 'exotic allele' which is not present in any of the other cultivars. These cultivars, Glenna, Morag and Nadine all express partial resistance to PCN which can be traced back in their pedigrees to an *S. vernei* accession. Formal proof that this band is linked to host genetic factors controlling the multiplication of PCN can only be obtained by segregation analysis on the relevant potato populations.

A foundation linkage map of barley (*Hordeum vulgare* L.) with particular reference to developmentally important genes

W. Powell, U.M. Barua, K.J. Chalmers, W.T.B. Thomas, C.A. Hackett, B.P. Forster & R. Waugh

Linkage maps are a fundamental biological resource for genetical analysis since they allow the phenotype of an organism to be related to its genotype. For this reason, the construction of linkage maps has been a major focus of human, mammalian and plant genome research. Techniques for map creation and genome analysis developed in one species can often be directly applied to another species. Thus, the molecular tools and concepts arising from the human genome mapping project are being rapidly adapted and

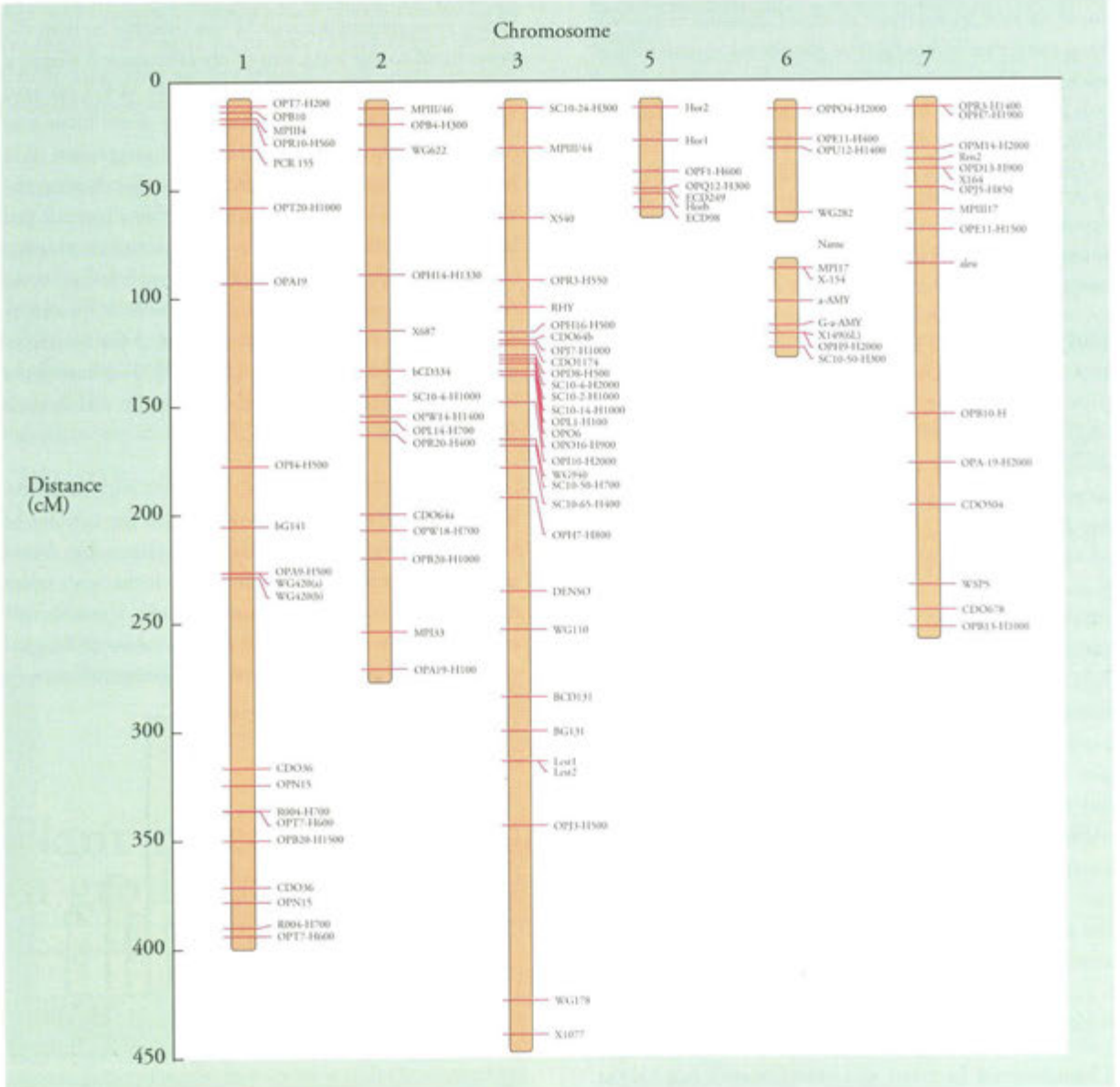

Figure 1 Current linkage map of barley at SCRI.

applied to plant genetic research. However, plants possess several attractive features which can facilitate both the creation and exploitation of genetic maps. These include tolerance of interspecific and indeed intergeneric hybridisation, availability of mutant phenotypes and several morphological markers, and large numbers of progeny from a single cross. Polyploidy which is far more common in the plant than animal kingdom has also allowed the creation and exploitation of aneuploid genetic stocks to rapidly locate genes to specific chromosomes. Plant biologists have also pioneered gamete based research to allow the regeneration of viable plants from microspores. This process of plant regeneration from microspore or anther culture represents a developmental switch from a gametophytic to a sporophytic mode of development. We have used this technology to produce a doubled haploid population for genetic mapping studies in barley.

The methodology and scientific rationale for the use of doubled haploids in plant genome studies has been reported previously (*Ann. Rep. 1991, 36*). In this report we focus on the production of a foundation linkage map of barley and its value in locating developmentally important genes.

Linkage map Genetic mapping is based on the co-segregation of alleles representing multiple polymorphic loci. The segregation of 130 morphological, isozyme, RFLP and RAPD loci was monitored in 59 doubled haploid genotypes derived from the F_1 of a cross between the barley cv. Blenheim and the breeding line E224/3. This data was analysed with MAPMAKER software and the current barley linkage map is shown in Figure 1. Total genome coverage is 1394 cM and six of the seven linkage groups have been assigned to chromosomes using chromosome specific RFLP markers. However, a further 40 markers remain unassigned and genome coverage is incomplete and clustering of markers is evident. For example, regions of chromosome 3 are well represented, but this reflects our attempts to saturate the genomic regions surrounding the *Rhynchosporium* resistance locus (*Ann. Rep. 1992*, p. 22). The mapping population is derived from adapted barley germplasm and the absence of markers on chromosome 4 and the relatively low number of markers on chromosomes 5 and 6 may reflect the lack of genetic variability in these regions of the barley genome.

Chromosomal location of genes determining height and loci controlling 'days to ear emergence' Detailed comparisons of primitive and modern barley cultivars have established that improvements in yield have largely been achieved by increasing resistance to lodging, reducing time to heading and incorporating disease resistance genes. Resistance to lodging and improved harvest index have been achieved through the introduction of genes for reduced plant height, and recessive alleles at the *denso* locus, which confer a semi-prostrate juvenile growth habit, have often been used to provide a source of short straw. The importance of this source of semi-dwarfism in barley breeding is reflected by the large number of cultivars that possess the *denso* gene, (74% of those grown in the UK possess this gene). The doubled haploid population used to create the barley linkage map segregates for alternative alleles at the *denso* dwarfing locus. Using co-segregant analysis, it was possible to map the *denso* locus to the long arm of chromosome 3 where it is located between RFLP markers WG110 and WG940. In this case, mapping the *denso* locus was possible because of its discrete segregation as a Mendelian character. The frequency distribution for height in the DH population is given in Figure 2 and is bimodal, corresponding to the presence or absence of the *denso* allele at the juvenile growth habit locus. A regression analysis indicated that over 80% of the variation in height could be accounted for by allelic variation at the *denso* locus (R^2 = 81.9%) but there was also considerable variation for height within each allelic class.

In order to identify and quantify other regions of the barley genome that may be involved in the control of height, a multiple regression approach has been employed. In addition to the *denso* locus, one other marker (OPL1-H1200) on chromosome 1 was shown to have a major effect on the expression of height.

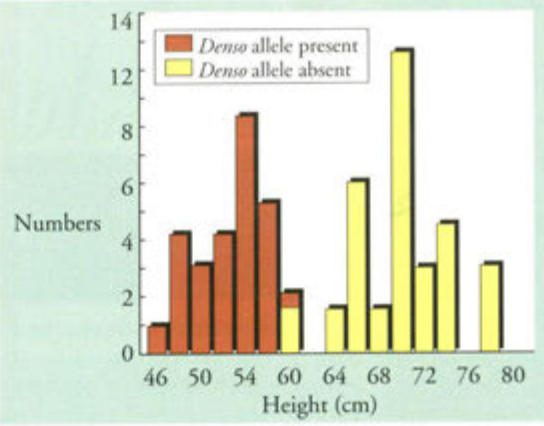

Figure 2 Frequency distribution for height in a doubled haploid population based on the presence or absence of the *denso* allele.

The best prediction of height (ht) was given by the following regression equation:-

$$ht = 56.3 + 14.7X_1 - 4.15X_2$$

where: X_1 and X_2 represent the *denso* locus on chromosome 3 and OPL1-H1200 on chromosome 1 respectively. The coefficient of determination was 86.0% indicating that the two markers account for a large proportion of the variation in height. The sizes and signs of the regression coefficients are also relevant. The absence of the *denso* allele in E224/3 on average increased height by 14.7 ± 1.08 cm whereas the E224/3 allele at the OPL1-H1200 locus on chromosome 1 (7H) decreased height by 4.2 ± 1.1 cm. Thus, in addition to the *denso* locus, there is at least one other region of the genome which controls the expression of height in barley. More importantly, E224/3 possess alleles which both increase and decrease height in barley.

The *denso* locus is also associated with days to ear emergence together with three other RAPD loci and one protein marker. The percentages of the phenotypic variation accounted for by allelic variation at these loci are given in Table 1. The protein locus, *Wsp5*, is linked to a RFLP probe (CDO 504) which is located on the long arm of chromosome 7 (Fig.1) whereas the RAPD markers are located on the long arm of chromosome 6 (Fig.1). The best multiple regression equation for days to heading is:-

$$HD = 22.0 - 1.67X_1 + 1.81X_2$$

where X_1 and X_2 represent the *denso* locus OPE11-H400 respectively. These two markers accounted for more than 60% of the variation in days to heading (R^2 = 60.3). Despite the fact that the *denso* locus is associated with lateness, there is at least one allele at another locus in Blenheim contributing to earliness.

Locus	Chromosomal location (L = long arm)	Percentage variation
OPU12-H1400	6(L)	24.9
OPE11-H400	6(L)	26.3
OPP04-H2000	6(L)	20.9
Wsp5	7(L)	13.0
Denso	3	41.5

Table 1 Percentage phenotypic variation associated with days to heading for the *denso*, *Wsp5* and three RAPD loci.

Conclusions and future research

1. Height and time to ear emergence are important development characters in barley and other members of the *Triticeae*. Major factors determining these traits have been located to specific regions of the barley genome.

2. At least two independent regions of the barley genome are involved in the control of height and the parental genotype E224/3 possesses genes which have both a positive and a negative effect. A similar genetical system exists for days to ear emergence.

3. Knowledge of the chromosomal location of genetic factors controlling height and days to ear emergence will allow greater precision in their manipulation in breeding programmes, providing a sound genetical framework for the deployment of dwarfing genes.

4. The establishment of the intrachromosomal location of important traits will allow saturation mapping of these regions using 'pooled DNA analysis'.

5. The available barley linkage map will allow other agronomic and quality related traits to be mapped.

6. Informative RAPD products linked to traits of importance will be cloned and the derived sequence used to develop robust PCR based assays.

Plant regeneration and transformation studies in groundnut (*Arachis hypogaea* L.)

S. Cooper-Bland, J. Watters & A. Kumar

Groundnut, *Arachis hypogaea* L., is the sixth most important crop in the world and is grown in many tropical and subtropical countries. It is an important source of oil seed, livestock forage and protein for human consumption, and provides a valuable export crop. Its cultivation improves soil fertility because atmospheric nitrogen is fixed by *Rhizobium* bacteria in the root nodules.

Figure 1 a) Multiple shoot bud regeneration from mature cotyledonary tissues of an Indian groundnut cultivar JL24. b) Multiple shoot development from buds derived from mature cotyledonary tissues.

Genotypes	Efficiency of plant regeneration: Mature cotyledons	Young leaflets
Indian		
JL24	60%	10%
TMV2	65%	12%
MK374	10%	0%
African		
Plover	85%	15%
IGGV 89323	82%	7%
55-437	49%	5%
Malimba	10%	0%
ICGS-11	8%	10%

Table 1 Plant regeneration from mature cotyledonary and young leaf tissues of African and Indian groundnut genotypes.

Conventional breeding has made a significant contribution to the improvement of groundnut cultivars. However, modern techniques of genetic manipulation are expected to play an increasing role in future groundnut improvement programmes. The ability to regenerate plants from cultured cells and tissues is a basic requirement for the exploitation of cellular and molecular techniques. This article describes the development of regeneration and transformation systems in groundnut.

Plant regeneration Studies on several legume crop plants, e.g. soybean, green and dry beans, and pea have shown that plant regeneration is possible from various explant tissues and in groundnut there are some reports of regeneration in a few genotypes. We have been developing efficient plant regeneration systems for Indian and African groundnut genotypes to use in *in vitro* genetic manipulation. Two explant sources have been used, namely mature de-embryonated cotyledon, and immature leaf. Table 1 shows the plant shoot regeneration response from mature de-embryonated cotyledons of 18 genotypes when cultured on Murashige and Skoog plant cell culture medium (MS30) with high cytokinin in the initial stage of culture followed by subculturing to a medium with a lowered cytokinin level. The results demonstrated that plant regeneration is highly influenced by genotype with Plover, TMV2, JL24, ICGV 89323 being amenable for transformation because they produced a high initial frequency of buds or shoots (Fig. 1). Rooting was observed in these experiments and is being optimised. Table 1 shows the frequency of determined bud and shoot regeneration from immature leaves which was also genotype dependent (Fig. 2). Again, many of the lines responded well, but some, e.g. 55-437, which responded well for cotyledon explants were not as responsive for leaves, while some lines, e.g. ICGS-11, were more responsive for leaves than cotyledons. The shoots produced are being rooted.

Genetic transformation There have been several recent reports of *Agrobacterium*-mediated transformation of leguminous plants, including soybean, green or dry bean and Faba bean. We have attempted to assess the *Agrobacterium*-mediated transformation method for groundnuts and initially used two virulent strains of *Agrobacterium tumefaciens*, C58 and A856, to assess their natural ability to infect wounded tissues and initiate tumours. Aseptically-grown seedlings of the Indian cultivar JL-24 were inoculated at wound sites on nodal tissues with *Agrobacterium* strains, and tumours were observed at the point of infection 3 weeks after inoculation (Fig. 3). The tumours were

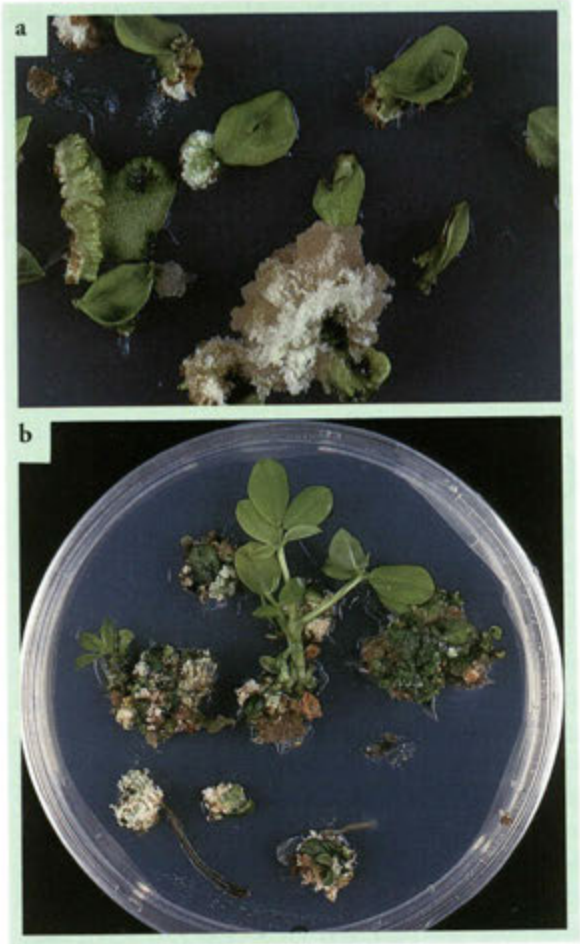

Figure 2 a) Callus development from young leaf tissues of an African cultivar Plover. b) Plant regeneration from callus derived of young leaf tissues.

excised from the plants and were freed from *Agrobacterium* contamination by repetitive subculturing on MS30 medium with 250 µg/ml cefotaxime antibiotic. The *Agrobacterium*-free tumour cells continued to grow on hormone-free medium for several months confirming their tumorigenic nature but attempts to regenerate transgenic plants from the calli were unsuccessful.

Another approach to obtaining transgenic plants employed a disarmed form of *A. tumefaciens* strain C58, possessing a binary vector, KIWI 105. The vector contains two marker genes, NPTII and GUS, under the control of the 35S promoter and NOS terminator. The expression of the GUS gene in this construct is negligible in *Agrobacterium*, making it ideal for studying expression of the gene in transgenic tissues and plants. Cotyledonary explants were inoculated with *Agrobacterium* C58 and GUS activity showed that the gene was expressed in the cotyledonary tissues

Figure 3 A typical crown gall tumour formation at the wound site of stem of a groundnut plant after inoculation with a wild strain of *Agrobacterium tumefaciens* C58.

48 h after inoculation and after 2 weeks in culture (Fig. 4). No GUS activity was observed in cotyledons that were cultured in the absence of *Agrobacterium* for 2 weeks. GUS fluorogenic and NPTII dot-blot assays confirmed the transgenic nature of calli and attempts are being made to regenerate plants from them. The sensitivity of JL-24 cotyledons to kanamycin and hygromycin was tested on a range of concentrations of the antibiotics. Kanamycin at 250 mg/l supported the formation of tiny buds from the cotyledons which did

Figure 4 GUS fluorogenic assay showing GUS gene expression in transgenic tissues of a groundnut cultivar Plover transformed with a disarmed strain of *A. tumefaciens* C58 carrying GUS genes as an identifiable marker.

not grow beyond the bud stage, while cotyledons were bleached and no regeneration was observed after 15-20 days culture on media with 20 mg/l hygromycin, suggesting that a hygromycin resistance gene may be better than a kanamycin resistance gene for the selection of transgenic tissues of groundnut.

We conclude from these experiments that *Agrobacterium* can be used to transform groundnut tissues and possibly to obtain transgenic plants. However, other transformation methods such as protoplast-mediated and the Biolistic gun-mediated methods are also being assessed. Our ultimate aim is to introduce agronomically important genes into cultivated groundnuts, including DNA complementary to the coat protein sequences of groundnut rosette assistor virus and Indian peanut clump virus, and the satellite sequences of groundnut rosette virus in the hope of producing transgenic groundnut plants with virus resistance properties.

This work is funded by the UK Overseas Development Administration in conjunction with the International Crop Research Institute for the Semi Arid Tropics (ICRISAT, India).

Removal of non-intron AU-rich sequences by splicing

C.G. Simpson & J.W.S. Brown

Successful intron removal by pre-mRNA splicing (*Ann. Rep. 1991*, 42-44) in dicotyledonous plants requires a 5' splice site, 3' splice site and AU-rich sequences. The importance of AU-rich sequences to plant intron splicing has been further highlighted by analysing the splicing of an AU-rich antisense intron sequence. By reversing the intron sequence in an expression construct, the complement of the intron sequence was transcribed. Although this sequence remained AU-rich by virtue of AT complementarity in the DNA, the authentic 5' and 3' splice sites were lost (Fig. 1). Nevertheless, an efficient splicing event involving the antisense intron sequence was observed and characterisation of the spliced product showed that 5' and 3' splice site sequences bordering the intron had been utilised. Thus, activation of cryptic splice sites on either side of the AU-rich sequence permitted the efficient removal of this essentially non-intron sequence (Fig. 1).

The ability of the plant's splicing machinery to remove non-intron sequences is an important consideration, not only in plant gene evolution, but also in genetic engineering strategies based on heterologous gene expression in plants. For example, the introduction of *Bacillus thuringiensis* (*Bt*) insecticidal toxin genes into transgenic plants has been a widely attempted strategy for genetically engineering insect resistance. However, the AT-rich *Bt* toxin genes are expressed at only low levels in transgenic plants and truncated polyadenylated transcripts have been observed[1]. These shorter transcripts were concluded to be a result of endo- and exonucleolytic mRNA degradation, but, based on the excision of an AU-rich antisense intron, the truncated transcripts may be the result of splicing events which excise regions of relatively AU-rich mRNA sequence. As a result the strategy of mutating the bacterial gene sequence to remove splice site sequences and reduce the AU content, without altering the amino acid sequence of the proteins has been applied to *Bt* toxin genes and other bacterial genes prior to introduction into transgenics. The demonstration that non-intron AU sequences can be spliced out of transcripts shows that while active transcription and some post-transcriptional processes can be assured by the utilisation of plant gene sequences, successful expression of these genes can be influenced by the foreign gene sequence itself which may affect the stability of the transcript and how it is recognised by the plant cell in terms of processing, transport and translation.

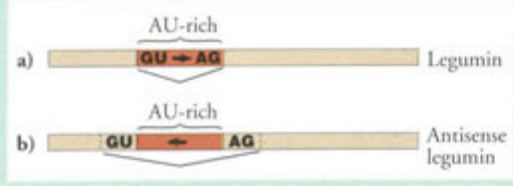

Figure 1 The legumin intron is removed at its authentic splice sites (a) and the AU-rich antisense sequence is removed by activation of cryptic -GU and AG- splice site sequences.

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An RNA helicase multigene family from potato

G. Clark, G. Asmar, P. Vaux, A.D. Turnbull-Ross, G.G. Simpson & J.W.S. Brown

RNA is involved in all aspects of gene expression, from gene transcription to the translation of proteins. As a single stranded nucleic acid, RNA is capable of forming defined structures through intermolecular base pairing interactions and such structures have been shown to be functionally important in, for example, transfer and ribosomal RNAs. In addition, RNA can associate with other DNA or RNA molecules intramolecularly and some essential post-transcriptional RNA processing events, such as pre-messenger RNA splicing (*Ann. Rep. 1991*, 42-44) and ribosomal RNA processing, are mediated by complex interactions with small nuclear RNAs (*Ann. Rep. 1990*, 28-30) or small nucleolar RNAs, and a vast array of protein molecules in the form of RNA-protein complexes. One important class of proteins which interact with RNA molecules, RNA helicases, has been identified relatively recently but is becoming increasingly well-documented in animal, yeast and bacterial systems. RNA helicases, which are related to DNA helicases and some viral proteins, catalyse alterations in RNA secondary structure and may have important regulatory roles in switching the conformation of complexes to allow assembly or reactions to occur. RNA helicases have already been shown to be involved in ribosome assembly, pre-mRNA splicing, translation and some aspects of development. Putative RNA helicases can be identified by the presence of a number of highly conserved amino acid motifs (Fig. 1). Although it is expected that a wide variety of RNA helicases with specificity for different RNA molecules and with different functions are present in the eukaryotic cell, the only plant RNA helicases to be isolated to date are homologues of the translation initiation factor, eIF4A, responsible for unwinding secondary structure at the 5' end of mRNAs to permit ribosome association and translation.

Three cDNA clones from potato which encode proteins containing several RNA helicase amino acid sequence motifs have now been isolated (Fig. 1). Two apparently full length clones (Heli 4 and Heli 6) would encode proteins of 62.5 kDa and differ in only 11 amino acids and in the length of their 3' untranslated regions. The third clone (Heli 9) is a partial cDNA missing 210 amino acids from its N-terminal end and is extremely similar in sequence to Heli 6, differing in only two amino acids. The minor changes in the conserved helicase motifs and the lack of extensive homology to other RNA helicases suggests that these genes encode a novel family of RNA helicases. Gene constructs for expression of these proteins in *E. coli* and for the expression of epitope-tagged proteins and antisense RNAs in transgenic plants are currently being made to analyse the function and cellular location of these putative RNA helicases. In addition these proteins contain a putative nuclear localisation signal which would conduct them into the plant nucleus and this important nuclear transport signal is under investigation. The largely neglected area of post-transcriptional regulation of gene expression in plants is now being addressed by research into two major classes of RNA-interacting proteins: RNA-binding proteins, containing RNP-80 motifs (*Ann. Rep. 1990*, 28-30) and RNA helicases.

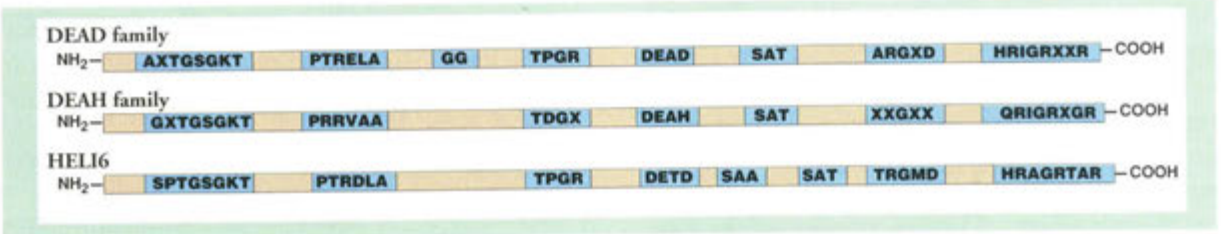

Figure 1 Schematic representation of conserved amino acid motifs in the two main subfamilies of RNA helicases (DEAD and DEAH) and the potato RNA helicase isolated at SCRI (HELI 6).

Cellular and environmental physiology

H.V. Davies

The past year has seen a significant increase in collaborative research both within the Institute and externally. This has been assisted through additional funding obtained, for a wide range of topics, from Industry, the European Commission, Scottish Office Agriculture and Fisheries Department, Research Councils and levy boards. The Department of Cellular and Environmental Physiology continues to place a substantial emphasis on the more strategic aspects of plant, soil and environmental research and to provide new insights into biological organisation and control through initiatives in mathematical biology.

In cell physiology, work on plant transport systems continues to break new ground in our understanding of compartmentation and cell-cell communication. An oat aleurone system has been used to quantify the uptake of a range of fluorescent xenobiotics into plant cell vacuoles and to study the developmental regulation of xenobiotic sequestration. Oat aleurone protoplasts possess two xenobiotic transport mechanisms, one on the plasmalemma and a second on the tonoplast. The functioning of the two mechanisms is temporally, as well as spatially, regulated and they show several similarities with the animal macrophage system. Both the plasmalemma and tonoplast transporters are sensitive to the drug probenecid, a potent anion transport inhibitor in mammalian cells. Also, as in the macrophage sequestration system, the plant transporters are competitively inhibited by antibiotics such as Penicillin G. In addition to the effect probenecid has on the uptake and sequestration of fluorescent dyes in plant cells, it also causes reversible vesiculation within the cytoplasm due to fragmentation of the vacuole membrane. Components of the cytoskeleton are also affected by probenecid.

Micro-injection of plant cells with fluorescent proteins and aldehyde-fixable fluorescent dextrans continues to be used to study the role and function of plasmodesmatal connections in the sieve element-companion cell (SC-CC) complex. In collaboration

with the Universities of Utrecht and Aberdeen, dextrans and proteins were shown to be capable of moving freely between the sieve element and companion cell, providing the first evidence that plasmodesmata at this interface have a molecular weight exclusion limit of at least 3000. Previously, 800 was cited as the upper exclusion limit of higher plant plasmodesmata. This supports the hypothesis that the companion cell may play an important role in maintaining the enucleate sieve element by synthesising and supplying it with the proteins necessary for its continued functioning. Plasmodesmatal functioning will be the subject of a review in the next Annual Report.

Novel nuclear magnetic resonance techniques have been developed to determine the specific activities of labelled substrates in metabolic pools, allowing the quantification of metabolic fluxes *in vivo*. A mathematical model of the metabolic conversion between sucrose and triose-P has been produced so that the extent of metabolite recycling and "futile cycles" during sucrose metabolism can now be quantified. In collaboration with others, the effects of manipulating the activities of key glycolytic enzymes in transgenic plants has been studied. The NMR approach will be supported by new HPLC techniques developed to separate hexose phosphates and sugar nucleotides. The latter have been used to investigate carbon fluxes through the pools of hexose-P and UDPglucose in a range of plant materials. Potato continues to be the chosen model system for studies on carbohydrate metabolism and new avenues are being explored to examine metabolic control. For example, stolon tips undergoing tuberisation rapidly switch from an invertase dominated pathway of sucrose degradation to one dominated by sucrose synthase and fructokinase. This is accompanied by substantial changes in the sugar balance and of the stolon's starch content. The presence of an alkaline invertase in the stolon tip has been demonstrated convincingly, but synthesis of the enzyme appears short lived during tuber development. It is never a dominant invertase enzyme. Work with antibodies raised against different classes of acid invertase proteins indicates that expression of the genes may be differentially regulated during tuber development and in the post-harvest storage period.

The programme on the molecular basis of tuberisation continues to develop (see p. 44) and will benefit from the appointment of additional staff funded through the EC Biotechnology initiative. Similarly, the identification of significant changes in gene expression during dormancy break in seeds of Douglas fir has been followed by the preparation of a cDNA library and a differential screening exercise to isolate ca. 15 different clones that are currently under scrutiny.

A programme has been initiated with the Soft Fruit Genetics Department on ripening processes in soft fruit, particularly in blackcurrant. Two dimensional electrophoresis of polypeptides and *in vitro* translation products have revealed significant quantitative changes in gene expression during fruit ripening and differential screening has been used to isolate ripening-related genes. The synthesis of the first blackcurrant fruit cDNA library has been a major achievement, given the difficulty of working with material inherently low in RNA (ca. 15 μg total RNA g^{-1} fresh weight) but high in components interfering with the isolation and purification protocol.

A new initiative on the use of stable isotopes to unravel the complexities of soil-plant-environment interactions was announced in the last report and has concentrated on studies on the temporal and spatial fate of nitrogen accessed by plant communities. New methods to analyse small, dilute samples of inorganic and organic nitrogen have had to be developed. Facilities are now available to measure gas samples on an ecological scale and the system is currently used to study microbial respiration and root turnover. A novel technique using ^{18}O-labelled water has also been developed to identify the capacity of defined parts of the plant root system to capture water resources and will be used together with analyses of ^{15}N to examine nitrate and water uptake simultaneously. The increasing use of non-invasive measurements of root activity (e.g. using stable isotopes) reflects the inadequacy of traditional destructive methods to provide unequivocal data at fine resolution.

A means to quantify simultaneously and continuously, the force exerted by roots and their elongation rate has been developed and permits, for the first time, measurements of how roots respond subtly to changes in mechanical impedance. Work on mechanical impedance is integrated with programmes on soil structure and is the subject of a review on p. 41 Mathematical biology plays an important role in helping to define the biological and physical constraints on transport processes in structured soil. For example, a geometric framework has been

developed linking soil diffusive properties across scale lengths relevant to microbial activity and a relationship between soil tortuosity and heterogeneity and soil diffusive properties has been derived.

In an important under-researched area, the organisational nature of mycelium in fungal colonies has been studied and a new theory of morphogenesis is being formulated to further our knowledge of the effect of soil environment on colony formation. Fungi show differential responses in colony expansion rate, the formation of reproductive structures and the translocation of nutrients between spatially discrete resources. Soil microbiologists have also revealed that qualitative and quantitative analyses of volatile components derived from soil bacteria have potential for mapping bacterial populations and their physiological activity. They have further shown the potential for using DNA hybridisation techniques to determine changes in microbial community structure, adding to the approaches with which to tackle a technically challenging problem. Studies on the spatio-temporal variation of nematode and protozoan grazers of microbes have revealed the importance of organic residues in the soil in modifying the number and activities of microbial flora and fauna. The effects of soil architecture and water relations are now being considered to integrate the relationships between soil, plants and microorganisms. Additional studies in mathematical biology includes work on the theory of disease spread to identify strategies for environmentally benign methods of disease control. Emphasis is placed on the influence of spatial arrangement of host plants on the impact of disease. The establishment of the Centre for Non-linear Systems in Biology within the Department has played a key role in attracting additional funds for research on microbial movement, disease epidemiology and the stability of metabolic pathways in plant cells and has brought together scientists from a wide range of disciplines.

In environmental physiology, the effect of prolonged exposure to chilling on growth and photosynthetic performance of three blackcurrant cultivars has been examined. Chilling reduced both the rate of leaf appearance and leaf expansion and, in some cases, delayed bud break. Base temperatures for leaf appearance and growth differed between processes and between chilling-tolerant compared with chilling sensitive genotypes. Photosynthetic rate (measured at 15°C and 5% CO_2) was unaffected by chilling but there were significant effects on stomatal conductance and photoinhibition. The effect of chilling on stomatal conductance was genotype dependent. This work will be developed further through the joint supervision of a MAFF-funded PhD studentship with Professor G Dixon, SAC, Auchincruive.

An existing simulation model has been used to compare different strategies of drought tolerance in potato. The model predicts that improving the relation between leaf expansion and soil moisture status has the greatest effect on tuber yield. Simulating an increase in either rooting depth or water use efficiency does not improve yields except under severe drought cycles. Whilst increasing the coefficient for the conversion of intercepted radiation into dry matter (C) increases yields under fully irrigated conditions, the effect diminishes with increasing severity of drought. Improving the relation between C and soil moisture status has no effect on yield compared with the standard.

In a comparison of the growing environment imposed by three plastic mulching materials used on potato crops, one material increased soil and air temperatures but reduced available photosynthetically active radiation (PAR) compared with the other materials used. Simulation modelling revealed that higher dry matter production at higher temperatures was offset by reduced interception of solar radiation soon after emergence. Two small scale experiments planted as a 'normal' and a second crop confirmed the physical parameters and the ranking of the simulated results. They also revealed that the highest temperature achieved under one mulch was damaging.

In the weed ecology programme, seedbank assessments were made 2-3 years after the start of a series of set-aside experiments conducted by ADAS, IACR and SAC designed to evaluate strategies for managing set-aside fields due to be returned to arable cropping after 3-5 years. The results showed large increases in the populations of many arable weed species and greater species diversity on plots where natural regeneration had been permitted. Sowing a grass cover crop considerably reduced seedbank expansion. In addition, funding has been gained to monitor seedbank changes in the ADAS TALISMAN experimental series. Joint work with the Crop Genetics Department has also been initiated on the origins and ecology of feral oilseed rape populations.

Comparisons of various strategies for reducing herbicides in cereal rotations at nine sites across UK

managed by ADAS, SAC and DANI have completed their fifth year. All herbicide programmes reduced weed seedbanks and maintained crop yields and broad spectrum herbicides applied at half normal rate, every year provided more sustainable weed control, at lower cost and with less risk than the use of spray thresholds to reduce the frequency of full-rate treatments. Seedbanks of untreated plots increase in the soil leading to substantial reductions in cereal yields after a few years.

Sodium monochloroacetate was given off-label approval as a raspberry cane desiccant in time for commercial use by growers, while fomesafen also continued to perform well. Calcium cyanamide failed to give adequate desiccation and will not be evaluated further.

The route to structure

I.M. Young & A.G. Bengough

Soil biophysics research at SCRI concentrates on the relations between the physical nature of soil and the biological processes occurring within it. Emphasis is placed on multidisciplinary research bringing together soil scientists, microbiologists, plant physiologists and mathematicians from within the Soil-Plant Dynamics Group and the work can be broadly divided into two areas, a) soil structure and b) root growth.

Soil structure Whilst relations clearly exist between soil structure and most soil processes, experimental results have been imprecise and qualitative because of the difficulties in quantifying soil structure. The problem has been to quantify specific structural parameters rather than rely on mere description, so that process mechanisms can be elucidated and predictions made. The lack of any available theory which adequately deals with the inherent structural heterogeneity of soil has led to a position of ignoring variability in favour of simple homogenised systems. Whilst this assumption allows the development of usable equations relating to the transport of substances through soil, e.g. Darcy and Fick's Laws, it has also meant that many of the derived equations are inappropriate to the field where temporal and spatial heterogeneity dominate and control microbial dynamics, gaseous and fluid flow, and plant development.

However, as with any theoretical framework, it is easy to identify the problems, but more difficult to provide an adequate solution. The scale of the problem is illustrated in Figure 1, and the answer resides in how to quantify the complex geometries present within soil, relating quantification to specific function. In this example there is significant physical heterogeneity in the size and shape of aggregates and pores. Of the several specific structural parameters that would be quantified, two have been given attention; structural heterogeneity, and pore morphology, both of which have been implicated as major influences of soil processes. *Functional quantification* of these parameters is the ultimate aim of the work at SCRI.

In any system the ease with which it can be explored has obvious importance. Examples in soil include protozoa and nematodes searching for food within and between aggregates, gas molecules diffusing away from decomposing organic matter in pore space, and water percolating through pores. *Heterogeneity* can be

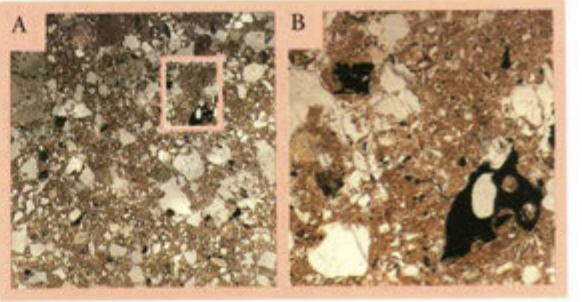

Figure 1 A, thin section of a soil aggregate. B, Higher magnification of enclosed area.

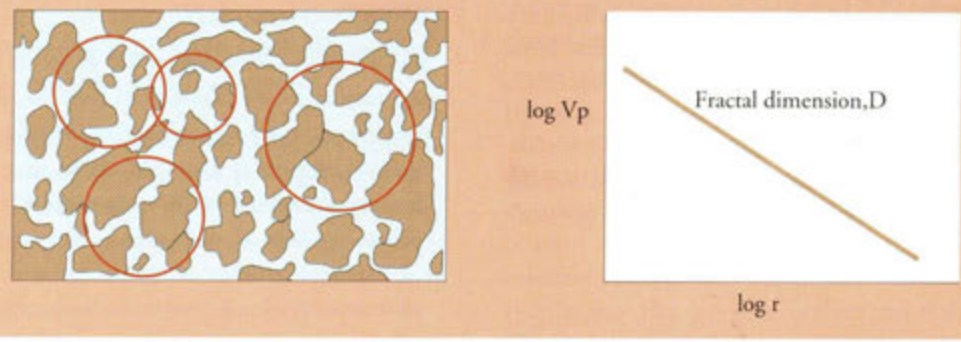

Figure 2 Calculation of the fractal dimension.

considered as introducing structural diversity that can vary over short distances and times in soil. In this situation, an average measure of structure would be redundant, since no one value would be representative of soil over all scales, and no causal link with function could be possible. Whilst heterogeneity indicates how variable or clumpy the structure is, knowledge of pore morphology is required to complete an adequate structural representation which could be of use in relation to soil processes. *Pore morphology* can be regarded as a measure of pore wall roughness and the path taken by a gas molecule diffusing in connected pore space. The use of fractal geometry allows functional quantification of these parameters.

Fractal mathematics is best defined as the geometry of complex structures. Whilst Euclidean geometry adequately deals with simple objects such as straight lines, circles and spheres, the geometry of rugged lines and heterogeneous soil aggregates are beyond its powers. Fractal geometry allows the quantification of such heterogeneous objects to be approached by fractional values of their Euclidean dimension.

One of the main characteristics of fractals is that new structure is revealed at higher resolutions, thus estimation of volume, area, or length must necessarily depend on the scale length of measurement. In real life there are upper and lower cut-offs, related to the sizes of the object and the smallest building block, respectively, beyond which this ceases to be true. In Figure 1, it is clear that new information is revealed concerning structural morphology at a higher magnification . Therefore, the answers to questions about surface area or porosity of soil will, if the soil is fractal, depend on the degree of magnification used for measurement. A simple example of the basic importance of fractal geometry to physical phenomena is found in the relation between aggregate mass, M, and radius, R, of a structure (e.g. a soil monolith)

$$M(R) = kR^{D} \quad (1)$$

where k is the constant of proportionality, and D is the fractal dimension. In the case of a fractal structure ($2 < D < 3$) density decreases with increasing size. For a non-fractal object $D = 3$ and density remains constant with size. Since numerous studies have related bulk density to many basic soil processes e.g. root growth, microbial dynamics, gaseous and fluid flow, the nature of the variance of parameters such as density have fundamental importance in soil studies. Figure 2 illustrates the calculation of the fractal dimension. Using images from soil-thin sections D is calculated for pore networks by counting the number, Vp, of pixels in a pore space within radius r. To relate this to equation (1) substitute Vp for M, and r for R. A linear fit through a plot of *log*Vp(r) vs. *log*r yields D. For a two dimensional thin section where $D \rightarrow 2$ a relatively uniform, non-fractal, distribution of pore space is indicated. When $1<D<2$ a heterogeneous fractal distribution is present. Typical values of D from a wide range of soil types from different countries reveal values in the range of 1.6-1.9. Assuming isotropy at the scales examined, values of D can be extrapolated to three dimensions by adding 1. Now that distribution of the pore space can be quantified, an assessment of the morphology of that space is required to complete the picture of soil structure. This is obtained through a companion measurement of the fractal dimension, the *spectral dimension*, which in qualitative terms may be related to pore tortuosity. Calculation of the spectral dimension is carried out on digital images of the pore space from soil-thin sections. A Monte-Carlo simulation is carried out for a randomly walking particle with each pixel acting as a potential site. The number of distinct sites visited,

S_{Vp}, as a function of steps taken, Vp, to visit those sites is recorded, and the spectral dimension d, is the resultant gradient of a plot of $\log S_{Vp}$ vs $\log$Vp.

The connection between the two dimensions and gaseous diffusion can be derived and is revealed in the relation

$$D(r) \sim r^{-\Theta} \quad (3)$$

where,

$$\Theta = 2(D-d)/d \quad (4)$$

$D(r)$ relates to the diffusion coefficient. The remarkable thing to note is that in equation (3) the constant diffusion coefficient, as used in Fick's Law, has been replaced by a scale dependent diffusion coefficient, the magnitude of which is directly related to the soil structural parameters of D and d. Thus for the first time an explicit quantitative link has been derived between gaseous flow and soil structure.

This relation allows us to explore the individual effects of structural parameters on gaseous flow. Soils with different D and d values were examined and the results showed that D and d had competing effects on flow, whereby increases in tortuosity, denoted by increases in $d(d \rightarrow 3)$ retarded flow; whilst increases in heterogeneity, denoted by decreases in $D(D \rightarrow 2)$ increased flow. The role of d in retarding flow is clear, since an increase in pore tortuosity retards forward movement. The role of D is slightly more complicated and involves the change from a homogeneous pore network ($D = 3$), where the pore space per unit volume remains constant, to a fractal network where the pore space per, unit volume increases with distance from the particles origin.

The fact that fractal geometry permits the functional quantification of soil structure offers soil scientists a process based framework for the examination of soil structure. Other research concentrates on the relation between soil structure and microbial dynamics. Whilst some problems do exist with the assumption of isotropy and the constancy of D over size scales, our research is developing to overcome these.

Root growth Crop growth and yield depend on the ability of a plant to obtain sufficient water and nutrients, which in turn requires the development of an adequate root system. In a moist field soil in the UK, soil strength may be sufficient to reduce the potential root growth rate by 30% or more and soil compaction caused, for example, by machinery and animals reduces crop yields. A collaborative experiment with SCAE showed that soil compaction

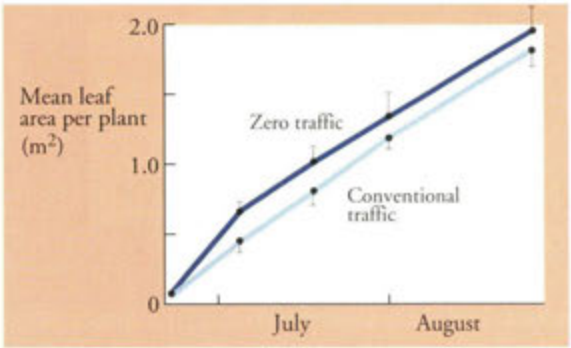

Figure 3 Potato leaf expansion can be slowed by soil compaction.

caused by vehicle traffic reduced the yield of a potato crop by reducing the rate of leaf expansion (Fig. 3). The amount of light intercepted by the crop canopy was decreased and associated with a reduced dry matter production and a 19% reduction in yield compared with zero traffic plots.

The effects of soil compaction on root growth can be due to a combination of factors including poor aeration, e.g. in compacted wet soil, and increased soil strength. Research has been concentrated recently on the effects of soil strength on restricting root growth by creating a high mechanical impedance to elongation. Soil strength can increase by an order of magnitude as soil dries and this information, when combined into models of root growth, can be used to determine the effects of soil drying on root distribution. Under conditions of high evaporative demand, soil water can be drawn upwards from below the deepest roots increasing the soil strength and limiting the maximum rooting depth achieved by a crop (Fig. 4). This work has demonstrated the importance of incorporating soil strength into models

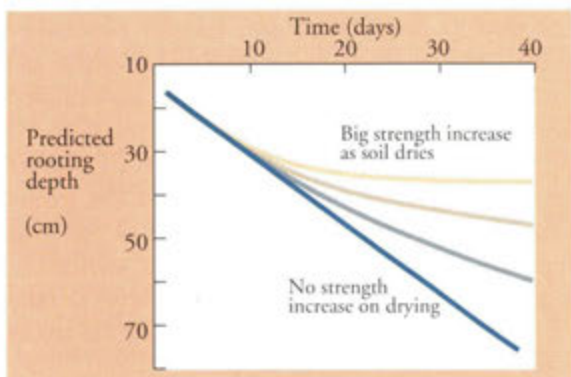

Figure 4 Soil drying can restrict the maximum rooting depth by increasing soil strength.

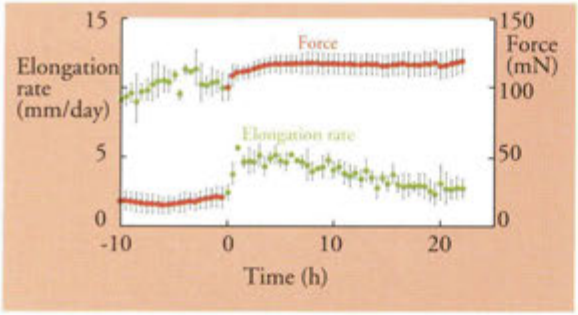

Figure 5 Increasing mechanical resistance to a seedling root causes an immediate reduction in the elongation rate.

of crop root growth so that better predictions of yield can be made.

To successfully model root growth in the field, it is necessary to understand how roots respond to changes in mechanical impedance that occur when roots encounter cracks or zones of compact soil. Experiments were performed in columns of soil with layers of different penetration resistance and the results showed that roots that have grown through compact soil may have a slower elongation rate for several days after they emerge into loose soil. To determine the changes in root growth rate that occur within minutes or hours of encountering a change in impedance, a new technique was developed that allows root elongation rate and axial resistance to root growth to be measured simultaneously. Sensors monitored changes in the force exerted by a root tip and its elongation rate as it grew inside a glass tube. Root elongation slowed immediately when the force on the root tip increased, and was followed by more than 10 h when the growth rate continued to decrease (Fig. 5). The opposite occurred when the force on the root tip was decreased, although the size of the immediate increase in growth rate was rather smaller. The study of root responses to impedance is valuable both in the context of predicting root behaviour under changing soil physical conditions, and in gaining and understanding of the basic processes controlling root growth.

The molecular basis of tuberisation in potato

M.A. Taylor, S.A. Mad Arif, H.V. Davies, L.A. George & A. Kumar

The potato is the fourth most important food crop in the World. providing a valuable source of essential vitamins such as ascorbic acid and an abundant supply of energy in the form of starch. Indeed, the potato has become a model system for research on carbohydrate metabolism, particularly as the plant is amenable to gene transfer technology using *Agrobacterium* as a vector. Commercially, the market for potatoes is becoming more sophisticated and producers will be required to meet the demands of processors as well as those of retail outlets requiring high quality produce in specific size grades. On the other hand, seed producers look for large numbers of high quality tubers within a smaller size grade distribution. Those involved in micro- or mini tubers will be concerned with accelerating the rate of tuber production (*Ann Rep 1990, 45*).

