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Scottish Crop Research Institute

Annual Report 1991

The Scottish Crop Research Institute is a major international centre for research on agricultural, horticultural and industrial crops, and on the underlying biological processes common to all plant science and crop growth. A broad multidisciplinary approach to research is a special strength of the Institute.

It is the lead centre for research on potatoes, barley, beans, brassicas and soft fruit crops in UK. In addition, research on a wide range of temperate, tropical and sub-tropical crops is undertaken commensurate with the skills available.

The Institute is housed in modern buildings with sophisticated equipment; it has an extensive range of controlled environment and glasshouse facilities; and 194 hectares land for field experimentation immediately adjacent to the laboratory complex.

SCRI is a Non-Departmental Public Body, with a Governing Body, grant-aided by the Scottish Office Agriculture and Fisheries Department and has charitable status. An increasing proportion of its income is derived from external sources. It is one of five Scottish Agricultural Research Institutes which, together with those of the Agricultural and Food Research Council, form the Agricultural and Food Research Service of the UK.

SCRI was established in 1981 by the amalgamation of the Scottish Horticultural Research Institute, founded at Invergowrie in 1951, with the Scottish Plant Breeding Station, Pentlandfield, Edinburgh, founded in 1920.

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Annual Report 1991

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

ISSN 0263 7200
ISBN 0 9058 7505 2
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Report of the Director

J.R. Hillman

Preliminary indices in the Quarterly Bulletin of Statistics issued by the Food and Agriculture Organization of the United Nations reveal that total agricultural and food production on a world basis fell slightly in 1991. This can be attributed largely to political decisions in those countries responsible for agricultural exports. With the exception of certain areas of the world suffering from war and strife, the less-developed countries generally experienced reasonable harvests and demand for food aid was less pressing than in previous years. *Per capita* food production in both developed and less-developed countries fell to a level close to that of 1988, but food stocks were apparently adequate to compensate for the shortfalls and suppress price rises. The record grain stocks accumulated in 1990 have permitted global grain use to exceed output in 1991. Much of the decline in grain production occurred in the former Soviet Union. World output of oilseeds and their by-products increased again in 1991, with rises in the production of rapeseed, cottonseed, palm kernels and soybeans. Raw sugar production also rose in 1991. Green coffee production was similar to that of 1990 but cocoa bean production declined. The collapse of the Soviet bloc in 1989 was followed in 1991 by the abrupt dissolution and disintegration of the Soviet Union itself. In turn, agricultural production was adversely affected, reflecting the breakdown in the arrangements for marketing and distributing food stocks and agricultural inputs such as seeds and agrochemicals. Throughout the cold war, there had been limited dialogue between scientists in the west and behind the Iron Curtain. Now there are pleas from large numbers of scientific organisations and scientific colleagues in the former republics for assistance. Few substantial support programmes aimed at promoting collaborative research and development in the non-medical life sciences have been implemented, despite the fact that a great deal of time and effort have been invested by the agricultural research institutes in establishing formal links. Undoubtedly, though, these links will lead to formal contracts in the near future.

Although recession in 1991 affected food processing and marketing, health and safety aspects remain paramount for Western consumers. New legal regulations such as the Food Safety Act 1990 restructured food legislation in the UK, influencing marketing operations and leading to the widespread application of analytical technologies at all stages in

the food production and retailing chain. Natural food flavourings, colouring agents, food irradiation, packaging, and freedom from contamination and imperfection are rapidly becoming crucial areas of research and commerce. In the UK, the food and drink industry became the largest contributor to the trade deficit. Import substitution will surely receive greater attention than hitherto.

As in 1990, the year ended with the inability of the United States of America and the European Community to agree on agricultural reform at the multilateral trade negotiations, suspending completion of the Uruguay round which fortunately does not have an official deadline. A package submitted at the end of 1991 by Arthur Dunkel, Director-General of the General Agreement on Tariffs and Trade, specified the major issues to be resolved in world agricultural trade. The issues included export competition and subsidies, market access and the conversion of non-tariff import barriers, special safeguards to prevent swamping of the domestic market by imports, reductions in domestic support, and future reforms. Successful completion of the Uruguay round would have a profound effect on the direction and priorities of agricultural research and development.

Growing awareness of the environment is manifest in the attention paid to the preparations by countries for the United Nations Conference on Environment and Development, the 'Earth Summit', due to be held in Rio de Janeiro in 1992. Strategies designed to reduce the threat of climate change arising from the so-called 'greenhouse effect' and the depletion of the stratospheric ozone layer have been linked to a range of other topics tied to pollution. Nonetheless, deep divisions have grown between the industrialised and less-industrialised countries over the financing and timing of environmental reforms. Establishment of the Global Environment Facility, which came into operation in 1991 through the cooperation of the World Bank, the United Nations Environment Program and the United Nations Development Program, may provide practical assistance in environmental protection and preservation of areas of rich ecological diversity.

Throughout the world, scientific research is under severe pressure. Scientists in the USA, for example, face sharply reduced grant-aid, greatly increased bureaucratic impedance, phenomenal political and legal interference, internecine disharmony, and a public perception mauled by ferocious activists and well-publicised accounts of fraud and failure to meet expectations. Most worrying of trends common to most countries, but especially prevalent in the USA, is the disillusionment of young scientists seeking a career in research. In truth, science has an insatiable appetite to consume all resources made available to it and thus judgements on priorities will be inevitable. The mechanisms to establish priorities, monitor progress and achievements, and avoid whimsical interference are central to the maintenance of a proper science base.

Unlike most research bodies in the UK, SCRI has experienced a period of growth commensurate with the transformation of its science and the importance of its projects in the context of global agriculture and the life sciences. The Scottish Office Agriculture and Fisheries Department (SOAFD) properly adopts a rigorous, clinical approach to the disbursement of its funding, carefully scrutinising all projects it finances wholly or in part. The support we receive from SOAFD and the rapid growth in contract income directly reflects the confidence of those that fund us. Detailed analysis of this Annual Report and previous reports reveals the major scientific achievements of SCRI and the associated Scottish Agricultural Statistics Service (SASS). The SCRI Interim Visiting Group Report was especially well received by the AFRC Plants and Environment Research Committee, providing independent assessment of the Institute. In drawing together the multidisciplinary skills of plant geneticists, pathologists, physiologists and agriculturalists, with molecular biologists, mathematicians, statisticians, physicists, chemists, and environmentalists, it has been possible to combine traditional and modern approaches to the life sciences. Valuable conventional expertise in agronomy, pathology, soil science and breeding have been retained and exploited at SCRI, in contrast to the situation elsewhere.

SASS received its first Visiting Group during 24-26 June 1991. The Group was comprised of Sir David Cox FRS (Chairman), Professor R Cormack and Professor B Morgan, with Dr M Carpenter and Dr P Maplestone of the AFRC Secretariat, and Dr T W Hegarty and Dr K Moore of SOAFD. In summary, the Group found that an extremely good organisation had been built up in the few years since the formation of SASS and commended the Director of SASS, Mr R A Kempton, for his hard work, leadership and initiative. A series of constructive recommendations

to strengthen SASS were presented in the report of the Group, and discussed at an implementation meeting with SOAFD and AFRC on 9 December 1991. Other organisations now look to SASS as the model system for multi-organisational research, consultancy and training in biomathematics and statistics. SASS is unique for it links the five Scottish Agricultural Research Institutes, the Scottish Agricultural College and SOAFD Agricultural Scientific Services, East Craigs, Edinburgh, enhancing the quality and value-for-money of the work in the 'Scottish System'.

Perhaps one of the most important changes affecting routine operation of research at SCRI during 1991 was the full implementation of the ROAME (Rationale, Objective, Appraisal, Monitoring and Evaluation) procedures as developed by SOAFD from recommendations of the Cabinet Office. Budgetary control of projects was transferred to Research Objective leaders of UG7 grade and above. Even greater emphasis is now placed on project management to retain flexibility, adaptation and most prominently, innovation. Science departments adjusted quickly to the change, their role evolving to sustain scientific disciplines, to act as centres for generating new ideas and proposals, and to attend to general administrative matters.

As universities and polytechnics undergo the transition to accommodate annual staff reviews, visiting groups, health and safety regulations, ROAME-based project management and redundancies, the institutes of the AFRS must share their experience as well as their science. SCRI collaborates closely at all levels with the higher education institutions, and our affiliation with Dundee University is probably the strongest, most productive linkage between an institute of the AFRS and a university in the UK.

SCRI houses several working genebanks of wild species, land races, derived research material, crop pests and diseases. New policies on the role of such genebanks are under consideration by government departments. That over 90% of the world's population depend on just 15 plant and 7 animal species for food grown on an ever-diminishing area of cultivated land poses enormous questions about the potential impacts of climate change, loss of genetic diversity and population growth. Population pressures, hence the demands on and perception of agriculture, differ according to region. Over 85% of the growth in human population occurs in the less-developed countries where the numbers of malnourished people have increased by 30% since 1980. In the tropics, the area cultivated *per capita* has declined from 0.28 ha in 1971 to less than 0.20 ha in 1990, a figure masking urbanisation, fragmentation of farms, and expansion of cultivation into virgin lands unsuitable in the medium-to-long term for arable farming. Deforestation, soil erosion, desertification, and loss of natural habitats must point towards social instability, emigration pressures and disruption of trade afflicting primarily those nations least able to cope. Employed in concert, conventional plant breeding and modern biotechnology have the capacity for the foreseeable future to meet the basic demands of mankind. Genebanks constitute the primary sources of genetic material for agriculture; those at SCRI have become a pivotal feature in the UK's contribution to world agriculture and biotechnology.

Professor T Blundell FRS was appointed Secretary to the Agricultural and Food Research Council (AFRC) on 1 January 1991. He followed Professor W D P Stewart FRS, FRSE who became Scientific Advisor to the Cabinet Office. Professor Blundell is an eminent molecular biologist noted for his application of modern biochemical, crystallographic and modelling techniques to determine the structure of complex organic molecules. His determination to build on the work of Professor Stewart, promoting the international excellence of AFRC research, fostering greater links with the higher education institutions, and focusing on science crossing traditional discipline barriers, will secure a leading role for AFRC in the UK scientific scene. By its association with the AFRC through the Agricultural and Food Research Service, SCRI will continue to develop closely similar precepts addressing the major agricultural and environmental problems through innovative science.

Two senior colleagues retired from the Virology Department during 1991. Professor B D Harrison CBE, FRS, FRSE was appointed to a Personal Chair in Plant Virology in the Department of Biological Sciences, University of Dundee, in mid-June following his retirement from the SCRI after 25 years as Head of the Virology Department. He first joined the then Scottish Horticultural Research Institute (SHRI) in 1954, leaving in 1957 to take up a post in the Plant Pathology Department at Rothamsted, where he stayed until 1966. In 1966 he returned to SHRI as head of the Virology Department and subsequently held the post of Deputy Director from 1979 to 1981 until he gained individual merit

promotion to Deputy Chief Scientific Officer (UG5). He was appointed an honorary professor in the University of St Andrews in 1987 and an honorary visiting professor in the University of Dundee in 1988. He was elected a Fellow of the Royal Society of Edinburgh in 1979 and a Fellow of the Royal Society in 1987. In 1990, he was awarded a CBE and in 1991 received an Honorary Doctorate of Agriculture and Forestry from the University of Helsinki. As an Honorary Research Professor at SCRI, Professor Harrison maintains close links with the Institute as supervisor of PhD students and of externally funded research projects. His personal research has the general aim of discovering the mechanisms underlying the biological properties of plant viruses. He is internationally acclaimed for his wide-ranging discoveries concerning viruses that are spread by soil-inhabiting organisms, virus resistance in plants, and virus diseases of potato, cassava and other tropical crops.

Mr W P Mowat, UG7 in the Virology Department retired in September 1991 after 32 years' service. Initially appointed in 1959 as a mycologist, he transferred in 1961 to the Virology Department to investigate fungal virus vectors. Thereafter he turned his attention to tobacco necrosis virus which is transferred by a fungus, and which affects tulips. This led to a long-term interest in bulbs. For the past ten years he pioneered the development of Scottish virus-tested narcissus bulbs, a notable achievement in raising phytosanitary standards and widely respected in international circles.

Staff at SCRI were deeply saddened by the untimely death in post of two highly dedicated colleagues. Mr M R Cormack, SSO in the Soft Fruit Genetics Department died in February 1991 after a long illness. He was appointed in 1962 to the then SHRI as Scientific Assistant in the Pomology Department to work on top fruit under the supervision of Dr (later Professor) C A Wood. Following a reorganisation and the reduction of research effort on top fruit he was given responsibility for a programme on techniques of establishing raspberry plantations and adaptation of various growing methods for mechanically harvesting raspberries. He was the prime mover in the formation of the UK Blueberry Growers' Group, and at the time of his death he was developing initiatives on woody plants and the introduction of horticultural crops into the Western Highlands. A talented sportsman, Mr Cormack played an influential role in social activities within and outwith the Institute. In December 1991, Mr G Pollock, P&TO in the workshop and garage, died suddenly. Soon after joining the Institute in 1963, he transferred to the vehicle workshop, eventually taking charge in 1983. There, he occupied a key position in the day-to-day organisation of a large and complex transport operation. In addition, he served in many capacities the local community of Longforgan. We extend our condolences to the families of both former colleagues.

Four staff deserve special mention. Professor N L Innes (Deputy Director) was elected Chairman of the Board of Trustees for the Centro Internacional de la Papa based in Lima, Peru, highlighting the growing interface between SCRI and the Centres (Institutes) supported by the Consultative Group on International Agricultural Research. Dr W Powell (Head of the Cell and Molecular Genetics Department) was awarded the Broekhuizen Prize by the European Cereal Atlas Foundation in recognition of an outstanding contribution by a scientist under the age of 40 to research in cereals. The prize was awarded at a ceremony in Wageningen, The Netherlands and cites Dr Powell's work on the regeneration of plants from microspores, and the integration of cellular and molecular methods of crop improvement with conventional approaches. Dr Powell and Dr A T Jones (Virology Department) were promoted to UG6 grade on individual merit. Mr D R Simpson, EWI in the Estate Department, received the Scottish Society for Crop Research Field and Glasshouse Staff Prize, coinciding with his retirement from the Institute after 37 years' service. This was the first occasion on which the prize had been awarded and was in recognition of the considerable contribution Mr Simpson has made to field experiments.

Detailed in this Annual Report are accounts of core-funded projects, new initiatives, and the numerous awards and grants made to staff throughout the year. Patents, new cultivars, reports, publications, participation in meetings and learned societies, and supervision of research students also indicate the buoyancy of the scientific effort which often involves coordination with several organisations. Such achievements are only possible by the unstinting effort and commitment of talented staff in every department and section of the Institute. By way of illustration, a major grant to Dr B A Goodman (Director's Group) for research on food irradiation has allowed SCRI to explore the use of electron paramagnetic resonance spectroscopy to examine the role of free radicals in

senescence processes. Another substantial grant involves the Departments of Cellular and Environmental Physiology and Cell and Molecular Genetics. Coordinated by Dr H V Davies, this grant by the European Community and the European Crisp and Snack Association Research Limited extends the research on genetic modification of carbohydrate metabolism in the potato. New projects include studies of plant ecology, physiology and biochemistry using stable isotope techniques at the natural abundance level.

The staff of SCRI gratefully acknowledge the contributions by the Governing Body to the increasing professionalism of the Institute and its scientific stature. We also thank the staff of SOAFD for their major investment in SCRI and the constructively high standards to which they operate. Grants, contracts and donations from the Scottish Society for Crop Research, governmental agencies, grower levy boards, local authorities, commercial companies, farmers and other individuals are also warmly appreciated. In its first year of trading, Mylnefield Research Services Limited was an outstanding success as the commercial arm of the Institute. After a remarkable year of change and growth, we look forward to the future with justified optimism.

Opening of the Crop Genetics Building

The Crop Genetics Building was formally opened on 31 May 1991 by Lord Strathclyde, Minister for Agriculture at the Scottish Office. Almost 100 distinguished guests attended the opening and subsequently toured the new building and potato glasshouses.

The Director, Professor J R Hillman, welcomed the guests in his opening speech and said that this was an important day for SCRI because it marked the final stage of the establishment of the Institute at Invergowrie.

"Following the decision to amalgamate the SHRI and the SPBS in the early 1980's, staff were transferred from Pentlandfield to Mylnefield. Any move will cause disruption, but in this instance it did not hinder the research programme and there has been a

The ceremonial opening of the Strathclyde Building. Left to right: Mr G.R. Mackay, Head of Crop Genetics Department; Mr. J.L. Millar, Chairman, SCRI Governing Body; Lord Strathclyde, Minister for Agriculture and Fisheries at the Scottish Office; Professor J.R. Hillman, Director SCRI.

Lord Strathclyde examines cooked potatoes with l. Dr M.F.B. Dale and r. Mr. J.L. Millar.

sustained increase in scientific productivity throughout the transfer period."

"The Scottish Office has made a major investment on this site and SCRI is of great importance to the economy of Tayside. Our Institute is unique in the United Kingdom because of the range of scientific disciplines covered and the number of crop species investigated which extend from potatoes and cereals through to cocoa, coffee and cassava. Furthermore, the Institute now has an international reputation for the excellence of its research in several areas."

Lord Strathclyde is shown the glasshouse facilities by G.E.L. Swan

"This new building and the related glasshouse complex will allow us to integrate modern biotechnology, chemistry and mathematics with conventional plant genetics. Our interface with our commercial partners will ensure that the technology is transferred to the market place."

The Director then paid tribute to the Scottish Office Agriculture and Fisheries Department. "They deserve a special commendation for they have been mindful of the value of scientific research and have supported the long term investment required at this site. I also thank my fellow Institute directors and the Principal of the Scottish Agricultural College for their co-operation and our industry and levy board partners for their confidence."

Lord Strathclyde then replied.

"Chairman, Professor Hillman, ladies and gentlemen; it is a great pleasure to be here today. Unlike many of you this is the first time I have had the opportunity to come and visit the Institute and already I have been greatly impressed by the dedication, professionalism and academic excellence of the work that you produce here."

"I am particularly gratified by the fact that there is such a great feeling of partnership between the work that the Institute does and the requirements that are laid down by government. In many fields of agriculture these may not always seem as being entirely compatible but I am delighted to say that here we have a tremendous centre of excellence and one which relies on providing the kind of quality in terms of research and development that not just this country needs but the whole world. I know that the main focus of today's event is to open this building. I am particularly glad to say that the main emphasis of the work on this building will be on potatoes although I gather that it will also include barley, beans, raspberries, blackcurrants and strawberries. It has cost nearly £3m and I hope that you recognise that is a measure of the commitment the government has towards the furtherance of these programmes and the commitment that it has in particular to this Institute."

"I congratulate you for all the work that you have done over the years and in advance for all the work you will be doing in this new building. Therefore, it is my great pleasure to declare this building, the 'Strathclyde Building', officially open."

Plant Genetics

R.J. McNicol & G.R. Mackay

Heritable variation is basic to the process of evolution and to the practice of crop improvement through plant breeding. Research on Crop and Soft Fruit Genetics at SCRI is targeted at increasing the variation available to breeders, as well as increasing our knowledge of how the various characters interact in terms of economically important traits, and increasing the efficiency and efficacy of selection methods by evaluating existing techniques and developing new ones.