Tubers develop initially from division in random planes and enlargement of pith cells of the stolon tip, followed rapidly by cell divisions in the stolon tip and in most parenchyma cells, particularly those associated with the inner phloem and cortical region. These divisions probably cease early in tuber development when the tuber has reached 30-40 g fresh weight. The final tuber size is determined predominantly by further increase in cell volume in the parenchyma associated with the inner phloem.

There is clearly a need for research aimed at manipulating the timing of tuberisation, the numbers of tubers initiated and hence final tuber size. Substantial amounts of data have been accumulated on the effects of nutrition, agronomic practice, daylength, hormone treatments and other features on tuber initiation but there is very little information on the cascade of events which result in (a) an inhibition of stolon growth at a relatively well defined stage in the life cycle and (b) the "switch" in the planes of cell division in the sub-apical region of the tuber which result in stolon swelling and tuber formation.

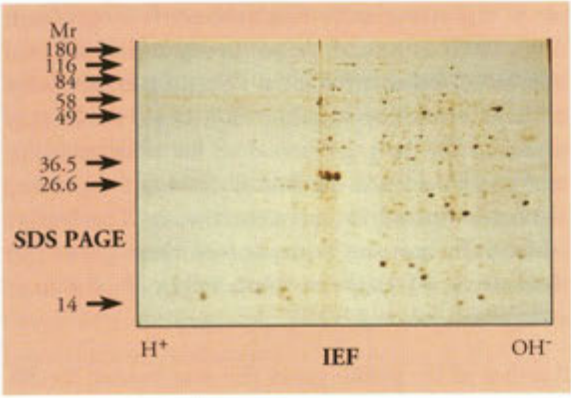

Figure 1 Typical 2 dimensional SDS/IEF PAGE separation of stolon tip polypeptides.

It is well established that changes in gene expression occur during tuber formation but work has concentrated principally on genes encoding the major storage protein patatin and protease inhibitors. However the genes involved in tuber formation *per se* have not been identified. As a first step towards achieving this goal at SCRI, changes in gene expression that occur during the early stages of tuberisation have been characterised in detail.

Analysis by two-dimensional electrophoresis Initially, the profile of polypeptides extracted from stolon tips was characterised by two dimensional SDS/IEF polyacrylamide gel electrophoresis and a number of quantitative and qualitative changes in gene expression were detected (Fig. 1). Analysis of the products of *in vitro* translation of RNA extracted from stolon tips confirmed that changes in gene expression were occuring in the very early stages of tuberisation. Using monoclonal antibodies raised against rat α-tubulin, two dimensional immunoblots revealed that different isotypes were expressed at the onset of tuberisation (Fig. 2). The tubulins are the major microtubule proteins which polymerise to form the cytoskeleton, essential for a variety of basic cellular functions. Distinctive microtubule arrays are formed during cell morphogenesis. The cortical array influences wall growth during interphase, the pre-prophase band defines the plane of cell division, the spindle is involved in daughter chromosome separation in mitosis and the phragmoplast affects the formation of the new cross wall during cell separation. It is likely, therefore, that microtubule organisation plays an important role in the onset of tuberisation when changes in division and growth pattern occur. It is further possible that specific tubulin isotypes have specific roles in development and the isotype that appears

Figure 2 Immunoblot of a 2D separation of stolon tip polypeptides using an α-tubulin monoclonal antibody. Panels A-D, stolon tips at different stages of development. (A=non-induced stolon tips, D= 0.5g tuber). Note the appearance of a second more basic isoform of α-tubulin that reaches a maximum in stage C (early swelling stage).

to be expressed specifically on tuberisation has an important role in the process. This area of research is being pursued in collaboration with Professor Mingel-Castel, University of Navarra, Spain.

Isolation of genes induced on tuberisation The preliminary study described above indicated that changes in gene expression, in addition to those detected using the tubulin antibody, occurred early in tuberisation. Differential screening of a cDNA library constructed from stolon tip RNA at an early tuberisation stage enabled the cloning of some of these genes. The rationale was that genes switched on at the onset of tuberisation possibly have an important role in the process. A cDNA library was constructed from RNA extracted from stolon tips at a very early tuberisation stage and was screened with probes derived from induced and non-induced stolon tips. cDNAs that hybridised to the induced probes but not to the non-induced were selected for further analysis. Seven cDNA clones with these characteristics were isolated and the pattern of the expression of these genes was investigated by RNA-blot analysis (Fig. 3). In all cases there was a large increase in gene expression (15-30 fold) in induced stolon tips compared to non-induced tips. As the tuber developed and increased in size, the expression of all but one of the genes diminished. The expression pattern was also investigated in tissues other than stolons and tubers and although they could be detected in leaves, roots and stems, the expression

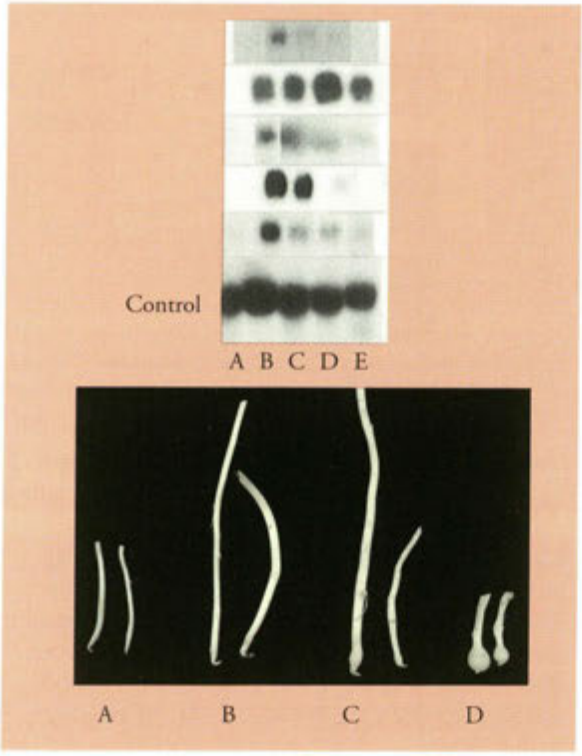

Figure 3 Expression pattern of cDNA clones isolated by differential screening. RNA was isolated from stolon tips at the stages shown in the lower panel (A-D) and from 25g fresh weight tubers (lane E) and probed with the cDNA clones isolated by differential screening.

levels were generally much lower. Analysis of the genomic organisation of these genes by Southern blotting showed that for most, but not all, there were several copies per genome. One of the genes was present as a single copy. Homologous sequences could be detected in the genomes of the non-tuberising *Solanum brevidens* and also in tomato.

The cDNAs obtained from the differential screen were sequenced and compared to known sequences in the EMBL database. On the basis of their sequence similarity it was possible to identify some of the potato genes from which the cDNAs were derived. Two that were strongly expressed at the onset of tuberisation encoded ribosomal protein genes, similar to the mammalian S19 and L7 genes, and were the first S19 and L7 genes to be cloned from a plant. In mammalian systems it has been shown that the protein encoded by S19 can be cross-linked to the initiation factor eIF-3, which is necessary for the binding of mRNA to ribosomes. This suggests that the S19 polypeptide is located at the site where the initiation factor binds and where translation of mRNA is initiated. The L7 protein has been localised on the surface of the 80S ribosome by biotinylation. It is significant that ribosomal protein genes have been isolated in a number of other differential screening experiments reported in the literature. In most cases, the level of expression of the genes encoding the ribosomal protein genes increased dramatically in rapidly growing tissues compared to quiescent tissues. The factors which influence the expression of these genes and their precise role in the ribosome will be of great interest to developmental biologists in general.

Another of the potato genes that was isolated by differential screening had strong sequence similarity to the S-adenosylmethionine decarboxylase (SAMDC) genes from human, rat and yeast. There was 53% similarity and 34% identity between the deduced amino acid sequences of the human and potato proteins with homology extending throughout a 330 amino acid overlap. This is the first plant SAMDC gene to be sequenced and the genomic clone corresponding to the SAMDC specific cDNA has been isolated recently at SCRI. The gene structure is currently being analysed and regulatory elements in the gene will be of particular interest. SAMDC is a key enzyme in polyamine metabolism as decarboxylated S-adenosylmethionine provides the propylamino moiety for the conversion of putrescine to spermidine and for the synthesis of spermidine to spermine (Fig. 4). Polyamines are known to stimulate plant growth and enhanced polyamine biosynthesis often occurs concurrently with rapid cell division. The isolation of the SAMDC gene provided the impetus to measure polyamine and associated enzyme activities in stolon

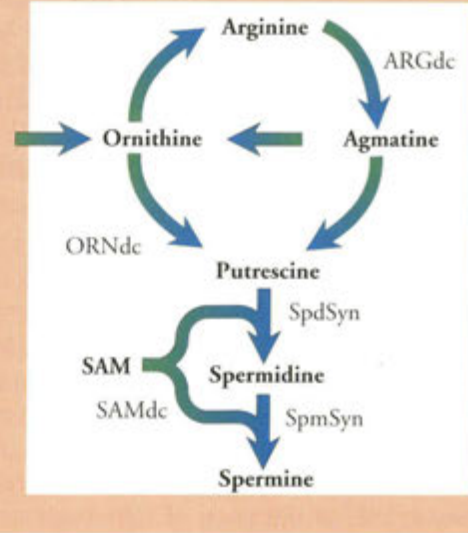

Figure 4 Polyamine synthesis from ornithine and arginine.

tip and tuber tissue during the early stages of tuberisation. Large changes in the activities of SAMDC and ornithine decarboxylase could be seen as the stolon tip began its radial expansion. An initial 2-3 fold increase in the activities of these enzymes was followed by a decline as the tuber increased in size. When free polyamine levels were determined, putrescine content declined in the initial stages of tuberisation, whereas the levels of spermidine and spermine increased. These data strongly imply that polyamine activity has some effect on tuberisation.

Other cDNA clones isolated by differential screening could not be identified on the basis of sequence similarity to known genes and therefore represent completely novel sequences. To determine the role of these genes in tuberisation their expression is being modified using "antisense" technology in transgenic plants. By substantially down-regulating the genes and studying the morphology of the regenerated plants it should be possible to determine their role, if any, in tuber formation. It has proved difficult to regenerate plants transformed with either the sense or antisense construct to the SAMDC gene probably due to the lethal effects of manipulating polyamine levels in the plant. However, supplementing the tissue culture medium with low concentrations of polyamines has improved survival rates in tissue culture. More sophisticated antisense vectors are now being used so that the antisense gene is only expressed when plants are treated with tetracycline.

Future prospects for research into tuberisation

SCRI currently leads the world in identifying the molecular events underpinning tuberisation. Although some inroads into understanding tuberisation have been made, it will require years of further study to fully understand the process. In order to accelerate and coordinate progress, a five-way European collaboration has been established, funded through the EC Biotechnology initiative. Pooling of resources will enable a wider range of genes that are expressed during tuberisation to be studied. Research will be expanded to analyse expression pattern in different cell types using *in situ* hybridisation and to identify any common responsive elements in promoter regions. At the same time *in vitro* tuberisation systems will be improved and the role of the cytoskeleton in tuberisation will be investigated in more detail.

Theoretical biology: beyond the straight and narrow

J. W. Crawford

While observation is the gathering of information, the synthesis of that information involves theory. A theory begins with some intuition about how a system works, and can be made quantitative by translating the concept into mathematical equations. The advantage of a quantitative theory is that it can be more rigorously tested against observation, since not only must it describe the trends in the data, but also the absolute values. Agreement with observation at this level lends far greater support for the theory. In comparison to physics where this approach has been used to great success, biology has, until recently, been far less obliging. The reason for this almost certainly lies in the fact that many physical systems from a pendulum to colliding subatomic particles, may be studied in near isolation from outside disturbances, and manipulated to reduce the complexity of their behaviour. In fact most of the historically significant discoveries of physics are limited to systems where this is possible. Newton's theory of gravity applied to two planets yields laws of motion which allow prediction of the position of the planets at any subsequent or previous moment. This predictable or 'clockwork' Universe was the Newtonian revolution. However, the disturbance from a third body destroys this

predictability and highly complicated and apparently random behaviour is possible. Biological systems by comparison cannot be as easily manipulated since the essence of their behaviour lies in their interaction with the surroundings. Furthermore, the 'physical laws' which control the numerous biochemical and biophysical processes are orchestrated by still more 'laws' which are laid down in the genetic code. Another simplifying trick used in physics is to study the system near its resting state, such as the motion of a pendulum undergoing very small swings, where the behaviour is often less complicated. Biological systems are almost never near to being in any one particular state, but are usually subject to a constantly changing external environment. The significance of these distinctions lies in the type of mathematics which is relevant. Physical systems may be deliberately manipulated in order that the resulting mathematics be tractable using contemporary techniques. This invariably means that the equations describing the behaviour are linear. Biology is the realm of non-linear mathematics, and only the last decade or so have the necessary tools become available to solve the resulting equations.

When less is more Linear equations describe systems which respond dynamically to change, in proportion to the magnitude of that change. For example, a waterwheel will deliver energy in direct proportion to its change in rotation speed. Furthermore, the behaviour of a linear system is equal to the sum of the behaviour of its part since two waterwheels deliver twice the power of one. However, a non-linear system is greater than the sum of its parts - a pineapple-sized lump of uranium delivers considerably more energy than two pieces of half the size. Because linearity is a far more restricting condition than non-linearity, it follows that most natural phenomena are likely to be non-linear. Indeed this does seem to be the case, and such non-linearities are the origin of far richer and more complex behaviour in non-linear systems.

As well as reproducing biological complexity, non-linearity can also produce regular behaviour. Non-linear feedback processes are thought to play an essential role in regulating the heartbeat. They can also give rise to spatial patterning and the application of non-linear systems theory to morphogenesis is a particularly active area of research. In fact, as more is understood about the mathematics, the number of potential applications is increasing in a strongly non-linear fashion.

The search for Methuselah Whilst many organisms live for a determinate period of time, some are capable of indefinite, or indeterminate, growth. An important factor in the stability of these organisms is their ability to adapt to a changing and heterogenous environment. The key to this adaptability lies in their complexity of form. A barrier to understanding indeterminacy has been the lack of an appropriate framework for capturing this complexity and understanding its capacity for optimisation of the organism.

Fungi are an important component of the soil biomass, both for breaking down organic material into products available to plants, and also for transporting otherwise immobile nutrients through the soil. They are also a classic example of indeterminacy since they can continue to grow indefinitely by orchestrated die-back and regrowth into new regions of soil. There are reports of a single organism extending across several kilometres. An understanding of the ability of the fungal colony to efficiently adapt to its soil environment and effectively exploit the nutrient base is clearly important in a wider context as well as in its relevance to sustainable soil fertility.

As can be seen in Figure 1, fungal colonies comprise a geometrically complex network of filaments or 'hyphae'. Organic material is processed via enzymes which pass through the hyphal walls, and some of the products are reabsorbed to be incorporated into new filament material. Despite this apparent complexity, we have been able to show that the hyphal structure is

Figure 1 A colony of *Trichoderma viride* growing on nutrient agar 49 h after plating. The colony measures approximately 1 mm across.

far from random. The structure can be quantified using a generalisation of high-school geometry known as fractal geometry, and fungi represent one of the many biological systems which are examples of fractals. The consequence of fractal structure in this instance is that the arrangement of hyphae is not random, as first appears, but is spatially correlated. Thus the distribution of hyphae in one part of the colony is not independent of the distribution in more distant parts. Furthermore, this fractal structure can be understood as arising as a consequence of the competition between exploitative and explorative growth strategies. A weakly branched colony requires fewer building blocks to explore for resources when nutrient is limiting, compared with a highly branched structure which invests a large amount of building material on more fully exploiting a nutritious volume. This optimising strategy combined with the correlated growth implied by the fractal structure sets severe constraints on the possible theories linking the mechanisms of growth morphology to the environment. Figure 2 shows that despite the underlying complexity, highly geometrically regular features can appear as density enhancements in the hyphal network. As a first step towards reaching some understanding, a theory has been proposed which links morphogenesis to non-linear calcium kinetics in the cytoplasm. We have shown that specific non-linear control of the free calcium concentration can lead to spatial patterning in the calcium distribution. This in turn can orchestrate the available transport system within the hyphae to lay down new precursors to growth in a way which leads to correlated growth. It is a major challenge for the theory to prove that such a mechanism is capable of reproducing the observed fractal structure, and work is continuing on this. However, progress has been made in showing that stable patterns such as those in Figure 2 can be produced on an underlying fractal network. Additional theories are also being developed, and together these ideas should provide a deeper understanding of the role of fungi in nutrient cycling in soil, and of the origins of indeterminacy itself.

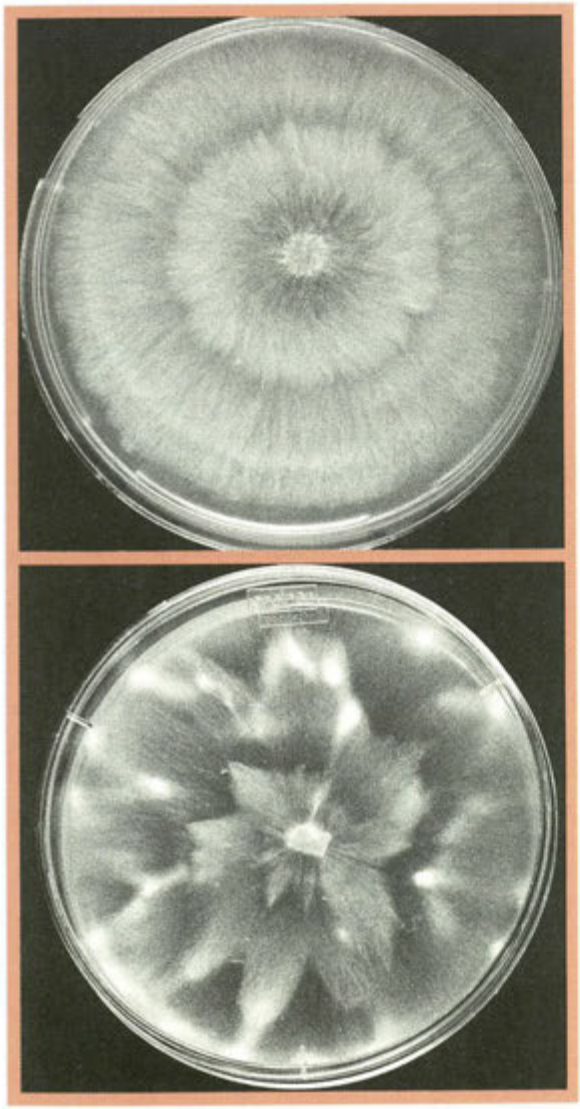

Figure 2 Colonies of *Mortierella elongata* Linnem. growing in potato dextrose agar. Upper colony, grown in continuous light, has formed ring-like density enhancements of hyphae. Lower colony, grown in continuous darkness, has formed a spiral density enhancement of hyphae. The diameter of the circular dish is 9 cm.

The benign control of crop disease With increasing environmental pressure to reduce chemical inputs to agricultural systems, there is a need to identify and optimise benign methods of disease control. Theoretical work at SCRI has focused initially on developing an understanding of the effect of planting cultivar mixtures on the progress of powdery mildew epidemics in barley. Results have shown that in many instances, mixtures are beneficial since they result in a reduction in the rate of evolutionary adaptation of the pathogen population to its host. They also result in a general reduction in disease levels and a consequent increase in yield compared to the same quantities of individual cultivars grown as pure stands. However, not all choices of mixtures demonstrate these positive benefits and disease progress is complicated (Fig. 3). The mixture combinations have to be chosen carefully. Through a thorough understanding of the interplay between genotypes of the host and pathogen, and

the complex spatial effects arising from random planting of the mixture cultivars, the critical factors may be identified. We have shown that informed manipulation of the mixture characteristics can result in a few dense patches of disease, or a more homogeneous and lower level of infection. This type of understanding together with knowledge of quality constraints on the mixture, can lead to more effective and efficient breeding strategies for the future.

From soils to cells A major initiative involving the application of non-linear systems theory is in understanding the interaction between soil structure and the physical and biological processes going on within. Fractal geometry is again used to characterise the structure and forms the basis of a theoretical framework for understanding gaseous diffusion, fluid flow and microbial movement. We have been able to show that these properties are highly sensitive to particular characteristics of structure. In particular, measured properties depend on the scale at which the measurement is made, and the theory shows how the behaviour across a wide range of scales relevant to the processes of concern may be inferred. This work is being carried out in collaboration with research groups at Kyoto University in Japan, and with the Institute for Soil Research, Müncheberg in Germany, and is described in more detail on p. 41of this report.

At the molecular level, non-linear systems theory is being applied with the aim of understanding the processes which control and stabilise the major metabolic pathways in plant cells. Present understanding of substrate partitioning in plants is limited to laboratory systems which aim to minimise complex behaviour and to understand control near to the systems resting state in the test tube. Under these simplifying circumstances, linear theory can be applied. However, plants are adapted to persist in a constantly changing environment where they are subjected to continual environmental 'shocks'. An understanding of how plants grow and adapt therefore requires an understanding of what processes act to stabilise metabolism when the system is far from its resting state.

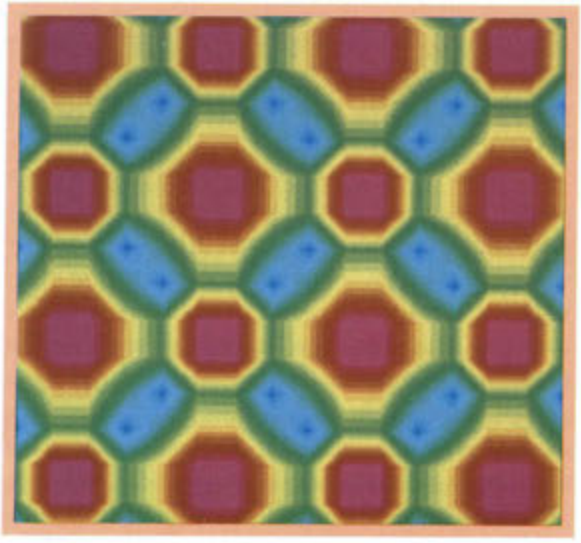

Figure 3 Distribution of disease levels in a field comprising a mixture of crop genotypes with varying resistance to the disease.

The traditional biochemical approach assumes a quantitative understanding of the whole, may be obtained from the sum of understanding of the parts. Non-linear systems theory tells us this is not true and so a new approach is required. We aim to understand the glycolytic pathway in plant cells by studying the non-equilibrium behaviour at two contrasting levels of detail. The variability of large-scale indicators of dynamic behaviour (such as pH or redox state) provide important information on the underlying control factors, their number and the degree of non-linearity. They provide information on the true level of complexity which underlies the observed behaviour. A greater level of detail may be obtained by attempting to identify the key reaction steps and formulating the equations which describe the coupling between the metabolite concentration and enzyme activities. Such non-linear couplings can elicit a stabilising influence and result in the emergence of large scale order from chaos at the small scale.

The Centre for Non-linear Systems in Biology In order to exploit the rapidly growing opportunities for close collaboration between experimentalists and theoreticians, The Centre for Non-linear Systems has been established. Based at SCRI, the Centre draws on expertise from the Department of Mathematics at the University of Dundee and the Scottish Agricultural Statistics Service as well as scientists from all departments within SCRI. The Centre has already won four research grant awards, and is seeking support from the public and private sectors. Associated with the work of the Centre is an international panel of nine distinguished research scientists who currently act as advisors.

By adopting new analytic techniques, and through genuine interdisciplinary collaboration we hope to unravel the mysteries which have hitherto evaded insight into that most subtle and problematical aspect of Nature - life.

Novel N_2-fixing cyanobacteria-plant associations

N.W. Kerby, M. Gantar[1] & P. Rowell[1]

The creation of plants with the ability to fix atmospheric N_2 has great environmental and economic potential to enhance plant growth and decrease the reliance of plants on nitrogen fertilizers. It would also reduce the reliance of agriculture on fossil fuels. Offsetting fertilizer applications to soil by fixing N_2 directly into plant tissues will lessen environmental problems associated with nitrate leaching to ground waters and emissions of nitrous oxide, a greenhouse gas, to the atmosphere.

The practical importance of symbiotic associations between plants and N_2-fixing microorganisms has long been known although the biochemistry and molecular biology of N_2 fixation has been elucidated only recently. Agronomically, the most important N_2-fixing association is that between legumes and the diazotrophic bacterium *Rhizobium*, while cereals such as rice, wheat and corn contribute more to human nutrition.

A long-term objective of many plant scientists is to introduce N_2 fixation into agronomically important plants and the insertion of nitrogen fixation genes (*nif* genes) into plant cells appears to be currently favoured. In addition to genes that specify the structure and synthesis of nitrogenase, other genes that respond to environmental signals are involved in transcriptional regulation. Although current methods are available for the introduction of DNA into plants, the technology is not sufficiently advanced to permit the introduction, integration and expression of the large number of genes required for N_2 fixation. Furthermore, the process of N_2 fixation would have to be integrated into plant metabolism.

An alternative approach is the introduction of N_2-fixing microorganisms into plants and efforts are being made to extend the range of plants capable of associating with *Rhizobium*. However, the formation of root nodules is complicated and consists of a variety of phenotypic steps controlled by a number of plant and bacterial genes whose products interact. The host range of certain rhizobial isolates has been extended and host-specificity barriers have recently been broken by the creation of transgenic plants. Additionally, artificial associations using either cell wall degrading enzymes or synthetic auxins have resulted in the formation of nodule-like structures following inoculation of rice with *Rhizobium*. However, N_2 fixation by these nodules has not yet been demonstrated.

Many plant-associated bacteria have been identified and their potential contribution to the nitrogen budgets of crop plants is being elucidated. *Azospirillum* is the best known diazotrophic microorganism in the rhizosphere and plant growth stimulation, attributed to the bacteria, has not always correlated with N_2 fixation. Other factors such as growth stimulants, enhanced uptake and assimilation of combined nitrogen or enhanced iron uptake may be involved. Only when *Azospirillum* is endosymbiotic, i.e. it develops intercellularly, is there a clear provision of atmospheric N_2 to the plant.

Nitrogen fixing cyanobacteria Cyanobacteria are photosynthetic prokaryotes which offer an alternative possibility for creating novel N_2-fixing associations with plants. Many species fix N_2 and they are unique in that two major processes, N_2 reduction to ammonia and oxygenic photosynthesis, are carried out within one organism and often within one cell. Despite being ideally suited to an independent existence, they are found in a diverse range of symbiotic associations with plants, fungi, animals, non-photosynthetic protists and bacteria. When comparing various plant-microbe associations it is evident that for successful and efficient utilization of atmospheric N_2 for plant growth, endosymbiotic associations are required, particularly for heterotrophic microorganisms which are dependent on the host plant for a supply of photosynthate for energy and carbon as well as protection of nitrogenase against O_2. However, cyanobacteria are either phototrophic or heterotrophic and can fix N_2 in the presence of high O_2 concentra-

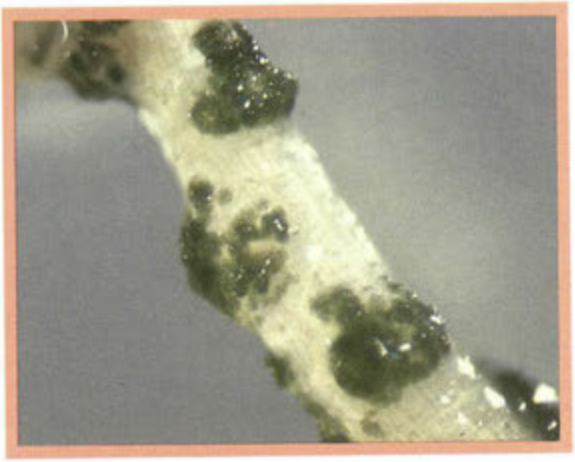

Figure 1 The surface view of a wheat root colonized by *Nostoc*.

[1] University of Dundee

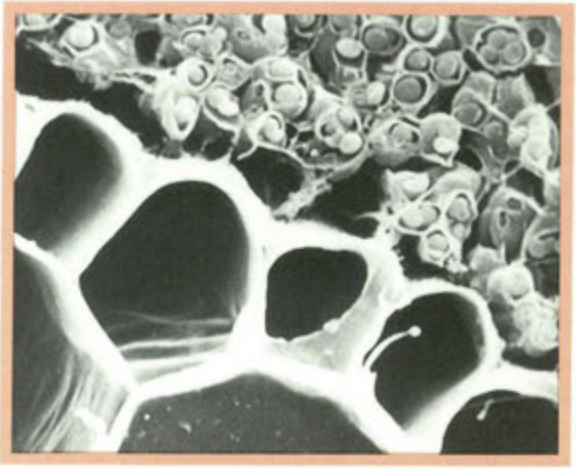

Figure 2 Scanning electron micrograph of wheat showing the intimate association of *Nostoc* aseriate packages on the surface of wheat roots.

tions. Such attributes may explain why cyanobacteria form natural associations with a wider range of organisms than any other diazotroph, and why novel associations with cyanobacteria may not be so severely constrained as those with other diazotrophs.

Associations between plants and cyanobacteria

Natural associations between cyanobacteria and plants range from epiphytic to intracellular and occur in almost all groups of plants. Cyanobacteria can invade structures such as leaf cavities that are normally part of the plant structure but in certain associations there are morphological modifications and those found in symbiotic associations are usually of the genus *Nostoc*. *Nostoc* has a unique life-cycle consisting of heterocystous filaments, hormogonia and aseriate packages. Heterocysts are the site of aerobic N_2 fixation while hormogonia are short motile filaments lacking heterocysts, which may be particularly important in the establishment of associations[3]. Artificial associations between wheat and N_2-fixing cyanobacteria have been created by co-culturing natural soil isolates of cyanobacteria with wheat seedlings grown in liquid culture[1,2] although not all isolates tested formed stable associations. Two types of associations have been recognised; 1) loose associations with roots, typical of *Anabaena* strains and 2) tight associations, typical of *Nostoc* strains (Fig. 1). The ultrastructure of a tight association has been characterised and cyanobacteria were found as hormogonia, heterocystous filaments, or aseriate packages, depending on their stage of development. The aseriate packages were in intimate contact with the root epidermal cells (Fig. 2) and cyanobacteria were found to penetrate both the root epidermis and cortex, forming packages in intercellular spaces (Fig. 3). Following inoculation of vessels containing growing wheat plants, certain strains appear to be specifically attracted to roots where their growth is supported and maintained (Fig. 4). Other cyanobacterial strains have also been found in stems and on the surface of leaves following inoculation of roots[2]. Our present studies reveal that only certain combinations can form viable associations suggesting that specific recognition factors are involved. In the early stages, the root surface is colonized by motile hormogonia that develop into immotile filaments. Such filaments become embedded in a mucilaginous envelope and develop into aseriate packages (Fig. 2). The filaments are composed of both vegetative cells and heterocysts and *c* 10 d after initial colonisation, aseriate packages started liberating hormogonia. We have also shown that factors produced and liberated by wheat stimulated the production of hormogonia in free-living *Nostoc* cultures and that this activity was more pronounced when wheat was grown in the absence of nitrogen[3]. Hormogonia promoting

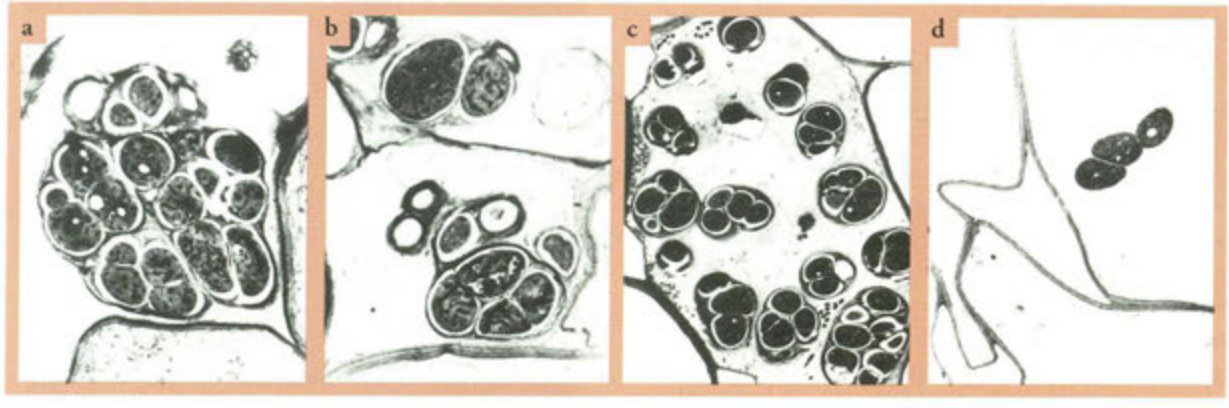

Figure 3 Transmission electron micrographs of wheat roots showing: (a), a cyanobacterial package in intimate association with an epidermal cell (x 3300); (b), cyanobacteria within an epidermal cell (x 7000); (c), an intercellular space within the cortex occupied by aseriate packages of cyanobacteria (x 2000); (d) cyanobacterial filament within a cortical cell (x 3000).

Figure 4 Certain cyanobacteria (strains LC2 and C3) are specifically attracted to wheat roots. Wheat plants were grown in agar in which cyanobacteria were uniformly distributed).

activity was also detected in root extract indicating that the wheat root contained some active substance that induced the developmental cycle of *Nostoc*. Hormogonia are the primary colonizers in many plant-cyanobacterial associations.

Effects of cyanobacteria on plants Associated cyanobacteria have been shown to contribute to the nitrogen budget of wheat[4] and greatly stimulate root growth (Fig. 5) both in the presence and absence of combined nitrogen. The increase in plant N concentrations appeared to be dependent on the wheat cultivar and the cyanobacterial isolate used[4]. Recently, using mass spectrometry, we have shown that the natural abundance δ^{15} N values of wheat roots colonized with cyanobacteria were significantly lower than control plants confirming that cyanobacterial N_2 fixation was contributing to plant N.

The nitrogenase activity of *Nostoc*, is maintained in the dark and in our studies *Nostoc* colonized roots of wheat grown in sand, in the absence of added combined nitrogen and light, and stimulated root and shoot growth, dry weight and nitrogen contents. Growth and nitrogenase activity of the cyanobacteria in the absence of light was presumably supported by plant exudates.

Growth stimulation of plants following addition of rhizosphere bacteria may not always be attributable to N_2 fixation but may be due to production of plant growth stimulating substances, increased ammonium or nitrate uptake and N-assimilation, or to effects on iron uptake via the production of siderophores. We have shown that cyanobacteria stimulate wheat root growth and it is known that they can produce plant growth stimulating compounds. However, our recent findings show that

Figure 5 Growth of wheat in the presence and absence of cyanobacteria for 15 days. Control plants (those without cyanobacteria) were grown in either Knop's medium or BG11 medium with ($BG\text{-}11_N$) or without ($BG\text{-}11_O$) NO_3-.

atmospheric N_2 fixed by cyanobacteria contributes to the N balance of wheat in these novel associations.

Conclusions Considerable effort has been made to introduce N_2 fixation into agronomically important plants by transforming *nif* genes into plants, extending the range of plants that can be nodulated by *Rhizobium* and by developing *Azospirillum* as a soil inoculant. However, little progress has been made in increasing crop productivity or in reducing the reliance of agriculture on chemical fertilizers by these methods. We believe that the creation of associations between plants and N_2-fixing cyanobacteria has been overlooked but may, on the basis of our preliminary results, offer a plausible solution to the problem. This is due, in part at least, to the ability of cyanobacteria to fix both carbon and nitrogen in an oxygenic environment, to grow heterotrophically as well as autotrophically and to naturally associate with a wide range of plants and other organisms. It is hoped that future collaborative studies between the Department of Biological Sciences, University of Dundee and SCRI, will concentrate on the transfer of metabolites between the partners and determine the viability of associations in the field.

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Chemistry

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

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

The role of the Chemistry Department is to develop and apply novel analytical and synthetic procedures for investigating factors that affect crop production and the quality of plant products. A new research programme, dealing with the relationships between lipid structure and function in plants and plant products, is being developed in which novel techniques for structural analysis of lipids from animal tissues will be adapted for plant products (p. 58). The effort will initially concentrate on the chemistry of heated frying oils financed by an EEC-AIR grant and, in particular, on the nature and mechanism of production of cyclic fatty acids. The interactions of such compounds with potato products is also being investigated. Another programme will be initiated on lipid oxidation in membranes or model membrane systems. This process is important in spoilage of foods and is also relevant biologically to such processes as senescence and interactions between plants and pests. There are many areas of research in the Institute that will benefit from this lipid expertise, including food irradiation, nematode metabolism, plant membrane research, stable isotopes in lipid metabolism, plant physiology and lipid functionality in cereal crops.

A new high-performance liquid chromatographic (HPLC) method, using an amino reversed-phase column, has been developed for the determination of the pyrimidinone glucopyranosides, vicine and convicine. The presence of both substances in the roots of *Vicia faba* has been confirmed and collaborative studies have been undertaken to determine their distribution in plant tissues taken at five growth stages between the onset of flowering and seed maturity.

Investigations have been undertaken to determine the effects of light on both the chlorophyll and total glycoalkaloid content of potatoes. The major alkaloids are shown in Figure 1. Statistically significant differences

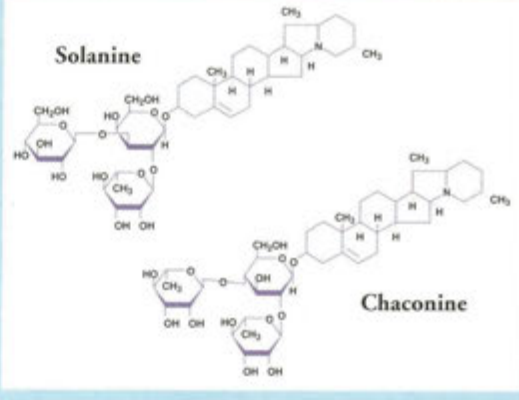

Figure 1 The stuctures of the two major glycoalkaloids present in potato (*Solanum tuberosum*) tubers.

were found between the rates of increase of both types of compound in six cultivars and, within a cultivar, both were highly correlated with each other. Induced changes in plant composition due to grazing or root damage continue to be investigated with particular emphasis given to changes in both the glucosinolate and carbohydrate contents of swedes after attack by turnip root fly. The results form the basis of a separate review (p. 104).

HPLC separations of the alcohol-soluble proteins extracted from six potato cultivars revealed small but significant differences in the profiles. These were maintained from year to year and from site to site, suggesting that they were independent of environmental factors. This may be a comparatively rapid means of identifying cultivars.

The range of volatile compounds capable of being analysed has been extended by using Tenax TA which is most efficient in the range 100 to 325°C. The volatile compound profile from the flowers of four raspberry cultivars showed aliphatic and aromatic aldehydes, ketones, alcohols, esters, monoterpenes, sesquiterpenes and several unusual nitrogenous compounds. The volatiles from the flowers of five varieties of oil seed rape included a series of C_4 substituted compounds, aliphatic aldehydes, monoterpenes, and dimethyldisulphide and dimethyltrisulphide. Glucosinolate breakdown products were noticeably absent. The epicuticular wax of raspberry leaves consists of a homologous series of long-chain (C_{40}-C_{60}) esters. Similar extracts from the leaves of a raspberry progeny, segregating for resistance to aphid attack, are now under investigation to determine a putative role of these compounds in mediating feeding behaviour.

The acquisition of a continuous flow stable isotope mass spectrometer, dedicated to natural abundance measurements, has permitted significant advances in the use of natural isotopic variations in plant physiology and ecology. The instrument, a Europa Scientific Tracermass with Roboprep CN and Roboprep G plus sample preparation systems, allows a wide variety of samples to be analysed for ^{15}N, ^{13}C and ^{18}O content. A number of projects in the Institute rely on these measurements and there is a need to improve existing methods to follow soil-plant nitrogen metabolism.

The low nitrogen content of plant material and soils presents a particular problem for the measurement of natural differences in ^{15}N. By carefully matching the chemical composition of isotopic standards to the carbon and nitrogen levels expected in plant samples and ensuring that consistent amounts of nitrogen are present in all samples, acceptable precision is routinely achieved on material containing only 1% nitrogen, providing 50 to 100µg of nitrogen is available for analysis. Soils present a greater challenge since they may contain less than 0.1% nitrogen. The large (>100mg) sample normally required for combustion analysis quickly fills the elemental analyser system with ash and may lead to incomplete combustion. The nitrogen blank derived from the oxygen pulse used for combustion can be separated from the sample-derived nitrogen by delaying the oxygen pulse till well after the sample is introduced into the combustion furnace. This method has normally been used for very small samples of material rich in nitrogen but it gives promising results with soil samples containing only 25µg of nitrogen. Such methods are required to achieve the rapid and reliable sample numbers needed for statistical significance in ecological studies.

Studies of total organic carbon and amino acid losses from roots of rape and wheat using first-generation plant growth and exudate collection systems have been completed. Following pulse-labelling of 25-day old forage rape (*Brassica napus L.*) with $^{14}CO_2$, approximately 17-19% of the fixed label was translocated to roots over 2 weeks, 30-34% was released into the rhizosphere and 23-24% was respired by roots. Of the ^{14}C released into the rhizosphere, 35-51% was assimilated and respired by rhizosphere micro-organisms.

Plant age was a significant factor in determining the effectiveness of roots as sources of microbial nutrition. Older plants released a higher proportion of nitrogen-rich amino acids, for which micro-organisms have a particular affinity. Amino acid loss from roots was limited by the amino acid concentration in the external medium. The compositions of root-derived acids and free amino acids within root tissues were similar. These results suggest that amino acid loss from roots occurs mainly by passive leakage (diffusion) rather than by active excretion. Future work will investigate the involvement of root-derived chemicals in interactions between plants and micro-organisms, using a second-generation model system employing trapping of both volatile and non-volatile chemicals.

During the year a DNA synthesis service was provided for the Institute, based on the Applied Biosystems model 391 DNA synthesizer, to produce primers for PCR, RAPDs and sequencing applications. A DNA sequencing service, centred on a Millipore Base Station with control and analysis software mounted

on a Sun SPARC station, will be provided. While sequencing reactions must still be done manually, the Base Station automates electrophoresis, DNA visualisation and base calling. Considerable savings in time are achieved and the need for radioactive isotopes is removed. The provision of this service represents a major investment by the Institute; additional funding was obtained from the Gatsby Charitable Foundation.

This is the first year in which the EPR and ENDOR facilities have been used and they have made a major impact on research activities in which free radicals are involved.

The project on the development of methods for the detection of irradiated food and the investigation of plant senescence processes has involved an initial screening of fruit and grains for stable free radicals. Cellulosic, lignocellulosic and other plant phenolics were studied as model compounds to help identify the sites of the free radical centres in the food products. Considerable progress has been achieved and potential improvements have been identified in the EEC-BCR method for the determination of irradiated materials of cellulosic origin. Attempts to elucidate free radical reactions in soft tissue have been made by irradiating fruit of strawberry and grape and following their transformations on annealing to progressively higher temperatures. The radicals $H^{\cdot}$ and $OH^{\cdot}$, formed initially by the fragmentation of water, then react with other molecules in the fruit to produce a number of new free radicals, all of which are unstable at ambient temperatures. In the case of grape, the free radical reaction products are similar to those obtained by irradiation of a solution containing equimolar amounts of glucose and fructose.

During the Institute Open Days on the 3-4 July a consumer assessment of irradiated and unirradiated strawberries was held. This exercise (believed to be the largest test of its kind to be held in the UK) showed that most participants could detect taste differences between the products, but roughly equal numbers favoured each type.

Work on the identification of the roles of free radicals in plant pathological processes has been commenced with the identification of the essential function of O_2 in the response of potato tubers to infection with *Erwinia carotovora*. Stable free radicals were detected in necrotic tissue formed as a result of infection and an unstable precursor that is produced immediately after contact of infected tissue with air was also trapped.

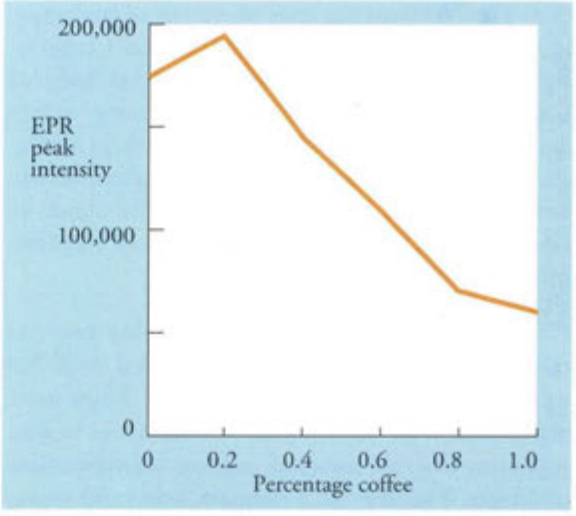

Figure 2 Influence of coffee on the intensity of the EPR spectrum from the O_2^- adduct of the TMPO spin trap.

Investigations of the free radical scavenging properties of various plant products have commenced. A study of the reaction of plant volatile oils from oregano, summer savory and thyme with the superoxide radical, O_2^-, has shown that reaction occurs with their carvacrol or thymol components, leading to the formation of free radical oxidation products. In a separate investigation it has been found that coffee, in addition to possessing stable free radical centres, has considerable free radical scavenging properties and is able to compete successfully with nitrone spin traps for O_2^- (Figure 2).

Using NMR microimaging, the sites of frost damage to blackcurrant flowers have been established non-invasively immediately after the freezing event and long before any external evidence of damage appeared. This technique will be used to investigate possible structural differences between frost tolerant and sensitive cultivars. In addition, extensive measurements have been made on blackcurrant and grape fruits with the dual objectives of improving our understanding of the developmental histology and authenticating the NMR imaging procedures. In ripe grapes it is possible to use chemical shift selective imaging techniques to produce separate images from water and sugar components. A substantial sugar gradient can be seen in ripening fruit (Fig. 3), although no such gradient exists in either unripe or fully ripened specimens. Finally, with the acquisition of new computer facilities for the NMR unit, it is now possible to produce complete 3-dimensional images. As a development of work carried out in the past two years, a complete

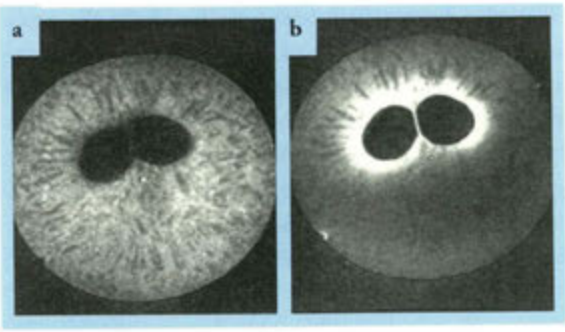

Figure 3. NMR microimage of sugar distribution in **a)** unripe and **b)** partially ripe grapes.

reconstruction of the vascular elements in a raspberry receptacle has now been achieved.

Work has continued on the isolation and characterisation of fibres from sources such as cereal straw, flax, nettles, brassicas, New Zealand flax, reeds and blackcurrant stems. In view of their agricultural interest, a series of oil-seed rape varieties have been assessed for their fibre potential. The lignin contents ranged from 5 to 8% while the fibre yield varied from 50 to 66% of the DM. The processing costs could, therefore, vary by *ca.* 50% even though the quality of fibre was not significantly different between varieties.

The facilities available for the characterisation of fibres has been improved by the acquisition of a Bruker IFS 66 FT-IR spectrometer. As well as improving the spectral quality, a microscope attachment has been obtained for identification of fibres *in situ* (Fig. 4).