Much of the emphasis in plant breeding in the past has been placed on increasing agricultural productivity in response to the pressure for an adequate food supply. Within the European Community there is now a trend away from increasing production at any cost, especially if that cost is deleterious to the environment. This is very much in accord with the overall objectives of SCRI breeding programmes which are aimed at maintaining productivity with a reduction in inputs like fertiliser, fungicides, pesticides and even water. While pest and disease resistance has always been a major target of SCRI breeding programmes, it is now increasingly urgent as many old chemicals are removed from use and new products are uneconomic to develop and gain approval for crops other than those that are widely grown. Resistance to blackcurrant gall mite and the associated reversion virus that it transmits is a case in point where there is no resistance in any commercially acceptable cultivar and the only control largely relies on the multiple use of one chemical. Breeding for drought tolerance in the UK has traditionally received a low priority. However, with global warming, increasing restrictions on water abstraction and problems of salinity where irrigation is being repeatedly used it is now receiving increased attention.

Diversification is a recent trend in agriculture and new plants are being grown in place of some traditional crops like cereals. For example, in soft fruit there is increasing interest in blueberries, cranberries and sea buckthorn. However, some of these crops are not adapted to growing on agricultural soils without major modifications of the pH and mineral status. Breeding and selection is therefore being directed towards the production of genotypes that are tolerant of agricultural soils. This has necessitated the collection of diverse germplasm from around the world and its establishment at SCRI where it is being evaluated prior to hybridisation. We gratefully acknowledge the contributions in both advice and kind that we have received from other breeders; some have sent unselected seedling progenies as well as parental material.

SCRI is the home of several plant gene banks, namely, the Commonwealth Potato Collection, the Potato Working Museum Collection, the Dihaploid Potato Collection, the Faba Bean Collection, the Cereals Working Collection and the Rubus and Ribes Soft Fruit Collections. These collections and their conservation is vital not only for the plant breeders, but also for those involved in associated research. The widespread growing, or even virtual monoculture, of modern elite cultivars has tended to result in a decline of genetic diversity. The very nature of breeding, which first requires the creation of variability before selection, and the nature of pests and diseases which develop resistance breaking strains, highlight the importance of maintaining and characterising these collections for the production of successful cultivars in the future.

Sometimes, however, the genes and their associated characters are not readily available to the breeder for many reasons, e.g. they are only available in another genus with which the crop species is incompatible. It is here that some of the new biotechnological methods that are being developed at SCRI may come into their own. Plant regeneration techniques have been developed which allow whole plants to be regenerated from a few or single cells. This technology, when used in conjunction with limited gene transfer techniques (genetic engineering), permits the insertion of potentially beneficial genes from various sources into crop species. To date, we have introduced genes for pest and disease resistance into brassicas, potato, raspberry, blackberry, blackcurrant and blueberry. Work is continuing to determine the stability and effectiveness of the genes within the crops. However, the release of genetically modified crops into the environment is subject to stringent statutory controls to avoid any risks, real or perceived and the traditional skills of the 'conventional' geneticists and plant breeder are being utilised to assess the risks associated with such releases.

Studies of pollen dispersal and gene flow have commenced and are yielding interesting information. A native wild species of raspberry is widely distributed throughout Tayside and the flow of a stable marker gene from commercial cultivars into the wild species has been studied at over 80 sites where over 4,000 phenotypes and 20,000 seedlings were examined. Only four instances of escapes were identified and all of these were found within 50 m of cultivated raspberry plantations. These are now being examined by molecular techniques to determine if they are vegetative escapes or the result of seed escapes. While further work remains to be carried out, the preliminary conclusions are that gene flow in raspberry from cultivated plants to wild or feral populations has been minimal, if not non-existent, over a 30 year period in the vicinity of SCRI. This research has now been expanded to encompass studies on gene flow by pollen dispersal in potatoes and oilseed rape by the initiation of a SOAFD funded project.

The production of commercial cultivars is now defined as 'near-market' and is not financed from the core budget provided by SOAFD. Some cultivars are developed at SCRI with external funding, while some strategic core funded research produces pre-release material which is passed on to commercial companies for completion, e.g. barley. External sponsorship of the Rubus breeding (red raspberry, blackberry and raspberry x blackberry hybrids) has been secured for two years from the Horticultural Development Council (HDC), Scottish Enterprise (Tayside) and Tayside Region Industrial Office. Funding for the blackcurrant breeding programme has come from SmithKline Beecham Drinks UK and its contract growers.

Production of finished cultivars of potatoes, barley and brassicas is primarily subject to the agreement with the Consortium of Nickersons Seed Specialists and Dalgety Produce and four new potato cultivars were added to the UK National List in 1991. However, the agreement is not totally exclusive and a number of contracts with other private companies are being pursued in attempts to ensure that other products of basic research do eventually reach the market place.

Progress in the development of progeny testing for disease resistance in potatoes continues to be made. It has now been possible to combine, experimentally, selection for resistance to late blight in the foliage and in the tubers, hitherto assessed independently, in the same population of potato seedling clones. Collaborative links with the Volcani Research Institute in Israel have been maintained and enabled the development of a glasshouse seedling test for resistance to early blight, *Alternaria solani*. Although this disease is not economically important in UK, our research has demonstrated it can cause substantial yield reductions in hot climates where it is endemic. Whilst access to cultivars resistant or tolerant to hot climate diseases is of immediate import to the British seed potato export industry, they may become increasingly relevant in North Europe if some 'Global warm-

ing scenarios' are correct. Nevertheless disease and pest resistance *per se* will not determine the success of a new cultivar. The UK potato industry continues to suffer substantial loss of tubers in store due to mechanical damage and we have shown recently that, because resistance to damage has a heritable component, selection for this important trait is feasible. Nutritional value of food crops is as important as disease and pest resistance and agronomic traits. The identification of six induced mutants of faba bean with reduced levels of the antimetabolites, covicine and vicine is an important step and the process of incorporating these variant genes into a range of agronomically suitable genotypes has been initiated. A genetic linkage map of faba bean is being made using a number of molecular markers and associated quantitative traits. *Inter alia* an interesting linkage between the markers and loci affecting autofertility has been discovered.

SCRI expertise in grain milling energy (GME) research has been extended to other grain crops including wheat and sorghum and is leading to a fundamental review of the factors affecting malting quality other than amylolytic enzyme activity. Linkage between biochemical markers and GME has been found and the *mlo* mildew resistance gene appears to be linked to loci affecting hot water extract. One SCRI spring barley line has been entered into National List Trials by our commercial partners.

Through all these efforts and through all these avenues of inter-disciplinary research, the Crop and Soft Fruit Genetics Departments seek to continue the genetic improvement and increase the genetic diversity of all the crops that are worked upon.

Progeny testing for resistance to diseases and pests of potatoes

R. L.Wastie, J. E. Bradshaw, M. F. B. Dale, G. R. Mackay, M.S. Phillips & R. M. Solomon-Blackburn

Increased levels of genetically-based disease and pest resistances are obvious prerequisites of sustainable agricultural systems, and are necessary to reduce the present reliance on control by agrochemicals. The development and use of progeny testing is an essential aid to the selection of resistant genotypes and to provide an insight into the genetics of resistance to enable genotypic selection to replace phenotypic selection. It is also essential, in a clonally-reproduced crop such as potatoes, that tests carried out on seedling or tuber progenies reflect the subsequent performance of clones in the field.

Recent research at SCRI has resulted in the development of a number of progeny tests which have been validated and are now being utilised to improve selection efficiency and hence increase the likelihood of superior cultivars emerging in the future, as well as providing information on the genetics of complex resistance traits.

Improved potato cultivars are conventionally produced by crossing parents with complementary desirable traits, including resistance to major diseases and pests. The ideal parent is one that transmits all of its desirable attributes to its offspring. With simply inherited traits, such as immunity to potato viruses X (PVX) and Y (PVY) and resistance to the potato cyst nematode, *Globodera rostochiensis* pathotype RO1, it is possible to produce such parents because in each case resistance is determined by a major dominant factor inherited in Mendelian fashion. However, for many other pests and diseases the inheritance of durable forms of resistance is more complex, and parents differ in the extent to which they transmit resistance to their progeny.

In order to increase the efficiency of production and selection of potatoes with resistance to several diseases and pests, it is necessary to identify the 'best' parents as sources of resistance. Such parents are more appropriately selected on the basis of their genotype rather than their phenotype, and their breeding value can only be estimated, and the most appropriate combinations of parents designed for future use, by assessing the average resistance of a sample of individuals from a particular hybridization.[1]

Figure 1 Seedling progenies being tested for resistance to late blight.

Another potentially useful feature of progeny tests is to enable resistant individuals within a progeny to be identified and selected either as parents or to subject to a further selection sequence for other attributes, such as yield, quality or resistance to other diseases and pests.

Figure 2 Six progenies showing different levels of resistance to gangrene.

Although progeny tests for tuber-borne diseases such as gangrene and dry rot can be done on clonally-propagated material, it is more efficient to test a seedling population from a sowing of true seed made within a year of crossing, rather than a smaller, probably unrepresentative, sample of clones which have been subjected to prior selection for other characteristics. The SCRI potato genetics improvement programme increasingly relies on 'in house' progeny tests which have been developed in recent years for a number of fungus and virus diseases and for cyst nematode.

Fungus diseases Resistance to late blight (*Phytophthora infestans*) in the foliage can be tested rapidly and successfully at the seedling stage with a good guarantee of agreement with subsequent field tests[2]. Seedlings are inoculated with a complex race of *P. infestans* 6-8 weeks after sowing, and blight is scored after 6 days incubation at 15°C[3] (Fig. 1). Resistant (surviving) individuals can be grown on and their tubers inoculated to assess tuber resistance. However, at present, separate sowings are made and tubers harvested as the plants are flowering, inoculated by dipping in spore suspension and the infected tubers counted after 2 weeks[4]. Standard cultivars or test progenies of known resistance are included in all late blight tests as reference points against which the seedling scores are compared.

Tests for resistance to gangrene (*Phoma foveata*) are done on tubers from a sample of the same population of glasshouse-grown seedlings as those used to test for blight resistance[5]. The tubers are rolled in a sand-based inoculum of *P. foveata* and incubated for 10 weeks at 4°C (Fig. 2). A seedling progeny test for resistance to powdery scab (*Spongospora subterranea*) has also been developed[6], and recent research suggests that dry rot tests (*Fusarium* spp.) can also be done on seedling tubers, but the necessity to wound each one before inoculation makes the procedure rather cumbersome.

Virus diseases Clonal progeny tests for resistance to virus diseases have been used for linkage[7] and other inheritance studies. Dominant major gene effects determining insensitivity to tobacco rattle virus (TRV)[8] and for restricted multiplication of potato leafroll virus (PLRV)[9] have been found.

Seedling progeny tests for resistance to PLRV infection transmitted by viruliferous aphids are effective for selecting resistant progenies but, in common with some other progeny tests, not for selecting the most resistant individuals within progenies. Seedling progeny tests for major gene resistance to PVY or PVX reveal the dosage of a resistance gene in a parent by recording the proportions of resistant and susceptible individuals in the progeny of test

Figure 3 Spray-inoculating seedlings with PVY or PVX.

Test-cross parental genotypes			Progeny Resistant : Susceptible (ratio)
Rrrr Simplex	x	rrrr Nulliplex (susceptible)	1:1
Rrrr Duplex	x	rrrr	5:1
RRRr Triplex	x	rrrr	All resistant
RRRR Quadruplex	x	rrrr	All resistant

R= dominant gene for resistance
r= recessive allele for susceptibility

Table 1 Segregation ratios in tetraploid potato progeny tests for major gene resistance at one locus.

crosses (Table 1). In these tests, seedlings are spray-inoculated with infected tobacco sap containing celite powder as an abrasive (Fig. 3) and susceptible seedlings develop mottle symptoms (Fig. 4) within a few weeks.

Selecting multiplex (triplex or quadruplex) genotypes as parents would improve the efficiency of breeding for resistance, because all of their progeny would be resistant even when the other parent is susceptible. By using such parents, virus resistance can be combined with other desirable attributes from susceptible parents without the need to screen the progeny or waste resources on raising susceptible seedlings. Multiplex parents are being produced by cycles of selfing or intercrossing resistant parents (Fig. 5), and test-crossing to identify duplex and multiplex clones (Table 1).

Figure 4 Seedling infected with PVY (left) and healthy (right).

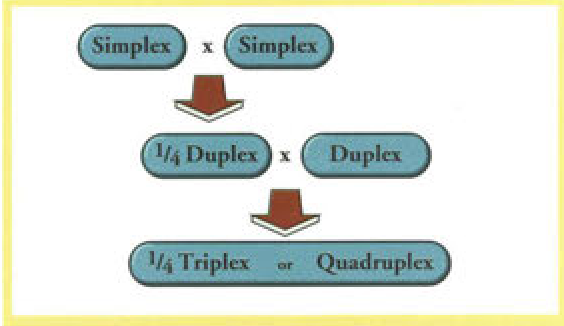

Figure 5 Procedure for producing multiplex resistant parents (single locus).

Potato cyst nematode Potato cyst nematodes (PCN) can cause considerable yield losses. Control methods include crop rotation, nematicides and resistant cultivars. The annual cost of chemical control in the UK is estimated at £5M, and there is increasing awareness of the environmental risks associated with intensive use of highly toxic nematicides. There are two important species of PCN in the UK, *Globodera rostochiensis* and *G. pallida*. The major dominant gene, H_1, derived from *S. tuberosum* ssp. *andigena* (CPC 1673) confers resistance to *G. rostochiensis* pathotypes Ro1 and Ro4, the former being the pathotype found in the UK. Testing for presence of the H_1 gene is rapid and effective on a clonal basis using closed plastic containers filled with infested soil (Fig. 6). To ascertain the status of the major gene in parental material, i.e. simplex, duplex, triplex or quadruplex (Table 1), resistant genotypes are crossed to a susceptible parent and the resultant seedling progenies grown in *G. rostochiensis*-infested soil in a glasshouse.

Resistance to *G. pallida* is inherited in a quantitative manner from a number of wild *Solanum* spp., e.g. *S. vernei* or *S. tuberosum* ssp. *andigena*. Non-additive variation for this character is an important factor in determining the resistance of progenies, indicating that progeny testing is necessary for effective selection

Figure 6 Container test for H_1 resistance to *G. rostochiensis*.

Figure 7 Testing seedlings for resistance to *G. pallida.*

of progenies with improved levels of resistance. Seeds are sown in John Innes compost containing 40 eggs g^{-1} of *G. pallida* Pa2/3, and the cysts on the exterior of the rootball are counted after approximately 11 weeks[10] (Fig. 7). The results enable the most resistant progenies to be identified and give information on the breeding value of parents[11].

Conclusions Plant breeding is genetic manipulation in practice but, of necessity, relies primarily on phenotypic selection using techniques which may appear somewhat empirical. However, research at SCRI has demonstrated that it is possible to increase the emphasis on genotypic versus phenotypic selection, thereby increasing the likelihood of producing disease and pest resistant cultivars without sacrificing quality and yield potential. The continued development, application and validation of the types of progeny tests reviewed here are also providing an insight into the genetic architecture of these important traits, thus decreasing the empiricism associated with breeding programmes. They are also producing unique combinations of genes essential to the progress of more fundamental studies at the cell and molecular level. Biotechnology and genetic engineering offer promise for the future, but require access to genetic variation, generated by the type of research outlined here, in order to fulfil that promise and usefully to supplement the dynamic progress being made in conventional breeding techniques. Ultimately, a cultivar cannot succeed unless it possesses acceptable yield and quality, no matter how disease or pest resistant it is.

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Identifying and exploiting resistance to potato late blight

R.L. Wastie, J.E. Bradshaw, J.G. Harrison & Helen E. Stewart

Late blight of potatoes, caused by *Phytophthora infestans*, is a recurrent scourge everywhere potatoes are grown. It can cause complete defoliation and death of the growing plant (Fig. 1), as well as infecting the tubers (Fig. 2), rendering them unsaleable. It is currently controlled in high-input agricultural systems by repeated applications of fungicides, but some failures in control, coupled to an increasing public awareness of the disadvantages of over-reliance on chemicals in general, have emphasised the necessity of breeding durably resistant cultivars.

The development of effective genetic resistance to late blight is dependent firstly on access to heritable sources of resistance to both the foliage and tuber aspects of the disease, either separately or combined; secondly, on methods of identifying such resistance; thirdly, on the means to exploit it in a programme of genetic enhancement; and fourthly, on being able to demonstrate its practical value and durability in the field.

Nature of resistance An extreme type of resistance, termed hypersensitivity, which virtually prevents

Figure 1 Potato crop extensively infected with late blight.

growth of the fugus within the plant, was discovered many years ago in native *Solanum* spp. It was transferred to *S. tuberosum* and found to be governed by one or very few major host genes called R genes. Unfortunately, the pathogen rapidly evolved new strains that were able to overcome this form of resistance, which was inherently unstable. Major gene resistance is now generally regarded as of little value in breeding programmes.

Within the last three decades a second mechanism, so-called horizontal resistance, has been utilised. This form of resistance is governed by many genes within the host; it is apparently stable and resistant to being overcome by changes in virulence in the pathogen. Horizontal resistance is expressed in several ways: by slowing the rate and frequency of penetration of the foliage or tuber by physical and/or chemical means; by offering physical or chemical barriers to spread within the tissues; and by reducing the rate of reproduction (i.e. sporulation) and hence slowing down the epidemic.

Sources of resistance Mexico is the most likely centre of origin of late blight and probably one of the best sources of resistant germplasm. The Mexican species

Figure 2 Blight-infected tubers.

Solanum demissum was extensively used in resistance breeding at the former Scottish Plant Breeding Station (SPBS, now SCRI), but unfortunately cultivars such as Pentland Ace and Pentland Dell, which were derived from crosses with *S. demissum*, contained R genes and very soon succumbed to new races of the pathogen. More recently, limited screening of material in the Commonwealth Potato Collection at SCRI revealed high resistance to foliage blight in some accessions of the Mexican species *S. papita* (tetraploid) and *S. polyadenium* (diploid) not previously used in our breeding programme. These wild species are being back-crossed to modern cultivars in an attempt to produce clones which, as well as being resistant to blight, are acceptable agronomically.

The Andean species of *Solanum* which gave rise to the modern cultivated *S. tuberosum* potato provided additional sources of resistance to blight. The neo-tuberosum programme at SPBS, which has now been terminated, re-created *S. tuberosum* type germplasm from *S. andigena* material. Several blight-resistant clones that were not complicated by R genes were produced from this programme, and one became a parent of the highly resistant cultivar Shelagh (1988).

Identifying resistance The ultimate test of the blight resistance of an individual genotype is its reaction to moderately severe disease pressure in an environment resembling that of a field crop. Unfortunately, such an environment is an inefficient means of assessing resistance to tuber blight, an impractical way of judging the resistance of material at an early stage in the selection procedure, and a laborious means of comparing seedling progenies. Progeny tests are therefore essential for breeding purposes and genetic analyses, where the value of parents needs to be assessed and many hundreds of individual seedlings generated and screened for resistance. Research at SCRI has produced and validated such tests (Fig. 3), both for foliage and tuber blight[1-4], and has shown that resistance to the two aspects of the disease is not closely correlated[5]. In 1991, financial assistance from the PMB enabled seedlings from crosses involving resistant parents to be screened for foliage blight and the survivors grown on in the glasshouse to produce tubers which were in turn directly tested for resistance. By this means, clones resistant to both foliage and tuber blight have been identified for further evaluation.

It is necessary in both field and glasshouse tests to be able to distinguish 'horizontal' or field resistance from the hypersensitive or 'vertical' resistance by using a

Figure 3 Accessions from the Commonwealth Potato Collection being tested for blight resistance.

complex race of the pathogen containing as many genes for virulence as possible in order to overcome any R genes in the host. If doubt remains concerning the presence of R genes in a particular genotype, it can be subjected to a detached-leaflet test *in vitro* using an appropriate race of *P. infestans.* The quantitative analysis of such a test can be assisted by using an ELISA developed at SCRI[6]. By using a complex isolate in the annual blight field test in Ayrshire and ensuring a favourable disease environment in which to make frequent assessments of the extent of infection on each genotype, as well as by using suitable control cultivars with which to compare them, a satisfactory discrimination between resistant and susceptible genotypes can be achieved[7]. It is also possible to test tubers for resistance by growing clones in a blight-free environment and inoculating the tubers in the laboratory. The assessment is complicated by the maturity class of the clones under test, and to obtain satisfactory results tubers must be harvested and inoculated at a similar stage of development, avoiding damage and allowing only the natural entry points (lenticels, eyes) to exhibit resistance[8].

There is evidence that the expression of field resistance in foliage may depend on the environment in which the plants are grown[9,10]. For example, in field trials the ranking of resistance of different genotypes often varies substantially with site or season[7], and the relative resistance of some genotypes may be highly variable, while of others it is more stable. Work at SCRI, using precisely-controlled environments and ELISA-based measurements of colonisation of leaves by *P. infestans*, is beginning to define the conditions under which resistance is expressed in a range of cultivars. Preliminary results suggest that both temperature and light are involved. Leaves of cv. Teena were resistant at 10°C but much less so at 20°C, and leaves of cv. Brodick were resistant when grown in a 20 h photoperiod but were more susceptible when the daylength was only 10 h. The resistance of cv. Torridon, however, appeared to be more stable and was expressed strongly in all the environments tested.

Exploiting resistance. In the last three decades we have been selecting for field (horizontal) resistance to late blight, and the emergence of cultivars such as Torridon (1988), Brodick (1990) and Stirling (1991) bears witness to the success of this approach. However, it should be recognised that the sources of this resistance are narrowly based, being derived from the same few accessions from Mexico, and for this reason other sources are being researched and introduced. With the availability of reliable screening tests, rapid progress is possible in resistance breeding. Cultivars such as Stirling[5], which transmit a high proportion of their resistance to their offspring, i.e. have good general combining ability (GCA), can be selected and crossed with genotypes that have good GCA for other traits to produce clones with both blight resistance and other desirable characteristics. Furthermore, even higher levels of resistance can be developed over a number of cycles of crossing and selection.

The reaction of clones to blight is normally assessed on a 1-9 scale of increasing resistance, a score of 3 being equivalent to 75% defoliation (Fig. 4), at which point growth ceases. The purpose of chemical control is to prevent infection reaching a level at which yield becomes affected, and the objective of resistance breeding is to do likewise, although in practice a score of 6 (representing about 33% of foliage destroyed) has been considered a good achievement. About a quarter of the 55 maincrop cultivars currently available in the UK have this level of resistance to either foliage or tuber blight, but only 9% to both[11]. Although inadequate on its own, it can usefully be combined with a reduced frequency and hence lower cost prophylactic spray programme.

Work at SCRI has explored the relationship between susceptibility to foliage blight and yield loss[12] using around 50 clones undergoing screening annually in Ayrshire. The difference in yield between the onset of blight (late July) and normal harvest (late September) is correlated with the foliage resistance of the genotype. A yield benefit is also obtained from genotypes which produce a high proportion of their total yield before the onset of the epidemic. A combination of early bulking, resistance to foliage blight and a high degree of resistance to tuber blight are thus the requirements of an ideal potato. Resistance to tuber blight is particularly relevant in a resistant cultivar

Figure 4 Plants showing (Lto R) foliage resistance scores of 1,3,5 and 7.

where a small amount of foliage infection is tolerated, because the spores produced, albeit at a low frequency, might infect tubers before harvest.

The value of blight resistance was demonstrated in 1991 in trials at four locations under organic farming conditions carried out in collaboration with the Henry Doubleday Research Association (Coventry), Elm Farm Research Centre (Newbury) and Earthwatch Expeditions Inc. (Massachusetts). Blight resistance is particularly important to organic growers because an effective chemical spray regime cannot be used to control blight. Seven of the newer SCRI cultivars which have high resistance to blight, and three or five commonly-grown cultivars with only average levels of resistance, were included at each site (Fig. 5). Two additional trials were situated on Scottish farms with traditional cultivation practices. At all the sites, some plants were defoliated by cutting off the foliage as soon as symptoms developed, whilst others were left to grow for several more weeks through the epidemic. The difference in yield between the two treatments provided a measure of the value of resistance in terms of the extra yield obtained. The mean increase in yield at the five sites shown in Figure 6 was 22% for the resistant cultivars and less than 10% for the more susceptible ones. At the Ayrshire site (not shown in Fig. 6), where blight arrived relatively much earlier in the season, increases in yield of four times in the resistant cultivars and of more than twice in the others were observed. The SCRI cultivars Brodick, Torridon and Stirling were consistently among the highest-yielding cultivars in the blighted plots. These and other cultivars exhibit scores of 7 or more for resistance to foliage and tuber blight, and are the products of many cycles of genetic improvement. If resistance proves to be stable, it augurs well for the future success of SCRI cultivars.

Figure 5 SCRI blight-resistant cultivars (right) adjacent to the susceptible cv. Maris Piper (left).

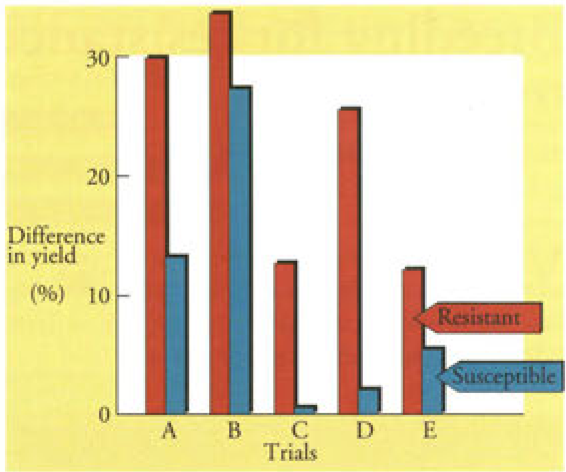

Figure 6 Percent increase in yield during 1991 blight epidemic of resistant and susceptible cultivars. Trials A-D on 'organic' farms, trial E at Mylnefield.

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Breeding for resistance to barley powdery mildew

W.T.B. Thomas, A.C. Newton & R.P. Ellis

Yield loss of barley due to one disease, powdery mildew caused by the fungus *Erysiphe graminis* f.sp. *hordei* could cost farmers in the UK at least £100M each year without control measures.

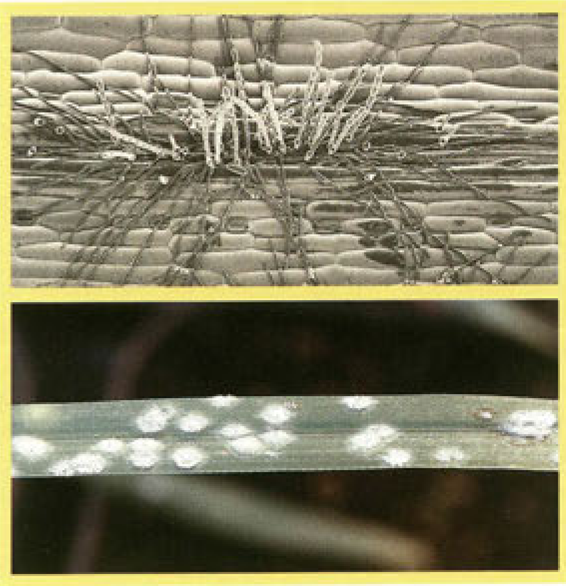

Figure 1a Scanning electron micrograph of a young powdery mildew colony on barley cv. Golden Promise 7 days after infection.
1b Barley leaf with pustules of mildew, each representing a successful infection. As the pustules grow, they coalesce covering larger areas of the leaf.

Life cycle The fungus infects green leaves when air-borne spores germinate on leaf surfaces, penetrating epidermal cells which supply nutrients for its growth. It then establishes a network of mycelium on the leaf surface producing spores (Fig 1a) which give the colonies a white fluffy appearance (Fig 1b). The asexual spores (conidia) are dispersed by wind and germinate when they land on other susceptible leaves. This life-cycle can be very short when favourably warm, dry conditions prevail. As the leaf senesces, the colonies produce small black resting structures, called cleistothecia, containing ascospores which are the result of sexual reproduction. Genetic recombination occurs during sexual reproduction and can lead to new races of the fungus. The cleistothecia are also a means whereby the fungus survives until the appearance of fresh green barley plants. Winter barley and barley volunteers in other crops provide a 'green bridge' to the next spring crop.

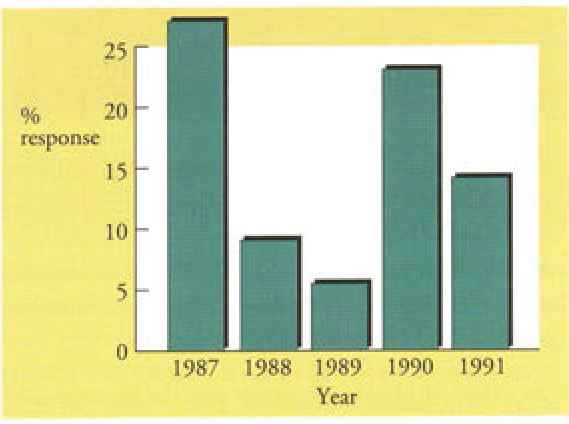

Figure 2 Fungicide response in spring barley trials at SCRI 1987 - 1991.

Effect on yield Estimates of the effect of powdery mildew on yield vary but application of fungicide to control the disease in spring barley trials of diverse material at SCRI has produced a yield increase of at least 5% and often more than 10% (Fig. 2). An early attack of the disease can restrict root development and tillering and it can also cause a reduction in grain size and increase in nitrogen content thereby reducing the quality of the crop. The yield advantage of winter over spring barley is largely due to increased ear and grain size but winter barley is vulnerable to mildew for a considerable period of time and an uncontrolled epidemic of mildew could be particularly damaging.

Fungicide control Recent SOAFD statistics show that the control of powdery mildew was the primary reason for applying fungicides to spring and winter barley. These figures are probably under-estimates as large areas of both crops were sprayed for no given reason which was probably a prophylatic treatment as most of the chemicals applied would have controlled powdery mildew. Whilst fungicides have successfully controlled the disease, the use of agrochemicals adds to the cost of production and is likely to decline due to environmental and economic pressures. The

decline will accelerate if grain prices fall and the cost of controlling the disease requires a greater increase in yield to offset fungicide application. Despite the wide range of fungicides available to control the disease, there is very little diversity in their modes of action. The pathogen can, and has, developed resistance to fungicides, such as those based on triazole compounds, and they are also able to combine multiple resistance to several fungicides.

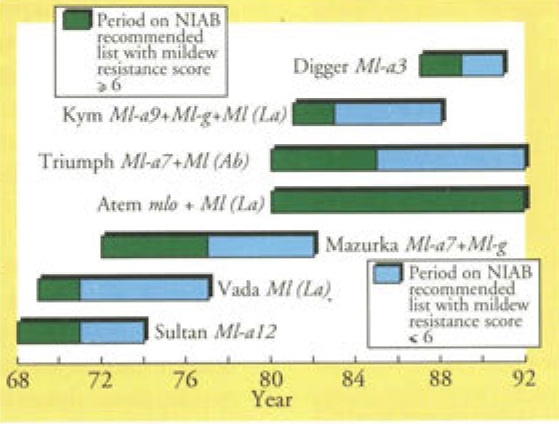

Figure 3 Durability of selected powdery mildew resistance genes in spring barley cultivars.

Major gene resistance Genetic host resistance to the disease was first recognised in the early part of the century and 15 different major resistance genes have been released in European cultivars over the last 40 years. However, the single major genes control resistances which are race-specific, i.e. their use has resulted in selection of pathogenic races with matching virulence genes. Matching virulence genes arise because the pathogen is very variable and new genetic races appear through natural mutation and genetic recombination following sexual reproduction. A new race that can overcome a resistance gene has a selective advantage, particularly if that gene is found in popular barley cultivars, and it will rapidly become the dominant race in the pathogen population. This results in formerly resistant cultivars becoming susceptible (Fig. 3) and farmers have to revert to the use of fungicides to control the disease. Resistance based on such genes becomes, in effect, a victim of its own success and has a short effective commercial life.

Plant breeders are constantly trying to isolate and incorporate new sources of resistance to powdery mildew but have exhausted most sources of resistance found in adapted germplasm. They are now incorporating resistances from more exotic sources such as collections of wild barley and its ancestor, *Hordeum spontaneum*. *H. spontaneum* has obvious deleterious characteristics, such as a brittle rachis which leads to shattering of the ear at maturity and breeders generally use a back-crossing programme to incorporate the resistance into a suitable genetic background (Fig. 4). Such programmes can produce material which is generally comparable with current cultivars but are more likely to yield parents for further crossing and selec-

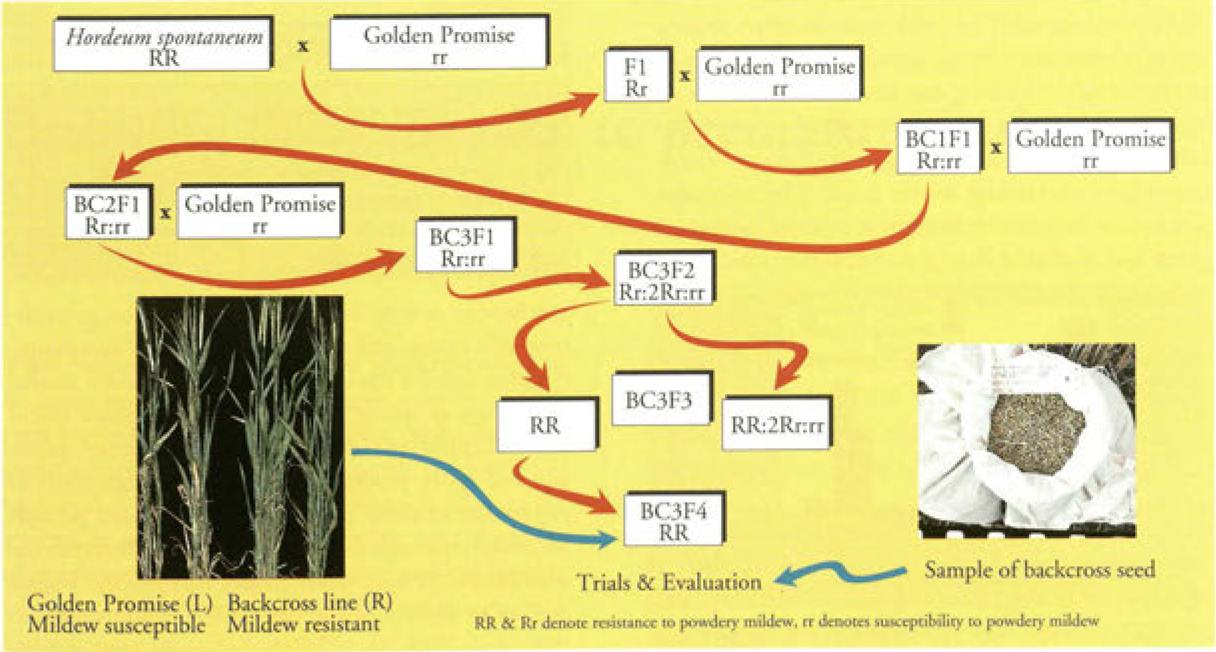

Figure 4 Typical backcrossing scheme to introduce mildew resistance.

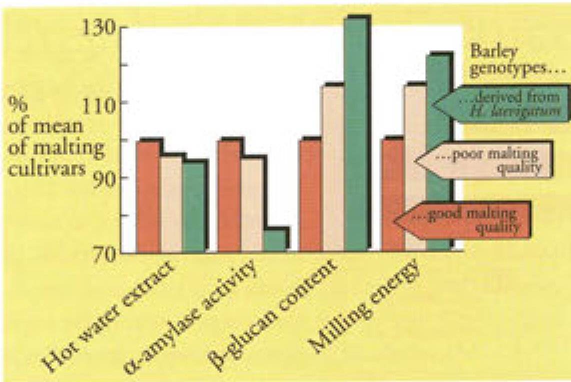

Figure 5 Comparison of barley cultivars derived from *Hordeum Laevigatum* with good & poor malting quality cultivars for a rawge of quality characters.

tion. Most of these resistances tend to be race-specific, like those that have already been described and they are unlikely to be durable. Resistances from exotic germplasm can also have deleterious effects upon other characters, for example, the Ml-(La) mildew resistance, derived from *Hordeum laevigatum*, in cv. Vada and its derivatives is associated with a number of deleterious effects on several quality characters (Fig. 5).

One major resistance gene that has proved atypically successful over the past decade is the Mlo resistance, which first became available in a commercial cultivar in the UK when the spring barley cv. Atem was recommended by NIAB in 1980. The resistance was still effective in 1991 and the cultivar remains on the NIAB recommended list, with more cultivars possessing this resistance being recommended, the most recent being cvs Chariot and Derkado. It has not yet been incorporated into any commercial winter barley cultivars, possibly due to linkage to the major vernalisation gene controlling winter habit. The Mlo resistance acts differently from other major resistance genes and probably is race non-specific, which may

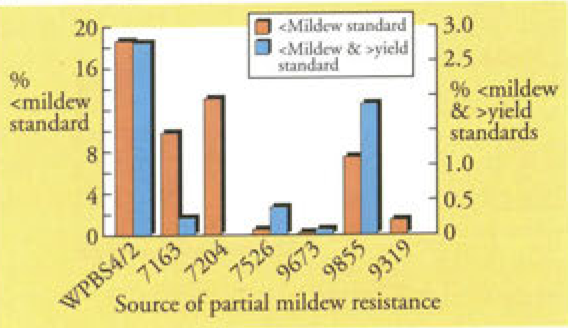

Figure 6 Percentage of inbred lines from Prisma x partial mildew resistance crosses predicted to exceed standards.

explain its durability. Races of the pathogen with increased aggressiveness on cultivars with the Mlo resistance have been found in the laboratory but not in the field. However, dependance upon a single resistance gene should be avoided and alternative resistances must be developed in case the Mlo resistance breaks down.