Most effort on the processing of fibres has concentrated on the peroxy systems, particularly oxone, and this subject is covered by a separate review (p. 61) which also discusses the results obtained with biomimetic

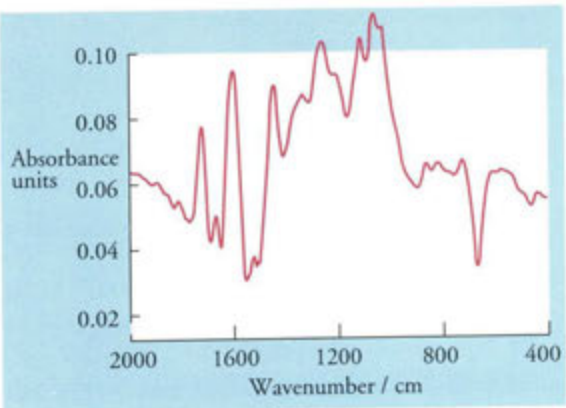

Figure 4 FT-IR microscopy spectrum of lignified section of blackcurrant stem fibres.

catalysts. Collaboration with the University of Dundee on X-ray diffraction for assessment of cellulose crystallinity has been initiated.

A scheme for the degradation and fractionation of plant cell walls by dissolution in trifluoroacetic acid has been proposed. The cellulose is solubilised but reprecipitated in water or methanol while the non-cellulosic polysaccharides (NCP) are partially hydrolysed and remain in solution. The reprecipitated cellulose regains a surprising extent of crystallinity. More importantly, many of the phenolic constituents still remain attached to the NCP fragments. The characterisation of these fragments should assist in establishing some aspects of cell wall structure.

Investigations on the control of differentiation and development in fibre cells has concentrated on lignification and the involvement of peroxidases. In flax, the level of peroxidase activity which is covalently bound to the cell wall increased as the tissues became lignified. Some of this activity can be solubilised by the action of a glycanase enzyme complex which is capable of extensive degradation of cell wall polysaccharides. These peroxidase isozymes have high affinities to catalyse the two final steps reported to be involved in lignin formation, the oxidation of NADH to hydrogen peroxide and the oxidation of coniferyl alcohol to phenoxy radicals.

In addition, the glycanase-insoluble wall residue still contains peroxidase activity that can be solubilised by limited proteolysis with trypsin. These trypsin-solubilised peroxidases have an enhanced affinity for the oxidation of coniferyl alcohol. It is postulated that the trypsin-solubilised peroxidases become insolubilised in

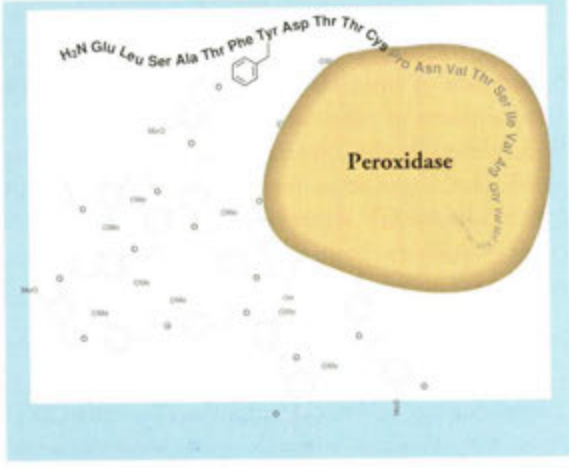

Figure 5 Possible linkage of a peroxidase to lignin in a plant cell wall.

the wall during, and as a result of, the free-radical induced polymerisation of lignin. Therefore, the peroxidases would be in the right location and would carry out the correct reactions at the relevant time to be considered as lignification-specific enzymes. Many peroxidases have an accessible tyrosine residue, near the N-terminus of the protein chain, which could be involved in a linkage to the wall. A possible linkage is shown in Figure 5.

The role of non-cellulosic polysaccharides in the structure and development of flax fibres is also under study. Antibodies have been raised against one of these components, a glucomannan, and their specificity tested. The antisera will be used to immunolocate glucomannan during development and will aid the biochemical characterisation of the enzymes which synthesize this polysaccharide.

Structural analyses of plant lipids

W.W. Christie

The chromatographic techniques used in lipid analysis are based essentially on three principles, namely partition, adsorption and ion-exchange. Two others, complexation (e.g. silver ion) and chiral chromatography, could perhaps be added, although both are usually carried out in conjunction with one of the main procedures. Gas chromatography (GC), utilizing the partition principle, and thin-layer chromatography (TLC), usually in the adsorption mode, have been the main instruments in bringing about the rapid increase in our knowledge of lipids in recent years. Although these still have much to offer, high performance liquid chromatography (HPLC), with which any of the chromatographic modes can be used, is now coming to the fore. In combination with mass spectrometry (MS), HPLC and GC are extremely powerful techniques.

Separation of lipid classes by HPLC Lipids lack chromophores of value for spectrophotometric detection, although the absorbance of isolated double bonds (and some other functional groups) at about 205 nm in the UV range can be used for some purposes. Among the limitations, only a few solvents are transparent at this wavelength.

Evaporative light-scattering detectors are relatively simple optical devices, and the first commercial instrument from A.C.S. Ltd (Macclesfield, U.K.) (Fig. 1) was utilized in much of the work described below. The eluent from the HPLC column passes into a heated tube where the solvent is evaporated in a stream of compressed air. If it is a lipid, the solute does not evaporate and passes as a stream of minute droplets through a light beam, which is reflected and refracted. The amount of scattered light is measured and bears a relationship to the amount of material eluting. As the response is not dependent on

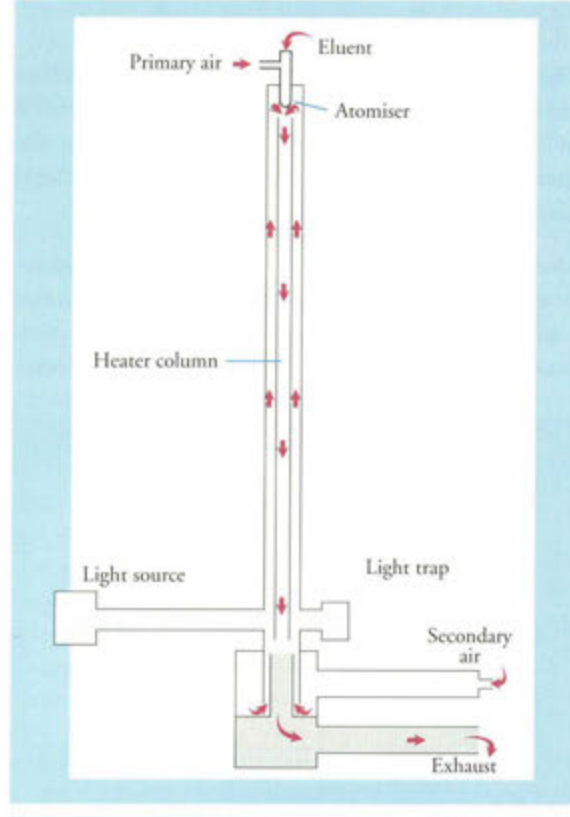

Figure 1 A schematic evaporative light-scattering detector.

particular chromophores, the detector is universal in its applicability. It can be used with any solvents and with complex gradients, and is relatively inexpensive and rugged. When used with a ternary solvent delivery system, it can permit some quite exceptional lipid analyses.

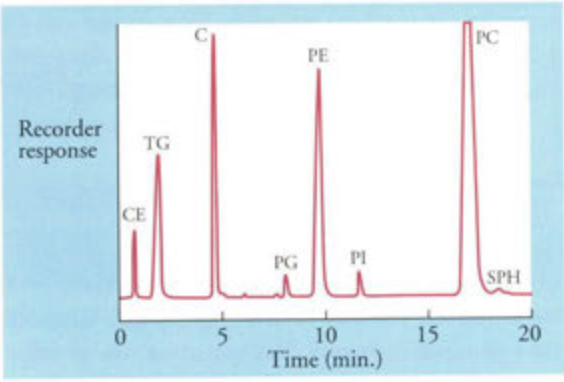

Figure 2 Separation of rat lipids by HPLC with evaporative light scattering detection.[1]
CE= cholesterol esters TG=triacylglycerols C= cholesterol PG, PE, PI, PC and SPH are various phospholipids

HPLC is of particular value in the separation of lipid classes, and we set out to devise an HPLC system in which all the common simple and complex lipids in animal tissues, ranging in polarity from sterol esters at one end of the spectrum to lysophosphatidylcholine at the other, were resolved and quantified in a single run. In fact, with the lipids of rat liver and related tissues, it proved possible to resolve all the main components in only 20 min. on a short column of silica gel (Fig. 2)[1]. The base-line was steady throughout the analysis, although there were abrupt changes of solvent at some points. In essence, hexane was used to bring off the cholesterol esters and triacylglycerols, then a gradient of isopropanol-chloroform (4:1, v/v) was fed in to elute cholesterol and other simple lipids, before water was introduced to separate each of the phospholipid classes. Finally the polarity of the gradient was reversed to remove all the water and to restore the column to its original activity over a further 10 min. This method has now been adapted to the analysis of the complex lipids in plants[2]. The response of the detector is not linear but tends to fall off in the low mass range, apparently because very small solute droplets do not perturb the light beam, and it is possible to overlook some minor components. However, with careful calibration, excellent quantitative results can be achieved.

Silver ion chromatography TLC with silver nitrate in silica gel has long been used for separations of lipid molecules by degree of unsaturation. The principle is simply that the π-electrons of double bonds in the fatty acyl residues of lipids react reversibly with silver ions to form polar complexes, the greater the number of double bonds the stronger the complexation effect. The technique initially proved difficult to translate to HPLC, but this has now been achieved by adapting ion-exchange columns to the purpose. A column of silica with bonded sulphonic acid groups was used, and silver ions were bound simply by injecting a solution of silver nitrate onto the column, while pumping water through it; the aqueous phase was then replaced with organic solvents[3]. As the column is long-lived and no silver ions elute with the compounds of interest, it can be very useful in small-scale preparative applications. With this silver ion column, the light-scattering detector is used and a stream-splitter is introduced between the end of the column and the detector, so that fractions can be collected for analysis by other means, e.g. for fatty acid determination. The separation of molecular species of triacylglycerols, the main constituents of most edible fats and oils, is an important problem in the food industry; the information permits a correlation of composition to physical properties. With oils containing a relatively small proportion of linoleic acid, the simplest elution scheme for silver ion HPLC was a gradient of acetone into dichloroethane-dichloromethane, that permitted the separation not only of the usual fractions with saturated and cis-monoenoic residues but also those with *trans* double bonds[4].

Most vegetable oils contain a high proportion of linoleic acid, and this was accommodated with a

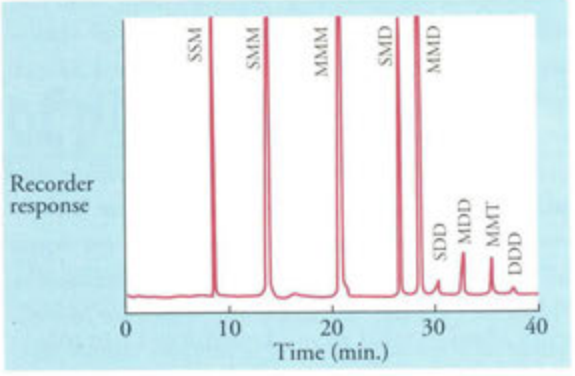

Figure 3 Separation of molecular species of olive oil by silver ion HPLC
S=saturated, M=*cis*-monoenoic, D=dienonic, T=trienonic fatty acyl residues

ternary gradient system simply by introducing acetonitrile into acetone after the first fractions were eluted, as demonstrated with olive oil (Fig. 3). There is excellent resolution of most fractions. With confectionery fats, a separation of the important disaturated-monoenoic species is rapidly achieved. The most highly unsaturated seed oil to be analysed was linseed oil where the most abundant component, trilinolenin, has nine double bonds.

Silver ion chromatography is not simply an alternative to reversed-phase chromatography, but should be considered as a valuable complementary technique. For example, the triacylglycerols of meadowfoam oil (*Limnanthes alba*) were resolved by HPLC in the silver ion and reversed-phase modes, and by the two techniques used in a complementary fashion[5]. Silver ion chromatography gave a distinctive resolution in which fractions, differing solely in the position of a double bond in one of the three monoenoic fatty acyl groups, were separated. Reversed-phase chromatography also gave fractions containing single positional isomers, but the pattern was less easy to discern, because of overlap of species containing unrelated fatty acids. When the two techniques were used sequentially, much better resolution was possible.

Gas chromatography-mass spectrometry in the identification of fatty acids Although the potential of MS for the identification of fatty acids in natural samples is enormous, there were relatively few reports of applications to real samples as opposed to model compounds for many years. A valuable new approach consists in the preparation of specific amide or ester derivatives, which stabilize the double bonds during ionization. Picolinyl ester derivatives of fatty acids permit the use of GC-MS for the simultaneous separation and characterization of components of real samples. Although surprisingly good resolution of such compounds is possible with non-polar GC phases and isomers of a given degree of unsaturation tend to be well resolved from each other, there may be overlap of fatty acid derivatives with a different degree of unsaturation. Some other form of fractionation is, therefore, necessary if a more comprehensive identification of the components of a complex natural mixture is required and the silver ion HPLC method described above is ideal, since even positional isomers can be separated[6].

As an example of an application to plant lipids[7], *cis*-4-hexadecenoic acid, 13-cyclopent-2-enyltridec-4-enoic acid (Fig. 4), C_{16} and C_{18} cyclopentyl and branched-chain fatty acids were found for the first time by means of this methodology in the seed oil of *Hydnocarpus anthelmintica*, used in folk medicine for the treatment of leprosy. Fatty acids of borage (*Borago officinalis*) and algae have also been investigated.

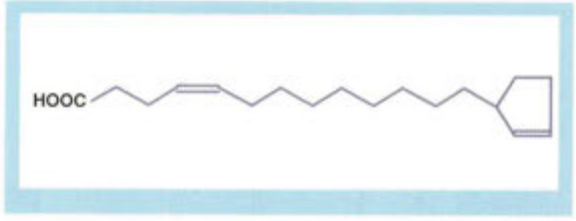

Figure 4 13-Cyclopent-2-enyltridec-4-enoic acid.

Stereospecific analysis of triacylglycerols Glycerol has a plane of symmetry, but when the two primary hydroxyl groups are esterified with different fatty acids, the resulting triacylglycerol will be asymmetric and can display optical activity. To simplify naming, a 'stereospecific numbering' system was recommended by a IUPAC-IUB commission on the nomenclature of glycerolipids. In a Fischer projection of a natural L-glycerol derivative, the secondary hydroxyl group is shown to the left of C-2; the carbon atom above this then becomes C-1, that below becomes C-3 and the prefix '*sn*' is placed before the stem name of the compound (Fig. 5). In plant and animal tissues, the major pathway for triacylglycerol biosynthesis is the *sn*-glycerol-3-phosphate pathway, which involves specific stereochemical intermediates. It ensures that natural triacylglycerols exist in enantiomeric forms with each position of the *sn*-glycerol moiety having a distinctive fatty acid composition. No lipase capable of distinguishing between position 1 and 3 of a triacyl-*sn*-glycerol is known, but some ingenious stereospecific analysis procedures, i.e. for determining the compositions of fatty acids esterified to each of positions *sn*-1, *sn*-2 and *sn*-3, have been developed. However, these involve complex series of synthetic and enzymatic steps and some difficult analyses.

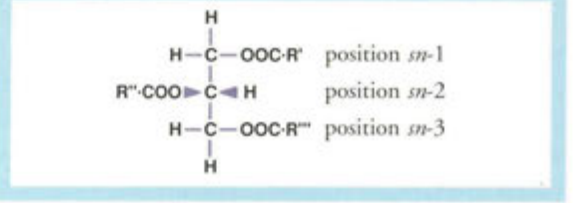

Figure 5 Fischer projection of a triacyl-*sn*-glycerol.

We recently showed that it was possible to resolve 1,2- and 2,3-diacyl-*sn*-glycerols as (S)-(+)- or (R)-(-)-1-(1-naphthyl)ethyl urethane derivatives by HPLC on a column of silica gel[8]. The elution order of components derivatized with the (*S*)-form of the reagent was 1,3- followed by 1,2- and finally 2,3-diacyl-*sn*-glycerols, and the elution order of 1,2-and 2,3-diastereomers was reversed when the (*R*)-form of the reagent was used. Molecular species of single-acid diacylglycerol derivatives were also resolved from each other and from the corresponding diastereomers.

This separation served as the basis of a novel procedure for stereospecific analysis of triacyl-*sn*-glycerols from natural fats and oils. The first step was to subject the triacyl-sn-glycerols to partial hydrolysis with a Grignard reagent to produce diacyl-*sn*-glycerols, which were converted to the (*S*)-(+)-1-(1-naphthyl)ethyl urethane derivatives for resolution into the diastereomeric forms by HPLC. The compositions of positions *sn*-3 and *sn*-l were calculated from GC analyses of the fatty acids of the intact triacylglycerols and of the 1,2- and 2,3-diacyl-sn-glycerol derivatives respectively; that of position sn-2 was obtained by difference.

Position *sn*-2 of seed oils tends to contain a relatively high proportion of unsaturated fatty acids, but the first samples analysed appeared to indicate little difference in the compositions of positions *sn*-l and *sn*-3. In olive oil, for example, the proportion of oleic acid in each of these positions was almost exactly 70%, tending to suggest that the triacylglycerols were not in fact asymmetric[9]. On the other hand when molecular species, isolated from olive oil by silver ion HPLC, were subjected to the stereospecific analysis procedure, marked asymmetry was observed in some fractions. The trimonoenoic species, which was half of the total, was of course symmetrical, but other species were not. It is evident that the structures of seed oils may be more complex than has been believed, but this will only be revealed if a wide range of methodology is applied to tackle the analysis.

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Chemical delignification of plant fibres

D. Stewart & I.M. Morrison

Virtually all industrial applications of plant fibres require some processing before the fibres can be used for industrial purposes. Fibrous products rely, to a major extent, on the strength and flexibility of the cellulose microfibril. The processing conditions, therefore, must be able to remove as much of the non-cellulosic components, particularly the lignin, as possible but retain the cellulose microfibrils in as undamaged a form as possible.

The special properties of cellulosic fibres depend, to a large extent, on the ability of the individual cellulose chains to bind together *via* hydrogen bonds. Such bonds are weak compared to covalent bonds but when large numbers are present, as in the cellulose microfibril, they act in concert to provide strength. The close alignment of a number of cellulose chains means that the microfibril exhibits crystalline characteristics. In celluloses which have not been subjected to alkali of

high molarity, the individual chains are parallel and the two crystalline forms which have been identified, Cellulose I_α and I_β, only differ in the dimensions of the unit cell. Cellulose chains which have been solubilised and reprecipitated result in Cellulose II which has the more energetically-favoured antiparallel arrangement. As well as these crystalline regions, there are portions of the cellulose microfibril with little or no order. These are the amorphous regions. The proportion of crystalline to amorphous regions in the native fibre varies between plant species and can change with different processing conditions.

The properties of a fibre determine its preferred use. The properties required from the long plant fibres which have textile applications are very different when used for clothing compared to those used for cordage. Similarly, the properties required to manufacture paper for documents which are expected to have a lengthy life are very different from those needed for paper tissues.

Processing of plant fibres removes non-cellulosic constituents. Removal of non-cellulosic polysaccharides (NCP) is essential for some applications such as rayon (viscose). However, retention of significant amounts of NCP is beneficial to applications such as the pulp and paper industry where they assist in binding individual cellulose fibres together. The necessity to remove the other major non-cellulosic constituent, lignin, is more widespread. For the long fibre applications, removal is a prerequisite when used for textiles but is not necessary in the manufacture of cordage. Short fibre applications, such as pulp and paper, almost always require low lignin contents.

The two most common delignification methods used in the pulping industry are the sulphite and Kraft processes. The former relies on the action of the sulphite and hydrogen sulphite ions to degrade the lignin to water-soluble lignosulphonates, while the latter uses sulphide and hydrogen sulphide ions in alkaline solution for degradation and dissolution. Although both processes have effluent disposal problems, the major environmental problems arise from the subsequent bleaching steps. Bleaching involves the removal of any residual or degraded lignin and has relied on chlorinated reagents. Their damaging environmental consequences are only now being realised and addressed.

There are many chemicals which can degrade the lignin component and bleach plant fibres at the same time causing limited damage to the cellulose microfibrils. Some of them are extremely efficient but are either too toxic or expensive to be used on a large scale. For example, laboratory experiments with acid chlorite and permanganate show that both chemicals react extremely efficiently and produce a very white product. Such methods can only be used as the standard to be attained by new and environmentally acceptable methods.

It has been shown that oxone, a triple salt with the composition $2KHSO_5.KHSO_4.K_2SO_4$, is a convenient source of the peroxymonosulphate ($HOOSO_3^-$) anion which can act as an oxidant under mild conditions. As such it can be used to delignify woods and the degradation products are less damaging to the environment than those from related procedures. Its potential for the delignification of herbaceous annual and perennial plants is currently being assessed.

Delignification of barley straw by oxone under acidic conditions has been determined over a range of oxone concentrations, temperatures and molarity of acid, and after subsequent extractions with 1M NaOH. Gross changes in the carbohydrate composition of the fibres were determined by conventional means, while changes in physical properties were monitored by FT-IR spectroscopy.

Increasing the oxone concentration alone whilst maintaining constant acidity (0.1M H^+) resulted in little detectable removal of lignin at low temperatures. Removal of lignin only occurred at temperatures >50°C and oxone concentrations >50 g l^{-1}. Higher concentrations of oxone were even more efficient but would not be practically feasible. In a typical experiment, no lignin was removed by 50 g l^{-1} oxone at 30°C, while 40% was removed at 70°C (Fig. 1).

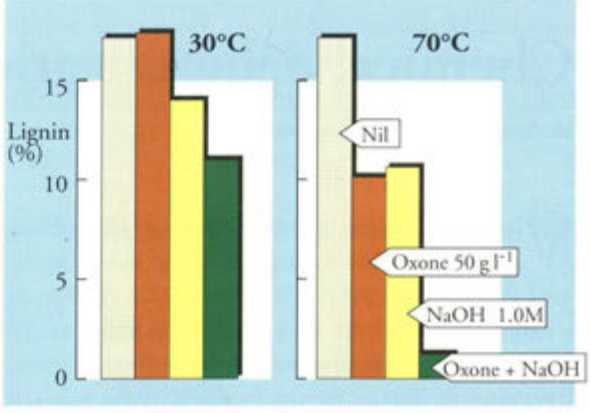

Figure 1 Removal of lignin by oxone treatment followed by 1M NaOH extraction at 30° and 70°C.

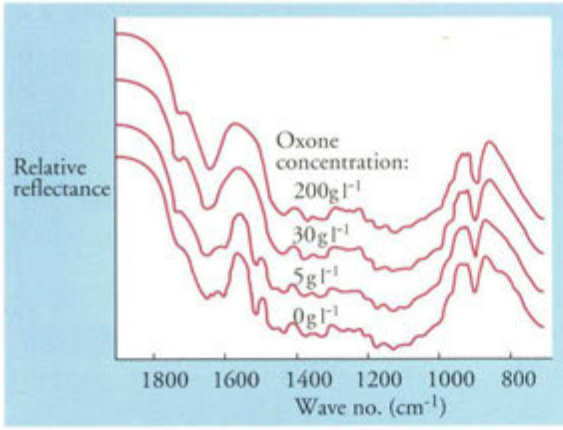

Figure 2 Effect of increasing oxone concentrations on the delignification of barley straw as shown by FT-IR.

A subsequent alkaline extraction removed 35% of the lignin at 30°C, while only 10% was left after treatment at 70°C. Alkali alone removed 17% and 35% lignin, respectively, at the two selected temperatures.

In conjunction with a subsequent alkaline extraction, increasing the acidity at 50 g l^{-1} oxone also increased the rate of delignification but the high molarity of the acid promoted a greater loss of NCP by hydrolysis. The L-arabinose side chains, which are present in the more acid labile furanose form, are readily removed. Even the main D-xylan backbone is attacked at higher temperatures although the loss of cellulose appears minimal. Treatment with oxone at 100°C did not bring about any advantages. Indeed, the chemical analyses, confirmed by FT-IR, indicated an increase in lignin content which must be due to some artefact.

Delignification in four concentrations of oxone at 70°C and 0.1M H^+ followed by extraction with 1M NaOH can be followed in the FT-IR spectra (Fig. 2). The frequencies to note are those at 1510 and 1595 cm^{-1} which are due to aromatic skeletal vibrations. The reductions in intensity correspond to lignin percentages of 11.1, 9.1, 1.5 and 0.3 for the 0, 5, 50 and 200 g l^{-1} oxone concentrations.

There is considerable interest in the use of other oxygenated species for delignification since they do not cause the same extent of environmental damage as the chlorinated compounds. Alkaline peroxide and ozone have both been investigated on barley straw as substrate. Peroxide alone appears to have little effect on the extraction of either lignin or NCP. However, FT-IR spectroscopy showed that reaction with lignin had occurred since the absorbances at 1510 and 1595 cm^{-1} were reduced in intensity. The spectrum of the peroxide treated sample shows spiking in the 1750-1630 cm^{-1} region. Since it is absent in other regions, it is not noise and due to the generation of carbonyl groups which are present in different environments and which absorb at slightly different frequencies in this region of the spectrum. Subsequent extraction with 1M NaOH caused loss of both lignin and NCP, the extent being greater in combination than with either reagent alone. The CP/MAS ^{13}C NMR spectra of plant fibres show some of the effects of treatment on the crystallinity and a comparison between untreated, chlorite- and peroxide-treated straw is shown in Figure 3. The chlorite treatment had little effect on the spectrum but peroxide appeared to have improved the crystallinity from the greater definition obtained.

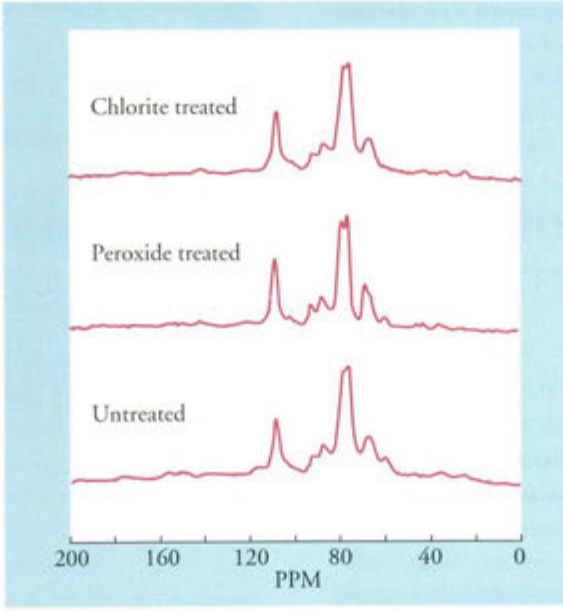

Figure 3 CP/MAS ^{13}C NMR spectra of untreated, chlorite- and peroxide-treated oat straws.

Figure 4 Effect of ozone on the delignification and bleaching of barley straw.

Ozone is ineffective under low moisture conditions but very efficient when bubbled through a stirred suspension of the fibre. When carried out in water, this form of oxidation generates high concentrations of H^+ ion but employing initially alkaline solutions reduces the hydrolytic effect of the low pH on the NCP. Visual changes in the treated straw are shown in Figure 4.

Novel chemical methods of delignification are also being investigated. One of these involves the use of porphyrin/metal ions as biomimetic catalysts. The ligninolytic enzymes reportedly contain a haem prosthetic group which is involved in its catalytic activity and the porphyrins may act in a similar manner. A number of these compounds have been investigated and a typical example is shown in Figure 5. Results with this compound and barley straw as substrate show that the lignin content can be reduced by *ca.* 30% but the work is incomplete and ways to improve the extent of delignification are being explored.

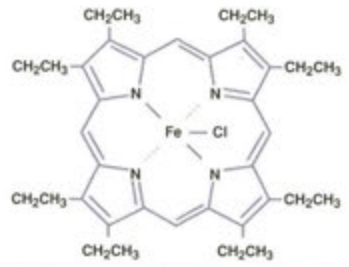

Figure 5 Structure of 2,3,7,8,12,13,17,18-octaethyl-21*H*,23*H*-porphine iron(III) chloride.

Applications of NMR spectroscopy

B.A. Goodman

Nuclear magnetic resonance (NMR) spectroscopy uses the fact that nuclei with non-zero spin exist in 2I + 1 (where I is the nuclear spin) energy states in the presence of a magnetic field, and that transitions between these states can be induced by the application of radiowaves of the appropriate frequency. Measurements of the precise energies of these transitions provide a great deal of information about the chemical environments of those nuclei. This article reviews three recent applications of NMR spectroscopy that have featured in research carried out at SCRI, namely (i) the metabolism of simple sugars by plant parasitic nematodes, (ii) identification and characterization of mobile elements on the surfaces of tobraviruses, and (iii) identification of the forms of phosphorus in organic soils (in collaboration with MLURI). All measurements were performed using either the Bruker AM300 or 500 spectrometers in the Chemistry and Biochemistry Departments at Dundee University. Previous articles have described the use of NMR spectroscopy at SCRI in elucidating sucrose starch interconversions in potato tubers (*Ann. Rep. 1991,47*) and on the isolation and identification of plant fibres for industrial uses (*Ann. Rep. 1991, 64*).

Sugar utilization by plant parasitic nematodes Recent experiments have demonstrated that it is possible to follow *in vivo* the utilization of food sources by plant parasitic nematodes with solution ^{13}C NMR spectroscopy. Transformations of specifically ^{13}C-labelled molecules can be monitored as the chemical environments of the label change and the uptake, transformation and utilization of glucose by *Longidorus elongatus* have been followed under conditions of moderate oxygen stress. Uptake was tracked easily by monitoring the decrease in the intensity of the peaks from the ^{13}C label in glucose in a solution that was in contact with a population of nematodes. After an initial rapid uptake, the demand tailed off rapidly and was followed, in some instances, by excretion of unaltered glucose molecules. After uptake, much of the glucose was converted into trehalose and glycogen with no change in the position of the label. Some lactate was also formed. After removal of any external glucose by thorough washing with either distilled water or phosphate buffer, the transformations of those products that were derived from the labelled glucose were determined. The results indicated that glycogen was used preferentially, probably via break-

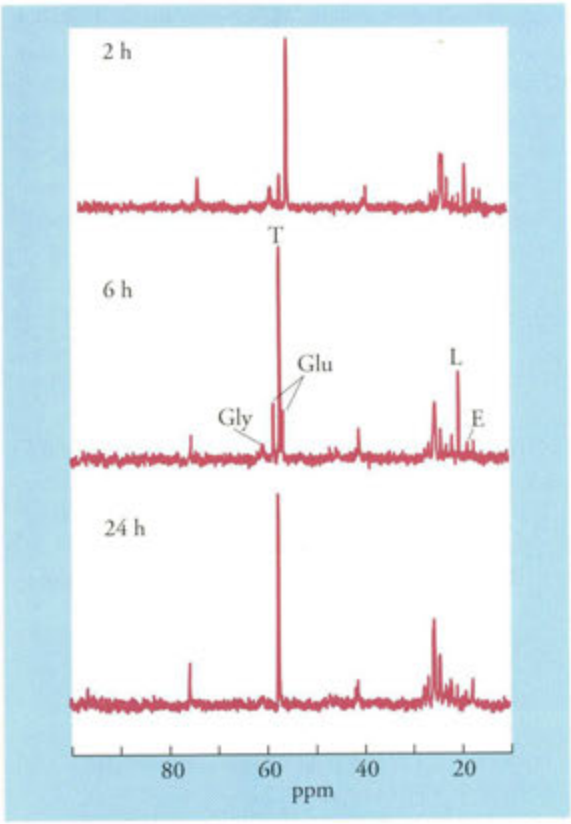

Figure 1 ^{13}C NMR spectra of the nematodes *Longidorus elongatus* taken 2h, 6h and 24h after removal from solution containing ^{13}C-1 enriched glucose. (Gly= glucogen, Glu = glucose, T = trehalose, L= lactate, E = ethanol).

down to glucose, and that trehalose was not utilized until both glycogen and glucose had been severely depleted. Typical spectra (Fig. 1) show that ethanol was also produced during glucose metabolism and that significant levels of both lactate and ethanol remained after thorough washing, demonstrating that both were located inside the nematodes. Thus both lactic acid and ethanolic fermentation processes operate in *L. elongatus* during glycolysis under conditions of limited oxygen availability.

Characterization of mobile elements in tobraviruses Virus transmission and infection are thought to often involve specific chemical bonding between virus particles and vectors or host cells. For example, tobraviruses are transmitted specifically by trichodorid nematodes; virus particles are bound to the nematode oesophagus, probably by chemical recognition of external parts of the coat protein. Recent work with tobraviruses has shown that solution ^{1}H NMR spectroscopy can identify mobile elements on the coat protein of virus particles. This solution NMR approach is effective because the limited mobility of the rigid, rod-shaped framework of the virus particles results in resonances from the structural elements that are too broad to detect.

The 1-D ^{1}H NMR spectrum of pepper ringspot tobravirus (PRV) shows the presence of several peaks from mobile residues (Fig. 2). These are difficult to interpret unambiguously because of extensive peak overlap, although tentative assignments are indicated on the spectrum. The residues alanine, asparagine, glycine, proline, serine and threonine, however, were clearly identified from their characteristic patterns in the 2-D COSY (homonuclear shift correlated or COrrelated SpectroscopY) spectrum of PRV (Fig. 3). These six residues are present without any intervening amino acids only in the last 38 C-terminal residues, and all six occur in the last 11 residues, where the sequence is:- H_2N-ser_{213} gly ala ala pro thr pro pro asn pro_{223}-COOH. Therefore, the NMR results suggest that the mobile element in PRV is an amino acid sequence containing between 11 and 38 amino acids close to the C-terminus of the coat protein. Thus the non-invasive solution NMR technique offers a novel approach to the detection of candidate elements involved in binding vector organisms to receptors on host cells.

Characterization of the forms of phosphorus in organic soils ^{31}P NMR spectroscopy has been used to characterise the forms of phosphorus in organic soils. Most of the measurements have involved the use of alkali extracts of soil, in which NMR was able to iden-

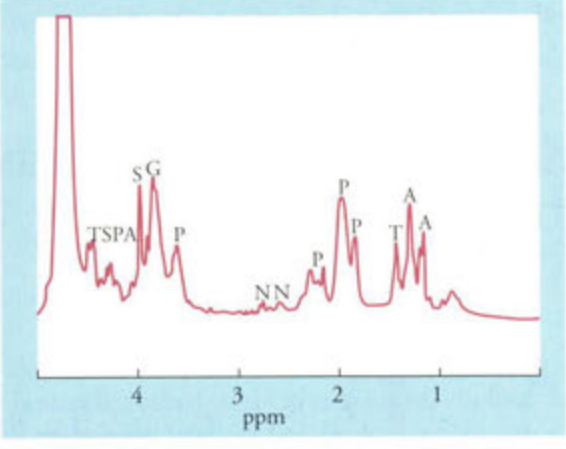

Figure 2 500MHz ^{1}H spectrum of PRV in D_2O/ phosphate buffer at pH7.0. (A = alanine, G = glycine, N = asparagine, P = proline, S = serine, T = threonine).

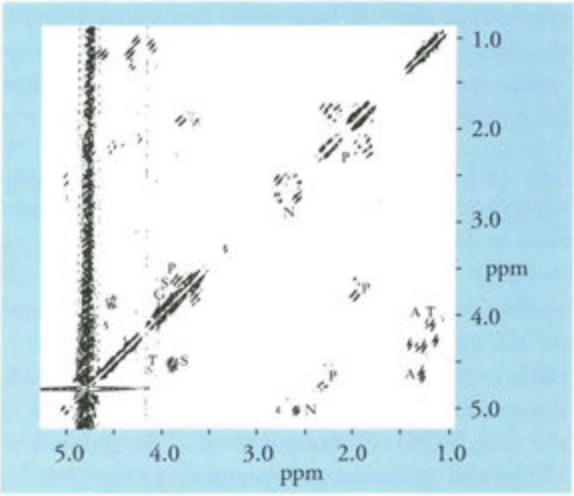

Figure 3 2-D phase sensitive COSY spectrum of PRV. (A = alanine, G = glycine, N = asparagine, P = proline, S = serine, T = threonine).

tify directly organic forms of phosphorus. The NMR procedure is simpler than partition chromatography techniques that are conventionally applied for this type of analysis. Different types of phosphorus compound can be identified because the different chemical environments result in resonances occurring at different frequencies (Fig. 4). The number of ester linkages and the presence or absence of direct carbon-phosphorus bonds are the major factors in determining the resonance frequencies in soil organic matter extracts. Structural variations remote from the phosphorus atoms have much smaller effects on the spectra but peaks characteristic of specific monoesters can still be distinguished.

Attempts to use solid state techniques to determine directly the forms of phosphorus in untreated soil

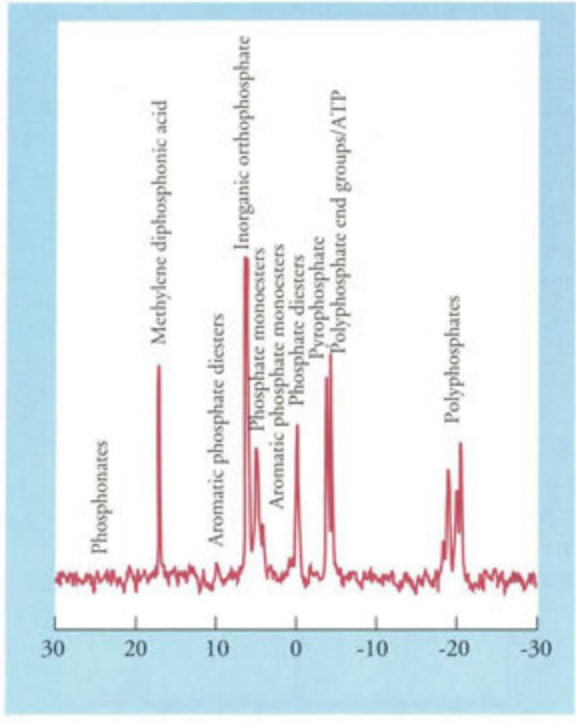

Figure 4 ^{31}P NMR spectrum of a 0.5M NaOD solution of a peat humic acid with assignments.

specimens have so far been unsuccessful. In samples with low iron content, spectra can be obtained easily using the CP/MAS technique, but the width of the resonance is too great for peaks from the individual components to be resolved. The use of peak narrowing techniques will be investigated as a potential route to overcome this problem.

Acknowledgements: This work was carried out in collaboration with W.M. Robertson, K.M. Brierley, M.A. Mayo, C.N. Bedrock (MLURI) and J.A. Chudek (Dundee University).

Characterisation of copper-containing enzymes by EPR spectroscopy

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

Approximately a dozen metal ions have well established essential roles in higher plants and animals. Some, such as sodium and calcium, are present in large quantities and are classed as bulk elements. Others occur only in small quantities and are known as trace elements or micronutrients, the most abundant of which is iron.

The biological functions of metals are numerous. Bulk elements are involved in the maintenance of structural integrity of macromolecules and the formation of ionic gradients across the plasma, and other membranes, whilst the trace elements, which are generally transition metals, are involved in catalysis and redox chemistry. The latter can for convenience be separated

into two categories; those which participate in electron transfer as in the mitochondrial electron transport chain, and those which form the active sites of redox active enzymes. Proteins belonging to the former group typically have extensive α-helical structures, where the local structure is modified by the presence of the metal ion. Those in the latter group are usually rich in β-sheet, have a more rigid structure, and the metal ions and adjoining groups usually have very limited mobilities. Metalloenzymes that catalyse chemical transformations are almost exclusively those metalloproteins with β-sheet structure.

Redox chemistry of transition metal ions Transition metal ions readily undergo reduction or oxidation reactions through the gain or loss of an electron. Some are, therefore, very good scavengers of free radicals through reactions such as

$$M^{n+} + e^- \rightarrow M^{(n-1)+}$$

Others may be instrumental in generating free radicals through reactions such as the Fenton reaction in which the highly reactive hydroxyl radical (HO·) is generated from hydrogen peroxide.

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + HO^- + HO\cdot$$

These two types of reaction form the basis of the catalytic activities of all enzymes that are based on transition metal ions.

In order to illustrate the major contributions that EPR spectroscopy has made to the characterisation of the active sites of metalloenzymes, this article will briefly summarise results obtained from proteins which contain copper as an essential cofactor.

Copper-containing enzymes Copper is generally limited to only two oxidation states in biological systems; Cu(I) which has the $3d^{10}$ outer shell electronic configuration and is therefore diamagnetic, and Cu(II) which has one fewer 3d electron and is therefore paramagnetic. EPR spectroscopy of Cu^{2+} is able to provide a wealth of information about the chemical environment of the metal ion. A typical spectrum is shown in Figure 1 where the four principal parameters $g_\perp$, $g_{||}$, $A_\perp$ and $A_{||}$ are indicated. The relationship between $g_{||}$ and $A_{||}$ can be used to determine the degree of covalency of the bonding between the copper and the ligands associated with it and also to determine the symmetry characteristics of the copper site. Additional splittings as a result of coupling to adjacent ^{14}N nuclei may occur, which can be invaluable in identifying the ligand nuclei that are bound to the copper. An example is depicted in Figure 2, which

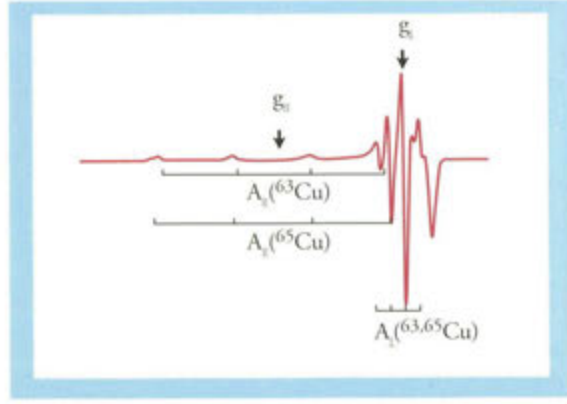

Figure 1 Principal EPR spectral parameters of Cu^{2+}. Separate peaks from the ^{63}Cu (69%) and ^{65}Cu(31%) isotopes may be resolved if spectral peaks are sufficiently narrow.

shows extensive spectral splitting as a result of coupling the unpaired electron to four ^{14}N nuclei.

Control of the superoxide radical is of paramount importance in all living systems, because of its ability to initiate oxidative and peroxidative damage to cell membranes. Two types of enzyme are involved in the first line of defence; the blue copper proteins which act directly as scavengers of superoxide and the superoxide dismutases (SOD), which catalyse the disproportionation of superoxide into oxygen and hydrogen peroxide

$$2O^{2-} + 2H^+ \rightarrow O_2 + H_2O_2$$

A wide variety of blue copper proteins have been isolated from higher plants, many from nonphotosynthetic tissue such as roots or latex. Several are glycoproteins and are closely related. For example in stellacyanin, plastocyanin and azurin the copper is coordinated to two histidines and two sulphur ligands in flattened tetrahedral structures.

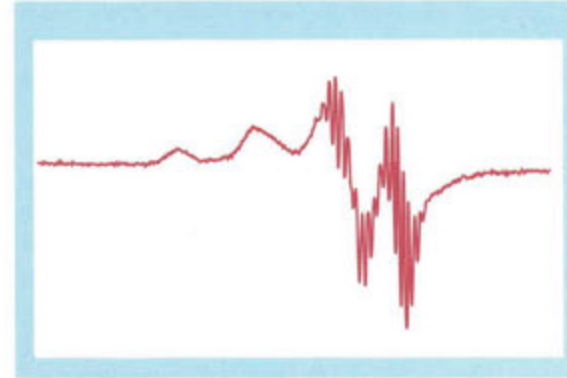

Figure 2 EPR spectrum of Cu : phthalocyanine.

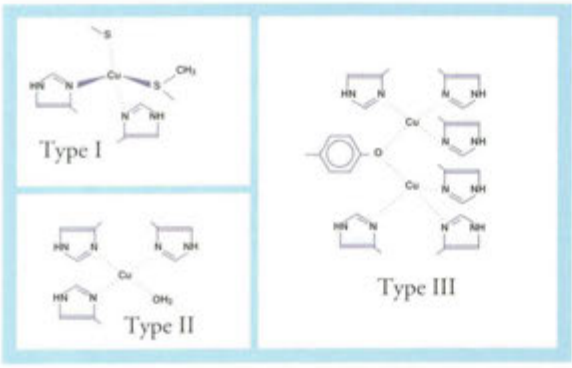

Figure 3 Proposed copper coordination environments in laccase.

Laccase (benzenediol: oxygen oxidoreductase) and ascorbate oxidase are members of a more complex class of blue copper oxidoreductases and bear close resemblance to mammalian caeruloplasmins, in which at least three different types of copper have been identified on the basis of their EPR and visible absorption spectra (Fig. 3). The Type I sites are similar to those in stellacyanin and plastocyanin and have relatively small copper hyperfine splittings in their EPR spectra, the Type II sites are tetragonal and have EPR spectra with large $A_{\|}$ and $A_{\perp}$ values and the Type III sites do not give EPR spectra and are thought to represent spin-coupled Cu(II) ions. The functions of plant laccases are only partially understood. Being a constituent of latex, laccase is released when a plant is damaged and specific phenols are then oxidised to free radical intermediates by the enzyme. The free radicals subsequently polymerise to create a physical barrier at the site of the injury. Laccase has been shown to contain the copper centres depicted in Figure 3 in the ratio 1:1:2 (Types I:II:III respectively). The EPR spectrum shows contributions from both the Type I and Type II Cu (II) ions (Fig. 4) and the technique has been successfully applied to the study of the stepwise reduction of the different sites by electrochemical means.

Three distinct families of **Superoxide Dismutase** are known with copper, manganese and iron as the prosthetic group. The copper enzyme also contains equimolar quantities of zinc, there being two Cu and two Zn atoms per molecule. The Cu(II) ions in the active enzyme give EPR spectra characteristic of Type II copper (Fig. 5) and the presence of histidine has been implicated in the coordination sphere. Substitution of other metals (*e.g.* cobalt) at the zinc

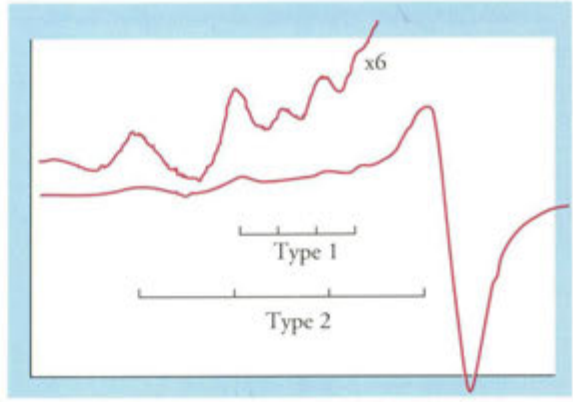

Figure 4 EPR spectrum of laccase - related mammalian enzyme, caeruloplasmin.

site has been used to show that the two metal sites are not in close proximity and by the use of oligonucleotide-directed mutagenesis it has been possible to show that subtle changes in the geometry of the metal sites can produce major changes in the electron transfer properties and specific activities.

In summary, many enzymes from higher plants and animals are known to require transition metal cations for their biological activities. EPR has been extensively used in the study of these systems and has been shown repeatedly to have distinct advantages over other spectroscopic techniques. Perhaps the most significant of these is the opportunity it presents to study the enzyme in question without the need for excessive purification.

SCRI is now fully equipped to undertake the study of metalloenzymes by EPR and many examples will be studied in the coming years.

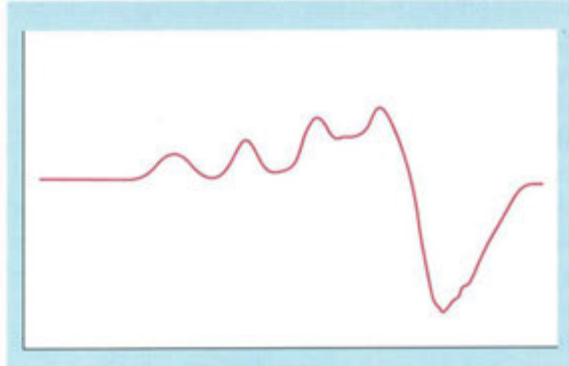

Figure 5 EPR spectrum of Type II copper in superoxide dismutase.

Fungal & bacterial diseases

J. M. Duncan

Previous contributions to the Annual Report have been concerned largely with aspects of the interactions of hosts and pathogens and, in particular, the genetics underlying compatible and incompatible combinations. In addition, the biochemical and physiological events underlying resistance are also being actively investigated at SCRI.