Partial resistance Partial resistance provides an alternative to major-gene resistance to powdery mildew and is more likely to prove durable. Partial resistance mechanisms are varied and act to restrict pathogen development, rather than exclude it completely, but they are inter-related and can be expressed at various stages of the asexual life cycle of the pathogen. One

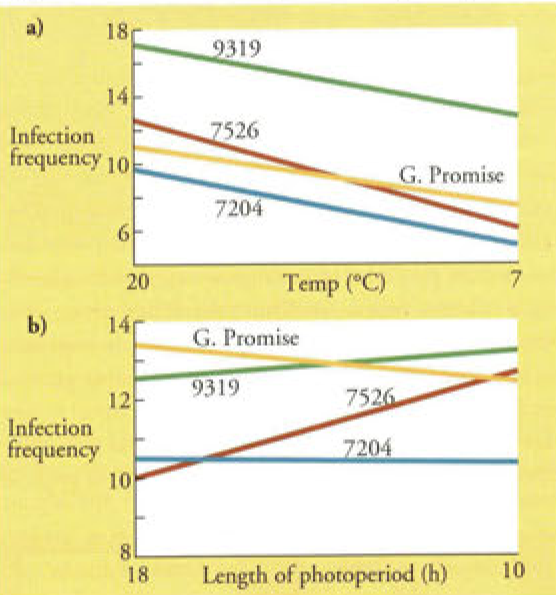

Figure 7 Effect of environmental variables upon infection frequency, a component of partial resistance.
a) Lower temperature reduces infection frequency.
b) Change in daylength does not affect mean resistance but alters the ranking of the cultivars indicating genotype x environment interactions.

mechanism acts by increasing time between germination of a spore and visual appearance of symptoms. Another reduces the infection frequency and a smaller percentage of spores landing on a leaf produce visible signs of infection. Other mechanisms restrict colony size and spore production. Combining high levels of expression of these mechanisms might well provide adequate resistance to powdery mildew. The predicted distribution of lines from crosses between cv. Prisma and seven sources of partical mildew resistance shows that nearly 20% would have an adequate level of resistance in the best cross (Fig. 6). Whilst this is promis-

ing, the number of lines with such a level of resistance and adequate yield is considerably reduced (Fig. 6) and combining both characters with good malting quality is even more difficult. Partial resistance is generally race-non-specific and, as it exerts little or no selection pressure upon the pathogen, should prove durable.

Unlike major-gene resistance, which is a qualitative character, partial resistance is a quantitative character. Qualitative characters are simply inherited and their selection is relatively straightforward but the inheritance of quantitative characters can be complex. Selection for partial resistance is therefore unlikely to be effective in the early generations of a breeding programme and is probably subject to the same difficulties as selection for other quantitative characters such as yield and malting quality. The environment can modify the expression of partial mildew resistance and the character is also subject to genotype x environment interactions (Fig. 7) which will cause problems not only in selection but also in the use of partial mildew resistance in winter and spring barley cultivars. Selection for partial resistance in the presence of effective majorgenes is a complex procedure and is problematic in the presence of ineffective major genes, unless there is widespread virulence towards them in the pathogen population. The development of biochemical and molecular markers may offer a practical solution to the problem if any can be found that are associated with partial resistance mechanisms.

Summary The use of major genes has, with one exception, failed to provide a source of durable resistance to powdery mildew and the breeding effort may be considered a long-term failure. However, they were successful until the pathogen developed matching virulence and they will remain a useful short-term means of controlling the disease. In the longer term, there is considerable potential to develop and increase levels of partial resistance. Unfortunately, this will be a multistage process as one cannot realistically expect a cross between an adapted barley and a source of partial mildew resistance to directly produce a line combining all the agronomic merits of the adapted barley with partial mildew resistance. At SCRI, our long term strategy is to develop breeding material which will produce cultivars with good quality, yield and partial resistance, and a commercial life which is not prematurely curtailed by the development of excessive susceptibility to powdery mildew.

Breeding for resistance to premature fruit shedding

R.J. McNicol

Premature fruit abscission in blackcurrant and apple has been studied extensively over the past 80 years but only recently has one of the most significant contributory factors been determined. In blackcurrant and redcurrant the condition is frequently referred to as 'run-off' and is manifest by a shedding of the flowers about 2-3 weeks after anthesis (pollen release). Yield losses in the UK, Scandinavia and Germany can be as high as 80%. In apple, pear and plum the term 'June-drop' has been used to describe the shedding of apparently well set but immature fruitlets from the trees. Again, yield loss can be considerable but is difficult to quantify in purely economic terms because the fruit that remain may be larger and of better quality.

Premature fruit loss has been attributed to poor self fertility, slow growth of pollen tubes, low temperatures, post harvest drought in the preceding year and even lack of solar radiation in the cropping year but there is no evidence that any of them are the major cause of the problem. We have found that infection

Variety	% Flower infection Styles	Ovules
Ojebyn	96	96
Ben Sarek	100	100
Brodtorp	98	98
Baldwin	98	98
Ben Alder	85	85
Ben More	83	83

Table 1 Fungal mycelium in pistils of newly dropped flowers naturally infected by *Botrytis cinerea.*

of the flowers by the fungus *Botrytis cinerea* is the main determinant of premature drop in blackcurrants and this new knowledge makes it possible to breed for resistance to the condition.

In the early stages of the investigation of 'run-off' in blackcurrant at SCRI many of the physiological and environmental causes that had been proposed, or were feasible, were examined. Plants were subjected to varied cold shock treatments at various stages of flowering, but maternal influences were always the most significant contributory factor. Pollen growth *in vitro* and *in vivo* occurred at temperatures as low as 2°C and fertilisation was successful as low as 3°C. Genetic studies involving a full diallel crossing programme of five blackcurrant parents also showed that flower drop was under the genetical control of the female parent, confirming that the source and quality of the pollen was not a contributory factor.

During the course of the histological observations, it was discovered that *Botrytis cinerea* was capable of germinating in the stigmatic fluid, growing down the styles and reaching the ovules within seven days. Three weeks after inoculating with spores of the fungus, a high proportion of apparently healthy fruitlets were shed, whereas uninfected flowers continued to develop and produce ripe fruit (Table 1). These observations were confirmed by field and glasshouse experiments (Table 2) and a similar response was found in some apple cultivars. Although substantially more inoculated than uninoculated flowers dropped, a high proportion of uninoculated flowers were lost

Variety	% Flowers dropped Uninoculated	Inoculated
Ojebyn	10	37
Ben Sarek	30	37
Brodtorp	30	77
Baldwin	47	63
Ben Alder	47	100
Ben More	47	100

Table 2 Flowers droppped after inoculation with *Botrytis cinerea.*

Days	% Flower dropped 1	3	4
Inoculated (I)	0	54	96
Non-inoculated (NI)	0	0	2
I + ethene	100	100	100
NI + ethene	100	100	100
I + NBD	0	0	40
NI + NBD	0	0	0
I + ethene + NBD	0	10	85
NI + ethene + NBD	0	15	58

Table 3 Effects of ethene, norbornadiene (NBD) and *Botrytis* infection on flower drop.

from 'run-off' susceptible blackcurrant cultivars e.g. Baldwin and Ben More and these latter flowers were found to be extensively colonised by *B. cinerea* when examined histologically.

Infection of blackcurrant flowers with *B. cinerea* induced the production of ethene gas which was measured by gas chromatography and inoculated flowers produced about six times more ethene than uninfected flowers. Ethene has long been known to induce flower abscission.

To confirm whether ethene was involved in abscission of blackcurrant flowers norbornadiene, an inhibitor of ethene production, was added to flowers in experiments which included both ethene and inoculation treatments (Table 3). The results confirmed that symptomless infection of blackcurrant ovaries by *B. cinerea* caused a marked increase in ethene production which was associated with flower drop. It should be feasible for the plant breeder to breed and select for resistance to *B. cinerea* infection of blackcurrant and possibly apple to develop cultivars that are not prone to premature fruit drop. However, having extensively screened a large number of blackcurrant genotypes, no differences in susceptibility of the flowers to infection have been found, but they do greatly differ in their

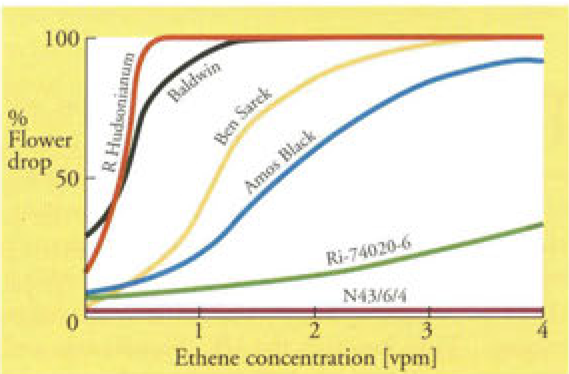

Figure 1 The calculated flower drop of blackcurrant genotypes in response to ethene.

response to ethene (Fig. 1). Results of tests with a wide range of genotypes and ethene concentrations showed that two Scandinavian selections were not prone to dropping their flowers even at relatively high concentrations of the gas.

We have now used these two genotypes in our breeding programme and found that their progeny segregate for the character. Selected seedlings have been clonally propagated for replicated experiments to determine if the lack of response to ethene is correlated with proneness to premature fruit drop.

This account illustrates how the lack of progress in breeding for resistance to a particular problem stimulated a physiological and pathological study that now permits the selection for an associated character which will result in new cultivars coming forward which are not prone to this serious disorder in blackcurrant. Preliminary evidence suggests that a similar result could be obtained in top fruit (apple, pear and plum) but as yet our investigations are incomplete.

New potato varieties

Stirling

Stirling is a high yielding early maincrop cultivar which was placed on the National List in 1991. It produces a bold sample of round to round oval, white skinned and white fleshed tubers. Stirling's principal strength is its ability to yield extremely well in many different areas throughout the UK and overseas, as well as in stressful conditions of such widely divergent environments as found in the Mediterranean area i.e. Israel and North Spain, where its resistance/tolerance to fungal pathogens, late blight, early blight and *Verticillium* have proved exceptionally good. It has repeatedly outyielded control cultivars in Israel and North Spain as well as performing well in more early districts such as Valencia in the south of Spain. In addition to the disease resistances listed, Stirling has also proved resistant (score 6) to soft rot and blackleg. It has also produced high yields, not only under normal agricultural practice, but also on organic farms in the UK.

Eden

Eden

Eden is a new potato cultivar which was placed on the National List in 1991. It is a high yielding, white skinned cultivar producing a moderate to heavy crop of medium-large ware potatoes with high dry matter. It has a good spectrum of disease resistance, notably resistance to *G. rostochiensis* (similar to M. Piper) and good levels of partial resistance to pathotypes of *G. pallida*. Eden has particular qualities for processing into crisps and some potential for chipping. One of its most important attributes is its ability to store at low temperatures (4°C) for prolonged periods without accumulating reducing sugars, thus producing a superior fry product (paler coloured) than cv. Record.

Glamis

Glamis is a high yielding, early maincrop cultivar which was placed on the National List in 1991. It produces an attractive 'bold' sample of uniform and regularly shaped parti-coloured (white/red splash) tubers, ideally suited for the prepack and 'baker' trade. In addition to its excellent performance in the UK where it has consistently outyielded the control cultivars Désirée, Pentland Crown and Maris Piper, it has also performed extremely well in many trials in and around the Mediterranean region (Spain, Cyprus, Israel and Portugal). The cultivar has excellent potential not only in the UK but also for export as seed to various countries for the production of high quality ware, e.g. the Canary Islands, where its earlier maturity provides a substantial advantage over other cultivars in those environments.

Glamis

Provan

Provan is a new early maincrop cultivar with good table qualities, which was placed on the National List in 1991. It has high yield potential and combines the same resistance to *G. rostochiensis* (Ro1) as Maris Piper, but with superior resistance to fungal and bacterial pathogens. It has a degree of partial resistance to *G. pallida* Pa1 and is moderately resistant (6) to soft rot. Provan produces a moderately heavy crop of medium-large white skinned tubers generally with a good skin finish. It is ideally suited to the prepack trade.

	Stirling	Eden	Glamis	Provan
Origin	83186 x 8204A4	10899AD14 x M. Piper	M. Peer x 3146A3	11234A816 x M. Piper
Year of Cross	1977	1979	1976	1979
Maturity	Maincrop	Early maincrop	Early maincrop	Maincrop
Wart	Susc.	Field immune - RG1	Field immune - RG1	Field immune - RG2
Late blight foliage	8	5	4	5
tuber	8	5	4	5
Gangrene	3	7	4	4
Dry rot	4	5	6	6
Skin spot	5	4	5	4
Common scab	5	3	3	5
Virus:				
PVA	Susc.	Sus.	Immune	Immune
PVY	5	3	3	3
PLRV leafroll	5	3	3	2
PVX	Susc.	Immune	Immune	Immune
PCN - G.rost.	Susc.	9	Susc.	9
G.pall.	Susc.	Pa1 partial res. 6 Pa2/3 partial res. 5	Susc.	Pa1 partial res. 7 Pa2/3 susc. 3

Molecular Biology

W. Powell

The astonishing developments in molecular biology together with an improved understanding of cellular processes has revolutionised experimental approaches to biological research. Recent research discoveries in biology rival and in many cases surpass those achieved in any other scientific area. Projects such as the human genome mapping project, gene therapy and genetic fingerprinting have captured public awareness. These impressive achievements are providing new opportunities and options for both basic and applied plant science research. Our research objectives reflect this dual theme of investigating important fundamental biological processes and, where appropriate, exploiting new approaches or information to extend our knowledge of the basic biology of the organism under study. This latter theme is well illustrated in our research on two very different organisms, namely parasitic eelworms and tropical trees. The biology of the two organisms are quite distinct but both are amenable to a common experimental approach. The synergism which emerges from the interaction between scientists involved in fundamental and strategic research is vital in our creative and productive research efforts in molecular biology and tissue culture.

Cell and tissue culture The relevance of research within the Cell and Tissue Culture Group is reflected in the number of research groups exploiting plant transformation technology for both fundamental and strategic research. The *in vitro* genetic manipulation of crops is dependent upon the availability of a successful cell and tissue culture system. This critical link between molecular and whole plant biology is of cen-

tral and crucial importance to our scientific efforts. Some examples of genetic transformation in plants are described in this section.

An improved knowledge of the basic biochemical pathways involved in plant metabolism has enabled key enzymes to be defined as targets for gene isolation. Antisense RNA technology in conjunction with genetic transformation allows the concentration of key enzymes involved in biochemical pathways to be significantly reduced. These technologies are currently being used to investigate carbohydrate metabolism in stored potato tubers (see article on 'Low temperature sweetening and invertase genes in potato'). Considerable progress has been made in the cloning of invertase genes from potato. In addition to the immediate goal of manipulating low temperature induced sweetening of potato tubers, these approaches will allow fundamental processes such as photo-assimilate partitioning and cell division to be investigated. Complementary research on directed mutation of invertase promoters will also enable key controlling DNA sequence elements to be identified.

Genetic markers Diagnostic molecular marker systems in the form of RFLPs and RAPDs are being used to create genetic linkage maps, in studies of bio-diversity and to analyse molecular phylogenetic relationships. Molecular mapping of the potato and barley genome has focused on locating genes controlling resistance to economically important diseases. For example, RAPD markers in conjunction with bulked segregant analysis have been used to locate the Rx gene in potato conferring resistance to potato virus X. Polygenic (durable) forms of resistance are also being considered. One outcome of these studies will be to improve both the speed and precision of gene transfer between sexually compatible species. Furthermore, the mapping of small nuclear RNA gene families in potato is being pursued and will improve our understanding of genome organisation and the evolution of gene families. In the long term, the development of linkage maps will form the basis of cloning phenotypically defined loci from a knowledge of their physical location in the genome.

The effective management of genetic resources is a key area in present and future plant improvement programmes. RAPD markers have been shown to be suitable tools to monitor and evaluate the extent and nature of genetic diversity in perennial tree crops. Future work will focus on studying the spatial distribution of such variability in relation to ecogeographical factors which has been already demonstrated in wild barley (see article on 'Measuring genetic diversity in crop plants'). Studies of this nature will have a major impact on the conservation and maintenance of plant genetic resources whether these exist as wild populations, primitive varieties or germplasm repositories. Restriction site mapping of nuclear and organellar genes have been used to study evolutionary relationships in a range of crop species. Difficulties involved in interpreting the underlying molecular basis of the polymorphism can be overcome by direct sequencing of PCR amplified DNA. Examples of our work on measuring genetic diversity are given later in this section. The dideoxy chain termination sequencing approach has been used to partially sequence mitochondrial small rRNA sequences of several *Theobroma cacao* genotypes. This represents a significant step forward since future molecular phylogenetic studies will involve sequence analysis of nuclear genes.

Gene expression Research within the pre-mRNA processing group concentrates on understanding the post-transcriptional processes involved in gene expression and regulation. During the past year major achievements have been made in the cloning and characterisation of the plant spliceosomal protein U2B" and the establishment of a sensitive assay system for splicing in plant introns. This system will enable differences in splicing between mono- and dicotyledonous plants to be studied. In addition to addressing problems of a fundamental, basic nature information emerging from this programme is also being used to design novel antisense vectors.

A major future challenge is to effectively harness complementary expertise and resources to tackle important scientific problems. This will undoubtedly demand international collaboration between multidisciplinary groups. In this context it is a great pleasure to acknowledge the vital contribution of our collaborators, post-graduate students and visiting scientists. Each of whom makes a special and stimulating contribution to our scientific activities and research environment.

Genetic transformation in plants

A. Kumar, J. Graham, P. Whitty & J. Lyon

Genetic transformation provides a means of introducing genetically engineered genes from any organism into a plant and increases the size of the gene pool from which useful genes can be isolated and transferred. Furthermore, a defined unit of genetic information (i.e. one gene or a few genes) is introduced into the plant with minimal interference to the recipient genome. This is important particularly for crop plants in which the genome is already fine tuned by centuries of breeding. Genetic transformation is also making significant contributions to the advancement of both basic and applied plant science research.

Several different methods of transformation are being evaluated at SCRI, including *Agrobacterium*-, protoplast- and biolistic gun-mediated transformation but this article will concentrate on our recent results from *Agrobacterium*-mediated transformation in potato and soft fruit crops. The soil-borne bacterium, *Agrobacterium tumefaciens*, is a pathogen of many crop species that transfers a small segment of DNA (T-DNA) from its tumour inducing plasmid (Ti-plasmid) into the nucleus of host plant cells. In nature, the integration of the T-DNA into the host genome and expression of the genes within T-DNA stimulates the host cells to divide resulting in the production of crown gall symptoms. This natural genetic engineering system has been exploited to transfer other foreign genes into plants. The Ti-plasmid has been modified using recombinant DNA technology to replace undesired tumour-inducing genes with desired genes from a wide range of organisms. The general features of the wild type Ti-plasmid, the disarmed Ti-plasmid vector and the process of transfer to plant cells are shown in Figure 1.

At SCRI we have developed efficient and reliable genetic transformation methods for potato and soft fruit plants (blackcurrant, blueberry, raspberry, strawberry) using *Agrobacterium*-mediated transformation. The two most important pre-requisites to ensure the success of the method are the availability of a plant regeneration system from explant tissues such as leaf,

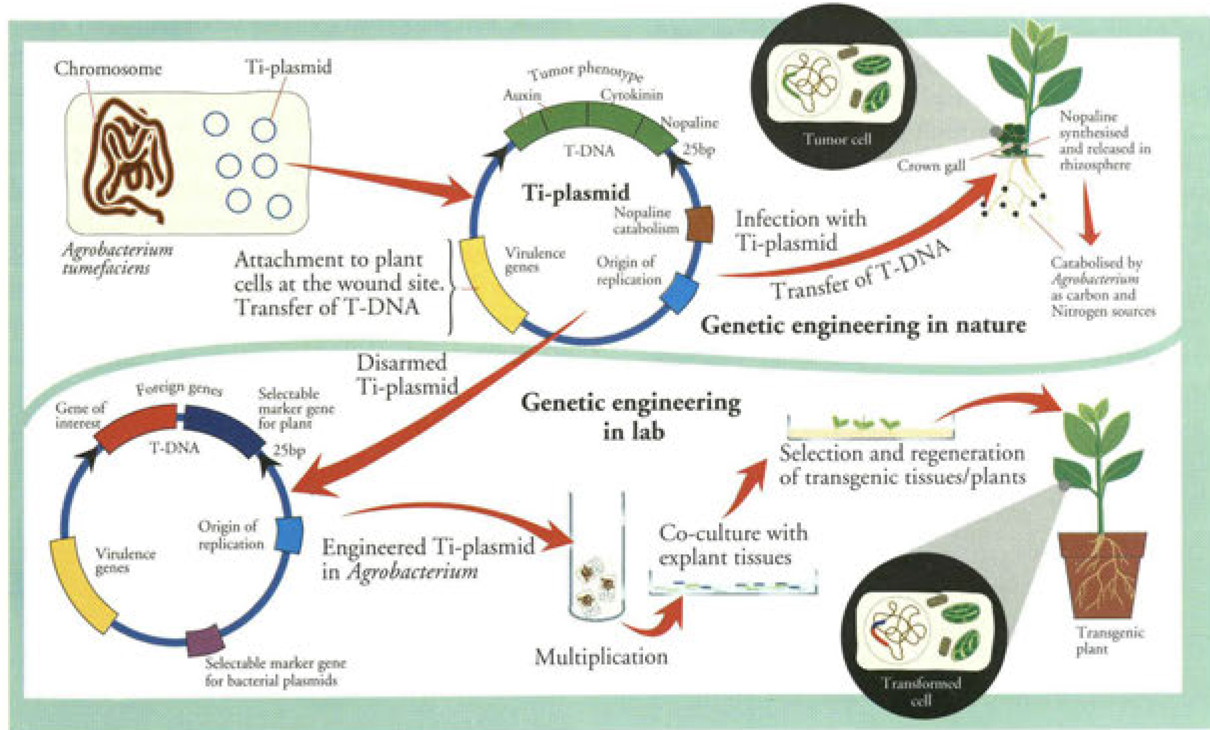

Figure 1 The general features of the wild type Ti-plasmid and the disarmed Ti-plasmid vector and the process of DNA transfer to plant cells by *Agrobacterium tumefaciens.*

Figure 2 Plant regeneration from explant tissues of potato cv. Pentland Squire.

stem, petiole and tuber, and suitable selection systems for obtaining transgenic tissues to produce transgenic plants.

Plant regeneration from explants We have recently developed an efficient plant regeneration system in potato using leaf, stem and tuber tissues, the success rate however from these explant tissues was found to be genotype dependent. A culture medium containing a specific combination of nutrients and growth hormones has been found to optimise regeneration from both stem and leaf tissues of several potato cultivars (Fig. 2). In soft fruit, plants have been regenerated from leaf, stem, and petiole tissues. Leaf tissue regeneration was most efficient for *Rubus* (raspberry), *Fragaria* (strawberry) and *Vaccinium* (blueberry), with stem tissue regeneration best for *Rubus* plants (Fig. 3).

Selection of transgenic plants Several selectable marker genes, such as neomycin phosphotransferase (NPTII) and hygromycin phosphotransferase (HPT) which encode proteins that confer resistance to the antibiotics kanamycin and hygromycin B respectively were assessed for their suitability for selecting transgenic potato and soft fruit plants.

Concentrations of 100 µg/ml kanamycin or 20 µg/ml hygromycin were found to be toxic to potatoes and could be successfully used to select transgenic tissues and plants containing NTPII or HPT genes (Fig. 4). However, most antibiotics, including kanamycin and hygromycin were extremely toxic to soft fruit plants and severely inhibited regeneration from explant tissues. Consequently, antibiotic based selection could not be used to select transgenic soft fruit plants. Other methods had to be developed and putative transgenic plants were identified by *GUS* fluorogenic and histochemical assays[1]. Genetically transformed plant tissues expressing the glucoronidase (*GUS*) gene turns blue or fluoresces blue under UV light when incubated with the substrate, 5, bromo-4-chlor-3-indoyl-1-glucoronide (X-Gluc) or 4-methyl umbelliferyl beta-D-glucoronide (MUG) respectively (Fig. 5). Several other methods such as Southern analysis, PCR analysis (Fig. 6), and NPTII assay, have been used to demonstrate the presence and expression of introduced genes .

Figure 3 Plant regeneration from explant tissues of *Rubus* species (raspberry).

Figure 4 Antibiotic selection scheme for obtaining transgenic plants of potato. Shoot regeneration from tuber discs, a) controls; b) with cefotaxime (cx) used to kill *Agrobacterium*; c) with cx plus kanamycin; d) inhibition of regeneration from untransformed discs in presence of kanamycin.

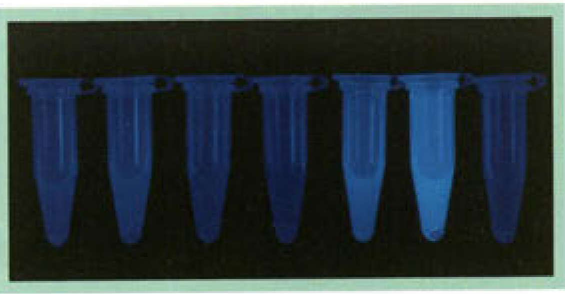

Figure 5 *GUS* fluorogenic assay showing different levels of *GUS* gene expression in transgenic raspberry plants.

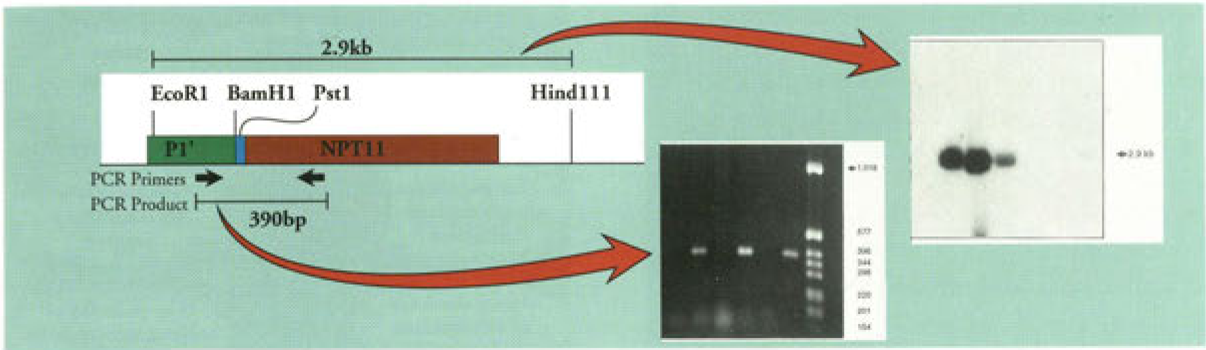

Figure 6 Molecular characterisation of the transgenic plants using PCR and Southern analyses, confirming the presence of the NPTII gene.

Established *Agrobacterium*-mediated transformation has been successfully used to introduce useful genes from plants, bacteria and viruses into potato and soft fruit plants and the technique is being applied at SCRI to assist projects in many scientific disciplines including physiology, virology and pathology as well as conventional plant breeding.

Isolating genes by transposon tagging Genes encoding unknown products have been isolated from maize and *Antirrhinum* by gene tagging using well characterised endogenous transposable elements. Endogenous transposable elements of the majority of crop plants including potato have not been sufficiently well characterised for use in gene tagging. To study the transposition frequency of the Ac element of maize in the genome of potato, we have introduced the Ac transposon using a Ti-plasmid based construct SLJ2995 containing a phenotypic marker, the rol C gene. The rol C gene expression is prevented by Ac insertion within the untranslated leader sequence of the gene. Excision of Ac leads to restoration of the rol C gene expression resulting in a pale green leaf phenotype (Fig. 7). Both the excision assay based on a marker gene (i.e. rol C) and the polymerase chain reaction (PCR) analyses using suitable primer pairs have revealed that a high frequency of Ac excision (>95%) occurs in potato. However, both PCR and Southern analyses have shown that less than 50% of the tested plants show continued presence of the Ac element in the genome after excision[2]. This result suggests that the Ac element can potentially generate insertional mutants in potato, and therefore can be used in isolating genes by transposon tagging. Initially, our target genes are the H_1 and Rx genes which confer resistance to the pathogenic potato cyst nematode (*Globodera rostochiensis* Ro1 pathotype) and potato virus X respectively. Both H_1 and Rx behave as dominant major genes in potato and this makes them suitable candidates for insertional mutagenesis using the Ac transposable element of maize.

Coat protein-mediated resistance to potato leafroll virus Some of the most successful applications of genetic engineering have been the production of virus resistant transgenic plants(*Ann. Rep. 1990,78*). Virus nucleic acid sequences encoding virus coat proteins (CP) when transferred to the host genome, have been found to confer resistance, termed "coat protein-mediated resistance", to the virus. A plant expression

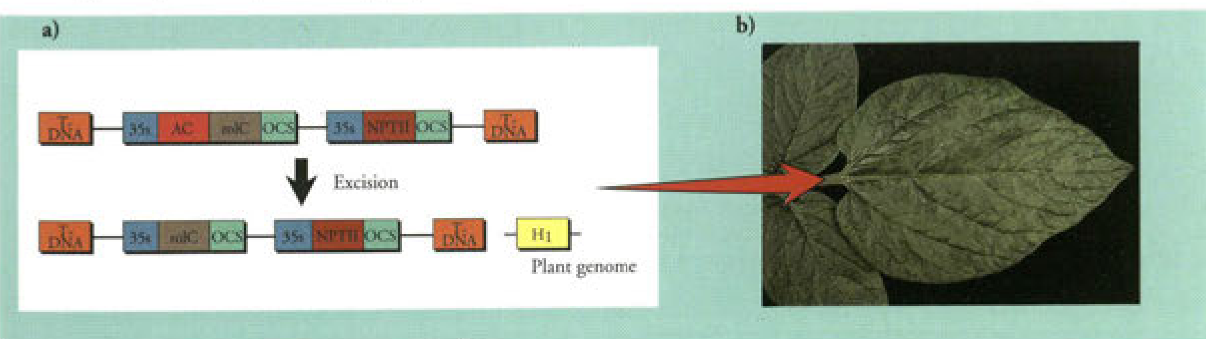

Figure 7 Excision assay for the Ac element in potato. a) Diagram of the construction of SLJ2995 b) Pale green sectors and spots in a potato leaf due to the expression of the rol C gene.

Figure 8 Trypsin inhibition due to expression of CpTi in transgenic soft fruit plants preventing breakdown of the substrate and therefore no yellow colour produced.

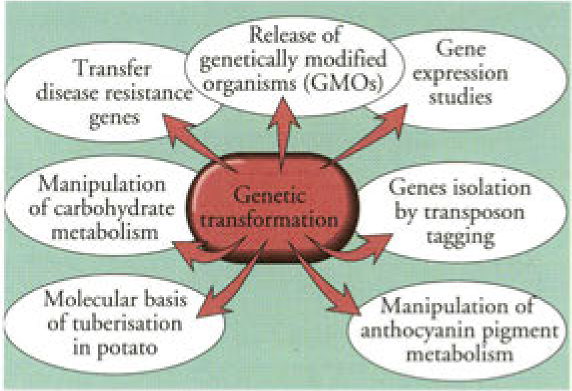

Figure 9 A diagram showing on-going research projects at SCRI.

vector was constructed which contains nucleotide sequences that encode the CP of potato leafroll luteovirus (PLRV) under the control of the 35S promoter of cauliflower mosaic virus (CaMV), and the nopaline synthase (*nos*) terminator. When this chimaeric gene was introduced into potato cv. Désirée and Pentland Squire by *Agrobacterium*-mediated transformation, a large amount of RNA transcripts from the integrated viral gene was detected in the transgenic plants. However, PLRV coat protein was detected in only some of the transgenic plants. When plants were inculated with PLRV, the CP gene induced a measure of resistance to virus multiplication at the secondary infection stage[3].

Cowpea trypsin inhibitor gene in soft fruit The cowpea trypsin inhibitor (CpTi) gene has conferred resistance to a range of insect pests when transferred into several plants. We have introduced a Ti-plasmid based construct (provided by the Agriculture Genetics Company) in which the expression of CpTi coding sequences is under the control of 35S CaMV promoter and *nos* terminator into *Rubus* and *Fragaria* plants. A trypsin assay was developed in which transgenic plants expressing the CpTi could be identified at an early growth stage (Fig. 8) and grown on for further analysis. Presently, the transgenic plants are being screened for resistance to a wide range of chewing insects. Genetic transformation has become an essential component of basic and applied plant science research and several projects at SCRI are utilising transformation methods (Fig. 9). The new challenge is to develop transformation methods for important and often recalcitrant plant species such as cereals and grain legumes. This would then allow the genomic manipulation of these species at the cellular and molecular level for use in both basic and applied research.

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[2] KUMAR, A., WHITTY, P., LYON, J., HOWARD, S. & JONES, J. (1991). *Second International Potato Molecular Biology Symposium (Abstracts).* St Andrews University, UK.

[3] BARKER, H., REAVY, B., KUMAR, A., WEBSTER, J. & MAYO, M. (1992).. *Annals of Applied Biology* 120, 55-64.

Measuring genetic diversity in crop plants

R. Waugh, B.P. Forster, E. Baird, K.J. Chalmers, J.R. Russell & W. Powell

Wild species and primitive cultivars (land races) are rich sources of genetic diversity which can be exploited to enhance the germplasm of crop plants and they frequently exhibit adaptations to extreme environments and resistance to diseases not possessed by their cultivated relatives. However, before such

sources of genetic diversity can be exploited, they must be systematically assessed. Traditionally, a combination of morphological and agronomic criteria have been applied but doubts over their validity have resulted in the increasing use of molecular and biochemical techniques. This article describes some of our work on evaluating genetic resources which may provide a basis for their effective utilisation in future crop improvement.

The application of new polymorphic assay procedures based on the Polymerase Chain Reaction (PCR) and short oligonucleotide primers of arbitrary sequence to determine genetic diversity was described recently (*Ann. Rep. 1990, 25*). The procedure termed Randomly Amplified Polymorphic DNAs (RAPDs) identifies polymorphisms in the DNA of individual plants and is proving exceptionally amenable to studies of plant genetic diversity. We have used RAPDs to estimate the degree of genetic variation within the cultivated potato genepool and,using a selection of primers, we have been able to discriminate unequivocally between a number of common potato cultivars. The procedure could potentially be developed for potato certification schemes and already the similarities and differences between a selection of cultivars have been compiled to illustrate the potential usefulness of RAPDs for cataloguing and describing potato germplasm.

The applicability of rapid methods for determining genetic diversity are especially relevant to perennial tree crops because many of the morphological descriptors which are currently used to discriminate and classify individuals are both limited in number and are expressed only in mature plants. Very little is known about the amount of variation present and how it is distributed in populations of many woody perennial crops. *Gliricidia sepium* and *G. maculata* are multipurpose leguminous tropical trees native to Central America and Mexico and in collaboration with the Oxford Forestry Institute, we have used RAPDs to monitor the genetic variability in 10 populations of *Gliricidia* from different geographical locations. Figure 1 shows the molecular profile obtained using one RAPD primer on five individuals from each of the population types. When several primers were employed, it was possible to partition the variation into within, and between population components. Most of the variation was found between populations although the method revealed some highly heterogeneous and some highly homogeneous populations. In addition, population specific markers were identified. RAPDs provided a cost effective method for the precise and routine evaluation of genetic variability. This information is of direct relevance to genetic resources programmes allowing the identification of areas of maximum genetic diversity which can be targeted during future collection missions.

The preservation of perennial tree crop germplasm resources, both *ex situ* and *in situ*, presents a range of problems. The short seed life (recalcitrance) of many species and their heterozygosity often necessitates the maintenance of germplasm as living tree collections. However, the logistics and financial commitment

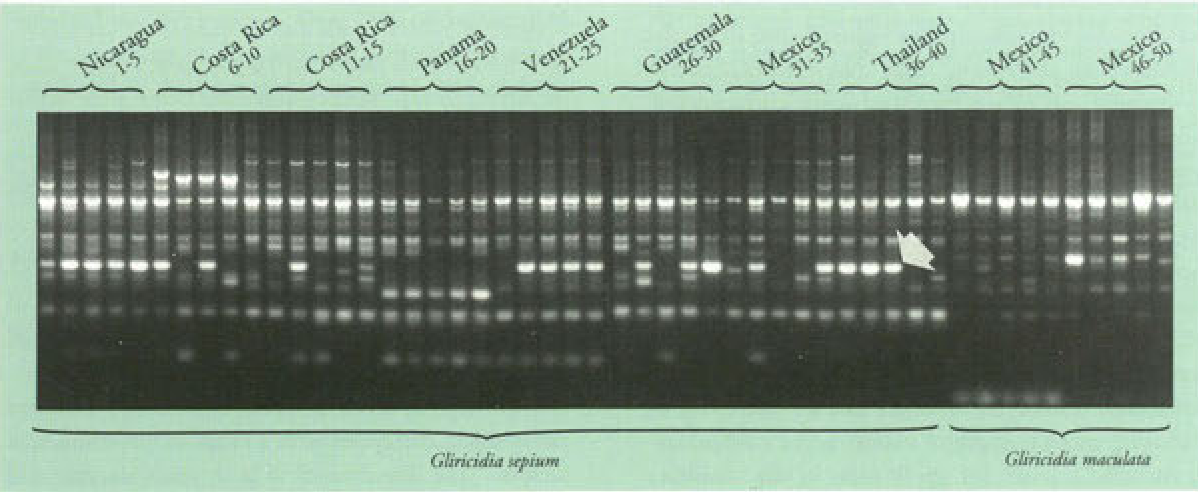


Figure 1 Fifty individual *Gliricidia sepium* or *G. maculata* plants from different geographical locations provided the DNA for an analysis of genetic diversity by RAPDs. The figure shows that there is a high level of diversity in some populations and relatively little in others, but most variation is found between trees sampled from different geographical locations.