Much interest over the past few decades has focused on resistance mechanisms which are reactive processes, i.e. changes that occur in the metabolism of the plant in response to a challenge from a pathogen, and these changes, or the end products of them, ultimately result in resistance. This form of resistance could be described as 'reactive resistance'. Indeed, responses of this type by plants to infection are often known as resistant reactions. Other forms of resistance based on the presence of pre-formed inhibitors in a plant have been reported but have in general received less attention. Such resistance, to adapt a fashionable adjective, could perhaps be described as proactive. The plants in which proactive resistance have been claimed to be present are as diverse as the pre-formed inhibitors themselves and both proteins and non-proteinaceous compounds have been implicated. A well characterized example of this form of resistance occurs in mango fruits which are subject to attack by *Colletotrichum gloeosporioides*. Antifungal resorcinols present in the rind of the fruit inhibit the development of the pathogen until the fruit is nearly ripe and then reduction in their concentration coincides with rapid expansion of hitherto quiescent fungal infections. Such protective mechanisms may be common in fruit where the immature seed need to be protected from infection until mature enough to be dispersed by animals, birds or insects that feed on the fruit. Despite the economic value of fruit production, mechanisms underlying quiescent infections and resistance to infection have rarely been elucidated.

A proactive defence mechanism which operates against *Botrytis cinerea*, the cause of grey mould in many fruits and plants, has been found recently in unripe fruit of raspberry. The fungus infects young fruits of raspberry and strawberry but does not become aggressive until the fruit starts to ripen. Williamson and Johnston have shown that the change from quiescence in young fruit to rapid expansion in

ripe fruit coincides with the disappearance of a protein present in young fruit which inhibits the endo-pectinases produced by *Botrytis*. This raises the prospect of a possible novel control system in which the expression of the genes coding the protein is altered so that resistance remains effective in ripe fruit. Proactive resistance is expressed in healthy plant tissue and may be linked to one or a few materials, while resistance is a consequence of a cascade of interrelated events within the host that may not be reversible. The former may therefore be simpler to manipulate.

The development of a pathogen on a plant is controlled by environmental factors as well as the internal response of the host and there is an optimum set of conditions for infection, spread within the host and sporulation for each pathogen. Surprisingly, these are often not well defined, even for the more important pathogens. The effect of environment on the expression of host resistance has been described previously by Harrison and in this report he examines the more direct effects on the pathogen by reference to the two aerial diseases, chocolate spot (*Botrytis cinerea*) of field beans and foliage late blight (*Phytophthora infestans*) of potatoes. The similarities and contrasts between the diseases are striking but the information that is produced in each case is vital for progressing our understanding of their aetiology and epidemiology, and for the development of predictive models that can be used in control strategies.

All of plant pathology involving pathogens is concerned with the development of one organism on another and their interactions. In the case of blemish diseases of potatoes, the process is known to be initiated by inoculum present in soil or on the mother tuber but the role of root infection has been largely ignored until now. This deficiency is addressed in an article that marks the end of the long career at SCRI of Pat Dashwood who has been one of the best mycologists at the Institute and employed her skills to identify fungi to clarify the importance of root infection in the development of tuber disease and to elucidate the relationships among pathogenic and other root inhabiting fungi. Her observations indicated that some of the saprobic fungi could have a role to play in the biological control of this group of diseases.

The final article by Pérombelon deals with methods for enumerating accurately and rapidly the numbers of pathogenic bacteria of the genus *Erwinia* on potato tubers. Imminent changes of controls on the movement of plant material within Europe and the possible consequences that it might have for the health of potato stocks throughout the Community makes this article timely and important, especially for the future economic health of the Scottish seed potato industry.

All aspects of research in plant pathology, from the most basic to the applied must impinge on the agricultural industry and the topics included in this report could contribute to the development of a healthier and more productive agriculture that would be less dependent on high chemical inputs for disease control. The need for inputs of skills and knowledge will remain at least as high as at present for the foreseeable future.

Quiescence and grey mould in raspberries

B. Williamson, D.J. Johnston, R.J. McNicol, V. Ramanathan & E.K. James*

Grey mould (*Botrytis cinerea*) is the most important post-harvest disease of soft fruits and vegetables worldwide and it is difficult to control satisfactorily with fungicides because the fungus is genetically variable and has developed strains resistant to many of the chemicals introduced in the last 20 years.

Early work at Invergowrie established that although only the ripe raspberry fruits develop the disease, the fungus infects the flowers and most fruits carry fungal inoculum by the time they begin to ripen. However, grey mould does not generally develop on flowers unless they have been damaged by late spring frosts, or on immature green fruits, except in extremely wet seasons.

Quiescent infections The fungus establishes a quiescent infection in newly opened flowers which ultimately gives rise to the disease in harvested fruit. Microscopical studies have shown that conidia of the pathogen germinate in the viscous fluid secreted by

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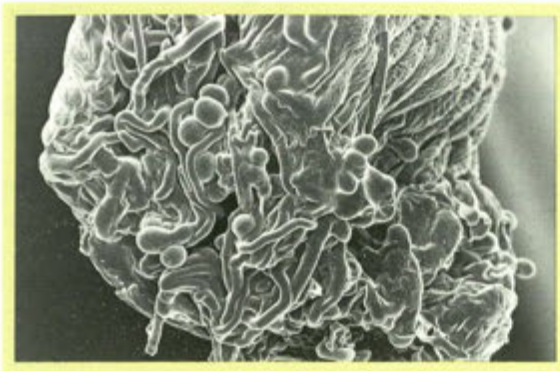

Figure 1 Conidia of *B. cinerea* germinating on the stigma of a newly-opened raspberry flower (low temperature scanning electron microscopy).

stigmas (Fig. 1) and the fungal hyphae grow slowly and symptomlessly amongst pollen tubes in the transmitting tissues of the styles (Fig. 2)[1]. Fungal growth is severely inhibited by stylar tissues, but the mechanisms involved are not understood. In some cultivars the fungus may reach the ovary walls, but grey mould rarely develops at this stage of fruit development. After fertilisation the styles always die and remain attached to the developing fruit. *B. cinerea* survives as mycelium inside the dead styles and, after the fruits ripen, the fungus spreads rapidly from the dead styles at high relative humidity to destroy the fruit (Fig. 3)[2]. Strong disease resistance mechanisms clearly operate in unripe fruit because the fungus does not cause rotting after inoculation of wounded berries, except at maturity.

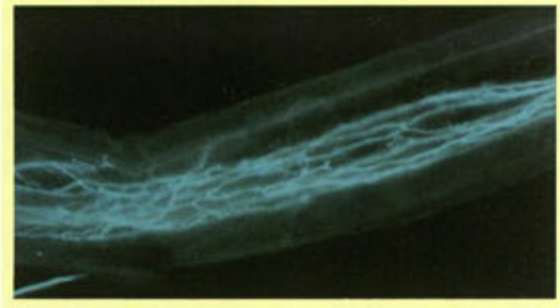

Figure 2 Fungal hyphae growing in transmitting tissue of a style (specimen stained by aniline blue and viewed by fluorescence microscopy).

Quiescent infection by *B. cinerea* occurs in all soft fruit crops we have examined, but the pathways for infection and the effect on the plant differ in each case. Fungicides are sprayed during the blossom period to reduce the serious losses due to post-harvest grey mould, but many flowers inevitably remain unprotected because current fungicides can only be applied at 7 to 10-day intervals.

Two aspects of the biochemical and genetic basis of the phenomenon of quiescence of *B. cinerea* in raspberry are being examined. Firstly, attention has been given to the secretion by the pathogen of polygalacturonase (PG) enzymes which digest the pectin component in plant cell walls. These enzymes play a key role in the early stages of infection in many fungal diseases of plants. Secondly, the nature of host plant resistance has been examined by studying the activity of a protein inhibitor of PG which is present in cell walls of green raspberry fruits.

Purification of fungal enzymes *B. cinerea* was grown in the laboratory on a pectin-rich liquid medium for 10 days and PGs were purified as shown in Fig. 4. Four isozymes were identified and characterised, two endo-PGs which cleave randomly the polygalacturonic acid 'backbone' of the large pectin molecule, and two exo-PGs which stepwise remove single galacturonic acid residues from the ends of the molecule [3].

A specific polyclonal antibody to one of the endo-PGs has been prepared and used to identify endo-PGs present in infected fruits after separation of the isozymes by preparative isoelectric focusing and SDS-PAGE. The antibody is also being used to study enzyme secretion at the ultrastructural level by means of immunogold technology. By culturing the fungus on media containing glucose or galacturonic acid (sugar from which pectin is largely built) as the sole carbon source, it has been possible to show by PTA-ELISA that the endo-PGs are constitutively expressed, whereas the exo-PGs are induced in the presence of galacturonic acid[4]. Further work will

Figure 3 Grey mould developing in ripe raspberry fruit as hyphae spread from attached dead styles.

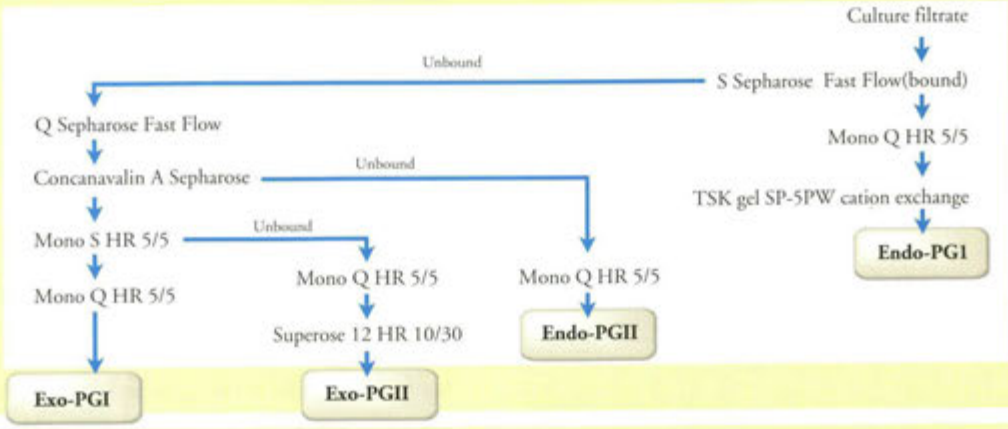

Figure 4 Separation protocol for purification of four polygalacturonases from cultures of *B. cinerea*.

include an examination of the precise role of the endo-PGs and exo-PGs in the infection process and the identification of the fungal genes which regulate their activity.

Enzyme-inhibitor protein from raspberry It was found that cell wall extracts from unripe raspberry fruits strongly inhibited the fungal PG activity of *B. cinerea*. Subsequently a 38.5 kDa PG-inhibiting protein (PGIP) with a pI value above 10 was purified from cell walls after extraction using high salt concentration (Fig. 5) and shown to specifically inhibit the endo-PGs, but not the exo-PGs[5]. The protein also inhibited an endo-PG from *Aspergillus niger*, but had no detectable effect on similar enzymes produced by *Erwinia carotovora* subsp. *atroseptica*.

The PGIP had highest activity in under-ripe fruits, but declined in activity in ripening fruits when susceptibility of the tissue to fungal attack can be shown by inoculation experiments to be increasing.

Figure 5 HPLC separation of PGIP extracted at high salt concentration from cell walls of immature raspberry fruits.

It seems likely therefore that the PGIP is involved in the resistance of fruits to grey mould and further work is needed to identify the plant gene responsible for its production. The N-terminal sequence of amino acids for the PGIP from raspberry shows strong homology with a similar molecule from beans, the only other PGIP to be fully characterised. We are cloning the PGIP from raspberry and propose to determine its function by means of recombinant DNA techniques.

Disease control by enhancing plant resistance By means of *Agrobacterium*-mediated gene transfer techniques we plan to incorporate the cloned PGIP gene into raspberries and other fruits susceptible to grey mould and by use of a constitutive promoter ensure that the protein is produced until fruits are ripe. These plants will then be evaluated for their resistance to *B. cinerea* and other fungal pathogens. This approach may enhance substantially the natural resistance of fruits to *B. cinerea* and thereby control grey mould without the need for fungicide spray programmes.

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Effects of humidity on *Phytophthora infestans* and *Botrytis cinerea*

J.G. Harrison, R. Lowe & N.A. Williams

Lesion expansion and spore production are important stages in the life cycle of pathogenic fungi affecting the aerial parts of plants. Expansion of lesions usually occurs as a pathogen colonises the host tissues. Fungal biomass increases leading to the potential to produce more spores as a lesion expands. Dissemination of spores spreads the pathogen, sometimes over long distances, and subsequent spore germination and infection of host plants results in a build-up of disease that is part of the development of an epidemic. Lesion expansion is often little-affected by atmospheric humidity, but sporulation is highly dependent on humidity close to the surface of a plant. Most pathogens require high humidities, often close to saturation, for sporulation, and spores are not formed in dry air. Many factors interact to determine the humidity adjacent to a plant. Except in saturated air, foliage continuously loses water vapour by transpiration, which humidifies the air close to plant surfaces. The humidity at any arbitrary point, where spores may be formed on a leaf surface, depends primarily on two factors, air speed and ambient humidity. As the rate of air flow across a leaf surface increases, so the transpired water vapour is removed more efficiently, reducing the humidity at that arbitrary point. For any given air speed, the humidity at that point is proportional to the ambient humidity, i.e. the humidity remote from the leaf. Clearly, air speed and ambient humidity interact to determine the humidity close to foliage, but other factors also affect humidity in the narrow zone of sporulation. For example, the pattern of air flow around a leaf, and therefore humidity, depends on its orientation, shape and surface topography. Sunlight falling on a plant heats the air adjacent to it, reducing the relative humidity (RH). No instrument is small enough to measure humidity next to a leaf and the complexities of the factors determining humidity make it very difficult to calculate, unless the ambient air is saturated and the leaf is in darkness.

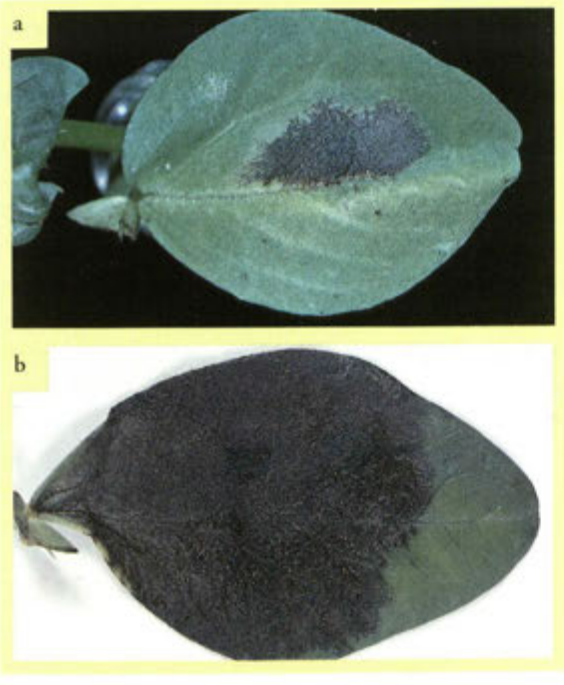

Figure 1 Lesions of *Botrytis fabae*. A) low humidity, B) high humidity.

***Botrytis fabae* on beans** Effects of humidity on expansion of chocolate spot lesions, caused by *Botrytis fabae* on leaves of field bean, were investigated, together with subsequent sporulation, as part of a wider epidemiological study of the disease at SCRI. Humidity was controlled within sealed systems containing infected leaves by bubbling air through saturated salt solutions before passing it over the leaves. The RH depends on the salt used and on temperature, which was controlled by immersing each system in a water bath in the dark. (Fig. 1a & b).

The rate of increase in lesion diameter was linearly related to humidity between 66% and 100% RH (Fig. 2). No conidia were formed at 86% RH, there were a few at 93% RH, but extensive sporulation occurred at 95 and 100% RH.

***Phytophthora infestans* on potatoes** Environmental factors affecting the development of late blight of potato were studied in detail. Like chocolate spot, blight is favoured by wet or humid weather and destroys the foliage. The interactions between ambient humidity and air speed were investigated and it was necessary to maintain ambient humidity at any

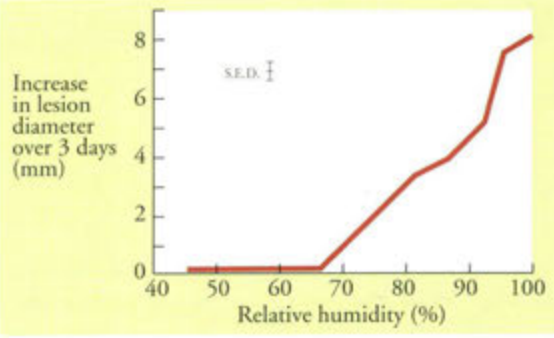

Figure 2 The effect of humidity on chocolate spot lesion expansion.

level, not just those generated by passing air through saturated salt solutions. A system for controlling the environment close to leaves was designed and built for this purpose[1]. Air is saturated by bubbling through distilled water in one water bath before passing it through a leaf chamber in a second bath. The temperature difference between the baths determines the RH. If both baths are at the same temperature, the air is saturated, and as the temperature of the first bath is reduced, the RH of air entering the leaf chamber decreases. The rate of air flow is controlled with an adjustable valve.

It is often difficult to distinguish blight lesions from healthy leaf tissue, and size measurements were found to be unreliable. Consequently, an alternative method of quantifying colonisation by the pathogen using an ELISA system based on polyclonal antiserum was developed. This method compared amounts of *P. infestans* in diluted homogenates of diseased leaves with a series of standards containing known quantities of the pathogen[2] and has proved to be highly reliable.

Ambient r.h. (%)	Air speed (mm/sec) 0.3	1.4	5.5	13.7
100	67,000	96,000	91,000	139,000
95	83,000	98,000	8,000	5,000
90	76,000	17,000	180	290
85	18,000	4,000	90	420
80	8,000	70	190	1,400

Figure 3 Numbers of sporangia of *Phytophthora infestans* that formed on detached potato leaflets incubated in air at different humidities and speeds.

Infected potato leaflets were incubated in ambient air at each of five relative humidities flowing at each of four speeds. Assessment of colonisation of each leaflet by ELISA showed that, in contrast to chocolate spot, humidity had no effect within the range tested (80-100% RH). Sporangia were washed from individual leaflets and counted with a haemocytometer. Ambient humidity and air speed interacted to determine numbers of sporangia produced (Fig. 3). Slow-moving humid air allowed the pathogen to sporulate profusely, but few sporangia were produced in faster-moving drier air. Intermediate levels of sporulation occurred in relatively slow-moving dry air or in faster-moving more humid air. (Fig. 4).

Figure 4 Sporulation of *Phytophthora infestans* on leaf.

Conclusions Both *B. fabae* and *P. infestans* require high humidities for sporulation on leaves. However, a complex interaction of factors precludes an accurate definition of the threshold humidities for spore production of air immediately adjacent to the pathogens.

Knowledge of effects of humidity on pathogenic fungi on aerial parts of plants is essential for the development of mathematical models describing disease progress that can be used to help formulate disease management strategies for reduced fungicide usage. The techniques developed at SCRI for the precise control of the environment around leaves and for quantifying the pathogen are powerful tools allowing rapid progress to be made in this important field of research.

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Blemish diseases of potatoes: roots as sources of inoculum

E.P. Dashwood & J.M. Duncan

Blemish diseases of potato are caused by a diverse group of unrelated fungi that sometimes cause a reduction in yield but primarily have a detrimental effect on tuber quality (Fig. 1 & 2). With an increasing demand from the consumer for uniform and blemish-free produce, these diseases have assumed increased economic importance for ware producers.

Both soil- and seed-borne inoculum have been shown to be sources of blemish diseases, although the relative importance of each varies among the diseases. However, the role of root infection in disease development on tubers had largely been ignored until recently. With the trend to micropropagation and a smaller number of propagation cycles in soil, root infection could be one method by which these diseases could build up to economically important levels in potato stocks.

To infect roots, a pathogen is required to compete with, or even displace the existing root microflora. Therefore, it is important that the major components of the flora on roots are identified and that any antagonistic or associative relationships with the pathogens is determined. Antagonistic associations could signal the possibility of manipulating root fungi to achieve biological control of blemish diseases.

Experimental approach Studies began at SCRI in the mid 1980s to determine the importance of soil, mother tuber and root infection on the occurrence and development of blemish diseases. Disease-free plants were produced by micropropagation in the glasshouse in either a perlite or peat-based substrate, and their roots were exposed or not exposed to inoculum in peelings from blemished tubers before being planted in the field. At the same time, healthy and blemished seed tubers were planted after being surface sterilized or not treated. A total of nine treatments were established and the development of disease in them was followed after harvest and during storage. Fungal isolations were made from the roots of the plants at three times during the growing season and the occurrence and associations of different fungi, non-pathogenic and pathogenic, were recorded.

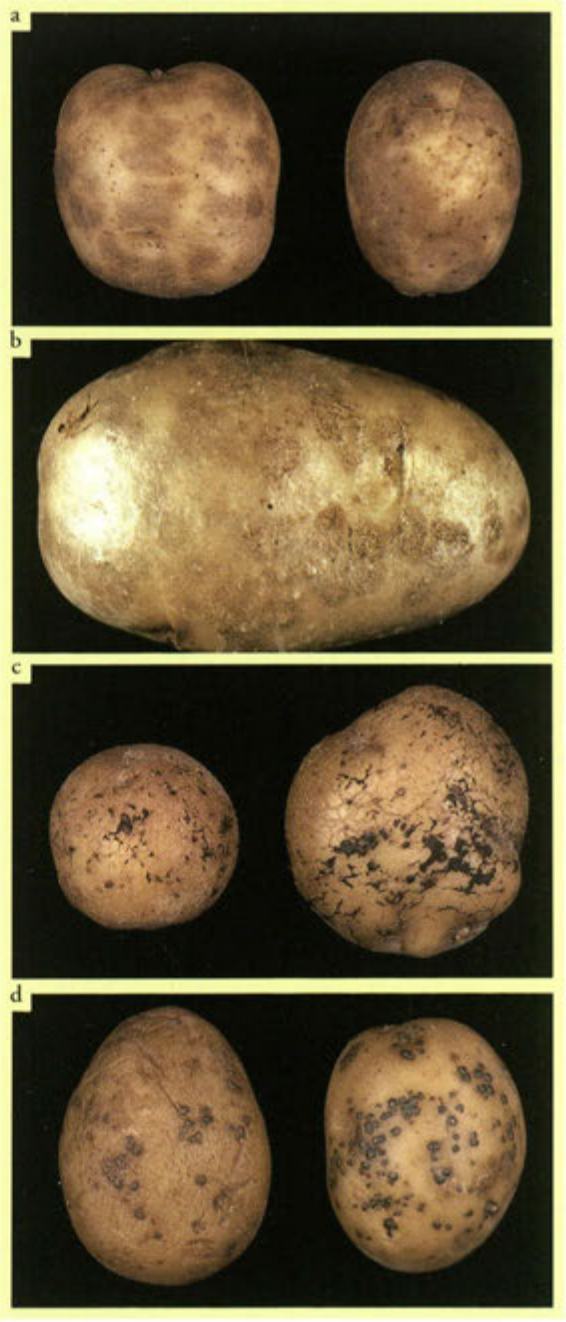

Figure 1 Blemish diseases of potato a) black dot (*Colletotrichum coccodes*), b) silver scurf (*Helminthosporium solani*), c) black scurf (*Rhizoctonia solani*) d) skin spot *Polyscytalum pustulans.*

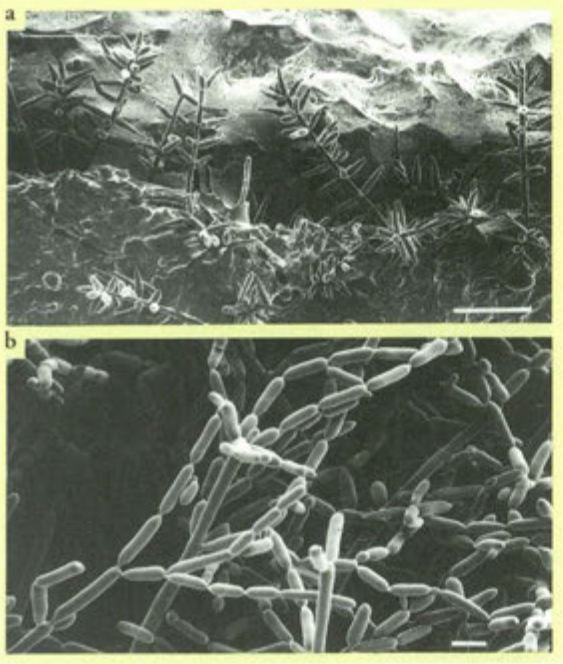

Figure 2 Surfaces of potato tubers infected with a) silver scurf (*Helminthosporium solani*) (scale bar 10 µm) and b) skin spot (*Polyscytalum pustulans*) (scale bar 1 µm) as viewed by scanning electron microscopy.

Occurrence of pathogens Three pathogenic fungi, *Polyscytalum pustulans* (skin spot), *Rhizoctonia solani* (black scurf) and *Helminthosporium solani* (silver scurf), occurred infrequently on roots suggesting that this source of inoculum was unimportant in the growing plant. However, root infection by the first two fungi can increase considerably after haulm senescence. Where root infection did occur during growth, it was clearly derived from contamination of the seed. *H. solani* was observed for the first time on roots in this study but it occurred very rarely at this site (Fig. 3), even from mother tubers that were severely contaminated. In all three diseases, symptoms were largely absent from progeny tubers derived from uninoculated, micropropagated plants, emphasizing the importance of seed-borne inoculum in initiating infection.

Figure 3 Potato root infected with silver scurf.

Levels of infection by *Colletotrichum coccodes* (black dot) were generally high in all treatments and increased considerably throughout the season. Although the highest levels occurred where inoculum had been added as tuber peelings or had been present on the original mother tubers, its frequent occurrence in other treatments indicated that soil was also an important source of inoculum. Common scab (*Streptomyces scabies*) was also soil-borne and it was commonest on the tubers from micropropagated plants, perhaps because they lacked antagonistic microorganisms to interfere with its development.

Verticillium dahliae was also isolated frequently from root segments in one of the experimental years. This fungus causes premature senescence of haulms in Britain, most commonly in dry years, and severe vascular wilt under Mediterranean conditions. Again, it occurred most frequently on roots of micropropagated plants, indicating that it was present in the soil and that infection might proceed more rapidly on roots that possessed few antagonists.

Occurrence of root infecting fungi Although more than 110 different fungal species were recovered from root pieces plated onto agar over the two years of the experiment, only five were recovered at a mean frequency of more than 5%. Three of the blemish fungi (*C. coccodes, R. solani* and *P. pustulans*) were amongst the fifteen most commonly isolated species from roots. When the occurrence of the fifteen was analyzed using Principal Components Analysis, they could be assigned to four groups (A,B,C, and D in Fig. 4). Group A consisted of fungi that occurred abundantly on roots from treatments where the microplants initially had been grown in peat-based composts, e.g. *Trichoderma viride, Chrysosporium pannorum, Aureobasidium pullulans* and *Penicillium* spp. that presumably were introduced in the compost. They are often antagonistic towards other fungi and there was low concurrence between members of this group with fungi in other groups, in particular Group B that contained several pathogens. Perhaps they were excluding or antagonising the pathogens. Group B represented an intermediate group between Groups C and D which largely consisted of general root-invading saprophytes that are common in cultivated soils. One pathogen, *C. coccodes* occurred in Group C and was the second most commonly isolated fungus from

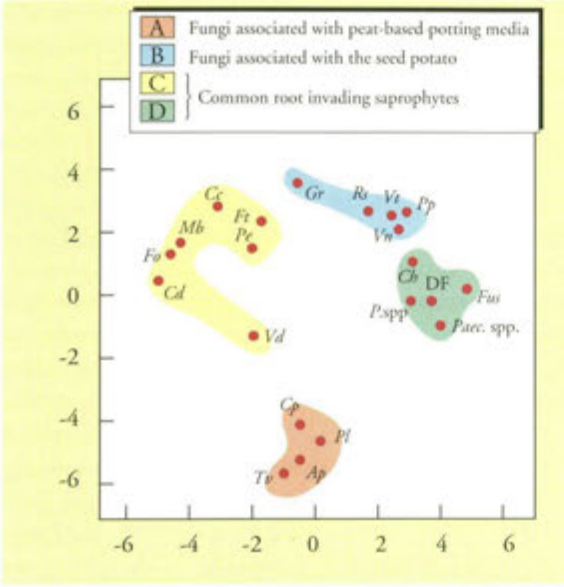

Figure 4 Spatial relationships of fungi found on roots of potato plants in August, 1985 as determined by principal co-ordinate analysis.
Group A: *Aureobasidium pullulans (Ap), Chrysosporium pannorum (Cp), Phoma leveillei (Pl), Trichoderma viride (Tv).*
Group B: *Gliocladium roseum (Gr), Polyscytalum pustulans (Pp), Rhizoctonia solani (Rs), Verticillium nubilum (Vn), V. tricorpus (Vt).*
Group C: *Colletotrichum coccodes (Cc), Cylindrocarpon destructans (Cd), Fusarium oxysporum (Fo), F. tabacinum, Microdochium bolleyi (Mb), Phoma eupyrena (Pe), V. dahliae (Vd).*
Group D: *Chaetomium spp., dark sterile fungus (DF), Fusarium spp. (Fus), Paecilomycespp. (Paec spp.), Penicillium (P. spp).*

roots after *Cylindrocarpon destructans*. Early in the season, its frequency was reduced in plants that had been grown in peat-based composts and it may have been antagonised by *T. viride* as there was a strong negative association between the two. Among other potential antagonists of *C. coccodes* that were recovered, *Gliocladium roseum* is known to parasitize the pathogen in culture.

Control of blemish diseases by antagonists and hot water treatment Fungi which are antagonistic towards or which exclude blemish pathogens from sites of infection on potato roots and tubers, have a potentially useful role in low input control of blemish diseases.

Many of the fungi isolated from roots were tested for antagonistic activity towards the blemish fungi in culture (Fig. 5), and 18 were tested further on pieces of potato tissue treated with antioxidant and in pot trials. *T. viride*, *T. harzianum* and *G. roseum* and *Penicillium* spp. all had some effect: both *Trichoderma* spp. and *G. roseum* inhibited growth of all four pathogens but *Penicillium* spp. were only effective against silver scurf

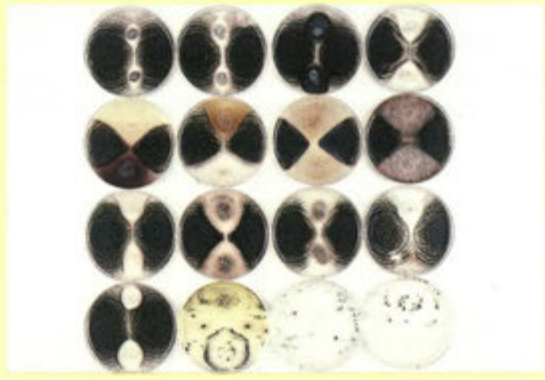

Figure 5 Screening potential fungal antagonists of black dot (*Colletotrichum coccodes*) on agar plates. Black segments which result from growth of the pathogen are absent from three dishes in the bottom row, each of which has been inoculated with a different isolates of *Trichoderma*.

on the discs. In pots of sterile soil, *T. viride* and *Penicillium expansum* controlled skin spot but only the former was effective in field soil.

Continuous-flow hot water treatment has effectively controlled the level of contamination of seed tubers by *Erwinia* spp. and has also had beneficial effects on blemish diseases. In trials, it reduced skin spot, silver scurf and black scurf infection from 92% to 2%, 29%

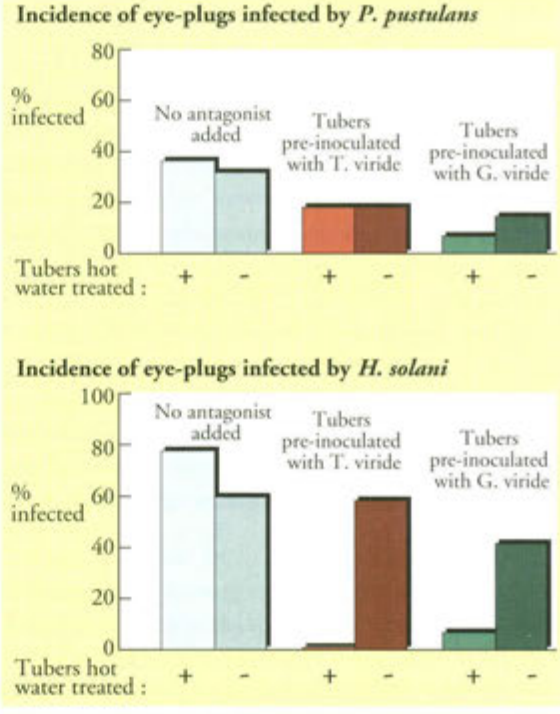

Figure 6 The effect of hot water treatment and antagonists on the incidence of *P. pustulans* and *H. solani*.

to 0% and 8% to 0% respectively, although it was only partially effective or ineffective against black dot. Potato tubers were subjected to hot water treatment and inoculated early or late in store with *P. pustulans* or *H. solani* and early or late with the antagonists, *T. viride* and *G. roseum*. The tubers were held in a potato store until April when eyeplugs were taken and assessed for the incidence of the two blemish diseases. Both antagonists reduced the incidence of the pathogens and the effects were greatest following early application of the antagonists and after hot water treatment (Fig. 6). However, even in the absence of hot water treatment some reduction in disease incidence was recorded.

Antagonists clearly have potential to control this troublesome group of blemish diseases. They may not eradicate them, but they could prove important in low input control systems when application of agrochemicals to foodstuffs is coming under increased scrutiny and the scope for control by fungicides is being restricted.

Assessment of blackleg inoculum on potato seed

M.C.M. Pérombelon, D. Jones & L.J. Hyman

Our knowledge of the etiology and epidemiology of potato blackleg has improved considerably and it has become clear why past and current measures have failed to control the disease. Furthermore, we can now formulate strategies which should achieve a satisfactory level of disease control. As blackleg is a seed borne disease, health of the seed is critical and this paper reviews methods to determine the blackleg potential of seed which have been studied at SCRI.

Epidemiology and etiology

BACTERIA. Although three soft rot erwinias are commonly associated with potatoes, namely *Erwinia carotovora* subsp. *carotovora* (Ecc), *E. carotovora* subsp. *atroseptica* (Eca) and *E. chrysanthemi* (Echr), only Eca and low temperature forms of Echr can cause blackleg in temperate regions. However, all three bacteria can cause tuber soft rot. Echr is not endemic on potatoes in Scotland although it is widespread in mainland Europe and occurs in crops grown in England from imported seed.

TUBER CONTAMINATION. It is generally accepted that tuber contamination by erwinias is unavoidable and the relative importance of several sources of the bacteria and pathways of contamination have been determined (Fig. 1). The soil is not an important source of the bacteria which survive poorly in the environment, nor are blackleg-affected plants, except to the daughter tubers which are usually systemically infected. The main sources of Eca in the field are contaminated and rotting mother tubers and, to a lesser extent, rotting leaves and stems following haulm kill prior to harvest. Wet conditions favour transmission of the bacteria to

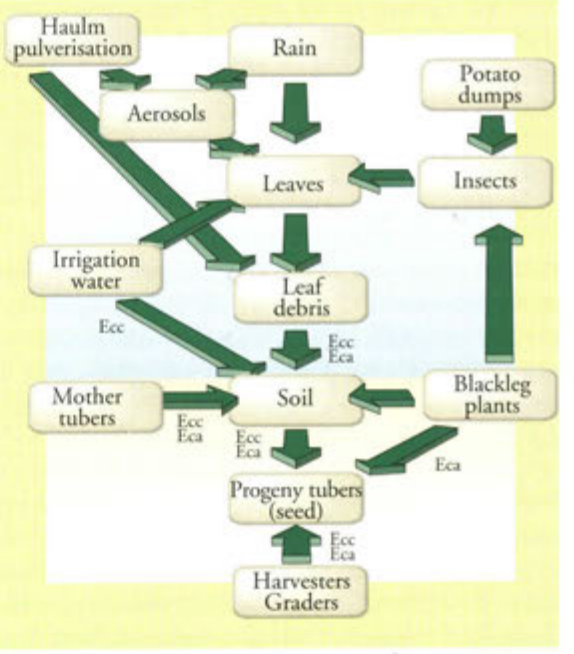

Figure 1 Pathways of contamination of seed potato tubers by erwinias.

the lenticels of progeny tubers. Extensive contamination also occurs during harvesting and grading when erwinias from occasional rotting tubers spread to fresh wounds. Once in lenticels and in suberised wounds, the bacteria can persist until planting time. Contamination levels, however, may vary between stocks depending on environmental and cultural conditions before and after harvest.

BLACKLEG DEVELOPMENT. Although most seed tubers are contaminated, usually only a small proportion of plants develop blackleg. Blackleg incidence can be affected by environmental factors, in particular, high soil water status favours rotting of the mother tubers, and more disease will develop if the seed is heavily than lightly contaminated in any given situation[1]. The threshold level of seed contamination below which blackleg is unlikely to develop under most conditions is *ca.* 10^3 Eca cells/tuber.

BLACKLEG CONTROL. Current control measures based on the etiology and epidemiology of the disease concentrate on producing 'clean' seed. They fall into two broad groups; (a) those aimed at avoiding tuber contamination in the field and in store, and (b) those that reduce the amount of inoculum on the seed. In the former, early harvesting before extensive rotting of mother tubers and spread of the bacteria to progeny tubers, dry storage conditions to reduce risks of erwinia multiplication in lenticels and wounds, removal of rotting tubers at harvest and grading coupled with measures to reduce wounding and the use of mini- or microtubers as initial propagative material reduce the risk of increasing contamination. These measures, alone or together, will not consistently produce healthier seed because they are less likely to be effective under wet than dry weather conditions in the field, and the standards of crop husbandry in the field and handling in store vary widely between farms. In the second approach, a continuous hot water treatment (53-55°C for 5 min) of seed tubers consistently reduced contamination level of sound tubers to below the threshold level for blackleg with no lasting adverse effect on plant growth or crop yield provided the timing of the treatment was appropriate to the cultivar used. However, the treatment can be applied only to high value seed stocks because of its cost.

Evaluation of seed health status

SEED CERTIFICATION. As blackleg incidence is related to seed contamination level and modified by weather conditions that are unpredictable, it is essential to plant seed with a low inoculum potential.

Seed certification has been the traditional method to ensure a satisfactory health status and blackleg has been a certifiable disease for 50 years in UK. However, certification schemes based solely on roguing and field inspections for blackleg have failed because they cannot detect or quantify latent infection in the progeny tubers, especially when contamination occurs after harvest. The measures were based on the erroneous concept that the pathogen is transmitted mainly in progeny tubers from diseased plants and therefore their removal would improve the health status, as is the case with many virus diseases. However, it is now common knowledge that seed from blackleg free crops can produce diseased plants and vice versa.

Although roguing will not eliminate the risk of blackleg, it will remove tubers with stolon end rot which are important post-harvest sources of the pathogen. Moreover, tubers from blackleg plants with latent vascular infection are more likely to produce diseased plants than those from healthy plants because the inoculum load tends to be greater. Field inspections for blackleg are still useful because the incidence of apparently healthy plants with systemic infection which later could develop blackleg is proportional to the incidence of active symptoms. Therefore, the lowest possible tolerance levels for roguing and blackleg in seed certification schemes should be recommended notwithstanding the fact that visual inspection alone is an unreliable indicator of seed health status.

QUANTIFICATION OF TUBER CONTAMINATION. In addition to inspecting crops for blackleg, methods are needed to determine the numbers of Eca present on the tubers to ensure freedom from the disease. Several microbiological and serological techniques have been developed and they must satisfy three main criteria, namely (a) Eca specificity, (b) adequate sensitivity, $<10^3$ Eca cells/ml and (c) speed and general user friendliness for large scale routine use. None of the methods meet all three criteria at present. The main difficulties arise from the presence of Ecc which is closely related to Eca and large numbers of saprophytic bacteria in tuber extracts.

VIABLE COUNT METHOD. Dilution-plating on a selective diagnostic crystal violet pectate medium is the oldest method for enumerating soft rot erwinias[2]. The erwinias can be identified according to the pattern of cavity formation when incubated for 24-48 h at differential temperatures. Cavities are formed by Eca only at 27°C, by Ecc at 27°C and 33.5°C but not at 37°C, whereas Echr forms cavities at all tempera-

Figure 2 Effect of incubation temperature on cavity formation by erwinias on pectate selective medium.
Eca = *Erwinia caratova* subsp *atroseptica*, Ecc = *E.c.* subsp *caratovora*, Echr = *E. chrysanthemi*.

tures (Fig. 2). Replicated inoculated plates can be incubated at the differential temperatures in parallel or, after incubating for 24 h at 20°C, the plates are velvet-replicated on to fresh plates and incubated at the differential temperatures. The latter is more precise as numbers of the different erwinias can be determined from the same inoculum sample. The method is simple but takes 48 h and the test material should be processed within 30 min to minimise cell death caused by phenolics in the tuber extract, even after addition of an antioxidant. More importantly, because of the inadequate selectivity of the medium, erwinias, especially Eca, can be overgrown at 27°C by contaminating bacteria which are often present at a concentration of 10^6 cells/ml tuber extract. For example, Ecc counts at 27°C are frequently less than at 33.5°C which inhibits growth of many saprophytes. Moreover, it is difficult to enumerate Eca precisely when large numbers of Ecc are present.

SEROLOGICAL METHODS. Several methods based on antibodies specific to the test organism are commonly used for routine identification of bacteria and many can be adapted for quantitative assays. The best known are different forms of ELISA and immunofluorescence staining (IF).

ECA SPECIFIC ANTIBODIES. Eca is serologically closely related to Ecc and less so to Echr. There are more than 40 serogroups of Ecc and Eca based on surface antigens and of these, serogroup I represents >90% of Eca strains in Scotland as in other countries. Although a high proportion of antigens are common to both subspecies, specificity appears to be conferred by cell wall components, lipopolysaccharides and flagellae. Polyclonal antibodies raised against Eca serogroup I live cells reacted specifically with Eca serogroups I and XXII, especially after absorption with Ecc in slide agglutination and immunofluorescence tests, but Eca could not be differentiated from Ecc in ELISA[3].

Monoclonal antibodies (MAB) specific for Eca serogroups I and XXII have been raised against live Eca serogroup I cells. The MABs are IgG and are directed against cell wall surface antigens, probably lipopolysaccharides and preliminary results indicate that they possess the desired specificity and usefulness in different immunological detection techniques. They should replace polyclonal antibodies for quantifying Eca in the near future. If necessary, MABs could be raised against other Eca serogroups and pooled to broaden Eca specificity.

ELISA METHODS. ELISA is an established method for detecting and identifying bacteria but is less commonly used to quantify populations. Three forms of ELISA have been used to assay erwinias, namely indirect ELISA, double antibody sandwich (DAS)-ELISA and dot-blot-ELISA. The sensitivity of both poly- and monoclonal antibodies in indirect and DAS-ELISA was at best 10^4 cells/ml and 10^5 cells/ml Eca in water and in tuber peel extract respectively, which are greater than the threshold level of 10^2-10^3 Eca cells/ml for blackleg development. The low sensitivity was attributed to loss of bacterial cells from the microtitre plate wells during repeated washing which leaves a relatively small amount of soluble and cell wall antigens adhering to the well wall. Moreover, binding of antigens to the wells and to antibodies was probably impeded by substances present in peel extracts. Techniques to improve the sensitivity such as enzyme-substrate system, amplification with biotin-streptavidin, heat treatment of assay material, use of different blocking agents, and make of microtitre plates had only a marginal effect. DAS-ELISA should be superior to indirect-ELISA as the primary antibodies coated on the plates capture antigens and allow interfering substances present to be washed away before adding the enzyme-linked antibodies. However, this was not observed in practice.

A modified DAS-ELISA developed in Spain and a modified dot-blot ELISA developed at SCRI have been compared and results are promising. An *in situ* erwinia enrichment step has been incorporated in the DAS-ELISA procedure; a semi-selective pectate-based growth medium is added with the test material to the primary antibody-coated well and incubated anaerobically for 48 h to favour erwinia growth before proceeding with the assay in the usual way. Erwinia numbers increase to *c.* 10^5-10^6 cells/ml even in the presence of other bacteria in peel extract provided that the initial Eca number is >10^2 cells/ml. The enriched Eca populations are readily detected using Eca specific MAB and an indication of the order of magnitude of tuber contamination can be obtained by testing decimal dilutions of the peel extract. The next lower positive dilution above a negative one could be expected to contain at least 10^2 cells/ml.

The modified dot-blot ELISA method involves immunomagnetically separating Eca cells from peel extract using the Immunicon system (Scotlab), filtering under vacuum on a low protein binding membrane and applying a standard indirect ELISA. The volume of test material can be 5-10 times greater than in ELISA in microtitre plates and coloration attributable to potato peel are greatly reduced. However, the results are semi-quantitative as intensity of coloration of the blot is compared visually with blots of a standard range of Eca concentrations. The sensitivity obtained using an alkaline phosphatase conjugate system is 10^3 and 10^4 cells/ml of Eca suspensions in water and peel extract respectively and one order of magnitude greater when the bacteria are detected by chemiluminescence. The whole procedure takes *ca.* 4 h with both the alkaline phosphatase and chemiluminescence systems and poly- or monoclonal antibodies can be used. In a further development of the method, the membrane can be pressed with the immunomagnetically separated and filtered Eca cells uppermost on a pectate-based, semi-selective solid medium and incubated overnight. Eca colonies formed can be visualised as above and enumerated.

IMMUNOFLUORESCENCE STAINING METHODS. Although Eca cells can be readily detected and numbers determined microscopically in fixed peel sap preparation by indirect IF using FITC conjugated IgG, the sensitivity is >10^4 cells/ml and counting is tedious. Results could be improved by using immuno-magnetically separated Eca.

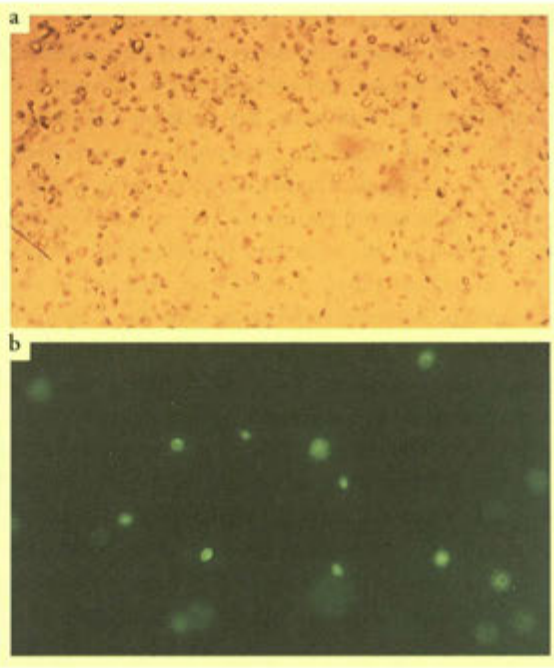

Figure 3 Immunofluorescence staining methods for detecting erwinias. a) low power light microscope. b) low power UV microscope.

Immunofluorescence colony staining (IFC) developed at IPO, The Netherlands has been used at SCRI. A test sample is mixed with molten semi-selective, pectate-based medium incubated for 24-48 h at 27°C and dried. The micro Eca colonies are stained with FITC conjugated antibodies overnight, washed and counted under a UV microscope[4](Fig. 3a & b). The method is sensitive and can detect as few as 50 Eca cells/ml but it takes 48-72 h to complete an assay. Counting colonies is tedious and slow but could be overcome by using an image analyser and photographs.

DETECTION OF VIABLE ECA CELLS. Serological methods detect dead cells and their debris in addition to live cells and only the latter are of interest when determining blackleg potential of seed. Of the four methods described above, only viable count and IFC methods detect solely viable cells. However, in practice, interference by dead cells is unlikely to play an important role in tuber health assessment. Dead cells and other antigenic substances tend to be lost within a month, probably through degradation in tuber lenticels.