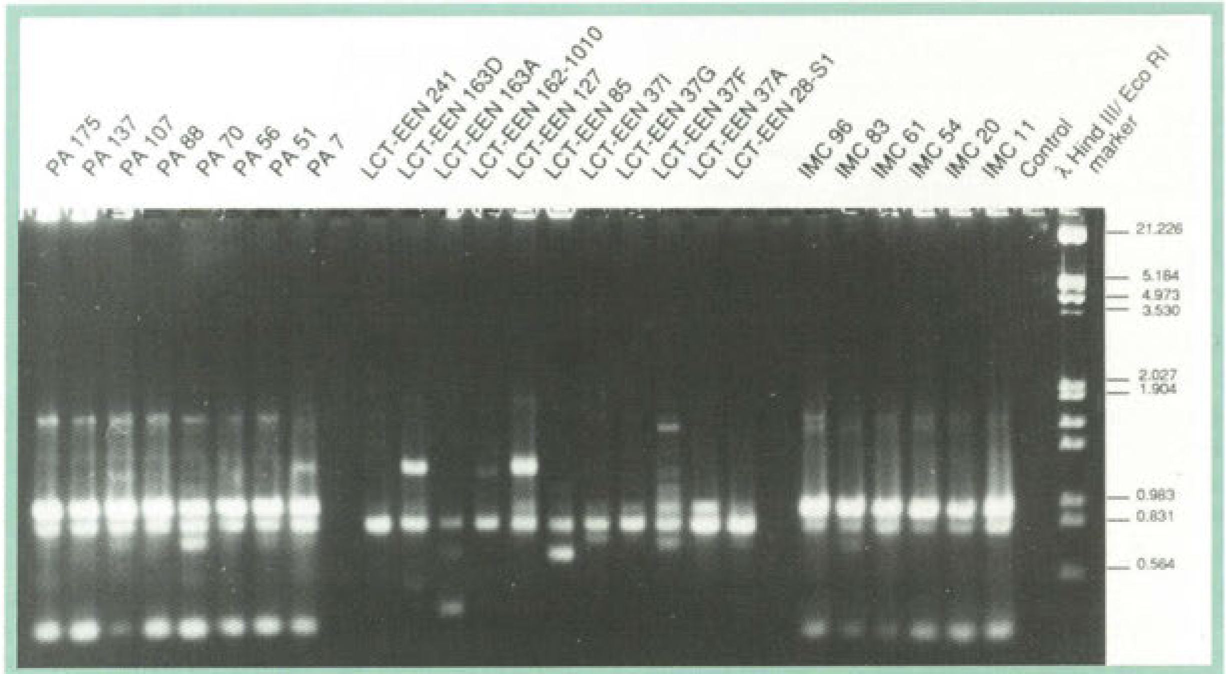
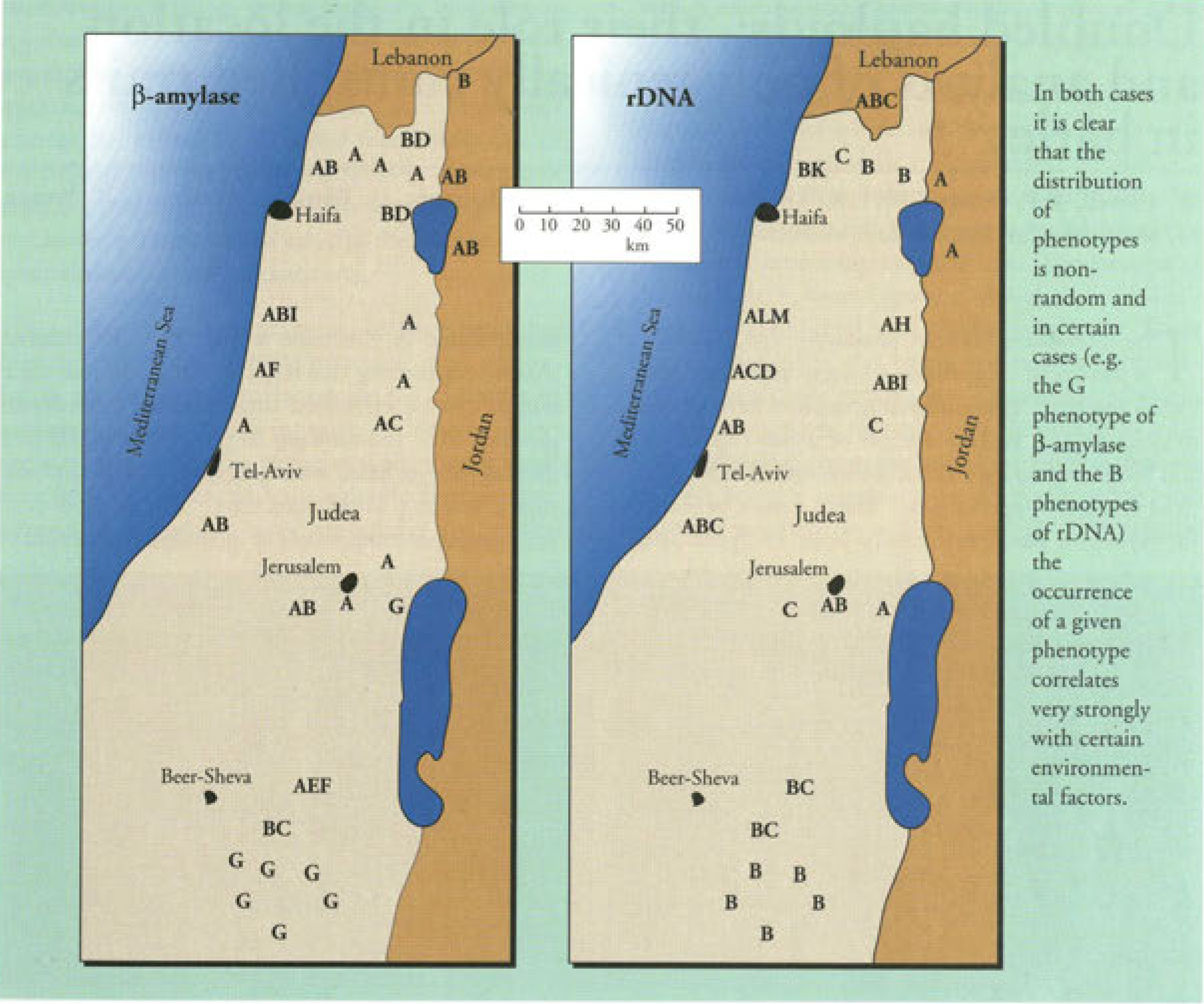


Figure 2 Cocoa accessions collected on three different expeditions to South America (PA, LCT-EEN and IMC) can be differentiated relatively easily using RAPDs. The accessions shown are impossible to distinguish by traditional methods.

involved in maintaining large collections of trees have lead to an urgent requirement for methods to accurately assess the level of variation within them in order to minimise duplication and to establish 'core collections'. In Central and Southern America, widespread deforestation has resulted in the designation of cocoa (*Theobroma cacao*) as a priority crop for conservation and a number of international germplasm collections have been established. We have used RAPDs to examine the variation in both closely and distantly related cocoa accessions. The closely related accessions could not be distinguished using conventional morphological or biochemical markers but 25 genotypes from a collection of individual cocoa trees from different geographical locations in South America could be distinguished or "fingerprinted" using a minimum of just three primers (Fig. 2). The RAPD markers therefore represent a series of descriptors which should facilitate the evaluation of cocoa germplasm and allow the rationalisation of existing genebanks. However, to utilise RAPDs effectively in managing cocoa germplasm resources, the methodology must be suitable for application in the countries which house the collections. Recently, we have been involved in transferring RAPD technology from SCRI to the International Cocoa Research Unit (ICRU) in Trinidad. This has involved training ICRU staff, purchasing and testing equipment, and supplying detailed protocols and troubleshooting guides.

Information gained by the use of marker technologies can extend beyond the synthesis of a genetic 'fingerprint'. For example, it may be possible to relate the variation found in wild species to ecogeographic factors and consider the spatial distribution of the genetic variability. Wild barley, *Hordeum spontaneum*, the progenitor of cultivated barley, exhibits considerable variability in its centre of origin in the Near East, including Israel, Turkey and Iran. The patterns of variability appear, at least in part, to be predictable both ecologically and climatically which suggests that the geographical distribution of this variation may be of adaptive significance. In collaboration with the Institute of Evolution, Haifa, Israel, we examined 135 accessions of *H. spontaneum*. Five plants from a total of 27 populations were chosen to cover the ecological and geographical range of the species in Israel. Variation at specific isozyme and rDNA loci was assessed and correlated with a range of environmental factors. The distribution of both grain isozyme and rDNA phenotypes (profiles) was non-random and particular phenotypes were restricted to specific regions of Israel (Fig. 3). In particular, the G phenotype of ß-amylase was restricted to the Negev Desert



In both cases it is clear that the distribution of phenotypes is non-random and in certain cases (e.g. the G phenotype of β-amylase and the B phenotypes of rDNA) the occurrence of a given phenotype correlates very strongly with certain environmental factors.

Figure 3 The two maps of Israel show the distribution of β-amylase and rDNA phenotypes in 27 populations of wild *Hordeum spontaneum*.

and the Dead Sea regions. Over 78% of the variation in the frequency of this particular phenotype could be correlated with the number of rainy days and the mean temperature in January. Variation at this particular isozyme locus may therefore be of adaptive significance and of value in the introgression of genes controlling abiotic stress tolerance from *H. spontaneum* into the cultivated *H. vulgare* genepool.

An improved understanding of population structure should therefore facilitate the effective exploitation of the wild relatives of cultivated crop plants. Genetic markers are extremely valuable in this work and can be used to monitor the extent and nature of the variability, predict the range of distribution and allow more effective sampling of the genepool.

Doubled haploids: their role in the location and analysis of polygenically controlled traits in barley

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The interaction between phenotype and genotype is of central importance to many aspects of biological research. Experimental geneticists have sought to describe and analyse sources of genetic variation and breeders have used this information to devise artificial selection programmes. The variation available for selection has traditionally been analysed and manipulated in two main ways. The first involves sharply contrasting characters or discontinuous traits which can be classified into discrete phenotypic groups; their transmission to progeny is highly predictable and is based on segregation and recombination following Mendelian laws. The second deals with genetical components of quantitative, metrical or

1 Hybridisation

The female parent is emasculated by the removal of anthers preventing self-pollination.

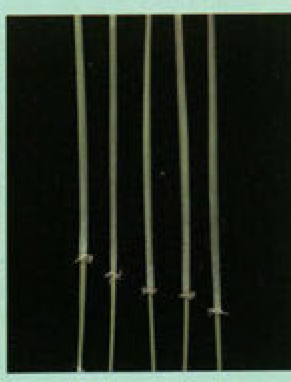
Ears of the male parent are induced to shed pollen.

Cross pollination is effected in a tube by inverting the male ear over the female.

Hybrid seed is allowed to develop and mature on the female ear.

2 Selection and treatment

Donor plants grown at 12°C.



Stems at the appropriate stage are harvested, leaf blades removed and surface sterilised.

The developmental stage of the pollen grains is checked cytologically. Only ears containing uninucleate pollen are pretreated.

Ears are pretreated under high humidity at 4°C for four weeks.

Figure 1 Procedure for producing doubled haploidss in barley via anther culture.

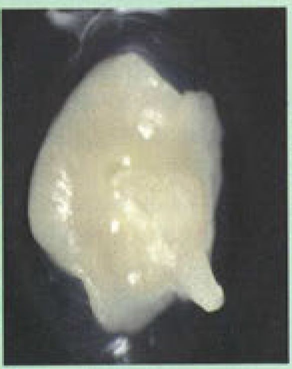
continuous variation which require a biometrical approach based on the means and variances of the populations under study. The genes responsible for quantitative variation are inherited in a Mendelian manner but cannot be identified individually due to the influence of environmental factors. This article describes our research at SCRI to identify regions of the barley genome which control the expression of quantitatively controlled characters.

The success of the programme on barley genetics has been dependent on two main strategies. First, a population which exhibits variation for the character of interest is screened using experimental designs allowing accurate partitioning of the total phenotypic variation into heritable and non-heritable components. Second, polymorphic assay procedures are applied which allow allelic variation independent of other genetical and environmental factors to be monitored.

Rationale for the use of doubled haploids Barley haploids are sporophytes which contain the gametic chromosome number (x = 7), whereas normal barley plants are diploid and contain seven pairs of chromosomes with each member of a pair being identical in a completely inbred homozygous line. The creation of haploids from heterozygous plants gives individuals with one member of each chromosome pair. These can be doubled to give completely homozygous individuals in a single generation. The absence of within family segregation, together with the ability to replicate individual genotypes results in doubled haploids (DHs) being a valuable genetical resource for both cultivar production and the analysis of polygenic

3 Tissue culture

Cultured anthers are induced to produce a mass of embryoids on a nutrient medium containing maltose and hormones.



Close-up of an individual embryoid.

Embryos develop shoots after about four weeks in culture.

Rooting is induced by lowering the maltose concentration of the culture medium.

4 Establishment of homozygous lines

Both haploid (n) and spontaneously doubled haploid (2n) plants are produced. Haploids produce sterile ears and continue to produce shoots (middle). The plant on the left shows the stage at which haploids are 'colchine-doubled' to produce diploids.

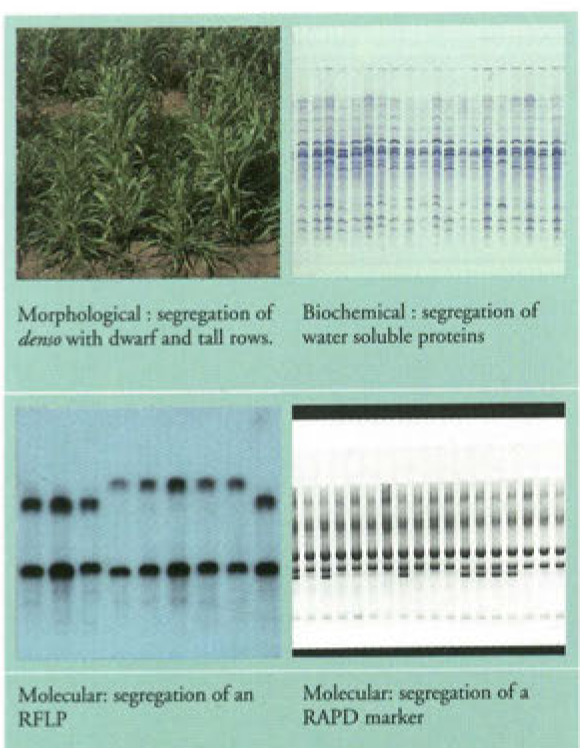
Individual doubled haploid plants are rapidly multiplied to produce homozygous lines. The populations are tested in the field for various agronomic traits.

%G	Heading date	Height	Yield	Milling energy	Hot water extract
erectoides	0	68	37	10	14
denso	31	72	0	0	0

Table 1 Percentage of the genetic variation (%G) associated with the *erectoides* and *denso* dwarfing genes for a range of agronomic and quality traits.

sources of variation. DHs can be derived from plants which have undergone just one round of recombination and therefore give maximal expression of linkage relationships. This is ideal for identifying associations between marker genes and regions of the genome controlling quantitative characters. These attractive features of DHs have prompted researchers to develop methods for the efficient production of DHs in barley.

Methods of producing doubled haploids in barley There are a number of ways of producing DHs in barley, but for them to be of use in genetic analysis, certain criteria have to be fulfilled, (a) it must be possible to produce large numbers easily and consistently, (b) the lines should be genetically normal and stable, and (c) ideally they should represent a random sample of parental gametes. These constraints have led to the exploitation of two routine methods for the production of DH populations in barley. The first of these techniques exploits the elimination of chromosomes in developing hybrid embryos after cultivated barley, *Hordeum vulgare*, is pollinated by *H. bulbosum*, a wild relative. The second technique, anther culture, involves the use of tissue culture technology to divert normal pollen grain development into the production of haploid embryoids. We have recently been successful in greatly increasing the efficiency of anther culture and it is now the preferred technique at SCRI. The breakthrough was made by the substitution of sucrose by maltose as the sole carbohydrate source in the anther culture medium. This resulted in a dramatic increase in the number of DH plants produced. Maltose also induced green plant production by promoting embryogenesis, thus bypassing the more conventional, and problematic callus phase. Callus formation is often associated with aberrant mitotic activity and gametoclonal variation resulting in the regeneration of unbalanced and unstable genotypes. The DHs produced via the embryogenic, maltose-induced pathway were found to be genetically stable when assessed by karyotype analysis, and by screening for genetic variation with biochemical and molecular markers. The methods of producing DHs in barley via anther culture are shown in Figure 1.



Morphological : segregation of *denso* with dwarf and tall rows.

Biochemical : segregation of water soluble proteins

Molecular: segregation of an RFLP

Molecular: segregation of a RAPD marker

Figure 2 Examples of genetic markers, morphological, biochemical and molecular.

Methods of detecting allelic variation The concept of using linked gene markers to predict the transmission of another gene complex is not new. However, this approach has been greatly facilitated by developments in methods to detect polymorphism, particularly at the molecular level. Marker genes fall into four general classes (a) morphological, (b) cytological, (c) biochemical, and (d) molecular (*Ann. Rep. 1990, 25*). Doubled haploid families extracted from F_1 hybrids heterozygous for marker genes will on average segregate in a 1:1 ratio. Classification of the DH families into two groups carrying the alternative allele allows the effect of that particular marker gene on quantitative traits to be assessed. The choice of which marker to use will depend on the precise objective of the study. For example, morphological markers such as dwarfing genes have been identified and used in barley breeding programmes to produce short-strawed lines resistant to lodging. In these circumstances, it is important to identify the effects of the genes on quantitative characters so that the consequences of using dwarfing genes in breeding programmes can be estab-

lished. The *erectoides* dwarfing gene found in cv. Golden promise and the *denso* dwarfing gene found in cv. Maris Mink and Triumph both confer distinct juvenile growth habits and have been widely used in spring barley breeding. A series of experiments have been conducted to identify genetic associations between these gene and quantitative traits. Estimates of the percentage of the genetic variation associated with the dwarfing genes and some quantitative traits are given in Table 1. Allelic differences at these loci are responsible for a large percentage of the genetic variation for yield, quality and certain agronomic traits. In many cases the dwarf allele exhibits an undesired expression of the associated trait and comparisons of this nature can assist in the formulation of breeding strategies targeted at reversing the situation.

The number of morphological markers available to locate quantitative trait loci (QTL) is limited and therefore there is a need to accumulate more polymorphic markers which segregate in a common cross. This requirement is met to a certain extent by isozymes. Associations between alleles at the *Amy1* locus (controlling formation of the enzyme α-amylase) on chromosome 6H and a QTL for single plant yield have been identified. More widespread coverage of the genome arising from the use of RFLPs and RAPDs have also enhanced the prospect of mapping QTL. Examples of the various marker techniques is given in Figure 2. Simply monitoring the segregation of genetic markers and searching for associations with quantitative traits is, however, a relatively inefficient procedure and strategies are needed to target markers in regions of the genome which are important in controlling the expression of quantitative traits.

Bulked segregant analysis for the rapid identification of QTL Continuous distributions that often characterise quantitative traits are represented by a range of individual genotypes each segregating for genes controlling the trait of interest. Genotypes found in the tails of the distribution are expected to differ at most of the loci controlling the character so that the alleles (increasing and decreasing) are highly associated. In addition to differing for QTL these genotypes are also expected to differ for individual loci linked to the quantitative trait of interest. This observation therefore provides a strategy to quickly map QTL. DNA from the extreme members of the distribution can be pooled to form two bulk samples which can then be screened with genetic markers to identify those which are polymorphic. We have used this approach to identify RAPDs linked to a QTL controlling milling energy in barley, illustrated in Figure 3. Four random primers were identified which detect polymorphism in the bulks and these were shown to be linked to the *Rrn2* locus on the short arm of chromosome 5H. The genetic map of this region of the barley genome is given in Figure 4 together with the likelihood plot for milling energy obtained from the genetic mapping package MAPMAKER/QLT. Additionally, analysis of variance shows significant differences between the mean values of milling energy for the two alleles at each marker. These results are shown in Table 2. For the linked markers on chromosome 5H the highest variance ratio is that of RAPDmarker OPD13-H900, suggesting that this is closest to the QTL. Normal

Marker locus	Variance ratio (df)	F probability
OPJ5-H850	16.20 (1,41)	2.4×10^{-4}
OPE11-H1500	29.34 (1,41)	2.9×10^{-6}
OPD13-H900	32.85 (1,41)	1.0×10^{-6}
Rrn2	20.58 (1,42)	4.7×10^{-5}
OPM14-H2000	23.88 (1,38)	1.9×10^{-5}

Table 2 Analysis of variance for variation of milling energy with marker loci.