TUBER AND STOCK SAMPLING. Regardless of the method used to quantify erwinia contamination of tubers, obtaining a representative sample for testing from a stock consisting of several tonnes in one tonne

boxes or 50 kg bags is an essential prerequisite. Most erwinias are located in the lenticels and suberised wounds on tubers and are efficiently recovered by peeling lots of 10 tubers by dry abrasion and expressing the liquid from the peel pulp. Alternatively, tubers can be sampled individually by taking a strip of peel from the stolon end to the rose end and expressing liquid from the strip in a suitable press. Results from individual tubers showed that variation in contamination between tubers may vary by a factor of 100 although when the figures from replicates of 10 tubers were considered, variation within a stock was not so great as to make sampling impractical. A one tonne box contains tubers harvested from >100 m of row and field variation is usually reproduced in each box unless the variation is related to topography or drainage. The logistics of how to collect tuber samples from box and bulk stored or bagged potatoes, when to sample during storage, before or after grading, and the optimum sample size, are best resolved empirically, taking into account of what is feasible in practice and the accuracy desired.

Conclusions

Blackleg potential of seed stocks is better assessed by determining the level of contamination by the pathogen on the tubers than by inspection for disease symptoms in the growing parental crop. The relationship between Eca seed contamination level and blackleg incidence was first established at SCRI in the early seventies[5] and its implication for seed health evaluation became evident soon later. Whereas appropriate testing measures were implemented in the early eighties in The Netherlands with 500,000 DAS-ELISA tests now carried out annually, and in Israel where >75% of imported seed stocks are tested using the viable count method, the technology transfer in Scotland has been slower but now such tests are being contemplated.

The introduction of health passports for plant commodities destined for export within the EEC would no doubt be a driving force towards a more rapid acceptance of testing for erwinia seed contamination. This in turn would result in a customer-led demand for healthier seed and a more rapid application of appropriate measures for an integrated disease control strategy to produce such seed.

Acknowledgements

Part of the work described above was financed by the Potato Marketing Board

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Plant viruses

T. M. A. Wilson

Plant virus research at SCRI has had a long and distinguished history, both nationally and internationally. It has been successful not only in addressing fundamental questions on virus structure, genome organization and expression, and genetic stability, variation and evolution, but also in applying this knowledge to improve the diagnosis, control and eradication of many viral diseases of major subsistence crops and high-value speciality crops in Scotland, the UK and overseas. The multidisciplinary nature of the SCRI site provides a challenging and unique environment for plant virologists to consider the broader implications of studies on these simple but often devastating pathogenic agents. For example, unravelling the exquisite specificity, complexity and efficacy of virus-vector interactions, determining the influence of susceptible or resistant host plant genomes on the viral replication cycle, pursuing mathematical modelling and statistical analysis of virus populations, epidemiology and evolution or seeking conserved patterns in viral genome sequences consequent upon their regular geometrical structures, shared vectors, hosts or tissue specificity. With so many collaborative opportunities, virus diseases of important crops, convenient model virus systems and intriguing biological questions to investigate, maintaining research focus is a daunting task. During 1992, we reviewed and reorganized all our programmes and research objectives. We have created multidisciplinary virus-oriented teams, each underpinned by expertise in recombinant DNA-based or conventional serology, electron microscopy and plant transformation technologies, themselves the subjects of further autonomous research programmes.

In addition to a new Head of Department, in October we welcomed Stuart MacFarlane to SCRI. Stuart brings molecular expertise in pea early browning tobravirus and will consolidate and expand our molecular virology and virus-vector relations programmes with tobraviruses and nepoviruses.

In the fungus-transmitted virus (Furovirus) programme, work at SCRI has provided the complete sequences of potato mop-top virus (PMTV) RNA 2 (3kb) and RNA 3 (2.5kb). PMTV RNA 2 has four open reading frames for proteins of 51, 13, 21 and 8 kDa, respectively. The first three resemble a triple gene block of cell-to-cell movement proteins, as found in potexviruses, carlaviruses and beet necrotic yellow vein furovirus (BNYVV). The last is rich in cysteine residues. PMTV RNA 3 contains the coat protein gene and a potentially suppressible UAG codon resulting in a 67 kDa readthrough protein, a feature common to most Furoviruses sequenced to date. In addition, there are features in the non-translated regions of these RNAs which are unique to PMTV. RNA 2 contains a long stem-loop structure at the 5' end of the molecule. Both RNA 2 and RNA 3 contain two short sequences, each of which is repeated

and one of which can form a short stem-loop in the 3' untranslated region. Indian peanut clump furovirus (IPCV) RNA 2 has been fully sequenced and encodes the coat protein, but with no readthrough domain. IPCV RNA 1 sequencing is underway. Research work has begun manipulating subclones and creating full-length cDNA clones of soil-borne wheat mosaic virus (SBWMV), type member of the Furovirus group. SBWMV RNA 1 and RNA 2 have been fully sequenced (Y. Shirako & T.M.A. Wilson, AgBiotech Center, Rutgers University, USA). Ultimately we expect to be able to manipulate full-length infectious cDNA clones and hence RNA copies of each component of each Furovirus to understand the mechanisms of acquisition and transmission by their respective fungal vectors [*Polymyxa graminis* (SBWMV, IPCV) or *Spongospora subterranea* (PMTV)], and to design new monogenic, pathogen-derived resistance gene strategies for crop protection by molecular interference. Unifungal cultures and sand or tissue culture transmission systems between infected source and healthy bait plants have been established for PMTV and *S. subterranea.* The others will follow. Common or unique features of genetic organization and expression support the view that the Furovirus group is extremely diverse and that the agent of sugarbeet rhizomania disease, BNYVV, and SBWMV typify this diversity.

As presaged by the 1991 Annual Report, panels of monoclonal antibodies (MAbs) against structural proteins have been prepared to an Andean isolate of potato leafroll luteovirus and to groundnut rosette assistor luteovirus (GRAV), highlighting our close collaborations with CIP, Peru and ICRISAT, India, respectively. MAbs to the picorna-like parsnip yellow fleck virus (PYFV), especially the 31 kDa capsid protein, are also now available.

The complete sequence of PYFV RNA (9871 nucleotides) has been determined. Detailed comparisons of amino acid sequence motifs confirm its taxonomic position between the bipartite *Comoviridae* (plant viruses) and the monopartite *Picornaviridae* (animal viruses). An RGD sequence has been identified in the 31 kDa coat protein domain which may interact with a plant cell surface receptor, by analogy with VP1 of foot-and-mouth disease virus and coxsackievirus A9, and animal cell surface proteins such as fibronectin. Alternatively, the RGD may have a role in binding to the vector during insect transmission, although PYFV relies on a helper virus, anthriscus yellows, for transmission by aphids. An RGD motif has recently also been found in the thrip-transmitted, negative-strand (ambisense) RNA virus, tomato spotted wilt virus. Details of these molecular and structural features and their implications for PYFV-helper-vector-plant interactions are described in more detail on p. 89.

Complex helper-dependent virus interactions and transcapsidation events involved in the groundnut rosette disease complex, and additional data on five GRV satellite RNAs which have recently been sequenced at SCRI are described in detail in the article on p. 85. GRV is the most devastating disease of this vital subsistence crop in tropical Africa, and unravelling the intricate dependencies of the viruses and satellites involved has been a major achievement by A.F. Murant, his group and overseas collaborators.

The RNA sequence, bipartite genome organization and unusual taxonomy of a resistance breaking strain (R15) of raspberry bushy dwarf virus (RBDV; an idaeovirus) has been described (*Ann. Rep. 1991, 83*). A full-length cDNA clone of RBDV (R15) RNA 2 has been prepared, and the coat protein gene inserted into a binary plant transformation vector for introduction into *Rubus* spp. Comparative sequence data now exist for RBDV strain D1 which fails to overcome the *Bu* resistance gene in many important *Rubus* cultivars. In the bicistronic RNA-2, only one amino acid change was detected in the 30 kDa coat protein, whereas there were multiple changes in the 39 kDa putative transport protein between strains D1 and R15. Even more changes were detected in the 190 kDa proteins encoded by D1 or R15 RNA-1, although only 60% of the nucleotide sequence of the RNA-1 of strain D1 is known so far. It is not possible to identify unequivocally at present the cause of the resistance-breaking character of RBDV R15. A PCR procedure was devised that discriminated between RBDV strains R15 and D1 but it will probably not distinguish all resistance-breaking from normal strains.

Studies on the tissue-specific distribution of graft-inoculated potato leafroll luteovirus (PLRV) in the vasculature of susceptible versus naturally resistant or PLRV coat protein-transformed breeding lines are described on p. 92. In both types of resistant plants, PLRV seems to be restricted to the adaxial phloem bundles. Four potato clones expressing high levels of host gene-mediated resistance to PLRV have been transformed with the PLRV coat protein gene to test for improved, additive or synergistic resistance.

During 1992, extensive gauze-house trials and the first field release in the UK of coat protein transgenic potatoes were made under extensive scrutiny by ACRE and the HSE Specialist Inspectorate. The aims of the experiments were to assess the agronomic characteristics and disease susceptibility of transformed potatoes in the field, and the efficacy of secondarily PLRV-infected coat protein transgenic plants to serve as sources for aphid transmission to healthy bait plants in the gauze-house. On the molecular front, changes in aphid transmissibility of PLRV were found to correlate with two or three amino acid changes in the coat protein and its readthrough domain sequence. Full-length clones of PLRV cDNA were constructed and transcripts tested for infectivity in protoplasts (work in collaboration with St Andrews University and CNRS IBMP, Strasbourg).

Virus detection and identification methods continue to be improved and simplified. A universal reverse transcriptase (RT)-PCR assay was developed for tobraviruses, both M and NM type infections, i.e. those with or without coat protein and RNA 2. Similarly, an RT-PCR protocol was designed to detect potato virus Y (PVY) not only in freshly harvested potato tubers, but also in stored dormant tubers, where the reproducibility of detection and quantification has been a problem as the virus titre diminishes. The design and use of degenerate PCR primers targeted to highly conserved sequences in a wide range of whitefly-transmitted geminiviruses has accelerated analysis of large numbers of samples and comparative sequence data collection.

Predictably, with global warming and the spread of a highly effective virus vector whitefly (*Bemisia tabaci*), came the first report of tomato yellow leaf curl geminivirus in field samples from Spain. PCR-based viral DNA fingerprinting studies on abutilon mosaic geminivirus from abutilon plants which had been propagated vegetatively for many years at SCRI or in Hamburg, Germany, revealed a higher than predicted rate of viral DNA mutation and evidence for mixed virus populations.

The groundnut rosette disease virus complex: aetiology, transmission, diagnosis, and novel approaches to control

A.F. Murant, D.J. Robinson, V.C. Blok, K. Scott, L. Torrance & M.J. Farmer

Several of the viruses studied at SCRI are important agents of disease in developing countries. Many of them are also of considerable scientific interest because of their affinities with viruses prevalent in the U.K. or because they possess novel properties. Among the most intriguing of these viruses are those responsible for the rosette disease of groundnut or peanut (*Arachis hypogaea*) in Africa. Funding for studies on the aetiology and diagnosis of this disease and the development of new forms of resistance has been provided over several years by the Overseas Development Administration. Throughout the work there has been extensive collaboration between SCRI and the International Crops Research Institute for the

Figure 1 Part of a field trial at the ICRISAT Research Station at Chitedze, Malawi, showing paired rows of rosette-resistant breeding lines on either side of a single row of the control susceptible cultivar, Malimba. All the plants were exposed to infection by releasing viruliferous *Aphis craccivora*.

Semi-Arid Tropics (ICRISAT), Hyderabad, India, and especially with their regional centre in Malawi.

The groundnut originated in South America, from where it was carried by the early colonisers to other parts of the world. However, only in Africa have crops become affected by rosette. Presumably the causal agents came originally from some native African host plant but its identity is unknown. Groundnut rosette disease (Fig. 1) can occur in devastating epidemics and is regarded as the most destructive virus disease of groundnut in Africa. For example, in 1975 it affected more than 1×10^6 ha in Nigeria and caused losses estimated at more than 0.5×10^6 t. There are two main forms of rosette disease: 'chlorotic rosette' (Fig. 2a), which is found throughout Africa south of the Sahara, and 'green rosette' (Fig. 2b), which is the prevalent form in West Africa. Plants with chlorotic rosette show a bright yellow leaf chlorosis which may affect the whole plant, or only some shoots or parts of shoots. The chlorosis may also affect only parts of leaves and when the green parts are extensive the symptom becomes a mosaic ('mosaic rosette'). In green rosette, the leaves are very dark green, or show a light green and dark green mosaic, and are much reduced in size. In all forms of rosette the plant is very stunted.

Symptoms of all these types of rosette are associated with infection by a manually transmissible virus, groundnut rosette virus (GRV). In nature, GRV is transmitted by *Aphis craccivora*, but only from plants that also contain a helper virus, groundnut rosette assistor virus (GRAV), which on its own causes no

Figure 2 Groundnut plants cv. TMV-2 showing symptoms of (a) chlorotic rosette from Malawi; (b) green rosette from Nigeria.

symptoms in groundnut. GRAV has isometric particles about 28 nm diam. and belongs to the luteoviruses, a group of plant viruses which includes those responsible for barley yellow dwarf, potato leafroll and many other important plant diseases. Like other luteoviruses, GRAV is not manually transmissible but is transmitted by the aphid vector in the *persistent* manner (i.e. aphids remain able to transmit the virus for many days after acquisition, and after moulting). No virus-like particles have been seen in sap from plants infected with GRV alone, but the plants contain infective single-stranded RNA (ssRNA) whose electrophoretic mobility suggests that it has a M_r of 1.5×10^6 (4.6 kb). In doubly infected plants some particles are produced that consist of GRV RNA within a shell composed of GRAV coat protein subunits, and it is probably in this form that GRV is transmitted by aphids.

The infective ssRNA of GRV has not been obtained free from host plant RNA. However, unlike healthy plants, infected plants yield abundant double-stranded RNA (dsRNA), and electrophoretic analysis reveals three major dsRNA species (Fig. 3). Two of them, dsRNA-1 (4.6 kbp) and dsRNA-2 (1.3 kbp), appear to be double-stranded forms of the genomic

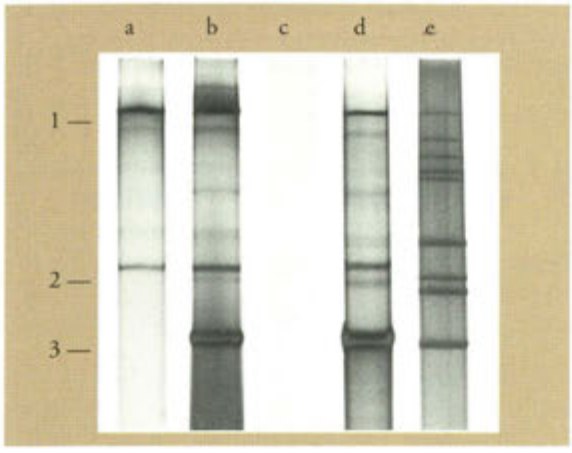


Figure 3 Electrophoresis in a 7% polyacrylamide gel of dsRNA preparations from *N. benthamiana* inoculated with: (d) GRV(NC), a GRV culture from plants with chlorotic rosette from Nigeria ; (a) G96, an isolate derived from GRV(NC) by eliminating the satellite RNA dsRNA (dsRNA-3); (c) dsRNA-3 alone (no infection); (b) G96 + dsRNA-3. Track (e): a preparation of dsRNA from rice dwarf virus used to provide standards ranging in *Mr* from 3.1×10^6 to 0.52×10^6. Arrows 1-3 indicate GRV dsRNA-1, dsRNA-2 and dsRNA-3 respectively.

ssRNA and a subgenomic ssRNA respectively. The third, dsRNA-3 (0.9 kbp), has been shown to represent a *satellite* RNA, i.e. it can be eliminated from GRV cultures (and so is not needed for the multiplication of GRV), but it cannot itself multiply except in the presence of the genomic RNA of GRV (Fig. 3). It is this satellite RNA that is primarily responsible for the symptoms of rosette disease; GRV isolates that lack the satellite induce no symptoms or only a mild transient mottle when inoculated to groundnut. Moreover, different variants of the satellite RNA are responsible for the different forms of rosette disease. Thus, when the dsRNA-1 and dsRNA-3 molecules associated with green or chlorotic rosette were separated and then reassociated in homologous and heterologous combinations, the symptoms induced in groundnut by the resulting reassortant cultures were those of the primary cultures from which the dsRNA-3 (satellite RNA) was derived (Fig. 4). Furthermore the 'mosaic' form of rosette seems to be caused by mixed infection of the groundnut plant with more than one satellite variant. It is possible to isolate from the green islands in the mosaic a form of GRV that causes only mild green mottle symptoms in groundnut, even though there is a satellite RNA associated with it. The yellow islands in the mosaic yield a mixture of this form and the normal form of GRV.

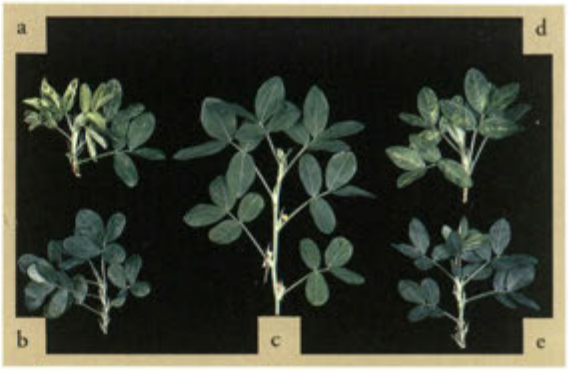


Figure 4 Shoots of groundnut cv. TMV-2 showing leaf symptoms and stunting induced by homologous and heterologous reassortants made from the dsRNA-1 and dsRNA-3 (satellite dsRNA) components from GRV(MC), a GRV culture from plants with chlorotic rosette from Malawi, or from GRV(NG), a GRV culture from plants with green rosette from Nigeria. (a) MC dsRNA-1 + MC dsRNA-3; (b) MC dsRNA-1 + NG dsRNA-3; (c) healthy groundnut; (d) NG dsRNA-1 + MC dsRNA-3; (e) NG dsRNA-1 + NG dsRNA-3.

The satellite RNA plays another important role in the aetiology of rosette disease: its presence in the plant has been found to be essential for the GRAV-dependent aphid transmission of GRV. If a source plant contains GRAV plus a satellite-free culture of GRV, *A. craccivora* can transmit only GRAV from it whereas the aphid transmits both viruses if the satellite is present (Table 1). This is the only example known of this phenomenon and the explanation is unknown. Clearly, although the satellite RNA is not essential for the multiplication of GRV in plants, it is required for the survival of GRV in nature. Figure 5 illustrates the interactions among the three causal agents of groundnut rosette disease.

Groundnut rosette is the only plant virus disease known to have such a complex aetiology, and because of its academic interest and economic importance, we have begun to study the molecular basis of some of

Viral components in groundnut source			Groundnut test seedlings		
GRAV	GRV	Satellite RNA	Number inoculated	Number infected GRAV	Number infected GRV
+	+	-	67	41	0
+	+	+	87	47	45
-	+	+	33	-	0

Table 1 Role of the GRV satellite RNA in mediating the GRAV-dependent aphid transmission of GRV.

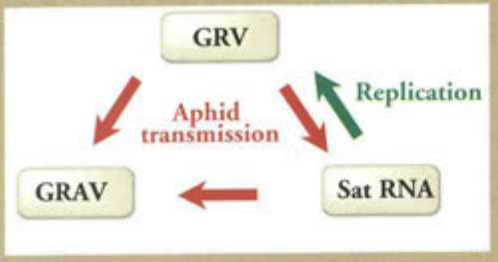


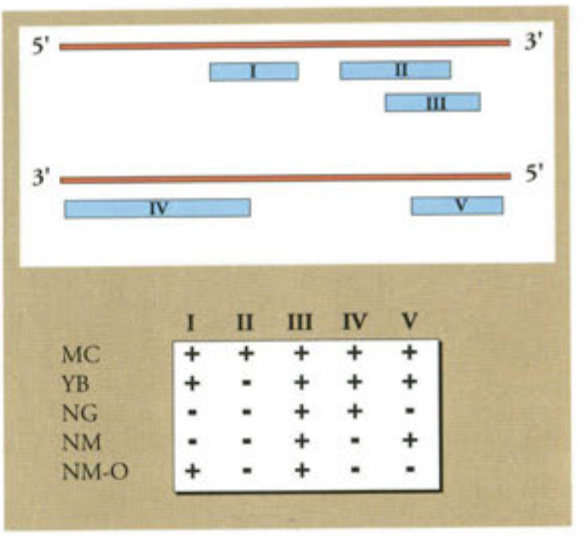
Figure 5 Diagram illustrating the interaction of the three causal agents of groundnut rosette disease: GRAV, GRV and the satellite RNA.

the observed phenomena in the hope of understanding them better and also of developing new forms of resistance in groundnut.

A start has been made on sequencing the GRAV genome, and the full sequence of the 28K coat protein gene has been determined. It has considerable homology with that of other luteoviruses, notably PLRV (Fig. 6). These findings are in line with evidence that GRAV has a moderately close serological relationship to PLRV and a more distant relationship to several other luteoviruses. Polyclonal and monoclonal antisera have been raised to GRAV and are finding use not only in laboratory testing but also in epidemiological studies, for example in tests to find possible native African hosts of GRV, and in tests of groundnut breeding lines for resistance to GRAV.

Figure 6 Comparison of coat protein amino acid sequences of GRAV and PLRV. Blue bars represent amino acids that are identical in the two viruses.

The GRV satellite RNA is proving to be a very interesting molecule. Nucleotide sequences have been determined for 10 clones representing five satellite variants associated with different types of symptom in groundnut or in the experimental host *Nicotiana benthamiana*. The sequence of the GRV satellite RNA has no close homology with any published virus or satellite sequences. Figure 7 represents diagrammatically the two strands of the satellite dsRNA molecule associated with chlorotic rosette from Malawi. One strand possesses two open reading frames (ORFs)



	I	II	III	IV	V
MC	+	+	+	+	+
YB	+	-	+	+	+
NG	-	-	+	+	-
NM	-	-	+	-	+
NM-O	+	-	+	-	-

Figure 7 Diagram of satellite dsRNA (dsRNA-3) from GRV(MC) showing the positions of ORFs I to V on the two strands. The table shows which ORFs are present in GRV satellite variants from GRV cultures MC, YB (a derivative of MC that induces yellow blotch symptoms in *Nicotiana benthamiana*), NG, and two isolates derived from NC (NM and NM-0), which induce, respectively, mild and very mild chlorotic rosette in groundnut.

(potential genes), and the other possesses three. It is not known whether any of these ORFs are expressed *in vivo*. Not all of these ORFs are present in all forms of the satellite that have been sequenced (Fig. 7). For example, both clones studied of the satellite RNA associated with green rosette from Nigeria (NG) lack three ORFs, including the largest, ORF IV. Further work is needed to show whether this is true for other clones of this variant and if so whether the lack of these ORFs is responsible for the green rosette symptoms.

Work on the sequencing of the GRV satellite RNA has led to the development of a complementary DNA probe which can be used to detect the presence of the satellite (and therefore of GRV itself) in plants (Fig. 8). This kind of probe will be especially useful for detecting mild or symptomless variants of the satellite RNA, but will also find use in epidemiological studies.

Natural resistance to GRV (though not to GRAV) is known in groundnut (Fig. 1). However, this form of resistance has several disadvantages: it is inherited as a double recessive character, which makes screening for virus resistance laborious; it occurs in long-season cultivars and is difficult to transfer to the short-season cultivars that are required in many areas; and 'resistant' plants can become infected under certain condi-

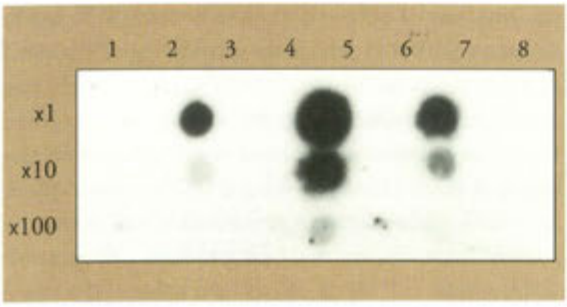


Figure 8 Detection of GRV satellite RNA in groundnut plants by using a complementary DNA probe. Dark spots correspond to samples from satellite RNA-containing plants. The samples were 5 µl aliquots of leaf sap (undiluted, or diluted x 10 or x 100) from groundnut plants as follows: (1) healthy; (2, 4, 6) infected respectively with satellite-containing isolates GRV(NC), GRV(NG) or GRV(MC); (3, 5, 7) infected respectively with satellite-free isolates derived from GRV(NC), GRV(NG) or GRV(MC); (8) infected with GRAV.

tions. The knowledge we are now accumulating on the molecular properties of the causal agents of groundnut rosette disease may therefore provide important tools for the development of new forms of resistance. Recent work with numerous other species of crop plant has shown that introduction into the plant genome of DNA copies of viral nucleic acid sequences can confer resistance to the homologous virus. In the hope that this can be done with the causal agents of rosette, a research project for genetic modification of groundnut has been established at SCRI.

In summary, this ODA-funded research programme has been rewarding in several ways: we have been able to unravel the uniquely complex and fascinating aetiology of groundnut rosette disease; we have obtained diagnostic probes that will be useful in epidemiological studies and in the search for naturally occurring resistance to the causal agents; we have developed ideas for new approaches to the control of the disease; and many of the results obtained will be applicable to other viruses and virus diseases of importance not only in Africa but in other parts of the world.

Parsnip yellow fleck, a possible 'missing link' between plant and animal viruses

B. Reavy, M.A. Mayo, A.D. Turnbull-Ross & A.F. Murant

Parsnip yellow fleck virus (PYFV) was first described at SCRI in 1968. In parsnip (*Pastinaca sativa*) it causes distinctive vein yellowing and mosaic (yellow flecks) on the leaves (Fig. 1). The form of the virus that infects parsnip occurs also in celery (*Apium graveolens*) and is widespread throughout the UK in the wild umbellifer, *Heracleum sphondylium* (hogweed or cow parsnip). A serologically distantly related virus which occurs in another wild umbellifer, *Anthriscus sylvestris* (cow parsley), infects carrot (*Daucus carota*) and is especially important in carrot seed crops.

PYFV has been studied extensively at SCRI because it has several features of considerable scientific interest.

Figure 1 Symptoms induced by PYFV in parsnip. (a) Vein-yellowing (primary symptoms); (b) Yellow flecks (secondary symptoms).

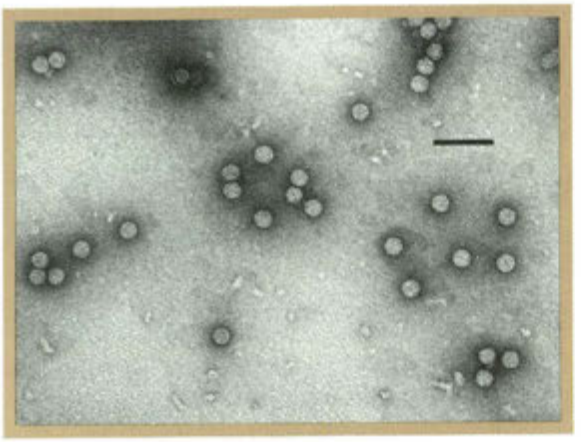


Figure 2 Particles of PYFV in 1% uranyl acetate. Bar represents 50 nm.

It is transmitted by aphids (*Cavariella* spp.) but only in association with an unrelated helper virus, anthriscus yellows (AYV). The virus complex is transmitted in the *semi-persistent* manner: aphids transmit the viruses for several hours to several days after acquisition but cease to transmit after moulting. This mode of transmission is unusual for viruses that have isometric particles *c.* 30 nm diam. as PYFV (Fig. 2) and AYV do. PYFV differs from AYV in several important respects: it is transmissible experimentally by mechanical inoculation, whereas AYV is not; it occurs throughout the mesophyll and epidermal tissue of the leaf, whereas AYV is confined to phloem tissue; and it induces the formation of vesiculated membraneous inclusion bodies adjacent to the nucleus of infected cells, whereas AYV forms inclusion bodies in which the particles are embedded in a proteinaceous matrix. However, both PYFV and AYV are unusual in having particles that have at least three coat proteins and a single large species of single-stranded RNA (about 10 kb for PYFV, about 12 kb for AYV). Most plant viruses with small isometric particles have one or two coat proteins and genomic RNA which, if 7 kb or less, is in the form of a single molecule, or if larger, is spread over two or three RNA molecules. In these particle properties, PYFV and AYV resemble some viruses of animals, such as poliovirus, foot-and-mouth-disease virus and the human rhinoviruses responsible for the common cold. Because these animal viruses are all classified in the picornavirus family, PYFV and AYV have therefore sometimes been referred to as 'plant picornaviruses'.

Because of its distinctive properties, PYFV has been made the type member of a new taxonomic group or genus, for which the proposed name is 'sequivirus', from the Latin *sequi*, to follow, accompany or attend (in reference to the dependent aphid-transmission). AYV is not regarded as a sequivirus, but may classify with rice tungro spherical virus (RTSV), which is transmitted in the semi-persistent manner by leafhoppers.

The similarities between PYFV and picornaviruses were reinforced when the nucleotide sequence of PYFV was determined. The PYFV genomic RNA molecule consists of 9871 nucleotides and, like those

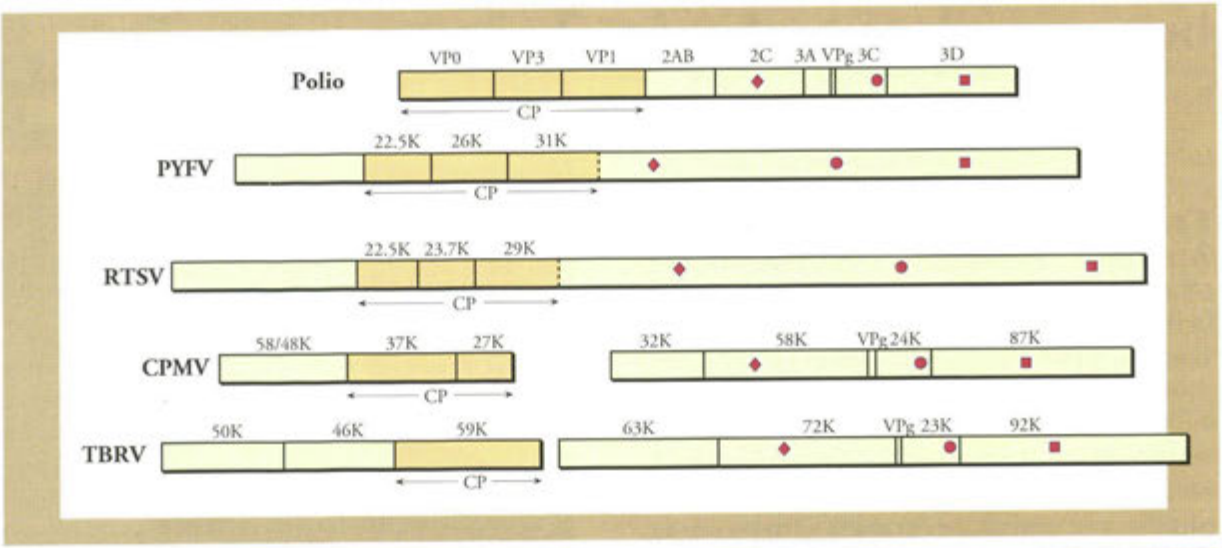


Figure 3 Arrangement of the genes in the RNA of PYFV in comparison with those of poliovirus, rice tungro spherical virus (RTSV), cowpea mosaic virus (CPMV) and tomato black ring virus (TBRV). CP = coat proteins, diamonds represent NTP-binding proteins, circles represent proteases, and squares represent polymerases.

of picornaviruses, encodes a single large 'polyprotein'. The PYFV polyprotein comprises 3027 amino acids and is of M_r 336000 (336K). The three particle (coat) proteins (31K, 26K and 22.5K) and other non-particle proteins are presumably cleaved from the polyprotein by viral proteases. The N-terminal sequences of the three mature coat proteins were determined and found to occur, as do those of picornaviruses, in the N-terminal half of the polyprotein (Fig. 3). However, PYFV differs from the picornaviruses in having polypeptide(s) of up to 43K on the N-terminal side of the coat proteins. Amino acid sequences characteristic of NTP-binding proteins, proteases and RNA polymerases are present in the PYFV polyprotein, and the arrangement of these sequences is the same as that found in the polyproteins of picornaviruses (Fig. 3).

No information of this sort is available for AYV but the nucleotide sequence of RTSV, which is thought to belong to the same genus as AYV, has recently become available. Although the single genomic RNA of RTSV is somewhat larger than that of PYFV (about 12.5 kb) it encodes a polyprotein that is laid out in the same way (Fig. 3). The three coat proteins are in the N-terminal half, and have additional protein(s) of up to 60K on their N-terminal side.

Figure 3 also shows that the same general genome organisation is found in two plant viruses with bipartite genomes, cowpea mosaic virus (CPMV: genus comovirus) and tomato black ring virus (TBRV: genus nepovirus) if their smaller genomic RNA molecules are depicted on the left of the larger ones. In these viruses too, the polyproteins encoded by the smaller RNA molecules have proteins of unknown function on the N-terminal sides of the coat proteins.

Some stretches of amino acid sequence in the 26K particle protein of PYFV are similar to parts of the VP3 particle proteins of animal picornaviruses. These stretches are in areas that have been shown in picornaviruses to be important in determining the three-dimensional structure of this protein and therefore that of the particle, suggesting that the particle structures of PYFV and picornaviruses may be similar.

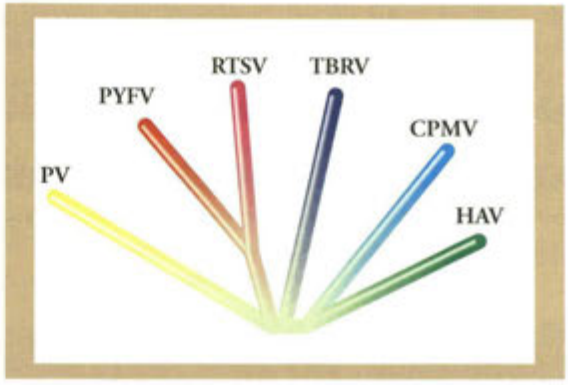


Figure 4 Diagram of an unrooted phylogenetic tree representing the relationships of the polymerases of PYFV, poliovirus (PV), rice tungro spherical virus (RTSV), tomato black ring virus (TBRV), cowpea mosaic virus (CPMV) and hepatitis A virus (HAV).

Some similarities in amino acid sequence are also found in the parts of the polyproteins of PYFV, RTSV, CPMV, TBRV and picornaviruses that have NTP-binding and RNA polymerase functions. Comparison of these sequences yields data that can be used to construct phylogenetic trees indicating possible relationships of PYFV to other plant and animal viruses (Fig. 4). The similarities observed suggest that PYFV and RTSV are more similar to each other than either is to any of the other viruses. We believe that PYFV and RTSV should be regarded as two distinct genera of a new plant virus family whose members have similarities in genome organisation to the picornaviruses. We have proposed the name *Sequiviridae* for this new family. It has been suggested that animal and plant viruses may have a common ancestor, and the similarities between PYFV and picornaviruses seem to support this idea. If such a common ancestor did exist, viruses in the family *Sequiviridae* are possibly closer to it than are viruses in any other plant family.

Long-distance movement and distribution of potato leafroll luteovirus within resistant and susceptible potato clones

P. M. Derrick & H. Barker

Potato leafroll luteovirus (PLRV) causes a damaging disease of potato. It is spread in crops by aphids but unlike many other plant viruses, it is not mechanically transmissible. The concentration of virus is relatively low in infected tissue because PLRV is confined to the phloem sieve element-companion cell complex. Disease symptoms undoubtedly result from damage to the phloem transport system. PLRV infection causes callose deposition in sieve elements of most potato cultivars and phloem cells may also exhibit a necrotic response.

There are two forms of resistance to PLRV in potato that are being investigated. The first (host gene resistance) occurs in a number of cultivars and SCRI breeding lines. Recent results suggest that in some breeding lines this resistance is controlled by a dominant resistance gene, designated as the Rl gene . The second form of resistance is obtained by transforming plants with sequences encoding the PLRV coat protein gene (transgenic resistance). In both forms of resistance, accumulation of PLRV is substantially reduced in comparison with susceptible plants. Potato clones developed at SCRI with these forms of resistance were described previously (*Ann. Rep. 1990, 78*). This report outlines work which aims to understand the mechanisms that underly these two forms of resistance.

In common with all solanaceous species, the phloem system in potato has a bicollateral composition, i.e. phloem bundles are located on both the inside and the outside of the xylem tissue, these being termed the internal and external phloem. In broad terms, it is thought that the direction of sap movement is upward in internal phloem and downward in external phloem, although connections between the two are present. The functional significance of the bicollateral arrangement is not, however, well understood.

PLRV was detected in susceptible potato plants grown from infected tubers by staining tissue sections with fluorescein-labelled antibodies and by tissue printing. PLRV was located within a proportion of phloem cells, chiefly companion cells, of both internal and external phloem bundles (Fig. 1). A greatly reduced proportion of phloem cells were infected in plants of resistant clones compared with susceptible plants. In addition to this, infected cells of resistant plants were largely restricted to the internal phloem bundles (Figs. 1 and 2), suggesting that movement of PLRV within phloem may be impaired. Interestingly, the restricted distribution of infected cells was observed both in plants with host gene resistance and those with transgenic resistance suggesting that a limitation of PLRV movement within plants was involved in both forms of resistance.

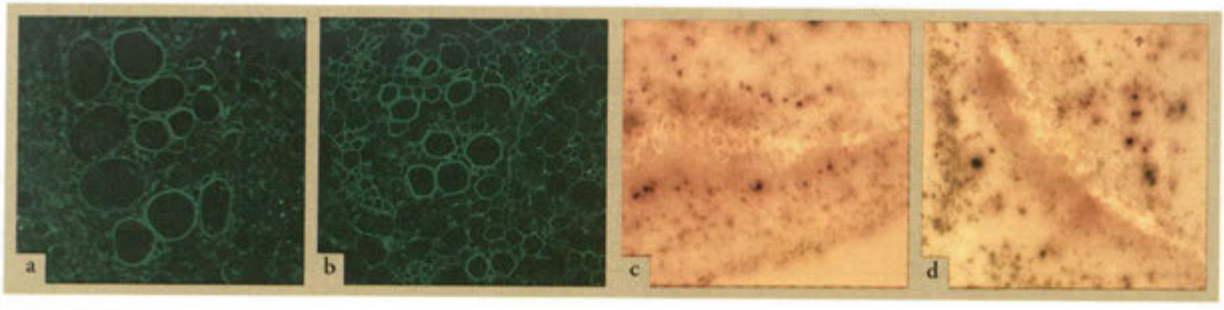


Figure 1 Localisation of PLRV-infected cells in potato clones. (a and b) Fluorescein-conjugated antibody labelling of PLRV-infected cells in petiole sections (c and d) location of PLRV infected cells in stem tissue on tissue prints. PLRV is located within both internal and external phloem in the susceptible clone G7714(1) (a) and cv. Maris Piper (c) but largely restricted to internal phloem bundles of the resistant clones G7445(1) (c) and C10 (d).

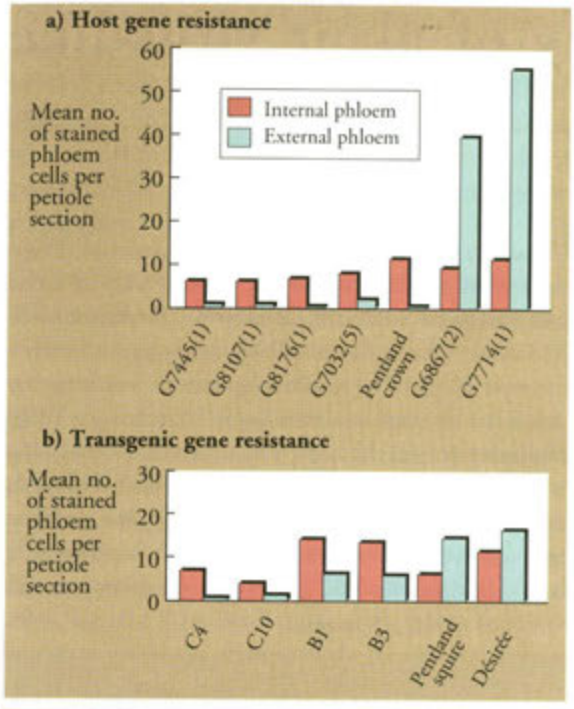


Figure 2 Distribution of PLRV-infected cells as revealed by fluorescein-conjugated antibody staining of sections from petiole tissue of potato plants with a) host gene resistance and b) transgenic resistance. PLRV is located primarily in external phloem bundles in susceptible clones (green bars) and internal bundles in resistant clones (red bars).

In experiments designed to test whether long distance virus movement was involved in PLRV resistance, stem segments of resistant or susceptible potato clones were grafted between PLRV-infected rootstocks and virus-free scions. The time taken for virus to move from infected rootstocks along the test stem segments and into the scions was estimated by detection of PLRV in young foliage by ELISA. PLRV moved through both susceptible and resistant stem segments at the same rate, infecting the growing tip foliage within 10 - 13 days following grafting. This was true whether the tested stem segments were two or four internodes in length.

The development of functional phloem connections across graft junctions was followed by observing the movement of the phloem-restricted fluorescent tracer 6(5)-carboxyfluorescein along phloem elements which traversed graft junctions (Fig. 3). The movement of PLRV across grafts and the development of connecting phloem traces capable of translocating the tracer across grafts occurred at about the same time, c. 10 - 14 days after grafting. Therefore, the delay in movement of PLRV through stem segments was due mainly to formation of new phloem connections across the graft. Once these connections were established, virus movement was very rapid.

Figure 3 Freehand section of potato stem illustrating phloem connections across a graft junction. The fluorescent phloem tracer 6(5)-carboxyfluorescein was loaded into leaves above the graft and is seen here below the graft in the (arrowed) external phloem cells.

Results of our experiments show that the restriction in the number and distribution of infected cells in resistant plants does not appear to be caused by an impairment of long distance virus movement through infected stems. It seems likely therefore, that both PLRV coat protein transgenic resistance and host gene resistance act either by suppressing virus replication or by inhibiting the movement of PLRV across plasmodesmata between sieve elements through which PLRV moves and companion cells in which PLRV replicates, thus limiting spread throughout infected plants. The very rapid movement of PLRV in the grafting experiments, once phloem connections across grafts had been established, indicates that long distance movement of PLRV may be a passive process.

This work was supported by funding from the Overseas Development Administration (Project 4561).

Expression and assembly of plant virus-like particles in bacterial cells

D-J. Hwang,[1] I.M. Roberts & T.M.A. Wilson

To protect the integrity and hence the infectivity of their genetic material, almost all viral pathogens of plants encode a coat (capsid) protein (CP) whose function is to assemble into a geometrically symmetrical protein shell surrounding the RNA or DNA genome of the virus, thereby making it inaccessible to degradative enzymes which are ubiquitous in cells, plant sap, vector secretions, and the environment. Viruses which persist in plant debris in soil, and in their insect, nematode or fungal vectors for effective and efficient transmission to fresh crop plants, do so as a consequence of the efficacy of CP in protecting their genetic material. In many viruses studied at SCRI, the CP also plays a positive role in acquisition, localisation and transmission between plants by a particular mobile vector, e.g. a nematode, fungus or insect.

The amino acid sequences of many hundreds of plant viral CPs have been determined, but for only very few has the precise three-dimensional structure of the protein shell been analysed by biophysical techniques such as X-ray crystallography. In even fewer cases do we have any indication of how the virus is assembled, what features of the CP are important, and how the viral nucleic acid is recognised in preference to cellular nucleic acids in the milieu of infected cells. In one or two cases, most notably tobacco mosaic virus (TMV), we can manipulate the virus assembly machinery *in vitro* to encapsidate the native viral RNA, or other foreign RNA species containing an appropriate TMV CP recognition signal, into stable virus or pseudovirus particles which can then infect or deliver the RNA into plants, even in the presence of nucleic acid degrading enzymes. The object of this research programme is to harness the essential components of a model plant virus assembly system (TMV) for expression and manipulation in a convenient intact cell system, the bacterium *Escherichia coli*. This should provide important new information on the mechanism and regulation of plant virus assembly *in vivo*, be easily adapted to other virus systems and begin to fill-in gaps in our knowledge of function between the many known sequences of viral CPs and the few static structures resolved by crystallographic analysis.

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Classically, TMV has been the model system of choice for studies on the spontaneous self-assembly of complex biological structures *in vitro*[1]. Preparations of TMV have remained remarkably stable and infectious for over 50 years at room temperature, retaining an intact 6.4 kb single-stranded (ss)RNA genome. TMV multiplies to very high copy numbers in plants, 1-5g virus kg^{-1} fresh leaf weight, which accounts for its use in early studies on plant virus assembly, the preparation of assembly competent viral CP and viral genetics. During the 1970s, the three-dimensional structure and polymorphic forms of TMV CP were studied extensively. A short sequence (a minimum of 75 nucleotides) of viral RNA which signals the initiation of virus assembly was mapped at about 1.0 kb from the 3'-end of the 6395 nucleotide genome of the common strain of TMV. An equivalent origin-of-assembly sequence (OAS) has now been identified in several fully-sequenced tobamovirus genomes.

Chimaeric single-stranded RNA molecules were created which contained a 440-nucleotide sequence spanning the TMV OAS but otherwise of foreign sequence. TMV CP purified from isolated virions encapsidated these foreign RNA species efficiently and completely *in vitro* and the resulting TMV-like pseudovirus particles had lengths predicted from the size of the RNA species they contained. Using stably transformed tobacco plants, we showed that, during a TMV infection, chimaeric nuclear gene transcripts containing the cognate TMV OAS were mistakenly packaged and accumulated as a substantial fraction of the progeny virus population. Consequently, expression of the chimaeric messenger RNA for chloramphenicol acetyl transferase (CAT) was diminished over three-fold during TMV infection, but only when the CAT mRNA contained a TMV OAS. This represented the first and only attempt to manipulate, in a predictable way, the assembly mechanism of a plant virus *in vivo*, in virus-infected cells. It confirms that no selective subcellular compartment or virus-specific inclusion body exists where OAS-dependent virus or virus-like particle assembly occurs.

Virus symptoms can often be attributed to interactions between the viral CP and the chloroplast.

The membranes of the light-harvesting thylakoid apparatus become disrupted by TMV CP and the efficiency and functions of photosystems I and II are affected. TMV CP can also encapsidate ssRNAs within infected cell chloroplasts, and the resulting pseudovirions comprise up to 2% of a normal virus preparation.

During experiments to investigate the molecular mechanism by which stable virus particles are disassembled following inoculation of plant cells, we prepared and used *in vitro*-made pseudovirus reporter gene particles to measure the extent of RNA uncoating and expression. During the early events of infection these non-replicative particles behaved as predicted for normal virus particles. However, to prepare these structures in quantity, *in vitro*, involved tedious, expensive and often inefficient protocols to clone, purify, digest and transcribe suitable bacterial plasmids, to propagate and purify virus, to isolate viral coat protein in an assembly-competent form, and finally to perform *in vitro* packaging reactions and recover pseudovirus particles of the predicted lengths. A simple and efficient procedure was required to maximise the technological advantages of preparing encapsidated reporter RNAs for early events studies with plant viruses, as well as to exploit a generic RNA-delivery system.

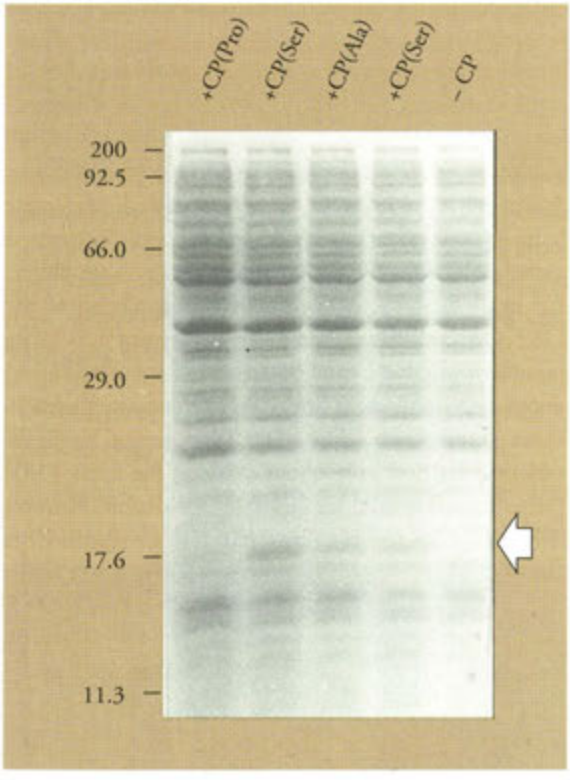

Figure 1 Expression of tobacco mosaic virus coat protein (arrowed band) in induced cultures of *Escherichia coli*. Total soluble bacterial proteins were separated by electrophoresis through polyacrylamide gel and the positions of known marker proteins together with their sizes are shown on the left. Extracts are from bacteria expressing no TMV coat protein (right lane), the native TMV coat protein [CP serine (Ser)] or either of two derivatives of the TMV sequence where the second amino acid position was changed from Ser to proline (Pro) or alanine (Ala).