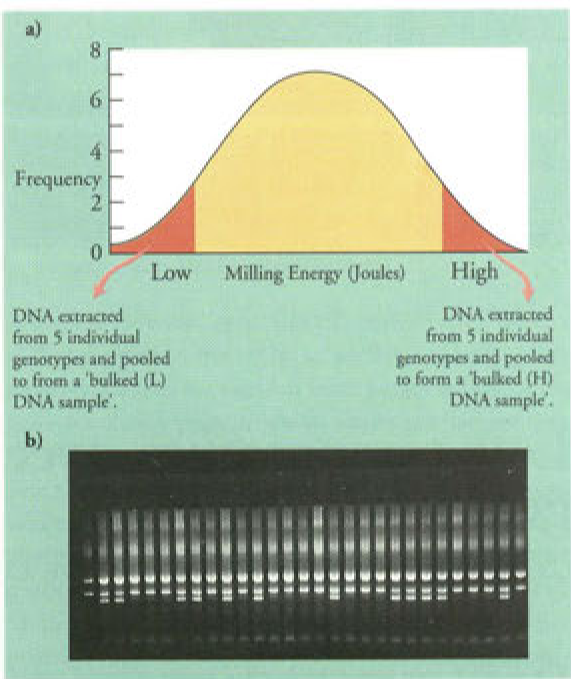


Figure 3 a) Frequency distribution for milling energy in a doubled haploid population of barley. The H and L DNA bulks are screened for polymorphism with a range of RAPD primers.
b)Polymorphism detected between the two bulks when screened with primer OPD13-H900.

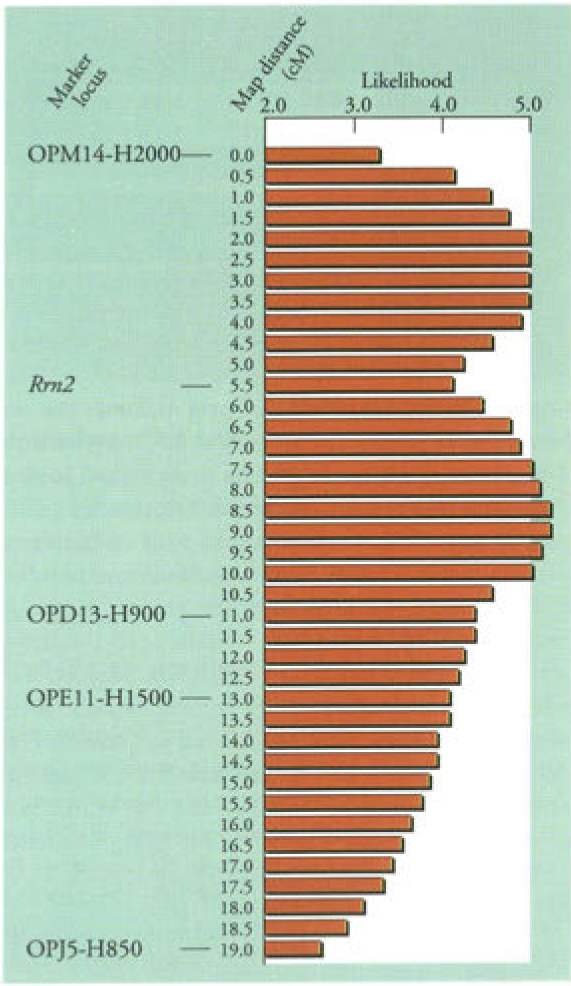


Figure 4 Genetic map of barley chromosome 5H showing the linkage and likelihood plot of a QTL for milling energy relative to the *Rrn2* locus and RAPD loci.

mixture models can also be used to analyse the association of the trait with the marker locus. They provide estimates of the recombination fraction between a QTL and the marker and show that the QTL controlling ME on chromosome 5H is almost certainly located between the *Rrn2* and the OPD13-H900 loci.

Conclusions and future prospects The approaches and methods outlined in this report are the outcome of collaboration between several different research areas with a common objective of understanding the genetic basis of continuously varying characters. The application of this knowledge will allow crop improvement programmes to proceed with greater precision and efficiency and, in particular, gene introgression from unadapted germplasm into adapted genotypes will be more precise. An improved understanding of the genetic organisation of quantitative traits will provide fresh insights into the mechanisms responsible for preserving and utilising this form of variation. The considerable gene synteny which exists between various crop groups will also facilitate the localisation of genetic traits in related organisms. In the long term, the localisation of QTL to specific regions of the genome in combination with physical or map based gene cloning techniques will open up the possibility of isolating the gene sequences responsible for the control of quantitative traits and lead to improved understanding of biochemical and physiological pathways.

Low temperature sweetening and invertase genes of potato

G.C. Machray, P. Hedley, R. Waugh, L. Burch, E. Cuthbert & H.V. Davies

Research into low temperature sweetening of potatoes forms part of a large multinational effort supported by industry and the European Commission under its ECLAIR program. Several key enzymes involved in carbohydrate metabolism in cold stored tubers, the reactions they catalyse, and the research groups involved in this study are detailed in Figure 1.

The overall objective is to modify the levels of enzymes in the cold stored tuber in order to minimise the accumulation of reducing sugars. When subjected to the high temperatures required to make crisps and chips, reducing sugars react with amino acids in a complex process known as the Maillard reaction which results in dark brown discolouration and

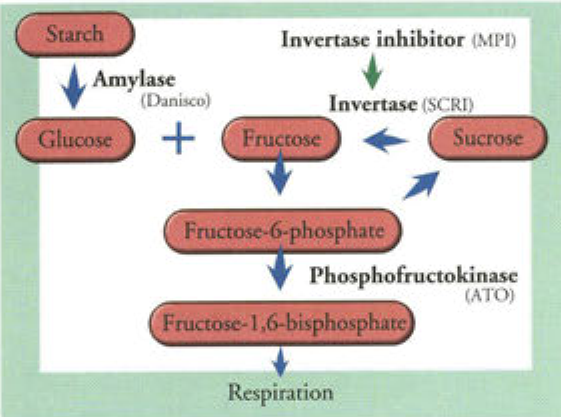


Figure 1 Selected carbohydrate interconversions in potato tubers. Shown are the enzymes under study and the participating groups in the program.
ATO = Agrotechnical Research Institute, Wageningen.
Danisco = Danisco A/S, Copenhagen
MPI = Max Planck Institute, Cologne
SCRI = Scottish Crop Research Institute, Dundee

unpleasant flavouring. This problem could be alleviated by reducing the concentration of these sugars by lowering levels of α-amylase, starch phosphorylase or invertase in the tuber while increasing the level of the invertase inhibitor. Also, incorporating a non-cold labile ATP:phosphofructokinase may prevent the diversion of hexose phosphates into sugar biosynthesis. The strategy adopted to effect these modifications is to genetically manipulate potato cultivars which are presently used for processing and retain other desirable characters previously bred into them.

There is evidence to suggest that the reducing sugars glucose and fructose present in the cold stored tuber are derived mainly from the direct cleavage of sucrose by acid invertase, an enzyme under study at SCRI. Using molecular and biochemical approaches we have identified and cloned two different invertase genes and purified invertase proteins from various potato tissues. One invertase gene (INV1) was selected from a genomic library of potato DNA by hybridisation to a cell wall invertase gene from carrot. The structure of this gene is shown in Figure 2. The coding sequences (exons) are interrupted by five non-coding regions

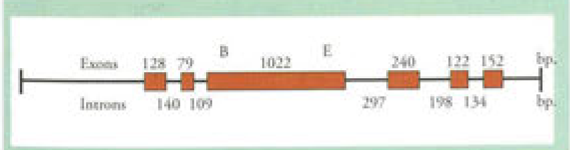


Figure 2 Structure of the INV1 gene. Exons are blocked, lines indicate introns. Sizes are given in base pairs.

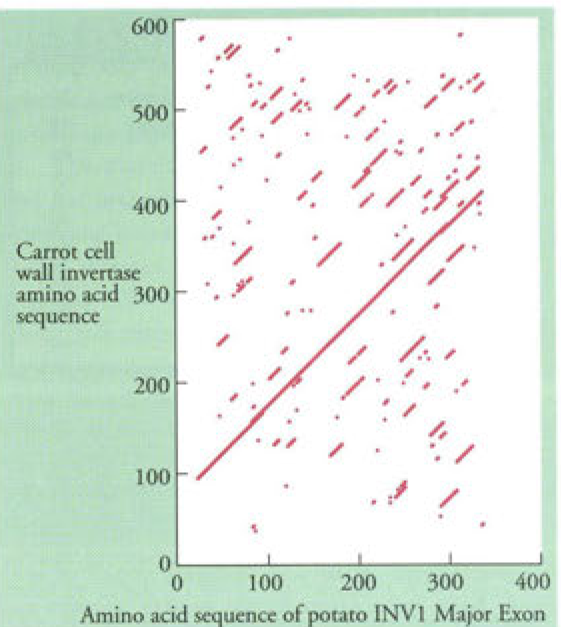


Figure 3 Dotplot comparison of the 341 amino acids of the major exon of INV1 to the entire carrot invertase amino acid sequence. The homology between the sequences is revealed as the continuous diagonal line.

(introns), as in the carrot gene. Significant homology exists between the potato and carrot genes shown in Figure 3 which illustrates the similarity between the derived amino acid sequence from the major exon of the potato gene and the carrot sequence. This potato gene clearly encodes an enzyme of the classic family of sucrose-hydrolysing proteins. In higher plants, the genes for the carrot and tomato acid invertases have been identified but the family also includes prokaryotic, fungal and animal representatives.

A second invertase gene (INV2) was selected from a cDNA expression library by an antibody raised against an invertase enzyme purified from potato tubers. This gene encodes a protein which shows similarities to a second set of enzymes, the ß-glucosidases. Only one plant member of this family has been cloned, from white clover *Trifolium repens*, but others have been cloned from other organisms (Fig. 4). Both invertase genes have been cloned into expression vectors (Fig. 5) in *Escherichia coli* allowing large amounts of the proteins they encode to be prepared, aiding biochemical and physiological studies of the enzymes which have proven difficult to identify and purify from tubers.

The second phase of the project involves an analysis of the expression of the cloned genes and modification of this expression using genetic manipulation and plant

Enzyme	Organism	% identity
β-glucosidase	*Caldocellum saccharolyticum*	50
	Agrobacterium tumefaciens	38
	Escherichia coli	46
	Trifolium repens	57
β-galactosidase	*Staphylococcus aureus*	46
	Streptococcus lactis	54
	Lactobacillus casei	42
Lactase	Human	50
	Rabbit	50

Figure 4 The β-glucosidase gene family. Figures given are the percent identical amino acid residues between the tuber invertase protein amino-terminal sequence (26 amino acids) and each family member.

transformation. Sense and antisense copies of both genes have been cloned into binary vectors and returned to potato plants via *Agrobacterium*-mediated plant transformation. While the primary goal is to determine what changes can be effected to carbohydrate metabolism in cold stored tubers, it will be interesting to examine wider effects on the physiology of the transgenic plants. Plants over-expressing heterologous invertase genes exhibit profound effects on source-sink relationships, carbohydrate partitioning and photosynthesis. The choice of promoter will be crucial for a directed modification to a specific process at a given time and temperature. Much progress has been made at the other centres involved in the research programme. Genes for α-amylase, starch phosphorylase, ATP:phosphofructokinase from a psychrophilic bacterium, and a putative invertase inhibitor have been cloned and transformed back into plants. Analysis of the effects, with respect to low temperature sweetening, of the appropriate modification to the level of each individual endogenous enzyme can now be determined. Future work will focus on combining any desirable effects achieved by genetic modifications to generate varieties whose low temperature sweetening and other characteristics can meet the requirements of the potato processing industry.

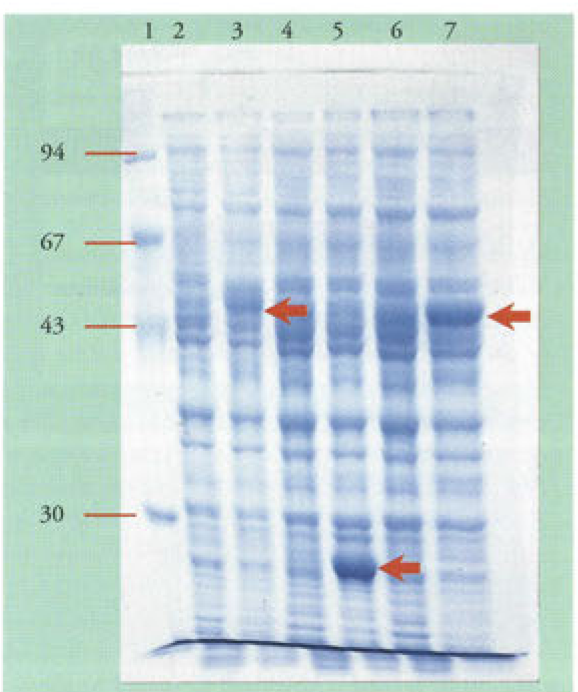


Figure 5 Protein extracts from *E.coli* showing GST-fusion proteins (arrowed) induced by IPTG from constructs including INV1 (lane 7) or INV2 (lane 3) sequences cloned into pGEX2T or the GST protein from the pGEX2T vector (lane 5). Lanes 2,4 & 6 contain the corresponding control extracts from uninduced *E.coli*. Lane 1 contains protein markers of indicated size in kDa.

Pre-mRNA splicing in plants

J.W.S. Brown, C.G. Simpson & R. Waugh

An understanding of how plant genes are expressed is fundamental to modern approaches to plant science in general and plant biotechnology in particular. Gene expression covers a multitude of processes beginning with the gene (DNA) and ending with proteins and enzymes which determine the final characteristics of the plant.

When a gene is turned on the DNA is copied into a precursor messenger RNA (pre-mRNA) by transcription. The pre-mRNA is processed further into a mature messenger RNA (mRNA) which acts as a template for protein production by translation (Fig. 1). Of the many different processing events which the pre-mRNA undergoes during its maturation the

removal of intron sequences is of primary importance (Fig. 1). Failure to remove introns by the process of RNA splicing would disrupt normal protein production and could have drastic effects on the biochemistry and physiology of plant cells and thereby plant development and differentiation.

The importance of this fundamental cellular process has been further emphasised in three observations. Firstly, when genes from monocotyledonous species (e.g. cereals) are introduced into dicotyledonous species (e.g. tobacco, potato) by genetic engineering, the genes are often poorly expressed and this has been shown to be due to inefficient intron removal. This inferred difference in splicing between monocotyledonous and dicotyledonous plants is an unexpected phenomenon and has important consequences for genetic engineering programmes. Secondly, the presence of certain introns in plant genes increases the levels of expression of the genes containing them. This phenomenon is being exploited in plant biotechnology programmes to produce higher levels of gene expression. Finally, alternative splicing systems whereby multiple mRNAs, and thereby proteins, are produced from a single pre-mRNA are well established in animal systems and are involved in key developmental and differentiation processes. There are only a handful of examples of alternative splicing in plants but, as more gene systems are examined, the importance of this phenomenon to post-transcriptional control of gene expression is expected to increase greatly.

To understand these phenomena, a detailed understanding of pre-mRNA splicing in plants is essential.

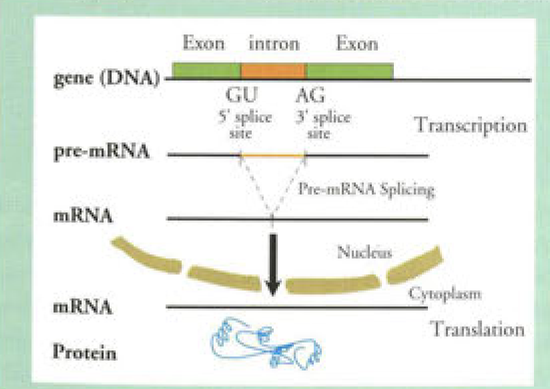


Figure 1 Genes are transcribed into pre-mRNAs containing exon and intron sequences. By the process of pre-mRNA splicing, introns are removed by excision at the 5' and 3' splice sites and exons are rejoined to give the mature mRNA. The mRNA transcripts then leave the nucleus to be translated into protein in the cytoplasm.

Pre-mRNA splicing has been extensively studied in animals and yeast, and the mechanism by which introns are removed and the protein coding regions (exons) are joined together has been elucidated (Fig. 1). The study of splicing in plants is more difficult and has necessitated the development of systems of expressing intron-containing genes in plant protoplasts and transgenic plants, and of analysing the RNA products. We have developed an expression vector which will transcribe a pre-mRNA containing different intron constructions when introduced into protoplasts and transgenics (Fig. 2).

Isolated RNA is analysed using specific oligonucleotides in reverse transcription and polymerase chain reactions (RT-PCR). RT-PCR is a procedure that utilises reverse transcriptase to transcribe a complementary DNA (cDNA) copy from a RNA template. The cDNA copy is then amplified by successive rounds of *Taq* DNA polymerase transcription in a PCR reaction. The oligonucleotides indicated in Figure 2 are used to prime the reverse transcriptase and *Taq* DNA polymerase reactions. They, therefore, demarcate the boundaries of the region of RNA under analysis. RT-PCR is highly specific, accurate and extremely sensitive permitting the detection of unspliced (intron-containing) and spliced (intron-excised) RNAs and an assessment of the accuracy and efficiency of the splicing reaction (Fig. 3).

Intron constructs based on the inefficiently spliced wheat amylase intron and the efficiently spliced pea legumin intron (Fig. 3) have shown that the difference in splicing between monocotyledonous and dicotyledonous plants does not lie at the level of intron splice sites but involves the content of adenosine (A) and

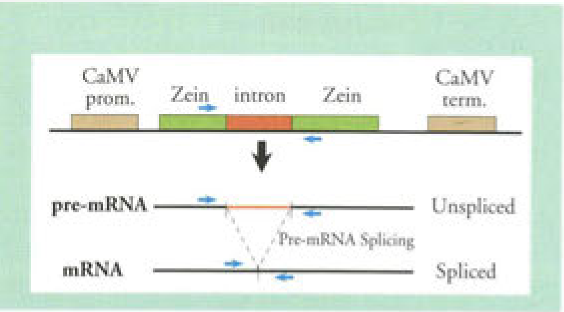


Figure 2 The expression vector used to study splicing consists of the cauliflower mosaic virus (CaMV) promoter/terminator regions driving transcription of a zein storage protein gene containing different intron constructs. RNA transcripts are analysed by RT-PCR using oligonucleotide primers bordering the intron sequence.

uridine (U) nucleotides in the intron sequence (Fig. 3). This is best illustrated in the case of the amylase intron where although splicing proceeds inefficiently, the correct 5' and 3' splice sites are utilised, even though other potential 5' splice sites are present. The importance of AU content to efficient splicing in dicotyledonous plants is highlighted by the increase in efficiency displayed by the amylase and legumin hybrid intron constructs over the amylase intron itself (Fig. 4). In these examples, AU-rich regions in either 5' or 3' halves of the intron have a dominant effect and effectively rescue the splicing efficiency of the AU-poor region. Therefore, a combination of AU-rich sequences and acceptable splice sites are needed to promote splicing in dicotyledonous plants. Thus, it appears that, as in animals, all the sequence information required for accurate splice site selection is present in the intron and the flanking exon sequences and accurate splice site selection occurs largely irrespective of the nucleotide composition of the intron (Fig. 3). Given the similarity of intron splice site sequences and transacting RNA and protein splicing factors, it is reasonable to expect that the basic splicing mechanism in plants will be similar to that of animals. However, any proposed mechanism of splicing in plants must take into account the obvious effect of AU-rich sequence content on the efficiency of splicing but it is not clear how AU-rich regions have their effect. It has been suggested that AU regions may be target sites for specific proteins. Our results which separate the process of splice site selection from the requirement for AU-rich regions indicate a role for such proteins in the formation of the large RNA-protein complex (spliceosome) in which splicing occurs

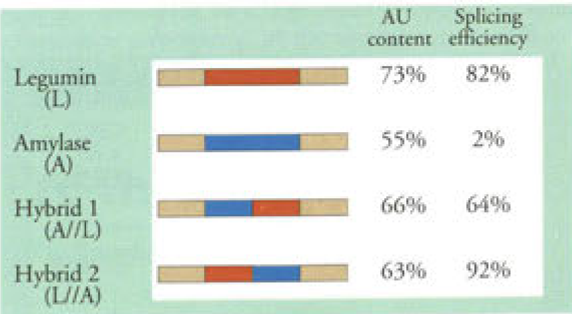


Figure 3 Splicing of monocotyledonous and dicotyledonous introns in tobacco protoplasts. The dicotyledonous pea legumin, monocotyledonous wheat amylase and reciprocal hybrid introns are shown schematically. Their AU content and splicing efficiency in tobacco protoplasts are given.