Definitive studies on molecular mechanisms and structural constraints on the CP amino acid and genomic RNA sequences for plant virus or virus-like particle assembly *in planta* would also benefit from an easily and rapidly manipulated *in vivo* model system, which was independent of virus replication. Whole plant infection is a complex, slow and asynchronous process. Non-viable virus mutants can be created and lost during reverse genetics experiments for many reasons other than inefficient particle assembly. Thus even the manipulation of available full-length infectious clones of some plant viruses to study the process of assembly *in vivo* would be tedious and slow. Conventional prokaryotic expression vectors and transformed bacterial cells offer a rapid, efficient, homogeneous and malleable system in which to study plant virus assembly. We have recently achieved the first self-assembly of pseudovirus or infectious plant virus particles *in vivo* in a convenient prokaryotic expression system.

Using a conventional, inducible *E. coli* protein expression system[2] we produced large amounts of either native TMV CP or several point mutants in the second amino acid position. Following induction of CP gene expression in exponentially growing bacterial cell cultures for 2-18 h, the cells were harvested, lysed and the insoluble debris was removed. Most of the TMV CP was recovered in the soluble fraction, size-fractionated by denaturing gel electrophoresis and seen by staining (Fig. 1). The identity, integrity and yield of the 17 kDa CP species were further confirmed by immunological cross-reaction with antiserum raised against native TMV (not shown). In separate experiments, CP yields were typically around 30-60 $\mu g\ ml^{-1}$ original bacterial culture, judged by staining intensity compared with known amounts of marker TMV CP (or 3-20 $\mu g\ mg^{-1}$ total bacterial protein, judged by immunoassay techniques).

Expression of plant viral proteins, including CP, in *E. coli* or yeast cells and their subsequent extraction and purification for functional assays, as antigens for

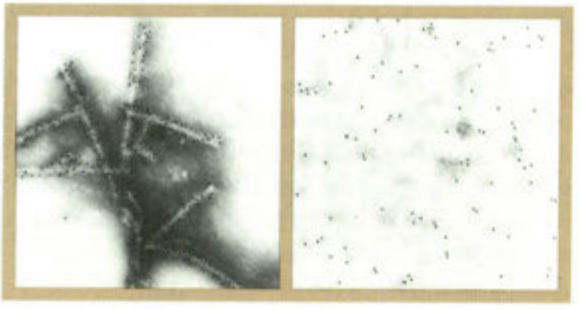

Figure 2 Native TMV coat protein purified from virions isolated from plants (left) or from bacterial cells (right) and treated at pH5 to promote helical aggregation. Samples were viewed in the electron microscope by negative staining and probed with a monoclonal antibody specific for helically assembled TMV protein. The black dots represent colloidal gold particles attached to the monoclonal antibody. In the left panel these are associated closely with the helical particles whereas in the right panel they are randomly distributed.

raising antisera or even for *in vitro* particle assembly studies, are becoming routine. We confirmed reports by other groups that TMV CP, and all the point mutants we made and tested, were unable to encapsidate native TMV RNA *in vitro* following purification of the proteins from bacterial cell lysates. In fact, all bacterially synthesized TMV CP, or its derivatives, precipitated during standard purification procedures and, in the electron microscope, a heterodisperse population of stacked disk-like structures replaced the long helical virus-like aggregates of CP expected at pH 5.0. The anomalous aggregation behaviour of *E. coli*-expressed TMV coat protein was confirmed by the failure of immunogold labelling with a monoclonal antibody-specific for helically assembled TMV CP (Fig. 2).

No conscious attempt has been made, however, to direct the specific assembly of virions or virus-like particles *in vivo*, in a bacterial cell culture. Two independent gene constructs were made to provide chimaeric foreign single-stranded RNA molecules as substrates for encapsidation by TMV CP through having a cognate TMV OAS. In contrast to the behaviour of *E. coli*-expressed TMV CP *in vitro* (above), co-expression of native or derivatives of TMV CP and an OAS-containing RNA *in vivo* resulted in accumulation of helical ribonucleoprotein rodlets (Fig. 3) of the predicted length, i.e. 66 nm for constructs containing a CAT-OAS mRNA of 1.4 kb. Protocols for bacterial cell lysis and efficient release of pseudovirus particles in the absence of the detergent Triton-X100 were developed. In contrast to the non-helical aggregates of TMV CP alone purified from *E. coli* (Fig. 2), the virus-like CAT-OAS rods were helical and could be immunogold-decorated with the (anti-neotope) helix-specific monoclonal antibody (not shown). In the absence of a TMV OAS, few if any virus-like rods could be trapped on polyclonal anti-CP antibody coated grids (Fig. 3a). An ELISA procedure was developed to quantify the yield of assembled particles using the helix-specific monoclonal antibody. Although background values recorded for crude bacterial cell extracts with several CP gene constructs alone were rather high, equivalent to *c.* 3 µg helical CP mg^{-1} total bacterial protein, co-expression of the CAT-OAS mRNA caused a rise of between 4.5-7.2 µg pseudovirions mg^{-1} total bacterial cell protein (Fig. 4) depending upon the TMV CP gene construct used in these experiments. It is clear from several replicate experiments that only about 20% of the total TMV CP expressed is used for RNA encapsidation, however the extent of pseudovirus particle production is

Figure 3 Assembly of TMV-like particles *in vivo* in *Escherichia coli* using the alanine derivative of the TMV coat protein gene and a CAT-OAS messenger RNA. (a) expression of TMV coat protein alone; (b) and (c) examples of the CAT pseudovirus particles assembled *in vivo* during bacterial cell culture. All samples were negatively stained for electron microscopy. The histogram shows the frequency-length distribution of pseudovirus particles taken from several independent electron micrographs. The predicted length for the CAT-OAS messenger RNA (1,400-nucleotides) is 66nm as confirmed by the data.

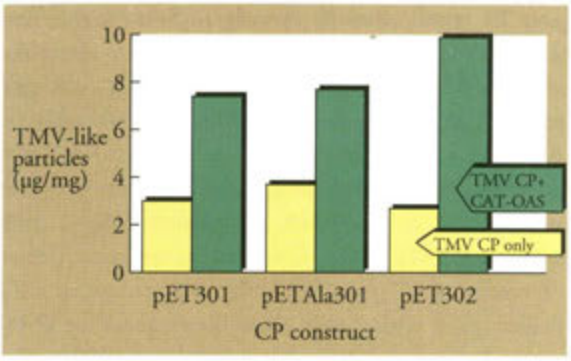

Figure 4 Quantification of TMV-like particles by enzyme-linked immunosorbent assay (ELISA). A mouse monoclonal antibody specific for helically-assembled TMV coat protein (i.e. virus-like particles) was used in the quantification of bacterial cell extracts. Values are expressed as micrograms TMV coat protein per mg total bacterial protein. Three different TMV coat protein constructs are represented. pET301 is the native viral coat protein sequence expressed using plant-optimised codons. pETAla301 is the same except that the second amino acid has been changed from serine to alanine. pET 302 is again the native TMV coat protein sequence, but expressed from a plasmid where an entirely synthetic gene was made using codons optimal for bacterial protein synthesis.

substantially more straightforward and less costly than previous methods. Various nucleic acid hybridization and PCR protocols were developed to confirm that the recovered rodlets contained the predicted chimaeric RNA species.

Two further observations of general significance to plant virology have been made using this system:

(a) When a 2.8 kb mRNA transcript with a 3'-terminal TMV OAS was expressed at high levels from a T7 promoter, and synchronously with the TMV CP gene, it seemed that the chimaeric mRNA was recruited into polyribosomes and began (5'→3') translation into protein before 3'→5' TMV CP-mediated assembly could be initiated in the opposite direction. In consequence, we observed curious and complex ribonucleoprotein structures in the electron microscope (Fig. 5). We interpret these to show that (pseudo) virus assembly *in vivo*, albeit in *E. coli*, cannot remove host cell ribosomes which are actively translating the 5' end of the chimaeric RNA. This observation reinforces a longstanding belief that some form of exclusive sub-cellular compartmentalisation must occur in the cytoplasm of infected plant cells. Progeny viral plus-strand RNAs transcribed from minus-strand RNA templates, representing 76% of all known plant viruses, must either be recruited for protein synthesis or encapsidated into daughter virions. The implications of this observation for later events in virus-infected plant cells are significant and amenable to further experimental study.

(b) We have detected expression and assembly of full-length infectious TMV particles in cultures of *E. coli* (described above) transformed only with a plasmid containing a full-length DNA copy of the TMV genome. Many years ago we showed that cell-free extracts of *E. coli* can translate full-length (6.4 kb) TMV RNA to produce one major polypeptide of 17.6 kDa[4]. This protein had many of the biochemical and biological properties of TMV coat protein and must be expressed by internal translation initiation at a fortuitous Shine-Dalgarno-like sequence. Our recent results confirm this observation since TMV RNA and CP were expressed *in vivo* from the plasmid pTMV212* which contained a full-length

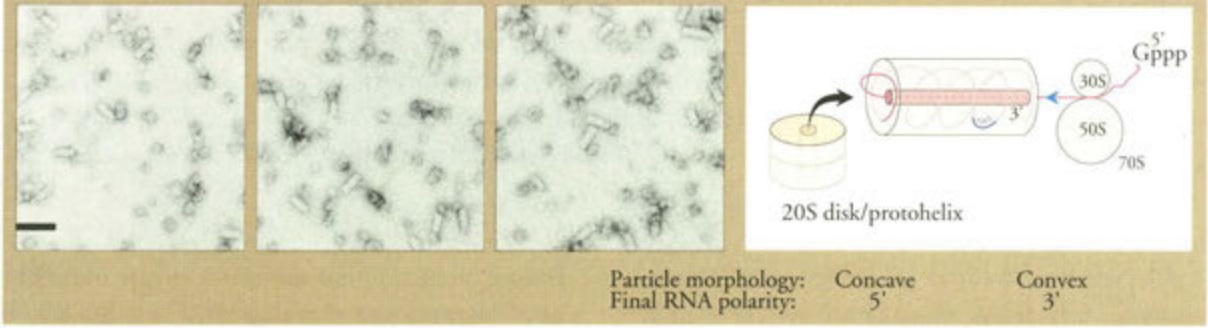

Figure 5 Partial assembly of TMV OAS-containing messenger RNA into virus-like particles under conditions in which the mRNA has been recruited by bacterial ribosomes before the initiation of assembly. Bacterial (70S) ribosomes are visible at the convex end of the growing virus-like helix where they block further 3' to 5'elongation by being unable to pass through the hole in the centre of the particle, as predicted by the "inside -out" mechanism of TMV self-assembly[3]. The right hand panel is a graphic representation the main structural features of this model.

*Gifted by Professor W O Dawson, University of Florida

clone of the viral genome with a T 7 promoter. High levels of TMV CP were seen following gel electrophoresis and immunoblotting. A proportion of this CP must have recognised the OAS on some full-length TMV RNA transcripts, as well as on TMV RNA fragments, and resulted in a low but infectious yield of full-length, uncapped, incorrectly 3'-terminated TMV genomes. This is the first report of the production of an infectious plant virus in bacterial cells. Naked TMV RNA did not survive exposure to cellular ribonucleases during bacterial lysis, clarification and storage of extracts at room temperature for several weeks.

In conclusion, we have established an easily manipulated bacterial system for packaging single-stranded RNA *in vivo* into virus-like particles. Further reverse genetic experiments should permit a detailed and high resolution analysis of the OAS-nucleotide and CP-amino acid sequence requirements for efficient TMV assembly *in vivo*, to confirm or refute predictions from X-ray structure models and extensive *in vitro* assembly data accumulated over the past 20 years. Results already indicate a role for molecular chaperone proteins in the efficient assembly of TMV CP into helical virus-like particles in *E. coli* cells. Thus we may find that plant virus assembly *in vivo* does not conform to the strict dogma of self-assembly, thereby explaining the discrepancy between *in vitro* and *in vivo* RNA encapsidation by *E. coli* expressed TMV CP. Conversely, we expect that expression of CP genes from other filamentous or rod-shaped plant viruses may allow their equivalent OAS region to be mapped, by co-expression of cDNA sub-clones and electron microscopic screening for rodlets in induced bacterial cultures.

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Nematode and insect pests

D.L. Trudgill

Control of damage by pests is vital for farmers but the pressure to reduce pesticide use is increasing. However, for many pests the introduction of effective alternatives will be a slow process. Genetic engineering offers many new possibilities, including the introduction of various defence genes into plants. However, the process is costly and money will have to be recouped from the farming industry. Also, most potentially useful genes will be effective over a much narrower range of crop and pest combinations than most pesticides. Similarly, introduced biocontrol agents are currently effective in only a limited range of crops and environments, and their use requires considerable expertise. Host plant resistance offers the best prospect for pest control in many crops, but the selection of virulent biotypes able to overcome resistance remains a threat. This diversity of approaches is reflected in research at SCRI where a substantial effort focuses on understanding the interactions between hosts and pests. To help lead these basic and fundamental studies, two additional molecular biology posts have been funded to study genetic diversity of insect and mite populations and support for molecular studies on potato cyst nematodes (PCN) has been obtained from the EC.

Molecular studies on aphids continue to be focused on the degree of heterogeneity in two important virus vectors, the large raspberry aphid, *Amphorophora idaei* and the peach-potato aphid, *Myzus persicae*. Analysis of ribosomal DNA has identified 13 genotypes in *A. idaei* biotype 2 which is virulent and overcomes resistance gene A_1 indicating the presence of considerable genetic heterogeneity. In contrast, only limited heterogeneity was found in six clones of *M. persicae*. Studies on gall mites from gooseberries, black and red currants showed they formed three distinct groups. The ribosomal genome is also proving useful in studies of variation in PCN and in root knot nematodes where the unusual occurrence of the 5S gene between

the 28S and the 18S genes has been confirmed in several species.

Routine applications of insecticides are the mainstay of raspberry beetle control but alternatives are being sought in an AFRC Link Programme involving the University of St Andrews. Behavioural studies using an olfactometer in the laboratory have shown that adult beetles are strongly attracted to specific volatile components of raspberry and hawthorn flowers and in the field, some raspberry cultivars were more attractive to beetles than others. Selection of the feeding and oviposition sites is influenced by differences in nectar production, time of day and by whether or not an egg has already been laid on the flower. Collaborative studies with IACR Rothamsted are seeking to identify the semiochemicals influencing beetle behaviour and hence how it may be controlled in Integrated Pest Management systems using less pesticides. Similar collaborative studies with groups in Switzerland, Denmark and the Royal Botanic Gardens, Kew on leaf surface oviposition stimuli for cabbage and turnip root flies have identified two further chemicals which are considerably more active than the previously identified glucosinolates. There is now potential to develop a biochemically targeted plant screen for root fly resistance in brassicas.

Temperature is a major environmental factor and recent studies on nematodes indicate that, within normal ranges, there is a linear relationship between temperature and life cycle duration. A base temperature and a constant number of degree days for development can be defined and comparisons of related temperate and tropical species indicate that the base temperature in the former is lower but the thermal constant is higher than in the latter. The effect of these differences is to give each species a competitive advantage in the environment to which it is adapted.

Research on PCN funded by the Potato Marketing Board and with colleagues in the advisory services has produced a simplified yield loss model and shown that the yield loss parameter is modified by a site and a cultivar component. The data will assist in the development of expert systems for better management of PCN by integrating tolerance of damage, partial resistance and rotation resulting in less dependence on nematicides.

Collaborative studies with the Royal Botanic Gardens, Kew, funded by the British Technology Group, on plant-derived chemicals for the control of nematodes has identified several biologically-active compounds. One of these, a sugar analogue, dihydroxymethyl dihydroxypyrrolidine (DMDP) has systemic activities and has been patented as an environmentally benign crop protection agent. This chemical also stimulates plant growth and can be applied as a foliar spray, soil drench or seed coating.

A survey of the occurrence of the New Zealand flatworm, a predator of earthworms, has shown that it is widely distributed in Scottish gardens, garden centres and botanic gardens, but that it appears to be relatively rare in farmland. Its known distribution suggests that it requires cool moist soils, and is intolerant of freezing. However, in favourable conditions it can almost eliminate certain species of earthworm important for maintaining good drainage in pastures.

Tolerance of potato cyst nematode damage

D.L. Trudgill & M.S. Phillips

A substantial proportion of ware potatoes in the UK is grown on land infested with one or other of the two species of potato cyst nematodes (PCN), *Globodera rostochiensis* or *G. pallida*. Unless very lightly infested, such crops have to be protected by pre-planting treatments with nematicides (Fig. 1) and currently, *c.* 22,000 ha land is treated annually in UK. Nematicides also control PCN population increase but resistant cultivars are more cost-effective and plant breeders have produced a range of cultivars with com-

Figure 1 Two strips of nematicide applied across a field of potatoes heavily infested with potato cyst nematode.

plete resistance to the multiplication of the only pathotype (Ro1) of *G. rostochiensis* found in the UK. However, the roots of such cultivars are still invaded and some that are relatively severely damaged are

Figure 2 Effect of the nematicide oxamyl (left hand plants) on the growth of tolerant cv. Cara and partially resistant but intolerant clone 11234 in soil heavily infested with *Globodera pallida*.

Figure 3 Growth of different graft combinations in soil free of potato cyst nematodes. Left to right/scion:stock; Cara on Cara, Pentland Javelin on Pentland Javelin, Pentland Javelin on Cara, Cara on Pentland Javelin, ungrafted Cara.

termed intolerant. Others such as cv. Cara are more tolerant, suffering relatively little damage in infested soil.

It has been more difficult to breed for resistance to the widespread pathotype of *G. pallida* (Pa2/3), and only a few cultivars with incomplete resistance have been produced. Some of them are also relatively intolerant (Fig. 2) although cv. Cara which is not resistant to *G. pallida* is tolerant of it. In contrast, the susceptible cv. Pentland Dell is consistently intolerant of both species of PCN. A series of studies were made to determine the basis of the difference in tolerance between cultivars.

Although PCN attacks the roots, it was suspected that the vigorous top growth of cv. Cara may contribute to its tolerance. Consequently, reciprocal grafts were

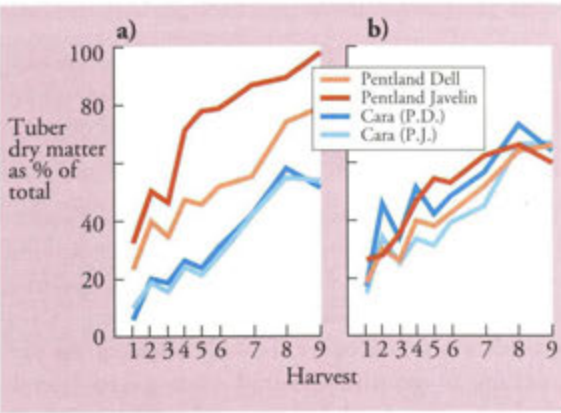

Figure 4 Tuber dry matter as a percentage of total dry matter of a) the scion genotype, and b) stock genotype over nine harvests of grafts between Pentland Javelin and Cara, and Pentland Dell and Cara.

made between cv. Cara and cv. Pentland Dell and the different combinations were grown in large pots or in field plots with high and low levels of *G. pallida*. In addition, grafts between cv. Cara (late maturing) and cv. Pentland Javelin (first early) were included in the pot experiment to determine the effect of stock and scion on overall growth. The results showed that scion genotype had a dominant influence on growth and maturity characteristics. Plants with Cara scions but Pentland Javelin stocks were late maturing with big tops (Fig. 3). The effects of Pentland Dell scions were intermediate, as shown by comparing the main effects of scion and stock on tuber dry matter as a percentage of total dry matter (Fig. 4). Stock genotype had relatively little effect on tuber dry matter even though the tubers were borne on the stock.

However, tolerance of PCN in the pot experiment was influenced most by stock genotype with Pentland Dell stocks tending to confer intolerance (Table 1). In the field experiment, Pentland Dell stocks again decreased tolerance but Cara scions contributed to increased tolerance. Consequently, Cara grafted on to Cara was the most tolerant combination in the field and Pentland Dell grafted on to Pentland Dell was the least tolerant.

Scion: / **Stock:**	Cara / Cara	Cara / P. Dell	P. Dell / Cara	P. Dell / P. Dell	LSD (5%)
Pot experiment (kg/plant)					
PCN					
Few	0.75	0.62	0.67	0.57	0.18
Many	0.52	0.43	0.55	0.33	
Field experiment (kg/plant)					
PCN					
Few	0.81	0.62	0.81	0.48	0.14
Many	0.78	0.49	0.57	0.29	

Table 1 Tuber yields of four graft combinations between cultivars Cara and Pentland Dell in a large pot and a field trial with soils lightly and heavily infested with potato cyst nematodes (PCN).

The agronomic basis of the tolerance difference between Cara and Pentland Dell was further explored at a field site where plots with a range of population densities of *G. pallida* had been prepared. Crop growth was measured by regularly assessing the percentage of ground covered with green leaves. Grouping plots into lightly, moderately or heavily infested (2-3; 10-18; and 36-63 eggs/g soil respectively) and plotting ground cover showed that the heavy infestation initially proportionally decreased the growth of the two cultivars to a similar degree (Fig. 5).

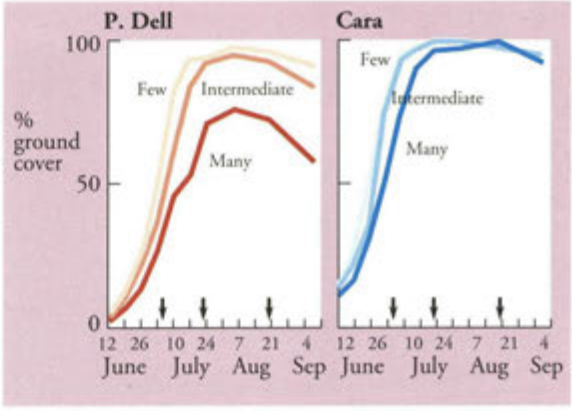

Figure 5 Effect of different levels of infestation with *G. pallida* on mean percentage ground cover for cultivars Cara and Pentland Dell.

However, Cara grew more rapidly and larger than Pentland Dell and hence still achieved 100% ground cover in the heavily infested plots whereas Pentland Dell did not. Regular destructive sampling confirmed the difference. At a plant harvest on 1 July, the slope of the regression of total plant dry matter on *G. pallida* population density was -0.113 for Pentland Dell but was larger, -0.251 for Cara. By the final harvest on 16 September the regression slope for Pentland Dell was much larger (-3.10) than for Cara (-1.00; Fig. 6). These results show that much of the tolerance of Cara was associated with its vigorous top growth enabling it to achieve 100% ground cover and light interception, in spite of damage by PCN.

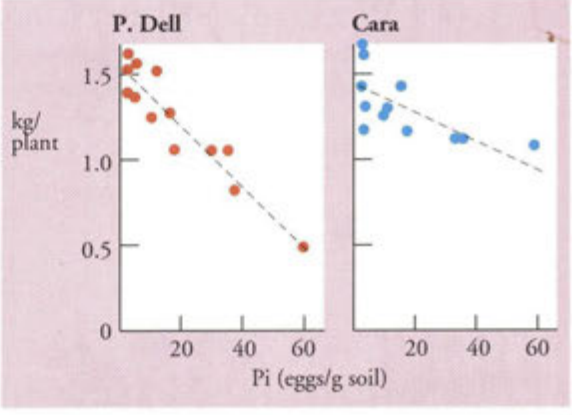

Figure 6 Yields of cultivars Cara and Pentland Dell regressed on pre-planting population density of *G. pallida*.

The value of tolerance as a means of reducing the need for nematicides was tested at a site uniformly heavily infested with *G. pallida*. In the untreated plots, the yield of Cara was decreased only 10% compared with Cara receiving the recommended rate of

	Rates of aldicarb (kg a.i./ha) 0.0	1.6	3.2	LSD (5%)
Yields (kg/plant)				
Cara	1.83	2.03	2.04	0.29
P. Dell	0.70	1.53	1.84	
Post harvest *G. pallida* (eggs/g soil)				LSR* (5%)
Cara	791	130	43	x3.16
P. Dell	94	117	66	

* Least Significant Ratio. Two means are significantly different if they differ by more than the multiplicative value of the LSR.

Table 2 Effect of a half and a full rate of the nematicide aldicarb on the yields of cultivars Cara and Pentland Dell and on final population densities of *G. pallida* at a heavily infested site (123 eggs/g soil).

aldicarb. In contrast, the yield of Pentland Dell was decreased by 38%. The yield of Cara was similar with a full or a half rate of nematicide but the yield of Pentland Dell given the half rate was still 17% less than the full rate (Table 2). Unfortunately, the population density of *G. pallida* increased more in the untreated Cara than in the untreated Pentland Dell (Table 2) and the full rate of nematicide was required to reduce it. Consequently, to be most useful, tolerance has to be combined with resistance.

Tolerance of PCN is a difficult character to screen in a breeding programme because of the substantial influence of agronomic characteristics. However, it is essential that resistant cultivars are tolerant as they will be grown at sites with PCN and consequently, methods of screening were sought. Initially, large trials at heavily infested sites compared yields in nematicide treated and untreated plots but further experiments showed that growth differences between single, replicated plants at a heavily infested site adequately discriminated between the extremes of tolerance.

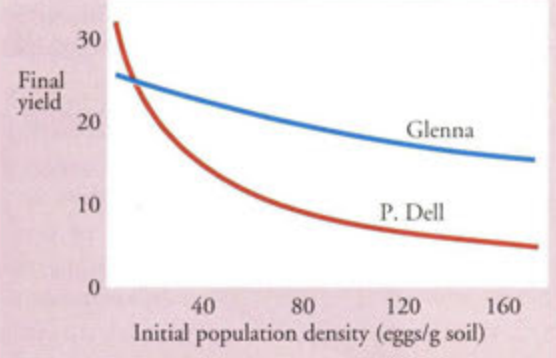

Figure 7 Yields of susceptible, intolerant Pentland Dell and partially resistant, tolerant Glenna regressed on pre-planting population densities of *G. pallida*.

	Pre-planting population density (eggs/g soil) 9	23	37	101	LSR* (5%)
Post harvest population density (eggs/g soil)					
P. Dell	13†	169	156	177	x2.3
Glenna	18	35	35	75	

* Least Significant Ratio.

† Means of four plots grouped according to previous cropping treatments used to modify nematode population densities.

Table 3 Relationship between pre-planting and post harvest population densities of *G. pallida* on susceptible cv. Pentland Dell and partially resistant cv. Glenna.

No table cultivars combining good levels of tolerance and resistance to *G. pallida* are available commercially but the second early cv. Glenna, bred at SCRI, was identified as combining both properties, together with good agronomic characteristics. In a field trial to determine the mechanism of its tolerance, the early growth of Glenna was more vigorous than that of Pentland Dell but increasing population densities of *G. pallida* decreased the growth of both to proportionally the same degree. However, because of its more vigorous growth in heavily infested soil, Glenna achieved 100% ground cover, while Pentland Dell did not and consequently its yield was less affected than that of Pentland Dell (Fig. 7).

Overall, the results suggested that high levels of tolerance of PCN are associated with vigorous top growth enabling crops to achieve 100% ground cover and high light interception despite nematode damage. Conversely, weak top growth is likely to confer intolerance but the grafting experiments with Pentland Dell point also to intolerance being associated with specific root-related factors.

Pest resistant brassicas; the role of leaf surface chemicals

A.N.E. Birch, R.J. Hopkins, D.W. Griffiths, W.H. Macfarlane Smith, R. Baur[1], E. Städler[1], T. Ramp[1], J. Hurter[1], M.S.J. Simmonds[2] & R.G. McKinlay[3]

Integrated pest management systems using pest-resistant crop cultivars are being developed at SCRI. Breeding for resistance to insect pests normally requires large numbers of plants to be screened in the field or glasshouse and is time consuming and erratic, being dependent on adequate numbers of the pest to exert a reasonably uniform selection pressure for resistance. To overcome these problems we are studying the mechanisms of pest resistance. The identification of biochemical or molecular markers can then be used in the laboratory to facilitate rapid selection of resistant plants at the seedling stage.

Recent field and glasshouse trials in Scotland identified several brassica cultivars and breeding lines which represent a wide range of resistance and susceptibility to turnip root fly (TRF, *Delia floralis*) and cabbage root fly (CRF, *D. radicum*), two important pests of brassica crops (Fig. 1). From the results of choice experiments in field cages and in a rotating choice-chamber to eliminate experimental bias, the major component of the observed resistance was found to be due to antixenosis, i.e. plant characters resulting in reduced numbers of eggs being laid by the adult fly. Four selected brassicas were then used to investigate the chemical factors involved in the resistance mechanism. A collaborative and multidisciplinary approach including entomologists, plant geneticists, chemists and electrophysiologists was employed to identify plant characteristics involved in host recognition, with specific emphasis on plant chemicals influencing oviposition preference.

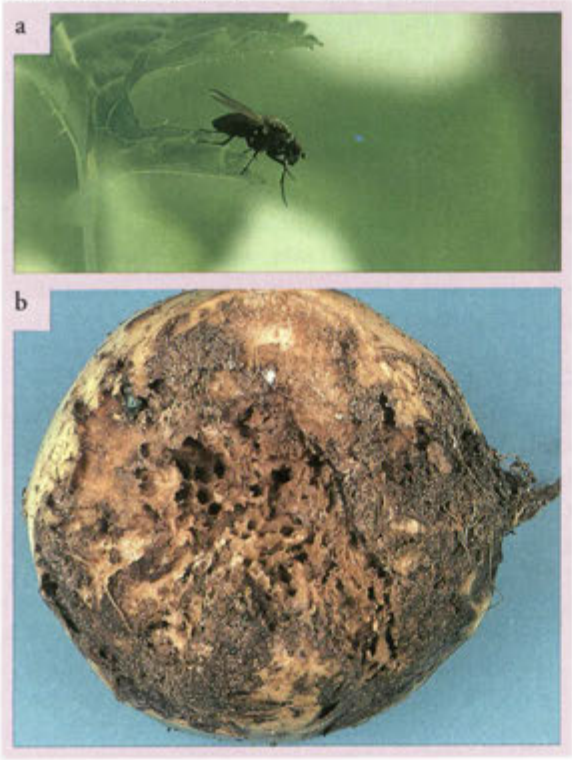

Figure 1 (a) Adult turnip root fly (*Delia floralis*); (b) Damage caused to swede root by root fly larvae.

Insect behaviour during egg laying Root flies go through a fixed sequence of behavioural events before selecting a suitable plant on which to lay eggs (Fig. 2). Detailed observations of the oviposition behaviour of TRF and CRF on the four brassicas indicated that both species discriminate between susceptible and resistant plants whilst on the leaf. For example, on the susceptible swede cv. Doon Major, 40% of the female flies that landed on the leaf went through the entire behavioural sequence to lay eggs, whereas on the resistant kale cv. Fribor, no flies progressed

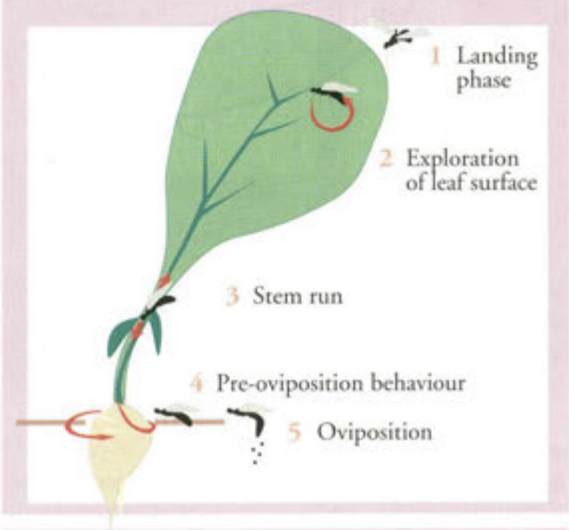

Figure 2 Behavioural sequence of adult female fly during oviposition.

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[2]Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS
[3]Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG

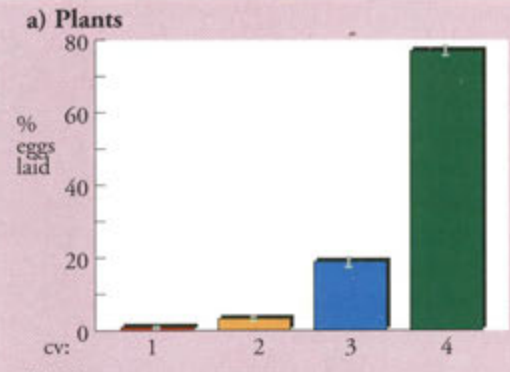

Figure 3 Percentage of total oviposition on a) whole plants; and b) artificial leaves sprayed with leaf surface extracts in separate choice tests.
1 = Fribor kale
2 = Dwarf green curled kale
3 = SCRI swede breeding line
4 = Doon Major swede

beyond the "stem run" stage and most flies rejected the plant soon after landing on the leaf. Resistance was therefore due either to a deterrent, a lack of suitable oviposition stimuli or both on the leaf surface.

Bioassay-guided chemical fractionation of the active leaf surface chemicals To determine the chemical identities of the active components and quantify their effects on root fly behaviour, leaf surface extracts were made by briefly dipping leaves sequentially into a non-polar and a polar solvent (dichloromethane and methanol respectively). The extracts were sprayed onto specially designed artificial leaves made from wax-coated, corrugated green card, then exposed to gravid female flies for 24 h in a rotating choice-chamber (Fig. 3b). The methanol-soluble fractions stimulated oviposition and the extent of the stimulus for each extract correlated well with results for oviposition on the whole plants (Fig. 3a). Since no evidence of deterrent activity was found in other fractions, this indicated that the resistance mechanism was due to a lack of specific oviposition stimuli on the leaf surface of the resistant plants.

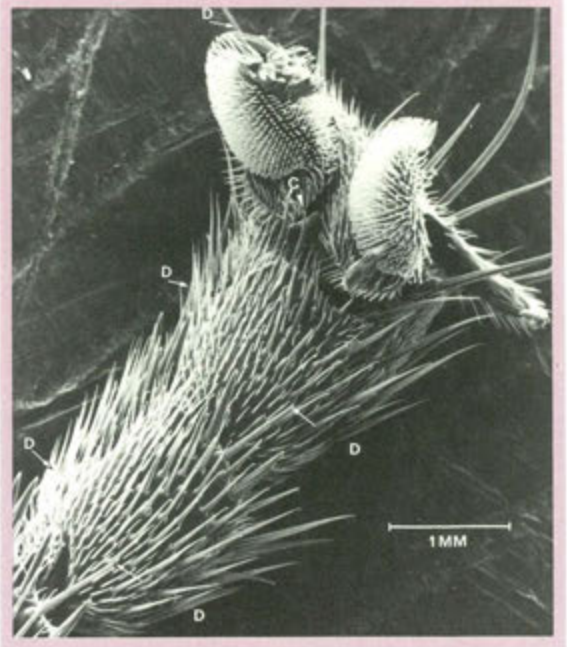

Figure 4 SEM of female cabbage root fly prothoracic tarsus, showing positions of different chemo-receptor hairs.

Electrophysiological activity of the leaf surface extracts on root fly tarsal receptors Root flies have several types of contact chemo-receptor hairs on their tarsi (Fig. 4) and labellum. These receptors are used by the adult female fly to detect different types of chemicals on the plant surface and assess the suitability of the plant as a site for egg laying. The responsiveness of the main tarsal receptors of TRF and CRF to leaf surface extracts of the four brassicas is being investigated in Kew and Wädenswil, using a sensitive electrophysiological bioassay (Fig. 5). This technique requires very small amounts of leaf surface extracts and provides a rapid screen for the chemical fractionation of the active components once the specific receptors have been identified. Tests on the four brassica extracts produced electrophysiological activities which correlated well with egg counts from oviposition bioassays. Current research is focused on fractionating the extracts using column chromatography, guided by the tarsal receptor bioassay and results indicate that although some glucosinolates present on the leaf surface are active as oviposition stimuli for both species, the main activity is due to one or more newly identified compounds first isolated at Wädenswil. These compounds stimulate specific tarsal chemo-receptors of both TRF and CRF and are several orders of magnitude more stimulatory than the most active glucosinolates tested in electrophysiological tests.

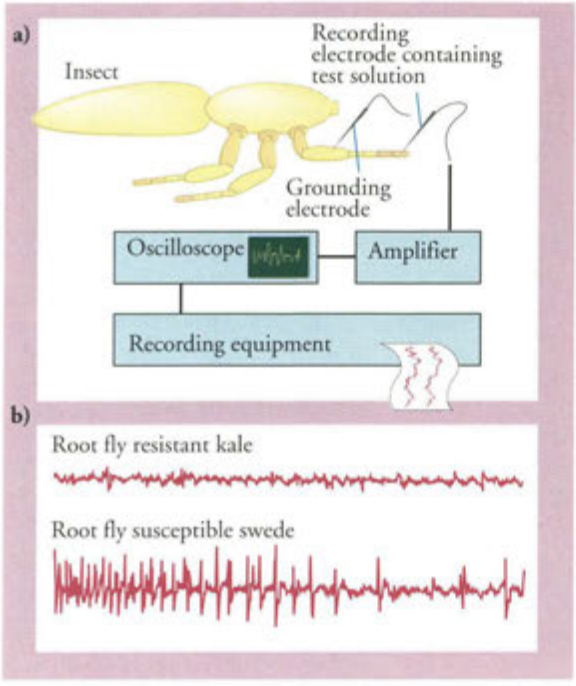

Figure 5 a) Diagram of the electrophysiological system used to record stimulations of root fly tarsal receptors; b) Contact chemosensory response (spikes in first second) following stimulation with leaf surface fractions of root fly resistant kale, and root fly susceptible swede.

Conclusions

Our collaborative studies comparing the behaviour and receptor sensitivities of the two *Delia* species have shown that, in general, TRF and CRF respond similarly to the four brassicas and to their extracted leaf surface chemical profiles. In addition, we have identified the mechanism of antixenosis and results indicate that resistance is due to lack of specific oviposition stimuli on the leaf surface. The good correlation between oviposition behaviour on plants, stimulatory activity in the artificial leaf bioassay and electrophysiological activity on tarsal receptors for extracts and derived fractions indicates that these techniques have potential in developing mor rapid screening methods for pest resistance in future plant breeding programmes.

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New Zealand flatworm in Scotland

B. Boag, R. Neilson, L.F. Palmer & A. Salowsky-Butcher

The New Zealand flatworm (*Artioposthia triangulata*) is considered to be an obligate predator of earthworms which was first reported from Scotland in 1965 (Fig. 1). Initially in the late 1980s, work in N. Ireland indicated that it could eradicate earthworm populations under agricultural conditions. Because of the potential threat to Scottish agriculture, surveys have been undertaken recently at SCRI in conjunction with the Biological Recording in Scotland Campaign, the National Museums of Scotland and SOAFD Agricultural Scientific Services to ascertain its present status. The results indicated that *A. triangulata* has a widespread geographical distribution, being recorded from over 400 separate sites from Orkney in

Figure 1 *A. triangulata.*

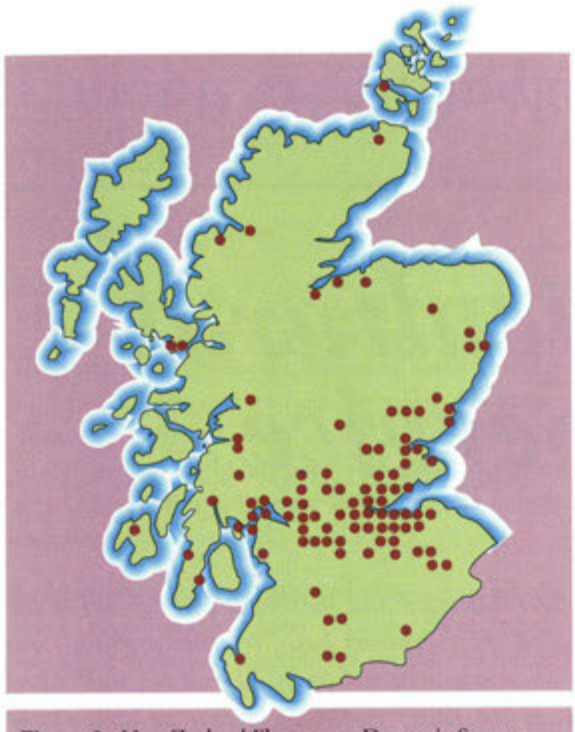

Figure 2 New Zealand Flatworm - Domestic Survey

the north to the Solway in the south (Fig. 2). Over 350 of these records were from domestic gardens and it was also found in botanic gardens, nurseries and garden centres. It was recorded on only one of the 600 randomly chosen arable farms in the survey although it has been reported on 13 farms in the country, of which six are in the Dunoon area where farmers have associated the disappearance of earthworms with poor drainage (Fig. 3).

Figure 3 A waterlogged field.

It is not known how the flatworm was first introduced into Scotland but it probably came with planting material from New Zealand or from Northern Ireland where it was recorded first in 1963. In 1987 it was reported from the Faroe Islands and while the first record from England was in 1965, the second confirmed report was in 1992. Within Scotland it was probably spread initially between botanic gardens, nurseries and garden centres and then to domestic gardens along with containerised plants. The very low level of infection in arable farmland would suggest there is little mixing of soil or planting material between gardens and farms and that it was unlikely to have a serious detrimental effect on agricultural output in the near future. In the long term, *A. triangulata* may not be a problem in eastern Scotland since there are relatively few earthworms in the intensively cultivated land and the flatworm, in certain circumstances, has been shown to co-exist with earthworms for over 10 years. However, in the west of Scotland where it is generally wetter and winters less cold, the flatworm probably has the potential to become a serious problem especially since most of the farming systems there depend upon grass or long term leys and the larger earthworm populations probably play a major part in maintaining structure and nutrient status. In view of its potentially harmful effects on agriculture and wildlife, *A. triangulata* was added to the Wildlife and Countryside Act in 1992 which makes it an offence to knowingly move the flatworm from one part of the country to another.

Although widespread in Ireland and Scotland, *A. triangulata* appears to be almost absent from England and Wales. Such a localised national distribution may have a detrimental effect on agricultural and horticultural exports if it is perceived that goods from Scotland and Ireland may be contaminated with the flatworm. Since little is known about the biology of *A. triangulata* and there are no approved chemicals for its control, prudent measures should be taken to limit its spread both within the British Isles and between Britain and the rest of the world.

Scottish Agricultural Statistics Service

R A Kempton

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

Additional SOAFD funding allowed three new programmes to be established during the year. That most relevant to SCRI is concerned with devising statistical methods for genetic mapping. An understanding of the arrangement of genes within the genome is essential for improving the potential of crop species. Until recently, mapping has been almost wholly restricted to major genes which give rise to discrete variation in phenotype. Many important characters, however, show continuous variation due to the cumulative effect of several genes, each with small phenotypic effect. Mapping of quantitative trait loci (QTLs) requires new methods of statistical analysis. These have been used at SCRI to map genes affecting height and heading date in barley and pod shape in field bean.

The other two programmes are in animal epidemiology (with MRI and SAC) and land use modelling (MLURI and ITE). These projects are highlighting how modelling can offer insight into the behaviour of complex systems. It is also clear that predictions can be very sensitive to the scale adopted for modelling the process, and the way stochastic variation is handled. An understanding of variability is also essential for efficient statistical estimation, particularly in complex experiments where variation may arise at several levels. Methods originally developed by the AFRC Unit of Statistics in Edinburgh provide pooled treatment estimates which give appropriate weightings to information from the different levels of the experiment. As a result of collaboration with the Statistics Department at Rothamsted Experimental Station, these procedures have now been made available in the Genstat statistical package. This has allowed a detailed examination of the methods in several areas of application. A course was also prepared and presented to scientists.

Observational data are the main focus of statistical work. However, an increasingly important area of research concerns the handling of information expressed as judgements and incorporated in what are termed expert systems. One EC-funded project to develop an integrated forecasting system is described in the article below. Another has involved developing a prototype system to model crop production and predict economic return using quantitative or qualitative rules and allowing knowledge to be represented at several levels of precision.

There was an increased demand for statistics training with over 40 courses given to SARIs and SAC during the year. Particular interest was shown in computational methods for sequence analysis, and a new course was developed in constructing phylogenetic trees.

Linking expert judgements with forecasting models

M Talbot & G.J. Gibson

Time series analysis methods can be powerful tools in extracting patterns from complex and noisy temporal data. However, forecasts based solely on such methods depend for their success on the patterns dominating future performance of the series. In practice this rarely happens and most important forecasts in industry, business and government involve judgements of some kind.

Judgemental expertise comes into play in forecasting in several ways. These include: choosing the appropriate modelling approach; selecting the variables to be used; determining the most effective model; adjusting the model parameters; and adjusting the predictions from the model. Of these the judgemental adjustment of model output is probably the most important and occurs for one of two reasons. The model may not have been performing adequately in recent time and it is easier to make *ad hoc* adjustments to the forecasts than to the model parameters. Alternatively, and more importantly, there may be knowledge of some external factors, not allowed for in the model, which are expected to influence future events.

In recent years there have been a number of major studies on the value of judgements in forecasting. Most of these have been concerned with the accuracy of expert judgement relative to statistical models and there is evidence that if individuals have expert knowledge then judgemental and statistical models can be of similar accuracy. However, the value of combining judgements and models has not been systematically investigated even though researchers have argued that there must be considerable advantages in the structured interaction of judgemental and statistical forecasting methods. Structure in this context means that the judgemental process is made explicit by revealing both the evidence on which judgements are based and the underlying logic. It also implies a formal link between judgements and the statistical model.

The importance of a structured approach to the use of judgements in forecasting lies in the need to justify and explain the basis of forecasts to others. Forecasts have a vital role in public and private sectors as a basis for decision-making. For this reason the use of systematic, defensible and best-practice techniques is more critical in forecasting than in many other applications of statistics. A valuable by-product of statistical methodology which is often over-looked is that it provides an audit trail for examination and replication whereas the judgemental process is inherently more difficult to describe, replicate and defend. At least one case is known of a government being sued for a forecast that involved judgemental adjustment of a quantitative model which if used on its own would have been more accurate.

For there to be a formal interaction between judgements and statistical methodology we must have a means of modelling judgements. In recent years developments in two areas, cognitive mapping and graphical models, provide elements which can help in the task of eliciting and modelling judgements.

Cognitive mapping Cognitive mapping attempts to describe a person's thought structures in the form of a network where nodes represent factors and lines join-

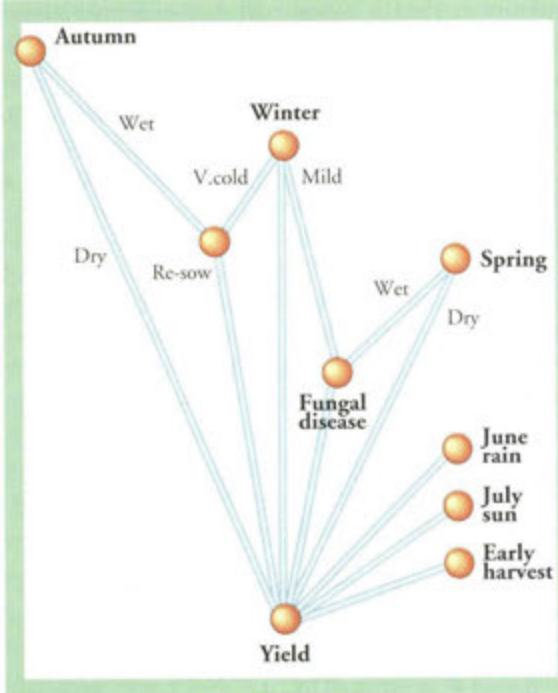

Figure 1 Factors affecting cereal yield - a cognitive map.

ing nodes represent the relationships between them. The factors can range from quantitative variables such as spot grain prices to qualitative variables such as possible consequences of a GATT agreement. A successful map will display all of the factors which an expert thinks are relevant as well as the important links between those factors.

Having elicited the context in which the expert views the forecasting issue, the next stage is to extract from the cognitive map a submap to be analysed more precisely. This could mean a multivariate time series model, an econometric model, a functional relationship, or an expert system-type belief model.

Figure 1 shows an example of a cognitive map developed for forecasting national crop yields. Cognitive maps can help in overcoming one of the major stumbling blocks in expert systems work, namely, the difficulties of extracting information from experts.

Expert systems and belief modelling At the heart of all expert systems lies an inference engine - a method of reasoning which acts on information to infer new knowledge from old. In the early days of artificial intelligence a number of *ad hoc* approaches were suggested but many of these have now been discarded in place of more soundly based methods.

Two broad strategies in expert system reasoning can be identified. One is the deductive approach where we attempt to find a logical path between statements which are believed to be true towards some assertion whose validity we wish to establish. One of the more successful implementations of this strategy, particularly in automated control systems, is based on what is known as fuzzy logic. In fuzzy logic, predicates such as "tall" or "resistant" are considered to lie on a scale between true and false.

The other broad strategy in reasoning with uncertain information is the inductive approach based mainly on probabilistic models. While deductive systems cannot easily deal with conflicts in evidence, probabilistic techniques are able to make predictions in the presence of conflicting evidence. These predictions will not always be true but they are good guesses that make effective use of the available information.

Bayesian reasoning is the classical probabilistic mechanism used to revise belief, given new evidence. In Bayesian reasoning the logical form of the statement "if A then B" is replaced by "if A then B with probability P". This conditional probability is written P(B/A) and can be read as "the probability of hypothesis B being true given that the evidence A is true". Probabilities are updated according to Bayes's theorem:

$$P(B/A) = P(A/B)\ P(B)/\ P(A).$$

An illustration of Bayesian modelling is given in Figure 2 with an advisory example concerning mildew in a barley crop. The components of a cognitive map for the problem are shown. Here the arrows indicate conditional relationships in a probabilistic sense and so this part of the cognitive map represents what is known as an influence diagram; that is a belief network with directed arcs and no cycles. To use the influence diagram the expert provides information on the conditional probabilities. Subsequently the system operates by taking information on the truth of propositions and adjusting the probabilities accordingly.

Influence diagrams The basic theory underlying influence diagrams is relatively simple. The main step forward from the computational aspect in recent years is the ability to identify groups of related nodes, called cliques, which can be solved separately, avoiding the need to tackle large systems of conditional equations.

The computational theory for evaluating causal graphs has been well established where factors take discrete values e.g. occur/not occur. However, in forecasting we need to be able to deal with continuous variables for which, until recently, the theory did not exist. In the past year work at the University of Aalborg,

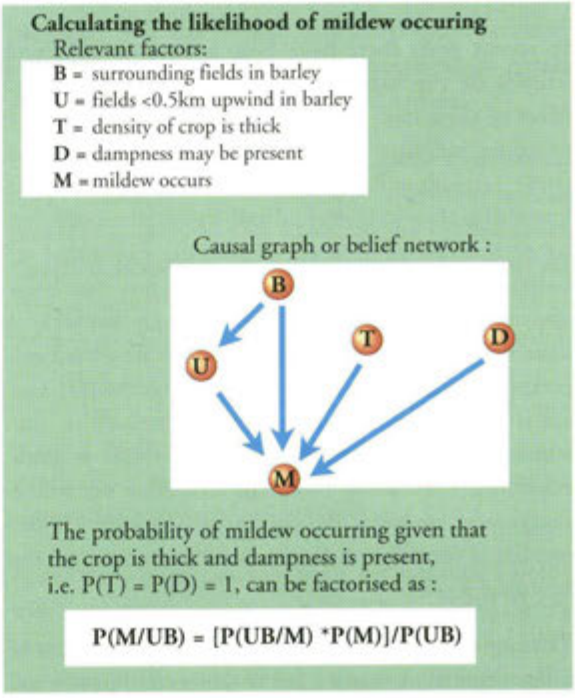

Figure 2 An example of Bayesian reasoning.

Denmark, and at the University of Edinburgh under the LIKELY project, has developed ways of combining discrete and continuous variables.

The work in Edinburgh has concentrated on a Monte Carlo simulation approach to modelling the conditional probability distributions. The approach assumes that the conditional distribution of each variable given all others can be specified, at least approximately. The estimation method, known as Gibbs sampling, is an iterative process taking random samples for each variable conditional on all others and repeating the process many times substituting after each cycle the values obtained in the previous cycle. It is known that the iterative sequence will converge to an equilibrium form of the multivariate distribution being modelled.

The Gibbs sampler is relatively easy to implement and has wide application in the study of systems with a large number of mutually interacting components, situations which are common in physics and biology.

Functional relationships Some of an expert's knowledge may consist of a theoretical understanding of their own field and this understanding can often be expressed in the form of functional relationships. An example from biology is that of dry matter yield in winter wheat where crop dry weight might be expressed as a function of intercepted radiation times a crop production efficiency constant. Functional relationships are classifiable into a range of types. At one extreme is the fully deterministic relationship. Next there is the short-run relationship where a stochastic dimension is included by adding a disturbance term in the equation. Then there is the situation, probably the most plausible in the forecasting context, where the expert may be able to specify a long-run, or equilibrium relationship between variables of interest, even though the short-term behaviour of the variables can deviate greatly from equilibrium.

One approach to the linking of functional and time series models is by means of error-correction or cointegration. Cointegration has become increasingly important as a tool in econometrics in recent years and is illustrated here with a simple example. Let y_t, x_t and z_t be time series which are believed to satisfy a law of the form,

$$\Delta y_t = a_1 \Delta y_{t-1} + b_0 \Delta x_t + b_1 \Delta x_{t-1} + c_0 \Delta z_t + c_1 \Delta z_{t-1}$$

where Δ indicates that the series are differenced to ensure stationarity. Suppose an expert believes that a theoretical law y=dx applies. Using a cointegration approach this might be incorporated in the model by adding a term, $g(y_{t-1} - dx_{t-1})$ (Fig. 3).

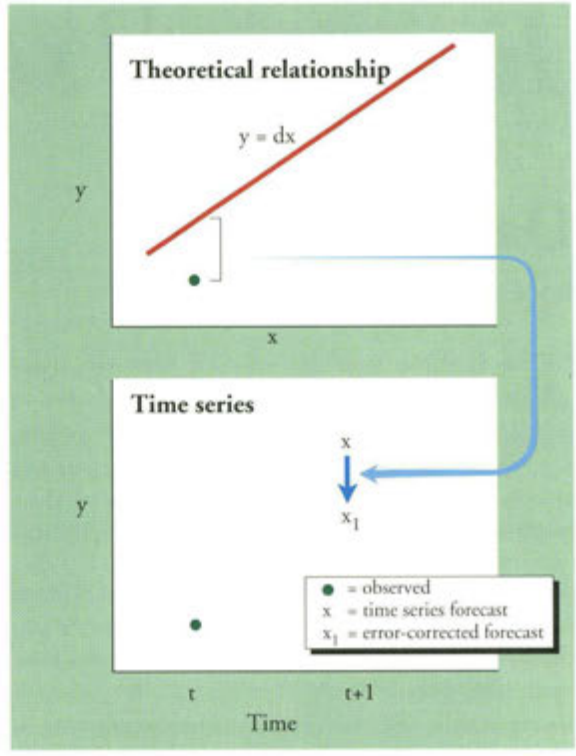

Figure 3 Linking theoretical and time series models by error correction.

The cointegrated term can be visualized as representing what happens when effects reach a long-term equilibrium and all differences go to zero, then the theoretical law is expected to come into play. Another way of viewing the error-correction model is to say that the y variable may be affected by both short-term and long-term effects. The short-term effects are not amenable to theoretical specification and are best represented by time-series models while the longer-term effects may be specified on theoretical grounds.

LIKELY project Linking Informal Knowledge and Expertise to Forecasting Models (LIKELY) is a multi-partner project supported by the EC Development of Statistical Expert Systems (DOSES) initiative and by SOAFD. The purpose of the project is to develop the theoretical basis and the computational tools to: capture the knowledge of experts in areas in which forecasts are required; evaluate these judgements; and combine the results in a structured way with statistical and functional forecasting models. The LIKELY software combines a number of relatively new concepts in the interface between artificial intelligence, statistics and cognitive science. The system is an open one allowing integration of external modelling packages and has been used to model a range of problems in economics and biology.

Research services

Data Processing

R.J. Clark

The SCRINet local area network, with Sun workstations at its centre, continued to increase in popularity as new computers were added. One of the new SUNS managed by the Unit, an image analysis system, was installed for accurate analysis of electrophoresis gels and DNA sequence autoradiographs.

Staff can access a wide range of computing services from desktop personal computers (PCs) connected to the network. A fibre-optic backbone connects nine main laboratory and office blocks, and thin ethernet wiring within each building provides connections to over 260 desktop outlets. Laser printers, graphplotters and lineprinters are attached as shared output devices. Over 120 PCs are currently installed and this number is increasing.

SCRINet is connected to the Joint Academic Network (JANET) wide area network for access to external mainframe services such as the Daresbury laboratory for DNA sequencing.

Software available on SCRINet includes statistical analysis packages such as Genstat and Minitab. Crop geneticists continue to use CHIP which is a package for managing genetic databases, generating experiment plans and labels, performing statistical analysis, and selecting progenies. Other UNIX software is used for modelling soil structures and graphics. However, services based on the Oracle relational database management system are increasing in popularity. The COSHH database, for example, allows any member of staff to view safety information on all chemicals on site.

Electronic mail facilitates collaboration between scientists at SCRI and co-workers throughout the UK and world-wide via JANET, BT Gold and CGnet.

Scientific Liaison and Information Services

D.A. Perry

The Library provides sources of reference to the staff of the Institute through a stock of specialised textbooks and current scientific periodicals. In addition, staff can access databases through the CABI CD-ROM system installed on site and by interrogating various on-line databases.

The Visual Aids Section provides a comprehensive photographic service using modern techniques with still and video camera equipment. The material produced is used for record, publication, display and publicity purposes. Graphics are produced on an Apple Macintosh system and are used to illustrate scientific results in publications and lectures and to create quality displays in exhibitions such as Scotgrow, Cereals '92 and PMB Open Day. Desk-top published documents ranging from simple leaflets to full colour productions such as this Annual Report are prepared for printing.

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

Estate, Glasshouse and Field Experiments Department

W.I.A. Jack

The Department provides a fully equipped and professionally expert service for the preparation of land, growing medium, sowing, planting, propagation, plant maintenance, harvest and clearing residues for field and glasshouse research experiments. Specialist teams equipped with a range of modern machinery handle a wide range of crops including cereals, potatoes and soft fruit.

The Institute has 194 ha of free draining, loamy soil at Mylnefield, Bullion, Gourdie and Lonsdale. The land rises from 15 m to 122 m, faces south to south-west and is exposed to westerly winds. Each year, 60 ha of land is used for experimental crops and trials are also carried out at the IAPGR farm at Blythbank and other off-station sites. The general crop husbandry is based on a long-term (20+ years) plan of land use and is consistent with good farming practice and sound business management. Land is divided into packages of 10-12 ha providing areas for arable crop trials with an 8-year break between crops of the same type and soft fruit trials with a 6-year break. Smaller designated areas of land are provided for specialist requirements. Water for field irrigation is provided from boreholes through underground ringmains with hydrants every 100 m. There are adequate crop drying, handling and storage facilities.

The Department maintains the UK virus-free nuclear stocks of *Ribes, Rubus* and *Narcissus* used for first-stage commercial production of planting stocks.

Glasshouses extend to 8000 m^2 and have fully automatic control of heating and supplementary lighting. Included within the glasshouse area are individual glasshouse cubicles ranging in size from 12 m^2 to 350 m^2 that provide facilities for out-of-season plant production and for specific purposes including quarantine and isolation. There are a further 1000 m^2 of cold glasshouses, polytunnels and net structures.

Controlled environment cabinets range in size from 0.25 m^2 to 5.0 m^2 and in complexity from simple incubators to growth rooms where temperature, lighting and humidity can be programmed to create a range of environments for growing temperate and sub-tropical plants.

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. Skilled inspections and corrective maintenance are frequently carried out to ensure that the expected performance and life of equipment, vehicle, plant and buildings are achieved.

The Department is divided into sections that specialise in electrical, electronic, refrigeration, heating and mechanical engineering. There is a garage section providing maintenance facilities for a substantial fleet of road vehicles, tractors and agricultural machinery. The Department also provides a general stores facility and a cleaning and security service.

The wide range of equipment and technologies present in the Institute is a challenge to Department staff and a very high percentage of repair work is carried out in-house. However, the complexity of some equipment and restricted access to spares, have necessitated negotiating service contracts with specialist companies and these are monitored by the Department.

Mylnefield Research Services Ltd.

N.W. Kerby

Mylnefield Research Services (MRS) Ltd is a separate subsidiary company wholly-owned by SCRI that has been established to handle the Institute's commercial activities while protecting its charitable status. MRS Ltd is both a technology transfer and project management company. Following payment of corporation tax, the net profit of MRS Ltd is transferred annually via the Gift Aid Scheme to SCRI thus allowing tax to be reclaimed from the Inland Revenue. During its first year of trading (1.4.91 - 31.3.92) MRS Ltd transferred £150,000 to SCRI through this mechanism, in addition to a management fee of £70,000. MRS Ltd appointed Dr Nigel Kerby as Commercial Manager in 1992, initially on secondment from the Department of Biological Sciences, University of Dundee.

MRS Ltd promotes, markets and delivers a range of products and services based upon expertise at SCRI namely, IPR and research in areas of new agricultural and horticultural cultivars; methods for diagnosing, treating and preventing diseases in crops; instrumentation and software; teaching, training seminars and conferences; consultancy; and providing specialised analytical services. Due to the large potential range of products and services, attention is given to areas where SCRI possesses the greatest scientific and commercial advantage.

The commercial strategy of MRS is to focus its marketing efforts into the following broad areas of activity: (i) income from external contracts; (ii) royalties from plant varieties; (iii) provision of scientific services; (iv) consultancy; and (v) provision of conferences, training and seminars. Primary target markets include: public sector organisations; private sector companies; associations and groups; and international organisations in the agriculture, horticulture food and raw materials industry. Markets for specialised products also exist in the medical, life sciences and biotechnology sectors. At present MRS is managing 50 external contracts and has 27 licensing agreements with private sector companies. New markets are continually being identified and we are regularly being approached by potential new customers. It is the intention of the Company to expand the funding base to benefit both the applied and basic science of the Institute.

SCRI is in a unique position to exploit its research findings from the laboratory, via glasshouse and field trials, to the market from its site in Tayside through the activities of MRS Ltd.

CAROS International Limited

D.J. Thomson

CAROS International Ltd. is a new company that was formed jointly by four Scottish Agricultural Research Institutes and the Scottish Agricultural College with the backing of Scottish Enterprise during 1992. CAROS is an acronym for Consortium of Agricultural Research Organisations in Scotland. CAROS International promotes and markets the international research, educational and consultancy expertise of the Scottish science base in agriculture and related disciplines and acts as a marketing agent for the members.

The Company is financed by subscription from each of the members, which in the case of SCRI is Mylnefield Research Services Ltd, and by Scottish Enterprise. It will pursue and negotiate contracts with funding agencies in the UK and abroad for international work, with priority given to projects where two or more of the members are involved. It will also promote collaborative agreements with research organisations in other countries. It is expected to act as a broker for European Commission research, development and education funds, and to negotiate research contracts from international agencies for the Third World. In addition, it will liaise closely with the offices of the British Council worldwide. The Company is unique for the range of biological, agricultural and environmental expertise available which includes crop production, animal and human nutrition, animal diseases, land use and the environment.

Furthermore, activities extend from fundamental research, through field investigations and extension work to education.

The Chairman of the Board of Directors is Lord Sanderson of Bowden and Dr David J Thomson, formerly Director of Technical and Commercial Marketing at the University of Bath, was appointed Chief Executive on 4 January 1993. A Management Committee composed of representatives from each of the members under the Chairmanship of Professor T J Maxwell, MLURI advises the Chief Executive. The Corporate Secretary is Mylnefield Research Services Ltd and the Registered Office is at Invergowrie, Dundee.

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 on 31 December 1992 was 320.

The AGM of the Society was held on 2 April when Mr James Millar C.B.E., Chairman and Chief Executive of Wm. Low & Company PLC took as his subject, "Farmers and Supermarkets - Who Needs Who?".

The Institute held Open Days on 3 and 4 July. On the Special Day for invited guests and Society members on the 3rd, the Peter Massalaski Prize for the most meritorious research by a scientist under the age of 36 was presented to Dr B.P. Forster.

The Committee of Management met on two occasions (2 April and 4 November).

Travel grants authorised from the General Fund by the Committee were:-

Dr B. Boag, Zoology Department, to Albufeira, Portugal.

Dr R Viola, Cellular and Environmental Physiology Department to Antwerp, Belgium.

Mr I.M. Roberts, Virology Department, to Granada, Spain.

Dr B. Williamson, Mycology and Bacteriology Department, to Italy.

A Soft Fruit Walk was held on Wednesday 22 July when 45 members heard an update on replacements for dinoseb-in-oil, grey mould resistance in raspberries, the practicalities of breeding a new variety of raspberry, the production of virus tested plants, and integrated pest management in raspberries.

There was a Potato Walk on Thursday 27 August where the latest varieties and advanced selections in the potato breeding programme were on view. Novel alternative potatoes aimed at the "gourmet market", late blight resistance breeding and optimising nitrogen applications were demonstrated.

Officers of the Society

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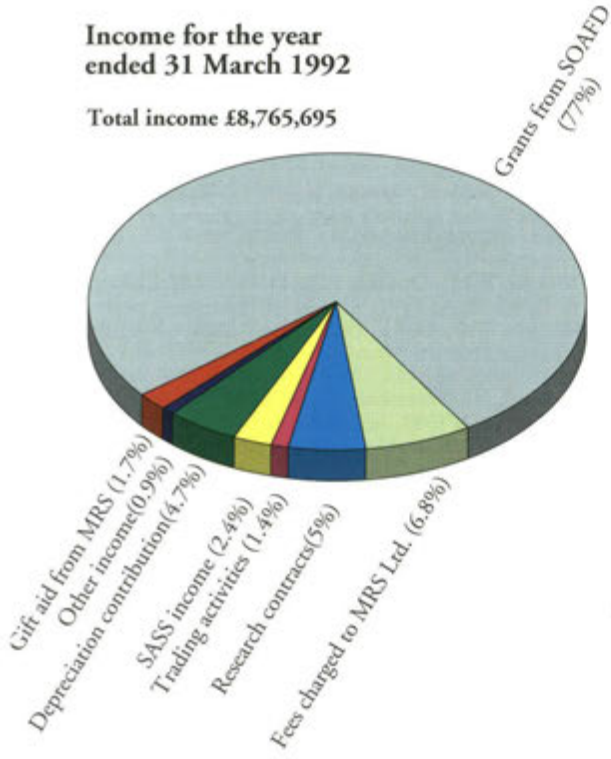
Summary of the Accounts

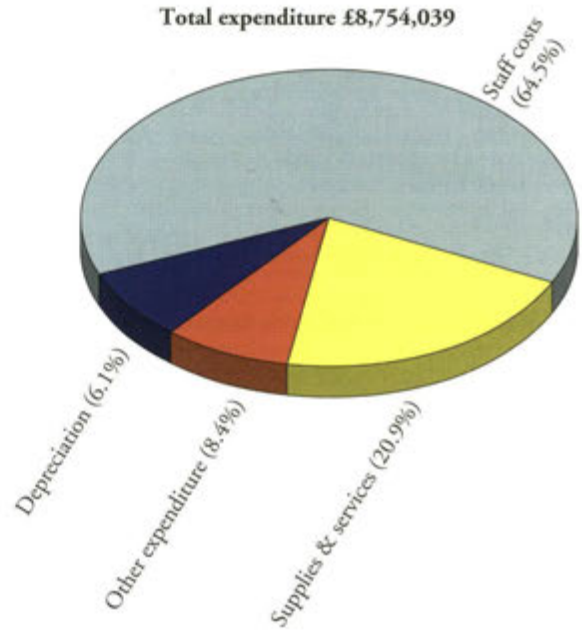
Income for the year ended 31 March 1992

Total income £8,765,695



Expenditure for the year ended 31 March 1992

Total expenditure £8,754,039



Balance sheet at 31 March 1992

Total value £11,921,432

Assets

Fixed assets	95 %
Stocks	0.9 %
Debtors	4 %

Liabilities

Capital reserve	87.3 %
Income & expenditure account	8.4 %
Current liabilities	4.2 %

Staff list

as at 31 December 1992

Director	Professor J.R. Hillman, B.Sc., Ph.D., F.L.S., C.Biol., F.I.Biol., F.R.S.E.[1,2,3]	UG4
Deputy Director	Professor N.L. Innes, B.Sc., Ph.D., D.Sc., C.Biol., F.I.Biol., F.R.S.E., F.I.Hort.[2,4]	UG5
Secretary	R.J. Killick, B.Sc., M.B.A., Ph.D., C.Biol., M.I.Biol.	UG7
Assistant to Director	T.J.W. Alphey, B.Sc., Ph.D., C.Biol., M.I.Biol.	UG7

Crop Genetics Department (CG)

Head : G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol.[5,6]	UG6
J.E. Bradshaw, M.A., M.Sc., Ph.D.	UG7
M.F.B. Dale, B.Sc., Ph.D.	UG7 (Prom. Apr)
R. Ellis, B.Sc., Ph.D.	UG7
W.H. MacFarlane Smith, B.Sc., Ph.D., C.Biol., M.I.Biol.	UG7
W.T.B. Thomas, B.Sc., Ph.D.	UG7
R.L. Wastie, M.A., Ph.D., F.I.S.P.	UG7
I. Chapman, B.Sc.	SSO
M.J. De,Maine, B.Sc., M.Phil.	SSO
G. Ramsay, B.Sc., Ph.D.	SSO
J.S. Swanston, B.Sc., C.Biol., M.I.Biol.	SSO
Ruth M. Solomon-Blackburn, B.A., M.Sc.	HSO
S.A.. Clulow, B.Sc., Ph.D.	HSO
S. Millam, B.Sc., Ph.D.	HSO (Tr. from CMG Jan)
Helen E Stewart, C.Biol., M.I.Biol.	HSO
M.J. Wilkinson, B.Sc., Ph.D.	HSO
A. Young	HSO
Jill Middlefell-Williams, H.N.C.	SO
G.E.L. Swan	SO
R.N. Wilson, N.C. H.	SO
G.R. Young	SO
Eva Bennett	ASO
A. Booth, O.N.C.	ASO
P. Davie, O.N.C.	ASO
Ann Donnelly, H.N.C.	ASO
Norma Dow	ASO
Michelle L.M.H. Fleming, H.N.D., B.Sc.	ASO
Frances Gourlay, H.N.C.	ASO
R. Keith	ASO
Karen I. McIlravey, O.N.C., H.N.C.	ASO
Jane McNicoll, H.N.C.	ASO
D. Todd	ASO
A. Wilson	ASO
M.P.L. Campbell	P&GS (E)
Alice Bertie	EWIII/II
J.D. Fuller	EWIII/II
Patricia E. Lawrence	EWIII/II
A. Margaret McInroy	EWIII/II
Moira Myles	EWIII/II
Joyce I. Young	EWIII/II

Soft Fruit Genetics Department (SFG)

Head : R.J. McNicol, B.Sc. [6]	UG7
R.M. Brennan, B.Sc., Ph.D.	SSO
Julie Graham, B.Sc., Ph.D.	HSO
Sandra L. Gordon, H.N.C.	ASO
Kay Greig, Dip. H.E.	ASO
Amanda J. Thomson, H.N.D.	ASO

Cell & Molecular Genetics Department (CMG)

Head :W. Powell, B.Sc., M.Sc., Ph.D.[5,6]	UG6 (IMP)
J.W.S. Brown, B.Sc., Ph.D.[6]	UG7
B.P. Forster, B.Sc., Ph.D.	UG7
A. Kumar, B.Sc., Ph.D.	SSO
G.C. Machray,B.Sc., Ph.D.	SSO (Appt. Jan)
R. Waugh, B.Sc., Ph.D.[6]	SSO
Vivian Blok, B.Sc., M.Sc., Ph.D.	HSO (Appt. Feb)
C.G. Simpson, B.Sc.	HSO
E. Baird, H.N.C., B.A.	SO
Gillian Clark, H.N.C	SO
B. Harrower, H.N.D., B.Sc.	SO
Jackie Lyon	SO
Nicky Bonar, H.N.C.	ASO
Diane Davidson	ASO
M. Macaulay, H.N.C.	ASO

Cellular & Environmental Physiology Department (CEP)

Head : H.V. Davies, B.Sc., Ph.D.[6]	UG6
H.M. Lawson, B.Sc., M.Agr.Sc., Dip. Agric., F.I.Hort.	UG7
D.J. Linehan, B.Sc., Ph.D.	UG7
D.K.L. MacKerron, B.Sc., Ph.D.	UG7
B. Marshall, B.Sc., A.R.C.S., Ph.D.	UG7
K.J. Oparka, B.Sc., Ph.D.[6]	UG7
J.W. Crawford, B.Sc., Ph.D.	SSO
B.S. Griffiths, B.Sc., Ph.D.	SSO
R.A. Jefferies, B.Sc., Ph.D.	SSO
K. Ritz, B.Sc., Ph.D.	SSO
D. Robinson, B.Sc., Ph.D.[6]	SSO
R.E. Wheatley, B.Sc.	SSO
I. Young, B.Sc., Ph.D.	SSO (Prom. Apr)
A.G. Bengough, B.Sc., Ph.D.	HSO
G. Goleniewski, B.Sc., Ph.D.	HSO
D.C. Gordon, H.N.C.	HSO
Heather A. Ross, H.N.C., C.Biol., M.I. Biol.	HSO
C. Scrimgeour, B.Sc., Ph.D.	HSO (Appt. Aug)
M. Taylor, B.Sc., Ph.D.	HSO
R. Viola, B.Sc., Ph.D.	HSO
J.S. Wiseman, S.D.H.	HSO
Katherine M. Wright, B.A., Ph.D.	HSO
Sandra Caul, H.N.C.	SO
Sandra E. Millar, O.N.C., H.N.C.	SO
D.A.M. Prior, H.N.C.	SO
Susan Verrall, H.N.C.	SO
Gladys Wright, H.N.C.	SO
D. Crabb	ASO
G. Dunlop, O.N.C.	ASO
Margaret Garland	ASO
Lesley George	ASO
Diane McRae	ASO

[1] Visiting Professor in the University of Strathclyde
[2] Visiting Professor in the University of Dundee
[3] Visiting Professor in the University of Edinburgh
[4] Honorary Professor in the University of St. Andrews
[5] Honorary Senior Lecturer in the University of St. Andrews
[6] Honorary Lecturer in the University of Dundee
[7] Honorary Lecturer in the University of Aberdeen
[8] Honorary Fellow in the University of Edinburgh

Chemistry Department (Chem)

Acting Head : D.W. Griffiths, M.A., Ph.D, C. Chem., M.R.S.C.,	SSO
G.W. Robertson, B.Sc., C.Chem., M.R.S.C.	SSO
H. Bain, H.N.C., L.R.S.C.	HSO
W. Matheson, B.Sc.	HSO
T. Shepherd, B.Sc., Ph.D.	HSO
Winifred M. Stein, H.N.C.	HSO
K. Taylor, H.N.C.	SO
Fiona Falconer, H.N.C.	ASO
Jean Wilkie	EWIII/II

Director's Group (DG)

Fibres	
I.M. Morrison, B.Sc., Ph.D.	UG7
J.M.S. Forrest, B.Sc., Ph.D.	UG7 (Tr. from Zoo June)
G. J. McDougall, B.Sc., Ph.D.	SSO
D. Stewart, B.Sc.	HSO
Fiona Carr	EWIII/II (P/T) (Appt. Jan)
Hazel Duncan	EWIII/II (P/T) (Appt. Jan)

Spectroscopy	
B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C.[6]	UG7
Anne Morrice, S.N.C., H.N.C.	ASO (Tr. from Chem Jan)

Mycology and Bacteriology Department (M & B)

Head : J.M. Duncan, B.Sc., Ph.D.	UG6
J.G. Harrison, B.Sc., Ph.D.	UG7
G.D. Lyon, B.Sc., M.Sc., Ph.D., D.I.C.[6]	UG7
M.C.M. Pérombelon, B.Sc., M.Sc., Ph.D.[6]	UG7
B. Williamson, B.Sc., M.Sc., Ph.D.[6]	UG7
A.C. Newton, B.Sc., Ph.D.	SSO
E. Patricia Dashwood, B.Sc., M.Sc.	HSO
Lizbeth J. Hyman, B.A.	HSO
Diana M. Kennedy, B.Sc.	HSO
R. Lowe	HSO
G. McMillan	SO
Jacqueline Heilbronn, H.N.C.	SO
D.J. Johnston, B.Sc.	SO
Naomi A.. Williams, H.N.C.	SO
D.C. Guy	EWIII/II
Evelyn Warden	EWIII/II

Virology Department (Vir)

Head : T.M.A. Wilson, B.Sc., Ph.D.[2]	UG6 (Appt. Mar)
A.T. Jones, B.Sc., Ph.D.	UG6 (IMP)
A.F. Murant, B.Sc., A.R.C.S., Ph.D., D.I.C., C.Biol., F.I.Biol., F.R.S.E.[6]	UG6 (IMP)
H. Barker, B.Sc., Ph.D.	UG7
M.A. Mayo, B.Sc., Ph.D., C.Biol., M.I.Biol.	UG7
I.M. Roberts, H.N.C., Dip.R.M.S.	UG7
D.J. Robinson, M.A., Ph.D.	UG7
Lesley Torrance, B.Sc., Ph.D.	UG7
G.H. Duncan, H.N.C.	SSO
B. Reavy, B.Sc., D.Phil.	SSO
S.A. MacFarlane, B.Sc., D.Phil.	HSO (Appt.Oct)
Maud M. Swanson, B.Sc.	HSO
G.H. Cowan, H.N.D.	SO
Sheila M.S. Dawson, H.C.	SO
Anne C. Jolly, H.N.C.	SO
E.W. Milne, O.N.C.	SO
Wendy J. McGavin, B.Sc.	SO
Kara D. Webster, H.N.C.	SO
Gillian L. Fraser	ASO
Ann Grant	ASO
Wendy Ridley	EWIII/II (Appt. Oct)

Zoology Department (Zoo)

Head :D.L. Trudgill, B.Sc., Ph.D., C.Biol., F.I.Biol.[5,6]	UG6
B. Boag, B.Sc., Ph.D.	UG7
D.J.F. Brown, B.A., Ph.D., C.Biol., M.I. Biol.	UG7
M.S. Phillips, B.Sc.	UG7
W.M. Robertson, N.H.C., F.I.S.	UG7
J.A.T. Woodford, M.A., Ph.D.[6]	UG7
A.N.E. Birch, B.Sc., Ph.D., C.Biol., M.I.Biol.	SSO
S.C. Gordon, H.N.C.	SSO
Gaynor Malloch, D.C.R., B.Sc.	SO (Prom. Jul)
R. Neilson, H.N.C.	SO
Ailsa Smith, B.Sc.	SO
Anne M. Holt	ASO
Sheena Lamond	ASO

Data Processing Unit (DP)

Head : R.J. Clark, B.A., M.B.C.S.	SSO
R. Kidger, B.Sc.	HSO
P. Smith, B.Sc.	HSO
I. Black, H.N.C.	SO
S. Clark, H.N.C.	SO

Scientific Liaison & Information Services Department (SLIS)

Head : D.A. Perry, B.Sc., Ph.D.	UG7
T. G. Geoghegan, A.B.I.P.P., A.M.P.A.	Senior Photographic Officer
S.F. Malecki, A.B.I.P.P.	Photographic Officer
G. Menzies	Photographic Officer
T.D.Heilbronn, B.Sc., M.Sc.	HSO
I.R. Pitkethly, H.N.D.	Higher Graphics Officer
Ursula M. McKean, M.A., Dip. Lib.	Assistant Librarian

Administration Department (Admin)

Secretary : R.J. Killick, B.Sc., M.B.A., Ph.D., C.Biol., M.I.Biol.	UG7
Accountant : S.L. Howie, C.A.	SEO
Assistant Secretary : D.L. Hood, B.Admin., Dip. Ed., L.T.I., A.I.I.M.	HEO
Personnel Officer : I. Paxton, H.N.C., M.I.P.M.	EO
Freida F. Soutar	HEO
Catherine Skelly	EO
Margaret Barnes	AO
Dianne Beharrie, Dip. Ed.	AO (P/T)
Maureen E. Campbell	AO (P/T)
Rhona G. Davidson	AO
Pam Duncan	AO (Appt. Nov)
Wendy A. Patterson, H.N.D.	AO
Sarah-Jane Simms, H.N.D.	AO
Kristy L. Grant, B.A.	AA
Barbara V. Gunn	AA
Loraine Galloway	SPS
Linda Butler	Typist
Joyce Davidson	Typist
Jean Findlay	Typist (P/T)
Sheena Forsyth	Typist (P/T)
Elizabeth J. Fyffe	Typist
Maureen Murray	Typist
Myra Purves	Typist
Elizabeth L. Stewart	Typist

Engineering & Maintenance Department (EM)

Institute Engineer : S. Petrie, B.Sc.	SP&TO (Prom. Feb)
D. Gray	HP&TO (Prom. Sep)
A. Low	P&TO (Prom. Sep)
K. Low	P&TO (Prom. Sep)
R. White	TG1
J. Anderson	Craftsman
D. Byrne	Craftsman
K. Henry	Craftsman (Appt. Jan)
F. Howie	Craftsman (Appt. May)
E. Lawrence	Craftsman
R.D. McLean	Craftsman (Appt. Jul)
R. Pugh	Craftsman
T. Purves	Caretaker
J. Rowe	Caretaker
C. Conejo	Handyperson
J. Oldershaw	Boiler/Handyperson
N. McInroy	EW1 (Tr. from EGF Sep)
Janice McDonald	AO (P/T)
G.C. Roberts	TG1
I.M. Scrimgeour	TG1
J.Flight	Storeman
D.L.K.. Robertson	Storeman (Tr. from EGF Feb)

Estate, Glasshouse & Field Experiments Department (EGF)

Head :W.I.A. Jack	SSO
G. Wood, B.Sc., Ph.D., F.E.T.C.	HSO
P.A. Gill, H.N.D.	HSO
J.R.K. Bennett	SO (Prom. Oct)
W.D.J. Jack, B.Sc.	SO (Regr. Jul)
B.D. Robertson	SO (Prom. Oct)
D.S. Petrie	SO (Regr. Jul)
C.C. Carrie	P&GS(E)
A.W. Mills	P&GS(E)
R. Ogg	P&GS(E)
D.G. Pugh	P&GS(E)
C.R. Dalrymple	EW1
E.A.M. Gardiner	EW1
L.A. McNicoll	EW1
J. Mason	EW1
D.A. Thomson	EW1 (Appt. Mar)
J.K. Wilde	EW1
A.J. Adams	EWIII/II (Appt. Mar)
J.T. Bennett	EWIII/II
E.J. Christie	EWIII/II (Appt. Mar)
C. Conacher	EWIII/II
G. Dow	EWIII/II
B. Fleming	EWIII/II
I. Fleming	EWIII/II
A.C.Fuller	EWIII/II
G. Lacey	EWIII/II (Appt. Oct)
C. McCreadie	EWIII/II (Appt. Oct)
T.A. Mason	EWIII/II
R. Murray	EWIII/II
Gillian Pugh	EWIII/II
M.J. Soutar	EWIII/II
Angela M. Thain	EWIII/II
C. Walker	EWIII/II (Appt. Mar)
Lorna Doig	AO (P/T)

Scottish Agricultural Statistics Service (SASS)

King's Buildings, University of Edinburgh

Director :R.A. Kempton, M.A., B.Phil. [8]	UG6
C.A. Glasbey, M.A., Dip. Math. Stats., Ph.D.[8]	UG7
E.A. Hunter, B.Sc., M.Phil.[8]	UG7
Janet M. Dickson, B.Sc.	SSO
G.J. Gibson, B.Sc., Ph.D.	SSO
G.W. Horgan, B.A., M.Sc.	SSO
M. Talbot, F.I.S., M.Phil. [8]	SSO
A.D. Mann, B.Sc.	HSO
I.M. Nevison, M.A.	HSO (Prom. Aug)
G.D. Ruxton, B.Sc., Ph.D.	HSO (Appt. Oct)
F.G. Wright, B.Sc., M.Sc., Ph.D.	HSO
Muriel A.M. Kirkwood, D.A.	ASO
Secretary :Elizabeth M. Heyburn, M.A.	EO
Diane Glancy	AA (P/T)
Karyn Linton	PS (P/T,)
Amy G. Stewart	Typist (P/T)

Ayr Unit

D.J. Hirst, B.Sc., Ph.D.	SSO
A. Sword, B.Sc., M.Sc.	HSO

Aberdeen Unit

Head :M.F. Franklin, B.Sc., M.Sc., Ph.D. [7]	UG7
S.T. Buckland, B.Sc., M.Sc., Ph.D.[7]	UG7
D.A. Elston, B.Sc., M.Sc.	SSO
Karen L. Cattanach, M.A., M.Sc.	HSO
Elizabeth I. Duff, B.Sc.	SO
Karen A. Robertson, B.Sc.	SO

Dundee Unit

Head : J.W. McNicol, B.Sc., M.Sc.	UG7
Christine Hackett, B.A., Dip. Math. Stats., Ph.D.	HSO

Short Term Contracts

SOAFD Flexible Funding

Cell and Molecular Genetics

A.D. Turnbull-Ross, B.Sc., Ph.D.	HSO

Cellular and Environmental Physiology

Yiqun Gu, B.Sc.	HSO
Susan Smith, B.Sc., Ph.D.	HSO
A. Anderson, H.N.D., B.Sc., M.Sc.	SO (Appt. Oct)
Ramance Peiris, B.Sc.	SO
A. Holmes	EWIII/II (Appt. Oct)

Crop Genetics / Soft Fruit Genetics

R.D. Butcher, B.Sc., Ph.D.	HSO (Appt. Oct)
Aileen Timmons, B.Sc., Ph.D.	HSO
Sharon Dubbels	ASO
Carol Taylor	EWIII/II (Appt. Jul)

Director's Group

N. Deighton, B.Sc., Ph.D.	HSO (Appt. Feb)
Sheila Glidewell, B.Sc., Ph.D.	HSO (Appt. Mar)

Mycology and Bacteriology

D. Cox, B.A., M.Phil.	SO (Appt. Oct)
S. Main	EWIII/II

SASS

S.D. Chasalow, B.A., M.A., Ph.D.	HSO (Appt. Dec)
D. Hitchcock, B.A.	HSO

Soft Fruit Genetics

Vasantha Ramanathan, B.Sc., Ph.D.	HSO

Zoology

Annette S. Salowsky-Butcher, Dipl. Biol	SO (Appt. Nov)

Department of the Environment

Mycology and Bacteriology

K. Harding, B.Sc., Ph.D.	HSO (Appt.Nov)
Anne Pack	Typist (P/T) (Appt. Dec)

Durham University

Mycology and Bacteriology

Lisa Fyffe	SO

EEC / ECSA ECLAIR

Cell and Molecular Genetics

P. Hedley, B.Sc.	SO

Cellular and Environmental Physiology

L.R. Burch, B.Sc., M.Sc., Ph.D.	SSO
Edna Cuthbert, S.N.C., H.N.D.	ASO
N. Ebblewhite	EWIII/II
Linda Sommerfield, B.Sc	EWIII/II

EEC

Cell and Molecular Genetics

K.J. Chalmers, B.Sc., Ph.D.	HSO

Crop Genetics

Lucy Payne, B.Sc., Ph.D.	n/a (Appt. Feb)

Virology

Sybil M. Macintosh, B.Sc.	SO

Gene Shears

Cell and Molecular Genetics

D.J. Leader, B.Sc.	HSO (Appt. Aug)
J.F. Sanders, B.Sc., Ph.D.	HSO (Appt. Aug)

Horticultural Development Council

Mycology and Bacteriology

A. Reglinski, B.Sc., Ph.D.	HSO

MAFF

Cell and Molecular Genetics

A.L. Craig	SO

Cellular and Environmental Physiology

Elizabeth A.Robertson	ASO
Sheena J. Rodger	ASO

SASS

Deena C. Mobbs, B.Sc.	HSO (Appt. Aug)

ODA

Cell and Molecular Genetics

Stephanie Cooper-Bland, B.Sc., Ph.D.	HSO
Sarah Fennel, B.Sc.	SO
Jennifer Watters, H.N.D.	ASO

Virology

P.M. Derrick, B.Sc., Ph.D.	HSO
P. F. McGrath, B.Sc., Ph.D.	HSO
J.S. Miller, B.SC	HSO (Appt. Dec)

ORSTOM

Virology

Michelle S. Leslie	ASO

PMB

Cellular and Environmental Physiology

G.J. Lewis, B.Sc., M.Sc.	HSO (Appt. Oct)
M. Young, H.N.D.	SO
SigrunHoldhus, Cand.mag	EWIII/II

Crop Genetics

Lisa F. Palmer, B.Sc	SO

Mycology and Bacteriology

D.A.C. Jones, B.Sc., Ph.D.	SO (Appt. Jan)
Alison C. Connelly, B.Sc.	SO (Appt. Sep)

Zoology

Jane Roberts	EWIII/II

United Biscuits

Cell and Molecular Genetics

I. Morrison	ASO

Miscellaneous funding

Cellular and Environmental Physiology

Elizabeth A . Murant, B.Sc.	HSO
Linda I.D. Marshall	EWIII/II (Appt. Mar)

SASS

D.I. Borchers, B.A., B.Sc.	HSO (Appt. Oct)
S.J. Beaney, B.Sc.	SO (Appt. Jul)

Soft Fruit Genetics

P. Lanham, B.Sc., Ph.D.	HSO
W.T.G. van de Ven, IR	HSO
N.J. Wilson, B.Sc., Dip. I.T.	ASO (Appt Jul)
Jane E. Fairlie	EWIII/II (P/T) (Appt. Mar)

Editorial Duties

Name	Position	Journal Title
A.G. Bengough	Editor (Joint)	*British Society of Soil Science Newsletter*
B. Boag	Editorial Board	*Annals of Applied Biology*
	Editorial Board	*Nematologia mediterranea*
D.J.F. Brown	Editorial Board	*Nematologia mediterranea*
M.F. Franklin	Editorial Board	*British Journal of Nutrition*
C.A. Glasbey	Editorial Panel	*Applied Statistics*
T.D. Heilbronn	Editor	*SCRI Annual Report*
	Editor	*SSCR Newsletter*
J.R. Hillman	Publication Committee	*Journal of HorticulturalScience*
	Editorial Board	*Agricultural Systems*
	EditorialBoard	*Journal of Agricultural Science*
N.L. Innes	Editorial Board	*AgBiotech News & Information*
H.M. Lawson	Associate Editor	*Journal of Horticultural Science*
D.K.L. MacKerron	Associate Editor	*Journal of Horticultural Science*
	Editorial Board	*Euphytica*
I.M. Morrison	Management Committee	*Journal of the Science of Food and Agriculture*
	Series Editor	*Advances in Plant Cell Biochemistry & Biotechnology*
A.F. Murant	Editorial Board	*Virus Research*
	Editor	*AAB Descriptions of Plant Viruses*
D.A. Perry	Editor	*SSCR Bulletin*
	Editor	*SCRI Annual Report*
W. Powell	Editor	*Heredity*
	Editor	*Potato Research*
D.J. Robinson	Editorial Board	*Journal of Virological Methods*
D. Robinson	Associate Editor	*Journal of Horticultural Science*
D.L. Trudgill	Editorial Board	*Nematologica*
	Editorial Board	*Fundamental and Applied Nematology*
	Consulting Editor	*Plant and Soil*
R.L. Wastie	Editorial Board	*Potato Research*
	Editorial Board	*Annals of Applied Biology*
B. Williamson	Editor	*Annals of Applied Biology*
T.M.A. Wilson	Senior Editor	*Molecular Plant-Microbe Interactions (APS)*
	Editorial Board	*Journal of General Virology (SGM)*
I.M. Young	Editor (Joint)	*British Society of Soil Science Newsletter*

Awards and Distinctions

Name	Dept.	Degree/Award/Distinction/Appointment
Dianne Beharrie	Admin	Certificate in Personnel Practice
B. Boag	Zoo	Honorary Lecturer, University of Dundee
B.P. Forster	CMG	Peter Massalski Award, 1992
Shirley Fryer	CG	Ph.D., University of Wolverhampton
A. Gardner	CEP	Ph.D., University of Dundee
J.A. Gonzalez-Perez	Zoo	Ph.D., University of Dundee
Frances Gourlay	CG	Higher Diploma of Education in Biological Science
Yiqun Gu	CEP	Ph.D., Heriot-Watt University, Edinburgh
N.L. Innes	Admin	Elected President, Association of Applied Biologists
G.R. Mackay	CG	Honorary Lecturer, University of Dundee
J.D. Madulu	Zoo	Ph.D., University of Aberdee
R.J. McNicol	SFG	Honorary Lecturer, University of Dundee, Department of Biological Sciences
A.J. Nisbet	Zoo	Ph.D., University of Glasgow
Wendy A. Patterson	Admin	Certificate in Personnel Practice
B.D. Robertson	EGF	Diploma in Management Studies, Dundee College of Further Education

Resignations

Name	Dept.	Grade	Month
Wendy Craig	CG	ASO	November
Jennifer Gorrod	DP	ASO	November
J.P.T. Grant	EGF	EWI	October
Joanne E. Hall	SASS	SO	November
S. MacDonald	CG	EWII	October
Catherine McDougall	Admin	AO	September
C. McKenzie	CEP	ASO	September
Lorna E. McLaren	SLIS	AO	August
Margaret M. Mills	Admin	AA (P/T)	January
A.D. Milner	SASS	HSO	September

Staff Retirements

Name	Dept.	Grade	Month
Rena Reid	Vir	EWII	July
Irene M.S. Terris	SASS	ASO	November

Deaths

Name	Dept.	Grade	Month
M.J. Allison	Chem	UG7	January
R. MacDonald	EM	HP&TO	October

Visits Abroad

Name	Country visited	Month of visit	Duration of visit
Eileen Baird	Germany	Sept-Nov	13 weeks
H. Barker	USA	June	1 week
A.G. Bengough	India	April	1 week
A.N.E. Birch	Holland	March	1 week
	Switzerland	March-April	1 week
Vivian Blok	N. Ireland	November	3 days
B. Boag	Portugal	April	1 week
	Finland	August	1 week
R.M. Brennan	France	July	4 days
	USA	August	9 days
D.J.F. Brown	Germany/Switzerland	March	2 weeks
	Portugal	April	3 weeks
	Canada	August	2 weeks
	Germany/Switzerland	September	2 weeks
	Greece	November	1 week
	Russia	November	1 week
J.W.S. Brown	USA	May/June	2 weeks
	Germany	Feb/March	3 weeks
S.T. Buckland	France	April	1 week
S.A. Clulow	France	January	1 week
J.W. Crawford	Japan	March	2 weeks
	Australia	March	2 weeks
	USA	April	1 week
M.F.B. Dale	France	January	1 week
H.V. Davies	USA	April	1 week
	France	July	1 week
	Denmark	November	2 days
	Denmark	December	3 days
M.J. De,Maine	France	January	1 week
J.M.S. Forrest	The Netherlands	February	5 days
	France	August	4 days
	Germany	September	2 days
B.P. Forster	Taiwan	August	1 week
	Pakistan	February	1 week
C.A. Glasbey	Switzerland	March	1 week
B.A. Goodman	Belgium	February	2 days
	Belgium	March	2 days
	Portugal	June	1 week
	Czechoslovakia	September	1 week
B.S. Griffiths	Denmark	July/August	1 month
	Brazil	September	10 days
W.D Griffiths	Switzerland	March	1 week
	France	June	3 days
Y. Gu	Germany	June	5 days
	France	September	4 days
Christine A. Hackett	Denmark/Germany/ Holland	March	8 days
Linda L. Handley	Spain	May	2 weeks
	Portugal	May	2 days
	Japan	September	10 days
	Spain	October	2 weeks
D.J. Hirst	Norway	March	2 weeks
	Norway	August	2 weeks
G.W. Horgan	The Netherlands	February	3 days
	The Netherlands	September	4 days
	Nigeria	November	10 days
E.A. Hunter	Spain	Feb/Mar	5 days
	Italy	April	4 days
	The Netherlands	June	1 week
	France	June/July	1 week
	France	Sep/Oct	4 days
	Belgium	November	5 days
L.J. Hyman	France	June	5 days

Name	Country Visited	Month of visit	Duration of visit
N.L. Innes	Indonesia	April	6 days
	Peru/Ecuador	August	5 days
	USA	October	9 days
D.J. Johnston	Crete, Greece	April	5 days
	Italy	October	8 days
A.T. Jones	Italy	July	1 week
	USA	August	1 week
R.A. Kempton	Australia/New Zealand	December	3 weeks
A. Kumar	The Netherlands	April	4 days
	Malaysia	August	10 days
H.M. Lawson	France	September	5 days
G.C. Machray	Holland	September	2 days
	Denmark	December	2 days
G.R. Mackay	France	January	1 week
	Poland	April	4 days
	Canada	September	1 week
D.K.L. MacKerron	Portugal	September	9 days
A.D. Mann	U S A	April	2 days
B. Marshall	The Netherlands	May	1 week
G.J. McDougall	The Netherlands	August	5 days
G. McMillan	France	August	1 week
	France	November	1 week
R.J. McNicol	USA	June/July	12 days
	Italy	October	6 days
A.D. Milner	France	June	5 days
I.M. Morrison	The Netherlands	August	5 days
A.F. Murant	Malawi/Zimbabwe	April	2 weeks
	The Netherlands	April	4 days
	USA	December	1 week
I.M. Nevison	Australia	March-July	4 months
A.C. Newton	Germany	September	1 week
K.J. Oparka	Germany	March	1 week
	The Netherlands	July/August	3 weeks
	The Netherlands	September	1 week
	France	September	4 days
Ramanee Peiris	Germany	June	5 days
M.C.M. Pérombelon	Israel	April	1 week
	France	June	5 days
	France	August	1 week
M.S. Phillips	France	January	1 week
W. Powell	Trinidad	September	1 week
	Costa Rica	September	1 week
	USA	November	1 week
G. Ramsay	Germany	March	3 days
	France	June	1 week
	France	September	3 days
B. Reavy	USA	June	1 week
	France	July	1 week
W.M. Robertson	Portugal	April	6 days
D. Robinson	Sweden	August	5 days
D.J. Robinson	Germany	February	1 week
	USA	September	1 week
G. Simpson	The Netherlands	July	1 month
	USA	May	1 week
Helen E. Stewart	Israel	May	10 days
J.S. Swanston	Spain	February	1 week
A.M. Sword	France	September	5 days
M. Talbot	U S A	April	3 days
	The Netherlands	May	3 days
	Switzerland	August	5 days
	Poland	September	4 days
	Luxembourg	October	2 days
	Belgium	October	1 day

Name	Country Visited	Month of visit	Duration of visit
Lesley Torrance	Peru/Bolivia	February	2 weeks
	Belgium	January	2 days
	Germany	June	3 days
	Austria	August	1 week
	USA	Ocober	2 weeks
D.L. Trudgill	Portugal	April	2 weeks
	France	June	3 days
	Pakistan	November	1 week
R.L. Wastie	Denmark	February	3 days
	The Netherlands	February	3 days
	Germany	July	1 week
	The Netherlands	September	3 days
R. Waugh	Germany	March	3 weeks
	USA	May/June	2 weeks
	The Netherlands	September	2 days
	France	November	1 day
	USA	November	1 week
R.E. Wheatley	Spain	May	2 weeks
	Portugal	May	2 days
	Spain	October	2 weeks
B. Williamson	Italy	October	4 days
T.M.A. Wilson	USA	July	2 weeks
	USA	September	1 week
	USA	October	1 week
	Germany	December	4 days
J.A.T. Woodford	Germany	March	1 day
	The Netherlands	March	5 days
	France	September	5 days
F.G. Wright	Germany	July	4 days
	Germany	October	8 days
I.M. Young	Japan	March	2 weeks
	Australia	April	2 weeks
	Germany	October	1 week

Service on External Committees or Organisations

Name	Position	Committee or Organisation
A.G. Bengough	Member	Scottish Soils Discussion Group
A.N.E. Birch	Member	Entomology Group Committee, Association of Applied Biologists
	Member	IOBC Working Group 'Breeding for Resistance to Insects and Mites'
R.M. Brennan	Member	NFT Blackcurrant and Bush Fruit Panel
D.J.F. Brown	Secretary/Treasurer	European Society of Nematologists
	Member	Society of Nematologists *ad hoc* Committee 'International Federation of Nematology Societies'
	Member	Institute of Biology 'IOBS Link Group'
S.T. Buckland	Member	Working Group on Integrated Population Monitoring, BTO
	Invited Participant	Scientific Committee of the International Whaling Commission
	Member	Review Group of the National Countryside Monitoring Scheme for Scotland, NCCS (Now SNH)
	Member	Scientific Program Committee for the 1994 International Biometrics Conference
J.W. Crawford	Member	Management Group of Centre for Non-linear Systems in Biology
R.P. Ellis	Member	BSPB Cereal Crop Group
	BSPB Representative	SAC Recommended List Consultative Committee
	Member	AAB Planning Committee for Conference 'Physiology of Varieties'
	Tech. Secretary	SSCR Cereal Sub-committee
J.M.S. Forrest	Member	Nematology Group Committee, Association of Applied Biologists
B.P. Forster	Co-ordinator	International Committee on Barley Chromosome (4) Genetic Mapping
P.A. Gill	Branch Secretary	Institute of Horticulture
	Member	Dundee College, User Liaison Group in Horticulture
S.C. Gordon	Session Organiser	BCPC Brighton Conference on Pests and Diseases
	Session Organiser	Crop Protection in Northern Britain Conference
T.D. Heilbronn	Publicity Officer	Association for Crop Protection in Northern Britain
J.R. Hillman	Member	AFRC Plants and Environment Research Committee
	Member	SOAFD Joint Management Board
	Member	ECRE Board of Management
	Chairman	SCRI/ASS/COSAC Liaison Group
	Member	SNSA Adviser to Committee
	Member	Senate, University of Dundee
	Chairman	Tayside Biocentre Group
	Member	University of Strathclyde Sub-Board for the Degree of B.Sc. in Horticulture
	Member	SSPDC Management Committee
	Member	Tayside Economic Forum
	Chairman	ADAS Gleadthorpe EHF Visiting Group
E.A. Hunter	Member	Management Committeee of ECFlair Concerted Action No 2 'SENS'
N.L. Innes	Chairman	Governing Board & Executive Committee, CIP, Peru
	President	Association of Applied Biologists
	Member	University of Dundee Botanic Garden Committee
	Member	UK Genetic Resources Committee
	Member	SARIC International Steering Group
	Member	ODA Research Review Group
	Member	MLURI Promotions Board
R.A. Kempton	Council Member	International Biometric Society
	Member	Environmental Change Network Statistics Working Group
R.J. Killick	Member	Continuing Vocational Education Advisory Group, University of Dundee
	Member	MBA Staff Student Liaison Committee
H.M. Lawson	Chairman	UK Weed Liaison Group
	Member	BCPC R&D Sub-committee - Weeds
	Chairman	Scottish Weed Group Experimental Committee
W.H. MacFarlane Smith	Member	BSPB Oilseed & Industrial Crop Group
	Member	AFRS Safety Officers Group
	Member	SARI Safety Officers Group
	Member	NPTC Plant Variety Development Panel
G.R. Mackay	Member	Interdepartmental and Users Committee, Breeders quarantine unit SASA
	Chairman	Potato Section, Eucarpia
D.K.L. MacKerron	Chairman	Working Group on Water Relations in Potato Production, EAPR Physiology and Agronomy Sections
	Secretary	SSCR, Potato Crop Sub-committee
	Coordinator	SOAFD Climate Change Research Group

Name	Position	Committee or Organisation
B. Marshall	Coordinator	Soil-Plant-Microbial Interactions, AFRC Soil Science Steering Group
	Deputy Head	Management Group of Centre for Non-linear Systems in Biology
U.M. McKean	Member	Tayside Chief Librarian Committee
	SARI Representative	AFRS Librarian Committee
R.J. McNicol	Member	NFT Raspberry Panel
	Member	NFT Strawberry Panel
	Secretary	NFT Scottish Soft Fruit Panel
	Member	SNSA Adviser to Committee
	Scientific Adviser	Scottish Soft Fruit Growers Ltd
A.F. Murant	Member	Plant Virus Sub-Committee, International Committee on Taxonomy of Viruses
A.C. Newton	Member	UK Cereal Pathogen Virulence Survey Committee
	Membership Secretary	British Society for Plant Pathology
	Secretary	Crop Diversity Group of the European and Mediterranean Cereal Rust Foundation
M.C.M. Pérombelon	Member	COST 88 - Bacteriology Committee
	Organiser	COST 88 - Workshop on Erwinia diagnostics at SCRI
D.A. Perry	Treasurer	Association for Crop Protection in Northern Britain
	Treasurer	British Society for Plant Pathology
	Secretary	CAROS International Ltd Board
W. Powell	Adviser	International Foundation for Science, Stockholm, Sweden
B. Reavy	Member	AFRC Protein Engineering Liaison Committee
K. Ritz	Member	AFRC Soil Plant Microbial Interactions Working Party
	Member	Management Group of Centre for Non-linear Systems in Biology
I.M. Roberts	Safety Representative	Royal Microscopical Society
	Chairman	AFRC Electron Microscope Advisory Group
D. Robinson	Member	AAB Plant Physiology Group
D.J. Robinson	Member	Advisory Committee on Releases to the Environment
	Member	Society for General Microbiology Virus Group Committee
M. Talbot	Member	Statistics Group of UK National List and Seeds Committee
	Member	Technical Working Party on Automation and Computer Programs of the International Union for the Protection of Plant Varieties
	Member	Statistics Committee of the International Seed Testing Association
W.T.B. Thomas	Member	AAB Plant Breeding Committee
L. Torrance	U.K. Rep.	COST-88 Management Committee
D.L. Trudgill	Chairman	EPPO *ad hoc* Committee on Potato Cyst Nematodes
	Member	Departmental Board of Biological Sciences, University of Dundee
B. Williamson	Organising Committee	Crop Protection Northern Britain Conference
J.A.T. Woodford	Regional Hon. Sec.	Royal Entomological Society
F.G. Wright	Member	AFRC Protein Engineering Liaison Group
I.M. Young	Member	SAC Soil Engineering Group
	Member	British Soil Science Council

Short term workers and visitors

Name	Country of origin	Dept.	Month/yr of arrival	Length of stay
A. Abruzesse	Italy	CEP	Jul 92	2 weeks
J. Aked	UK	CEP	Jul 92	2 weeks
R. Alonso	Spain	SASS	Aug 92	1 month
S. Anwar	Pakistan	Zoo	Apr 92	1 year
M. Armstrong	UK	CMG	Aug 92	14 weeks
G. Asmar	UK	CMG	Oct 92	1 year
M. Athwal	UK	Zoo	Aug 92	3 months
R. Barlow	UK	CMG	Oct 92	3 months
A. Boyd	UK	CG	Apr 92	6 months
D. Carter	UK	M&B	Oct 92	3 months
D. Chader	Poland	CG	Oct 92	3 months
J. Clive	UK	CG	Aug 92	4 months
P. Colorado	Spain	CEP	Nov 92	7 weeks
W. Einig	Germany	CEP	Oct 92	1 week
H. Elangwe	Cameroon	CEP	Nov 92	11 months
L. Espen	Italy	CEP	Jun 92	2 months
N. Florey	UK	DG (Fib)	Jul 92	3 months
V. Flores	Peru	Vir	Jan 92	1 year
F. Franklin	UK	SASS	Jul 92	7 weeks
A. Franz	Germany	Zoo	Aug 92	2 weeks
L. Giles	Australia	SASS	Mar 92	3 months
M. Grainger	UK	M&B	Jun 92	10 weeks
Gera Grit	The Netherlands	CEP	Sep 92	4 months
C. Haggarty	UK	SLIS	Apr 92	3 months
L. Hakim	Bangladesh	CMG	Mar 92	1 year
C. Hancorn	UK	SASS	Jun 92	2 months
K. Harding	UK	CMG	Jul 92	3 months
G. Hill	UK	CEP	Jun 92	5 months
C. Hole	UK	CEP	Nov 92	2 weeks
T. Isam	UK	M&B	Sep 92	3 months
H. Jansen	The Netherlands	CG	Oct 92	5 months
R. Jones	UK	M&B	Mar 92	6 weeks
M. Joschko	Germany	CEP	Dec 92	2 weeks
M. Joschko	Germany	CEP	Sep 92	2 weeks
R. Kempers	The Netherlands	CEP	Jul 92	4 months
H. Koike	Japan	CEP	Dec 92	2 weeks
M. de Koort	The Netherlands	CEP	Jan 92	6 months
D.L.M. Kyessi	Tanzania	Vir	Jun 92	5 months
R. Lance	Australia	CG/CMG	Jul 92	3 months
N. Leach	UK	Zoo	May 92	10 weeks
N. Legesse	Ethiopia	CMG	Jun 92	4 months
Rachel Legg	UK	Zoo	Aug 92	1 year
J. Love	UK	CG	Aug 92	4 months
Janie Lyon	UK	CG	Jan 92	3 months
N. MacKinnon	UK	SASS	Jun 92	10 weeks
T. Mahmond	Pakistan	Vir	Oct 92	2 months
S. Main	UK	M&B	Jun 92	6 weeks
Wanda Marczynska	Poland	CG	Sep 92	3 months
N. Matsui	Japan	CEP	Oct 92	2 months
Alison Maxwell	UK	CG	Jul 92	2 months
A.McLeod	UK	M&B	Jun 92	3 months
S. Mey	UK	CEP	Jun 92	5 months
J-B. Morel	France	M&B	Jan 92	2 months
Barbara Murray	UK	SASS	Jun 92	10 weeks
Kirsty Murray	UK	CMG	Oct 92	15 weeks
A. Norris	UK	Zoo	Jun 92	6 weeks
Catherine de Nova	New Zealand	SFG	Oct 92	6 months
E. Okirima	UK	CMG	Aug 92	14 weeks
R. Overall	Australia	CEP	Nov 92	1 week
T. Pagella	UK	CG	Aug 92	8 months
C. Palivan	Romania	DG (Spec)	Jul 92	1 month
J. Passioura	Australia	CEP	Apr 92	5 months
H. Pedersen	Denmark	CEP	Jan 92	4 months
A.Pesnyakevich	Belarus	M&B	Nov 92	3 weeks
M. Prescott	UK	SASS	Apr 92	5 months
Vasantha Ramanthan	Sri Lanka	M&B	Jun 92	6 months
N.S. Rao	India	CG	Feb 92	5 weeks
R.T. Robbins	USA	Zoo	Apr 92	2 weeks
R. Schaffer	UK	CEP	Jul 92	4 months
K. Sinclair	UK	Zoo	Aug 92	3 months

Name	Country of Origin	Dept.	Month/yr of Arrival	Length of stay
H. Syed	UK	CMG	Oct 92	3 months
J. Szabo	Romania	Zoo	Jun 92	1 week
B. Thomas	UK	M&B	Jun 92	10 weeks
G. Thottappilly	Nigeria	CMG	Mar 92	6 months
P. Tien	China	Vir	Nov 92	6 months
I. Tobias	Hungary	Vir	Oct 92	3 months
T. Uribe	Spain	CG	Jun 92	2 weeks
G. Venner	The Netherlands	CEP	Jul 92	5 months
T. C. Vrain	Canada	Zoo	Apr 92	2 weeks
M. van Vuuren	The Netherlands	CEP	Nov 92	4 months
N. Wales	UK	SASS	Aug 92	1 year
R. Walker	UK	M&B	Jul 92	10 weeks
Caroline Waridel	Switzerland	M&B	Mar 92	3 months
H. Watson	UK	M&B	Mar 92	6 weeks
A. Wheelwright	UK	SASS	Dec 91	9 months
P. Whitty	UK	CMG	May 92	1 year
G. Yeates	New Zealand	Zoo	Aug 92	2 weeks

Longer-term visitors and Research Fellows

Name	Country of origin	Dept.	Month/yr of arrival	Length of stay
Jill Ellis	UK	CG	Nov 92	3 years
Linda L. Handley	UK/USA	CEP	Jun 91	5 years
Dorothy Spencer	UK	CG	Aug 89	3 years
Angelika Ziegler	Germany	Vir	Jan 91	2 years

Postgraduate Students

Name	Dept.	Subject
I. Abdalla	SASS	Automatic detection of tissue boundaries in ultrasound scans of pedigree sheep.
M. Arif	Vir	Potato mop-top furovirus transmission.
Siti A. Mad Arif	CMG	Plant genetic transformation and gene expression.
J. Allainguillaume	CG	Accelerated gene localisation in potato.
A. Anderson	CEP	Quantification and evolution of qualitative theory of soil structure.
G. Asmar	CMG	RNA helicase genes.
E. Baird*	CMG	Potato molecular biology.
R. Bargota	CEP	Starch synthesis in *Vicia faba.*
S.N.B. Barr	CG	Somatic hybridisation of tetraploid and wild potato.
U. Barua	CMG	RAPD methods of detecting polymorphisms in barley.
Annette Baty	DG-fib	Control of cell wall biosynthesis during differentiation of fibre cells.
M. Biggs	SFG	Factors influencing the development of cold tolerance and dormancy in woody perennials.
Wendy Breese	M&B	Downy mildew of *Rubus* cane fruits.
Karen Brierley	DG-spec	NMR spectroscopy for characterisation of the coat protein of pepper ringspot virus.
J. Chen	Vir	Molecular biology of fungus-transmitted cereal viruses.
F.A. Comerford	CMG	Lamins in the plant nuclear membrane.
D. Cox	M&B	Developement of non-linear mathematical theory of plant disease epidemiology.
I. Dawson	CMG	Molecular diversity of tropical tree species.
Lisa Duncan	Zoo	Study of the surface molecules of plant parasitic nematodes.
G.J. Evans	Vir	Directed down regulation using antisense RNA and RNA self cleavage.
Sarah Fennel	CMG	Biochemical and molecular markers of *Arachis.*
Valerie Godfrey	M&B	Pectin lyase production in *Erwinia carotovora* ssp. *atroseptica.*
Mary Gray	CEP/SFG	Regulation of anthocyanin gene expression in blackcurrant.
B.E. Harrower*	CMG	Molecular biology of nematodes.
P.E. Hedley	CMG	Genetic manipulation of sugar metabolism in tubers of potato.
Jackie Heilbronn*	M&B	Protease from *Erwinia* and elicitation of defense mechanisms in potato.
R.J. Hopkins	Zoo	Resistance to cabbage and turnip root fly in swedes.
D-J Hwang	Vir	Expression and assembly of plant viral coat protein into virus like particles.
Lizbeth Hyman*	M&B	Characterisation of pectolytic bacteria by monoclonal antibodies.
D.J. Johnston*	M&B	Latent infection of flowers by *Botrytis cinerea.*
Anne Jolly*	Vir	Comparison of potato leaf roll virus strains in the P 5 gene.
D.J. Leader	CMG	U5snRNA genes from potato and maize.
F.J. Legorburu	Vir	Surface features of tobacco rattle virus particles.
G.J. Lewis	CEP	Methods for simulation of water and nitrogen use in potato.
I. Manoussopoulos	Vir	Mechanisms of aphid transmission of potyviruses.
Pauline McConway	M&B/CMG	Molecular biology of potato resistance to erwinias derived from *Solanum brevidens.*
R.J. McNicol*	SFG	Investigations into running off in blackcurrants.
Jane Miller	Vir	Potato leafroll virus in protoplasts.
Elizabeth Murant	CEP	Endocytosis in plant cells.
F. Nabugoomu	SASS	REML estimation in a series of varietal trials.
R. Neilson*	Zoo	Ecology and effect of pollutants on marine nematodes.
N.E. Nyange	SFG	Breeding for resistance to coffee berry disease and coffee rust.
C. Orozco-Castillo	CMG	Molecular diversity and genetic linkage mapping of *Coffea* sp.
Denise W. Pallett	Zoo	Epidemiology of potato virus Y.
L.G. Pereira	Vir	Monoclonal antibodies to potato mop-top virus.
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.
W. Ribeiro	CMG	Genetic variation in *Phaseolus vulgaris.*
Heather A. Ross*	CEP	Investigation of the control of sugar breakdown.
Jessica Searle	M&B	Population Genetics of *Rhynchosporium secalis.*
J. Shaw	SASS	Techniques for discrimination of seed types using imaging measurements.
C.G. Simpson	CMG	Transposable elements from maize.
D. Stewart*	DG	Physico-chemical studies of plant fibres.
J.S. Swanston *	CG	Malting and brewing properties of novel barley starch combinations.
R.E. Wheatley*	CEP	Nitrogen transformation in cultivated soils.
Joanne Wilde	CMG	Genetic fingerprinting of cocoa.
A. Wilson*	CG	Gene position in a synthetic *Brassica napus* .
M.W. Young	CEP	Predictive models for the nitrogen requirements of potato crops.

* Permanent members of staff

SCRI Research Programme
1992-1993

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. 100 research projects undertaken on behalf of various bodies, including other Governmental bodies, commerce and levy boards.

040 SCR/006/91	ROA Genetic architecture of tetraploid potatoes and production of enhanced germplasm	Bradshaw J E
045 SCR/007/91	ROA Develop, improve and use screening methods for resistance to diseases and pests of the potato	Wastie R L
046 SCR/008/91	ROA Develop and use screening tests for biochemical compounds in potatoes	Griffiths D W
047 SCR/017/91	ROA Maintenance, improvement and evaluation of the Commonwealth Potato Collection	Wilkinson M J
066 SCR/060/91	ROA Interactions between tolerance, resistance and potato cyst nematodes	Phillips M S
109 SCR/064/91	ROA Biology and population dynamics of agricultural pests especially plant-parasitic nematodes	Boag B
114 SCR/061/91	ROA Analysis of the inheritance of resistance to and complementary virulence of potato cyst nematodes	Phillips M S
152 SCR/042/91	ROA Identification and exploitation of genetic markers in crop improvement	Forster B
157 SCR/028/91	ROA Physical and chemical characteristics of fibres from fibre-producing herbs, shrubs and trees	Morrison I M
158 SCR/029/91	ROA Control of differentiation and development in plant fibre cells	Morrison I M
159 SCR/030/91	ROA Novel and conventional processes for the extraction and modification of fibres from plant sources	Morrison I M
180 SCR/062/91	ROA Mechanisms determining specificity and efficiency of transmission of tobraviruses by (Para)Trichodorus nematodes	Brown D J F
181 SCR/063/91	ROA Mechanisms determining specificity and efficiency of nepovirus transmission by longidorid nematodes	Brown D J F
187 SCR/009/91	ROA Investigate the genetics and biochemistry of the low temperature sugar stability characteristics of potato for use in fried food products	Mackay G R
205 SCR/031/91	ROA Evaluate the potential of NMR spectroscopy for the determination of the composition and structure of plant fibre and fibre products	Goodman B A
215 SCR/067/91	ROA Biology and ecology of pests and beneficial arthropods associated with cane and bush fruits	Woodford J A T
231 SCR/NON/	IFS Genome organisation and expression of plant picornaviruses	Turnbull-Ross A D
260 SCR/NON/	IFS Construction and evaluation of UsnRNA based transformation vectors for the delivery of antisense RNAs to plant cell nuclei	Guerineau J F

261 SCR/007/90	ROA Introduction of exogenous DNA into *Rubus, Ribes, Fragaria* and other soft fruit genera using *Agrobacterium tumefaciens*	McNicol R J
262 SCR/008/90	IFS Characterisation of the particle protein of pepper ringspot tobravirus by Nuclear Magnetic Resonance spectroscopy	Goodman B A
263 SCR/004/90	ROA Incorporation of the Birmingham Potato Collection into the Commonwealth Potato Collection	Wilkinson M J
264 SCR/005/90	ROA Correlating glasshouse and field performance of true (botanic) potato seed populations	Clulow S A
265 SCR/006/90	ROA Development and evaluation of methods for specific applications of high-technology instrumentation for the SCRI research programme	Griffiths D W
271 SCR/010/90	IFS Endocytosis in higher plants: the potential for uptake and targeted transport of foreign molecules	Oparka K J
274 SCR/002/90	FF Sensitivity analysis of crop performance with development to aid crop management in an altered climate	Crawford J W
275 SCR/001/90	FF To determine the factors modifying the transcription of genes controlling carbohydrate metabolism	Davies H V
281 SCR/053/91	ROA Investigation of the genetic control of characters determining crop performance in barley	Ellis R P
282 SCR/054/91	ROA Development of improved methods of generating and evaluating variation in barley for a range of important characters	Thomas W T B
283 SCR/055/91	ROA Investigation of the genetical determination of biochemical components that relate to cereal quality with the aim of improving selection procedures in breeding programmes	Swanston J S
284 SCR/056/91	ROA Anther and isolated microspore culture in cereals and legumes	Ramsay G
285 SCR/058/91	ROA Anti-nutritional factors in faba beans	Ramsay G
286 SCR/057/91	ROA Tissue culture and transformation in legumes	Ramsay G
287 SCR/059/91	ROA Biochemical markers in faba beans	Ramsay G
288 SCR/019/91	ROA Devise techniques for modifying the competitive relationship between fruiting and vegetative phases in raspberry	Lawson H M
289 SCR/020/91	ROA The collection, evaluation and conservation of genetic resources of perennial soft fruit genera	McNicol R J
290 SCR/021/91	ROA The development of molecular and biochemical markers in woody perennial fruit crops	Brennan R M
291 SCR/022/91	ROA Investigations of the genetics and mechanisms of pest and disease resistance in *Ribes, Rubus* and other soft fruit genera	McNicol R J
292 SCR/023/91	ROA Investigation of mechanisms and genetic control of low temperature tolerance in perennial fruit crop genera	Brennan R M
293 SCR/024/91	ROA The floral biology of perennial soft fruits	McNicol R J
294 SCR/025/91	ROA Gene flow from cultivated to feral populations of soft fruit species and its implications for the release of genetically engineered plants	McNicol R J
295 SCR/026/91	ROA Study the properties, relationships and resistance mechanisms to viruses and virus-like diseases of soft fruit crops [*Rubus, Ribes* and *Fragaria*]	Jones A T

296 SCR/027/91	ROA Produce and maintain virus-tested stocks, assess resistance and index British and imported *Ribes* and *Rubus* for virus infections	Jones A T
297 SCR/010/91	ROA Development and exploitation of tissue culture techniques, in particular microspore culture technology in *Solanum tuberosum* L.	Millam S
299 SCR/012/91	ROA Exploitation of protoplast technology in the development of new material and in the introduction of new genes into existing material	De,Maine M
300 SCR/013/91	ROA The mechanisms of dihaploid formation following pollination of tetraploid potatoes with dihaploid inducer clones	Wilkinson M J
301 SCR/014/91	ROA The production of dihaploids and their use in improving the efficiency of germplasm enhancement and genetical study of *Solanum tuberosum*	De,Maine M
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
303 SCR/015/91	ROA Inheritance of resistance to potato virus diseases and production of resistant enhanced potato germplasm	Solomon-Blackburn RM
304 SCR/016/91	ROA The production, maintenance, distribution and associated management of facilities to produce disease-free tubers of genetic stocks of potato clones	Chapman I M
305 SCR/034/91	ROA Genetic studies within the family *Brassicaceae,* as model systems for the study of cytotaxonomy, polymorphism and gene introgression	Millam S
309 SCR/033/91	ROA To determine the biochemical and molecular mechanism associated with seed dormancy in woody species	Davies H V
310 SCR/068/91	ROA Effect of nematophagus fungi on plant-parasitic nematodes and the elucidation of factors influencing trap formation	Boag B
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
315 SCR/073/91	ROA Use of biochemical and molecular techniques to characterise aphid populations	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
319 SCR/051/91	ROA Examination of the hypersensitive response induced *in planta* by nematode elicitors and internal image anti-idiotype antibodies	Forrest J M S

320 SCR/052/91	ROA A microscopical investigation of the secretions of potato cyst nematode and their distribution within the host cell of resistant and susceptible potatoes	Forrest J M S
321 SCR/077/91	ROA Chemical characterisation and properties of the cuticle in plant parasitic nematodes	Robertson W M
322 SCR/081/91	ROA Molecular analysis of species and virulence group relationships in *Meloidogyne* spp	Trudgill D L
323 SCR/078/91	ROA A molecular and biochemical study of collagen differences associated with speciation in *Meloidogyne* spp., and host specificity of the nematode bacterial parasite *Pasturia penetrans*	Robertson W M
324 SCR/079/91	ROA Involvement of carbohydrates in retention and release of virus particles in vectors	Robertson W M
325 SCR/080/91	ROA Mechanisms of nematode damage and tolerance in relation to resistance and better strategies for control without using nematicides	Trudgill D L
326 SCR/050/91	ROA Physical and physiological constraints on the growth and activity of plant root systems	Robinson D
327 SCR/049/91	ROA Relating soil structure to biological function	Young I M
329 SCR/047/91	ROA Strategies of drought tolerance in arable crops	Bengough A G
330 SCR/082/91	ROA Dynamics of microbial populations in relation to environmental factors	Griffiths B S
331 SCR/046/91	ROA Transport of substances through soil: regulating and mediatory role of microbes	Ritz K
332 SCR/045/91	ROA Interactions between environment and microbial transformations in root zone soils	Wheatley R E
333 SCR/044/91	ROA Identification and quantification of root exudates	Shepherd T
334 SCR/032/91	ROA Monitoring and prediction of weed and other wild plant populations in and vegetation management strategies for crops, uncropped areas and rotations	Lawson H M
339 SCR/043/91	ROA Mechanisms of uptake and transport of xenobiotics	Oparka K J
340 SCR/004/91	FF Computation of safe isolation distances for field-grown genetically modified crops	Mackay G R
341 SAS/005/91	FF Binary image restoration at subpixel resolution from multi-level data	Glasbey C A
344 SCR/002/91	FF Investigate techniques for identification of foodstuffs of plant origin subjected to ionizing radiation, determination of received radiation dosages and elucidation of the role of free-radicals in senescence related processes	Goodman B A
357 SCR/357/92	ROA To determine the regulation of cell-to-cell transport and carbohydrate flux in sugar and starch-storing tissues	Davies H V
358 SCR/358/92	ROA Molecular mechanisms involved in tuberisation in potato	Taylor M
359 SCR/359/92	ROA Quantifying the benefits of genetic resistance to late blight and other diseases of the potato in pesticide-free (organic) farming systems	Mackay G R
360 SCR/360/92	ROA Develop techniques of molecular spectroscopy and utilize them in the resolution of problems involving composition, structure and/or biochemical processes in biological species of importance to agricultural science	Goodman B A

361 SCR/361/92	ROA Genetic control of pathogenicity and host specificity at the molecular level in the fungal pathogens *Phytophthora* and *Rhynchosporium*	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*)	Lyon G D
363 SCR/363/92	ROA Biochemistry of pectic enzymes produced by *Botrytis* and soft-rotting *Erwinia* spp. and related saprophytic bacteria	Williamson B
364 SCR/364/92	ROA Epidemiological and etiological studies of bacterial and fungal pathogens of potatoes, cereal crops and raspberries	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
368 SCR/368/92	ROA Molecular genetics of plant-parasitic nematodes: exploitation of the *Caenorhabditis elegans* model	Blok V C
369 SCR/369/92	ROA Genetic manipulation in higher plants: *in vitro* regeneration, genetic transformation and gene isolation in plants	Kumar A
370 SCR/370/92	ROA Study mechanisms of virus transmission by aphids with special reference to umbravirus, sequivirus and closterovirus complexes	Murant A F
371 SCR/371/92	ROA Structure and expression of viruses with novel or unusual properties	Mayo M A
372 SCR/372/92	ROA Study the molecular biology of tobacco rattle virus and the basis for diversity among strains	Robinson D J
373 SCR/373/92	ROA Enhance virus resistance by transforming plants with virus-related nucleic acid	Barker H
374 SCR/374/92	ROA Methods and techniques for electron microscopy of viruses and virus vectors	Roberts I M
375 SCR/375/92	ROA Application of transmission and scanning electron microscopy to the study of viral and fungal diseases of plants and their vectors	Roberts I M
376 SCR/376/92	ROA Serological analyses of virus proteins	Torrance L
377 SCR/377/92	ROA Structure and function of the genome RNA of potato leafroll luteovirus	Mayo M A
378 SCR/378/92	ROA Investigation of the molecular biology, serology and transmission of potato mop-top virus	Torrance L
379 SCR/379/92	ROA Virus resistance in potato: a study of mechanisms, expression and inheritance	Barker H
380 SCR/380/92	ROA Investigate molecular mechanisms underlying patterns of variation in white-fly transmitted geminiviruses	Robinson D J
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 Characterisation of pollen, volatile chemicals and fungal spores emitted from oilseed rape fields as possible causes of human allergy	MacFarlane Smith WH
383 SCR/383/92	ROA Molecular biology and vector relationships of cereal Furoviruses	Wilson T M A
384 SCR/384/92	FF Distribution of the New Zealand flatworm in Scottish farmland with emphasis on farms where it has already been found	Boag B
385 SCR/385/92	FF The selection of improved genotypes of *Rubus*	McNicol R J
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
391 SCR/391/92	FF Minimising the risks of transformed pollen escape from glasshouse containment	Wilkinson M J
392 SCR/392/92	FF Transformation of antisense and fusion constructs of sliceosomal genes into potato	Brown J W S
393 SCR/393/92	FF Combined NMR and mathematical study of major metabolic pathways in higher plant cells	Viola R
394 SCR/394/92	LINK A molecular approach for the detection and diagnosis of the agent of reversion disease and of other virus-like agents of blackcurrant	Jones A T
395 SCR/395/92	LINK Detection of *Phytophthora* diseases in horticultural planting stocks by the Polymerase Chain Reaction (PCR)	Duncan J M

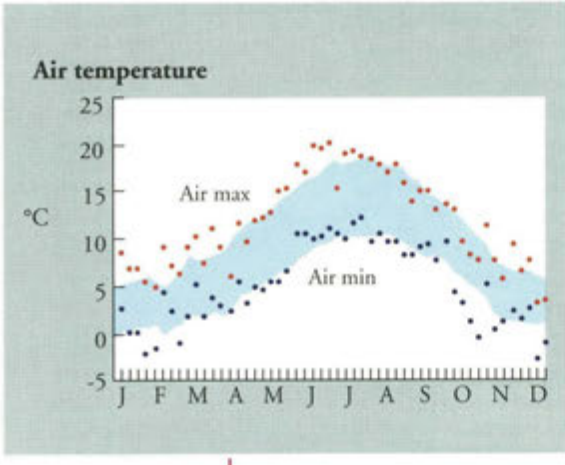
Scottish Agricultural Statistics Service

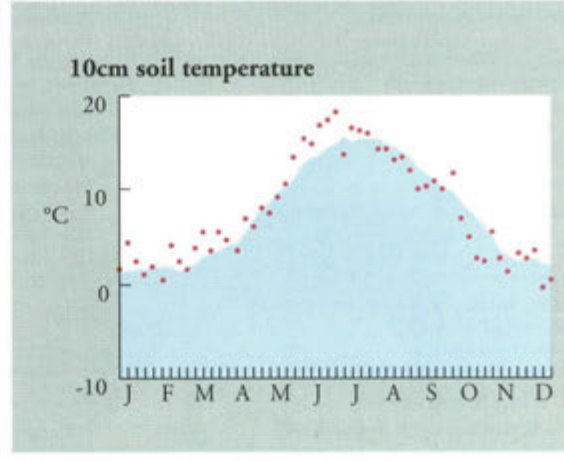
001 SAS/001/92	ROA Research, consultancy and training in statistics and mathematics in support of SOAFD-funded agricultural R & D	Kempton R A
002 SAS/002/92	ROA Development of statistical methods for modelling spatial distribution with special reference to Geographic Information Systems	Buckland S T
003 SAS/003/92	ROA Application of mathematical and statistical modelling to epidemiology, with particular reference to *Listeria monocytogenes*	Gibson G J
004 SAS/004/92	ROA Image analysis and spatial processes in microscopy, medical scanning systems, remote sensing and photography	Glasbey C A
005 SAS/005/92	ROA Statistical aspects of genetic linkage and chromosome mapping with specific reference to quantitative traits in barley, field bean and potato	McNicol J W
006 SAS/006/92	ROA Appraisal of mathematical modelling and risk analysis methods in veterinary epidemiology	Gibson G J

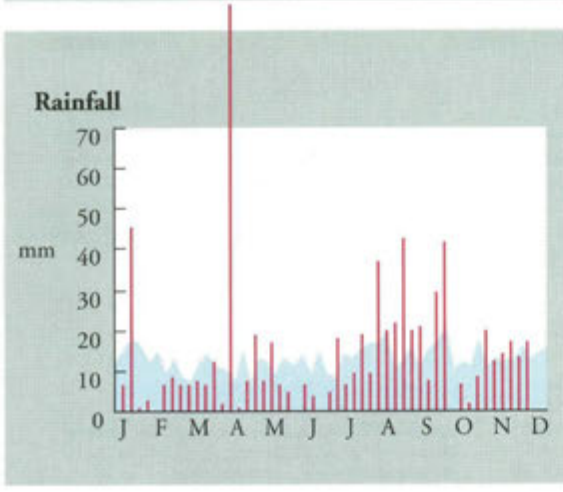
Meteorological Records

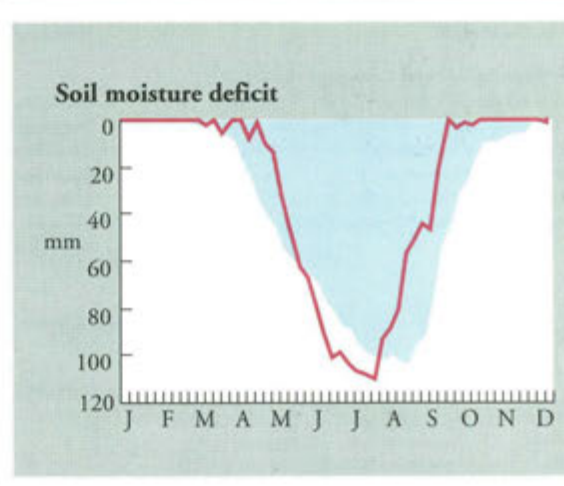
D.K.L. MacKerron

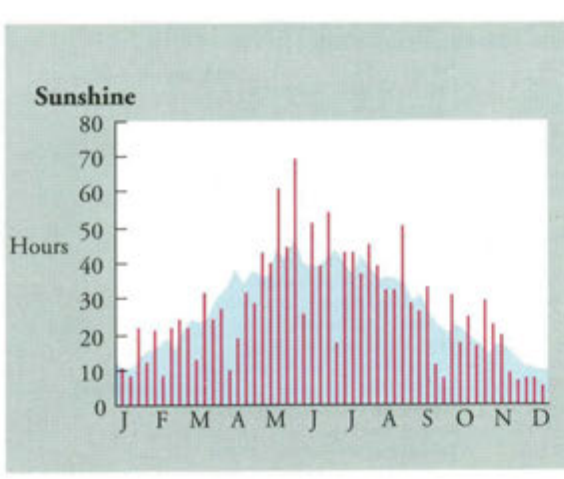
Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 1992 and the long term average for 1961-1990( ).

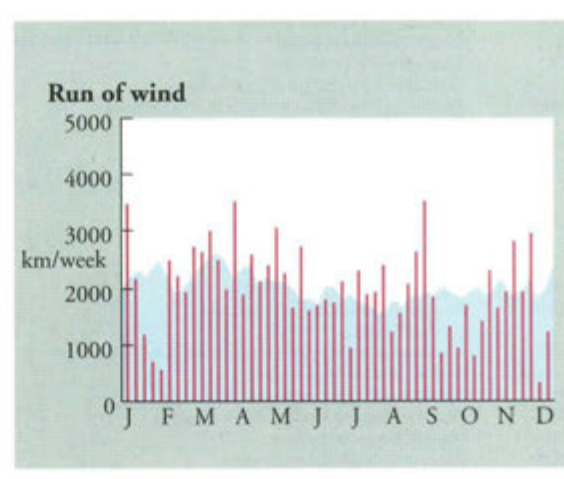












Agricultural and Food Research Service Institutes

AFRC Institutes

AFRC Institute for Animal Health	Compton, Near Newbury, Berkshire RG16 0NN	0635-578411
Compton Laboratory	Compton, Near Newbury, Berkshire RG16 0NN	0635-578411
Pirbright Laboratory	Ash Road, Pirbright, Woking, Surrey GU24 0NF	0483-232441
AFRC & MRC Neuropathogenesis Unit	Ogston Building, West Mains Road, Edinburgh EH9 3JF	031-667-5204
AFRC Babraham Institute	Babraham Hall, Babraham, Cambridge CB2 4AT	0223-832312
Laboratory of Molecular Signalling	Dept of Zoology, University of Cambridge Downing Street, Cambridge CB2 3EJ	0223-336600
AFRC Roslin Institute		
Edinburgh Research Station	Roslin, Midlothian EH25 9PS	031-440-2726
AFRC Institute of Grassland and Environmental Research	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	0970-828255
Aberystwyth Research Centre	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	0970-828255
North Wyke Research Station	Okehampton, Devon EX20 2SB	0837-82558
Bronydd Mawr Research Station	Trecastle, Brecon, Powys LD3 8RD	0874-636480
Trawsgoed Research Farm	Trawsgoed, Aberystwyth, Dyfed SY23 4LL	09743-615
AFRC Silsoe Research Institute	Wrest Park, Silsoe, Bedford MK45 4HS	0525-60000
AFRC Institute of Food Research	Earley Gate, Whiteknights Rd, Reading RG6 2EF	0734-357055
Norwich Laboratory	Norwich Research Park, Colney, Norwich NR4 7UA	0603-56122
Reading Laboratory	Earley Gate, Whiteknights Rd, Reading RG6 2EF	0734-357000
AFRC Institute of Arable Crops Research	Harpenden, Herts AL5 2JQ	0582-763133
Long Ashton Research Station	Long Ashton, Bristol BS18 9AF	0275-392181
Rothamsted Experimental Station	Harpenden, Herts AL5 2JQ	0582-763133
Broom's Barn Experimental Station	Highham, Bury St. Edmunds, Suffolk IP28 6NP	0284-810363
AFRC Institute of Plant Science Research	John Innes Centre, Colney Lane, Norwich NR4 7UH	0603-52571
Cambridge Laboratory	John Innes Centre, Colney Lane, Norwich NR4 7UH	0603-52571
John Innes Institute	John Innes Centre, Colney Lane, Norwich NR4 7UH	0603-52571
Nitrogen Fixation Laboratory	University of Sussex, Brighton, Sussex BN1 9RQ	0273-678252
AFRC Computing Division	West Common, Harpenden, Herts AL5 2JE	05827-62271
Horticultural Research International	Wellesbourne, Warwick CV35 9EF	0789-470382
HRI, East Malling	West Malling, Maidstone, Kent ME19 6BJ	0732-843833
HRI, Littlehampton	Worthing Road, Littlehampton, West Sussex BN17 6LP	0903-716123
HRI, Wellesbourne	Wellesbourne, Warwick CV35 9EF	0789-470382

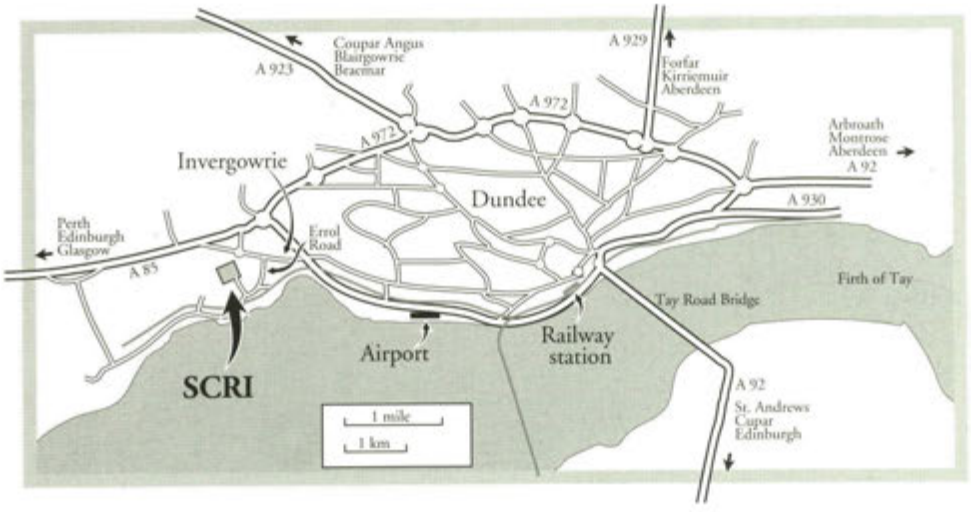
Scottish Agricultural Research Institutes

Hannah Research Institute	Ayr, Scotland KA6 5HL	0292-76013
Macaulay Land Use Research Institute	Craigiebuckler, Aberdeen AB9 2QJ	0224-318611
Moredun Research Institute	408 Gilmerton Road, Edinburgh EH17 7JH	031-664-3262
Rowett Research Institute	Greenburn Road, Bucksburn, Aberdeen AB2 9SB	0224-712751
Scottish Crop Research Institute	Invergowrie, Dundee DD2 5DA	0382-562731
Scottish Agricultural Statistics Service	University of Edinburgh, James Clerk Maxwell Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JZ	031-650-4900

List of Abbreviations

AAB	Association of Applied Biologists
ADAS	Agricultural Development and Advisory Service
AFRC	Agricultural and Food Research Council
AFRS	Agricultural and Food Research Service
ASS	Agricultural Scientific Services (SOAFD)
BCPC	British Crop Protection Council
BSPB	British Society of Plant Breeders
BTG	British Technology Group
CIP	International Potato Centre - Peru
COSAC	Council of Scottish Agricultural Colleges
COST-88	European Co-operation in the field of Scientific and Technical Research
EAPR	European Association for Potato Research
EC	European Community
ECLAIR	European Collaboration Linkage of Agriculture and Industry through Research
ECRE	Edinburgh Centre for Rural Economy
ECSA	European Chips and Snacks Association
EEC	European Economic Community
EHF	Experimental Husbandry Farm
ELISA	Enzyme linked immunosorbent assay
FF	Flexible Funding (SOAFD)
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
ISHS	International Society for Horticultural Science
ISPP	International Society for Plant Pathology
IVEM	Institute of Virology and Environmental Microbiology
MAFF	Ministry of Agriculture Fisheries and Food
MLURI	Macaulay Land Use Research Institute
MRI	Moredun Research Institute
NERC	National Environmental Research Council
NFT	National Fruit Trials
NIR	Near Infra-Red
NMR	Nuclear Magnetic Resonance
NPTC	National Proficiency Test Council
ODA	Overseas Development Administration
ORSTOM	Organisation for research in science and technology over-seas
PMB	Potato Marketing Board
PVRO	Plant Variety Rights Office
RFLP	Restriction Fragment Length Polymorphism
RRI	Rowett Research Institute
SAC	Scottish Agricultural College
SARI	Scottish Agricultural Research Institutes
SASS	Scottish Agricultural Statistics Service
SCRI	Scottish Crop Research Institute
SDA	Scottish Development Agency
SNSA	Scottish Nuclear Stocks Association
SOAFD	Scottish Office Agriculture and Fisheries Department
SSCR	Scottish Society for Crop Research
SSPDC	Scottish Seed Potato Development Council
TRIO	Tayside Regional Industrial Office

Access to Scottish Crop Research Institute



SCRI is on the east coast of Scotland, midway between Edinburgh and Aberdeen.

It is located at Invergowrie on the main A85 road 6km west of the centre of Dundee.

British Rail has direct InterCity services between Dundee and London, Edinburgh and Glasgow and other UK cities.

Flights are available to Dundee Airport from Manchester and Aberdeen, and scheduled services operate from many domestic and international destinations to Edinburgh and Glasgow