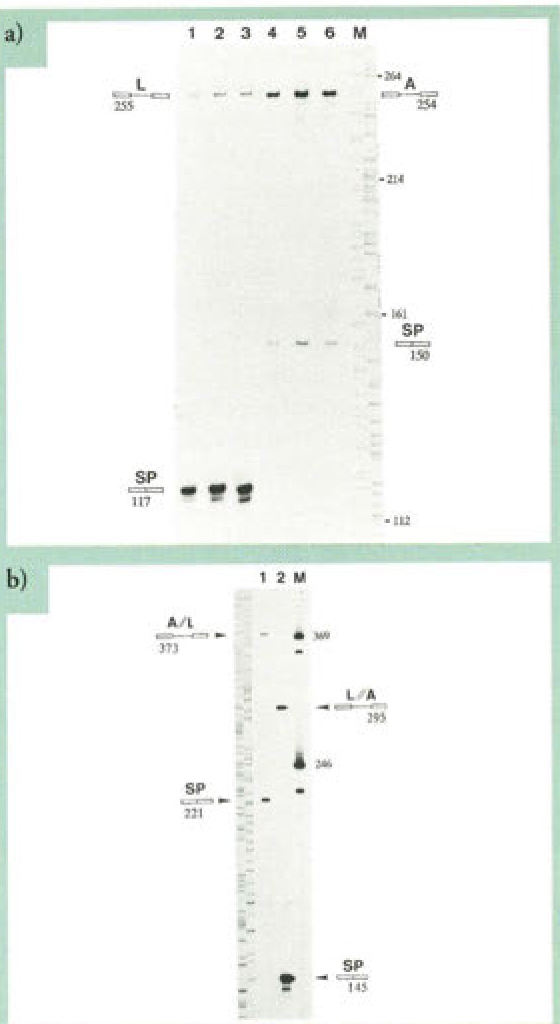


Figure 4 RT-PCR analysis of splicing of monocotyledonous, dicotyledonous and hybrid introns in tobacco protoplasts. (a) The monocotyledonous intron is poorly spliced in tobacco protoplasts (lanes 4-6) compared to the dicotyledonous intron (lanes 1-3). (b) When regions of the monocotyledonous intron are fused to regions of the efficiently spliced dicotyledonous intron, splicing efficiency is rescued (lanes 1-2). The unspliced products (L, A, A//L and L//A) and spliced products (SP) are indicated.

(*Ann. Rep. 1990, 28*). Alternatively, AU-rich regions may have a non-specific role in splicing by limiting the formation of RNA secondary structure allowing splicing factors to assemble and participate in splicing more efficiently. The expression and analysis system described here is being exploited in a detailed examination of how plant introns are defined and recognised by the factors involved in plant pre-mRNA splicing. Information currently being generated is greatly enhancing our knowledge of this area of molecular biology and gene expression.

Cellular and Environmental Physiology

H.V. Davies

The research objectives within Cellular and Environmental Physiology are aimed at providing a quantitative and qualitative understanding of the processes which govern plant growth, development and quality. The empirical, field-based approach has been largely replaced by highly targeted objectives which have gained substantial financial support from outside agencies. However, the potential to respond to market forces is retained, as evidenced by the award of contracts from commercial companies for rigorous and professional field trialling of agricultural products.

Two new research initiatives have been identified for support through "seedcorn" funding. These are, specifically, on the use of the natural abundance of stable isotopes in biological research (see p. 59) and an initiative on woody species. As far as the latter is concerned, a project on molecular mechanisms underpinning dormancy break in seeds of forest species is already underway and this will be extended to include, amongst other things, dormancy of perennating buds in woody shoots and hardening and subsequent de-hardening of over-wintering woody shoots. The initiative will involve several Departments in a coordinated effort.

A mechanistic understanding of stress tolerance continues to have high priority. The mathematical model describing water-constrained yield in the potato crop has been used to provide a quantified assessment of the value of specific plant strategies in tolerating drought conditions. The model of water-constrained growth in potato will be refined further during the next few years by incorporating detailed experimental data from a collaborative programme with ADAS and Cambridge University Farm (funded by the PMB). Given restrictions in the use of water for irrigation purposes in parts of the UK, it is imperative that the effects of soil moisture regime on crop growth and development are quantified. The programme will also focus on limiting nitrate pollution in groundwater by tailoring nitrogen inputs to meet crop demand. The interaction between water and nitrogen supply and its effect on growth will also be modelled. It is hoped that additional funding will become available to support the specific areas of strategic work required to strengthen the overall programme. A hydroponic system is being developed for growing potato plants under conditions of salt stress. This will be used to

determine the degree of variation in salt tolerance with species.

A pressure probe-microinjection system has been developed and used to demonstrate that plasmodesmata function as pressure-sensitive valves. The valves close in response to pressure differentials induced between adjacent cells, a finding which has implications for wounding responses (induced mechanically or by pathogens). The first response to cell damage following the loss of cell turgor may be the pressure-generated closure of plasmodesmata to isolate the damaged regions. The mechanism may also regulate symplastic phloem unloading in storage sinks. This will be explored in collaboration with the University of Utrecht. Fluorescent probes continue to be used effectively to monitor the sequestration of xenobiotics by plant vacuoles. Attention has focused recently on the observation that the drug probenecid, a potent inhibitor of anion transport in mammalian cells, blocks the sequestration mechanism at the vacuole membrane causing accumulation of specific fluorescent probes in the cytosol.

Research continues on the process of sucrose-starch interconversion in storage sinks, using potato tubers and cotyledons of developing faba bean as model systems. The programme continues to place emphasis on a combined approach encompassing plant metabolism (pathways of carbon flux determined, for example, using NMR), enzymology (protein purification and kinetic characterisation) and the isolation of specific genes. The article on low temperature sugar accumulation and the isolation of acid invertase genes (p. 47) represents an excellent example of the success of an interdepartmental, multidisciplinary approach. This work will be extended to isolate invertases from other commercially important crop species. Alkaline invertase and sucrose synthase have been purified for the first time from *Vicia faba* cotyledons and the enzymes characterised. The first cloning of a plant fructokinase gene has resulted from previous work on protein purification. As with the other cloned genes associated with carbohydrate metabolism, transformation technology will be used to determine the effects of modifying enzyme levels on carbohydrate fluxes. This includes a trans-European effort to determine the role of specific pyrophosphatases in regulating pyrophosphate concentration in subcellular compartments.

Substantial progress has been made in isolating novel genes showing increased expression in the stolon tips of potato during the tuber initiation process. The expression pattern of seven cDNA clones isolated by differential screening has been thoroughly characterised. Transcript levels of all seven increase substantially during the early stages of tuberisation. The genes are also expressed in leaf, stem and root, but the level of transcript in these tissues also changes in plants induced to tuberise. Five of the seven clones have been sequenced to completion. One has been identified as the S-adenosylmethionine decarboxylase gene (involved in polyamine biosynthesis), another two are highly homologous to eukaryotic ribosomal protein genes (S19 and L7). Polyamine biosynthesis during tuberisation is currently being determined. The function of other genes will be assessed using an antisense approach to down regulate transcript levels. A collaborative project on identifying ripening-related genes in blackcurrant has been initiated.

Long term investigations into the persistence of, and seedling emergence from, seven arable weed species under continuous winter crop rotations have been completed. This work forms part of a continuing joint project with the IACR on modelling weed population dynamics. In collaboration with IACR, ADAS, SAC and DANI, analysis of the soil seedbanks at the start of long term field experiments involving reduced herbicide inputs, set-aside, headland management and organic farming have established base lines from which changes in seed population can be assessed. Results have demonstrated the size, diversity and species composition of the original seedbanks and their evenness of distribution across experimental sites. Statistical techniques have been developed for the effective analysis of data of this type. The data has revealed, amongst other things, that an initial sowing of clover on set-aside plots prevents a major increase in the seedbank of arable annual weeds.

An application has been made, using SCRI data, for off-label approval for the use of sodium monochloracetate as a raspberry cane desiccant to replace dinoseb. If successful, this could allow growers to re-introduce cane vigour control in their crop management system. Work continues on other alternatives, including fomesafen. The herbicide, desiccant and growth regulator database MICROHERB, developed jointly with Queens University, Belfast, is now widely used by agricultural advisers, agrochemical companies and agricultural colleges throughout the UK.

Research into soil-plant dynamics falls into three basic areas: plant-environment interaction, soil-plant

microbial interactions and non-linear mathematics. All of these are concerned with tackling the inherent variability (spatial, temporal and species) which is a feature of the soil-plant system. As referred to earlier, facilities for measuring the natural abundance of stable isotopes in the environment are being upgraded and will provide a greater insight into the major pathways of elemental flow at levels of complexity ranging between molecular and agro-ecological levels. The technique has clear relevance for many projects, for example, exploring trophic interactions in the soil-plant system and tracing and quantifying carbon movements between plants via mycorrhizas (exploiting ^{13}C discrimination between C3 and C4 species). ^{13}C discrimination will be used to measure the relation between plant growth and the N and water costs of that growth. Techniques for following nitrogen transformations in soil using variations in the ^{15}N:^{14}N ratio are being developed. A system is also under construction to permit control of CO_2 concentrations in the atmosphere. The system will allow relatively unrestricted rooting depth while allowing non-destructive access to the root system. Effects of manipulating carbon supply to the plant on carbon allocation to, and within, the root can then be quantified.

Volatile gaseous products are being used to characterise microbial populations and their physiological activity, while DNA hybridisation techniques are used to characterise microbial community structure. Nematode and protozoan grazers of micro-organisms are quantified in the rhizosphere, and at sites of decomposition, to determine their effect on nitrogen availability.

Non-linear mathematics and fractal geometry are applied to topics ranging from diffusion in heterogeneous media to the spatio-temporal dynamics of microbial populations, morphogenesis and epidemiology. A unified theoretical framework is sought with the goal of understanding the processes which govern transport and cycling of nutrients and xenobiotics in the soil. Relevant geometries are being developed to integrate with dynamic theories of gas flow, microbial population growth and movement. On a larger scale, theories of spatio-temporal dynamics of airborne pathogens are receiving attention with special emphasis on host patchiness and spatial spread. To strengthen the interdisciplinary nature of such programmes the Dundee Centre for Nonlinear Theory in Biology has been established incorporating individuals from Dundee University Mathematics and Computer Department, SCRI and SASS. The objective is the development and application of novel mathematical and statistical techniques to biological research. The mathematical approach is further strengthened by the use of artificial intelligence techniques to integrate uncertain knowledge based on climate models and qualitative information relating to pests and diseases and to determine the likely impacts of climate change on Scottish agriculture. This work forms part of a larger programme funded by SOAFD and coordinated by SCRI.

Sucrose starch interconversion in potato tubers

R. Viola & H.V. Davies

Sucrose produced in the photosynthetically active parts of developing potato plants is translocated into the growing tubers where it is rapidly converted into starch. The conversion of soluble sucrose into starch, a highly dehydrated polysaccharide in the tubers, drives the accumulation of carbon in this tissue. Starch biosynthesis represents by far the most important metabolic event during tuber growth and starch commonly constitutes 65-70% of the dry matter at tuber maturity (Fig. 1). There is considerable interest in understanding the mechanisms regulating "sink" strength in plants and elucidating the key factors limiting the efficiency of photosynthate conver-

Figure 1 Fluorescence image of isolated cell from mature potato tubers showing starch granules (courtesy of K.J.Oparka).

sion into starch because they may provide a means to improve starch yield in commercially important crop species. In spite of the obvious importance of starch in the human diet and in a range of industrial processes, the precise pathway of starch synthesis in starch-storing organs is not yet known.

Sucrose metabolism The mechanisms regulating unloading of sucrose in the developing potato tubers and its entry into the storage cells have been described previously[1]. Sucrose unloading in developing tubers occurs via the symplastic network but it is unknown whether, under normal circumstances, a significant proportion of sucrose is transported into the vacuole when it enters individual cells. However, the activities of enzymes involved in sucrose breakdown are very high in developing tubers. In particular, sucrose synthase activity increases markedly in developing tubers at the onset of rapid starch deposition and it is likely that most if not all incoming sucrose is catabolised by this enzyme. As sucrose synthase activity is confined to the cytosolic compartment, the majority of incoming sucrose is readily metabolised upon its entrance into the storage cell.

The rate of sucrose influx into the tuber seems to be, in itself, an important factor determining the potential for starch synthesis. For example, when sucrose import into developing tubers is terminated by detaching tubers from the mother plant, the capacity of the tuber tissue to convert exogenous metabolic precursors (sucrose, glucose and fructose) into starch is markedly reduced. This is associated with significant declines in sucrose synthase (90%) and ADP glucose pyrophosphorylase (50%) activities in detached tubers[2]. Thus the activities of enzymes involved in converting photosynthate into starch appear to be related to the import of photosynthate itself. Indeed, the decline in sucrose synthase in detached tubers can be prevented if sucrose is supplied directly to tubers through the cut surface of the stolon.

The conversion of sucrose into starch is not the only mechanism driving sucrose import into the tuber. When starch synthesis in the tuber tissue is reduced experimentally by detaching tubers or inhibited by treating tuber storage cells with sodium fluoride, the uptake of metabolic precursors, glucose or fructose, was not reduced significantly. Instead, glucose and fructose are converted into sucrose rather than starch, i.e. sucrose rather than starch biosynthesis becomes the primary metabolic sink for the hexose-phosphates derived from glucose and fructose metabolism. It is presumed, but not yet proven, that the sucrose is then stored in the vacuole.

Carbon flux into the amyloplast Whilst sucrose breakdown takes place in the cytosol, starch biosynthesis is confined to the amyloplasts, which are highly specialised, starch-storing plastids. The physical separation between the enzymes of sucrose and starch metabolism is important for the regulation of carbon metabolism in plant cells. The complex interaction of feed-back and fine control mechanisms which regulate carbon flux between the chloroplast and the cytosol in green cells have been studied in detail but the regulation of carbon flux into the amyloplasts in cells of non-photosynthetic starch storing organs is largely unknown. This is mainly due to the difficulties associated with the isolation of intact and functional amyloplasts. The lack of information on the transport properties of these organelles represents a serious obstacle to the definition of the precise pathway of sucrose conversion into starch. In particular, little information is available on the form in which carbon is transported into the amyloplast. If carbon flux into the amyloplasts occurs in the form of three-carbon (C3) compounds (by analogy with the chloroplasts) additional regulatory steps are likely to be involved compared to the situation where six-carbon (C6) compounds are transported. In order to investigate this problem we have adopted non-destructive ^{13}C-NMR techniques which can provide a detailed description of the intramolecular partitioning of ^{13}C isotope following the introduction into the cell of a substrate labelled in a specific carbon position (e.g. [1-^{13}C]glucose). This is particularly useful as the sequence of reactions involved in the conversion of C3 or C6 compounds in the plastid will result in different isotopic redistribution within the C6 compound eventually incorporated into starch. If C3 compounds are transported into the amyloplast, extensive equilibration between C_1 and C_6 in glucose released from starch would be expected as a result of the isotopic rearrangement brought about by the reaction catalysed by the cytosolic and plastidic forms of triose-phosphate isomerase (Fig. 2A). Extensive redistribution in starch would also be expected if interconversion of C3 and C6 compounds took place in the cytosol (triose-phosphate recycling) prior to transport of C6 compounds into the amyloplast (Fig. 2B). However, no isotopic redistribution in glucose released from starch would be expected if C6 compounds are transported directly into the amyloplast without interconversion with C3 metabolites in the

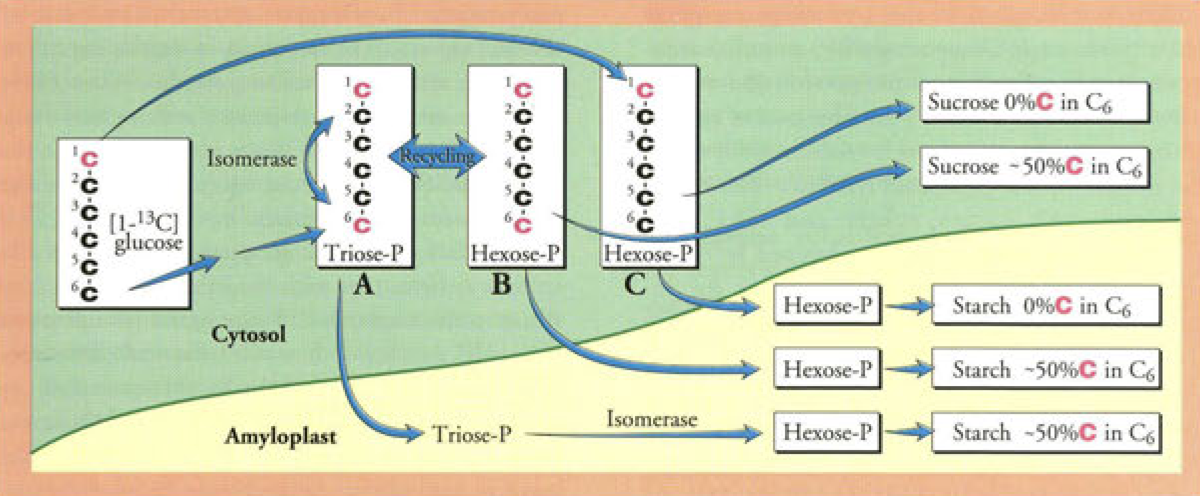


Figure 2 Redestribution of label from [1-^{13}C] glucose in starch or sucrose following the transport of either C_3 molecules (A) or C_6 molecules with (B) or without (C) hexose phosphates to triose-phosphates interconversion in the cytosol.

cytosol (Fig. 2C). In addition, isotopic redistribution in C6 compounds incorporated into sucrose would be expected if the pathway illustrated in Fig. 2B occurs *in vivo.*

When tissue excised from developing potato tubers or faba bean cotyledons is incubated with [1-^{13}C]glucose or [6-^{13}C]glucose, partial isotopic redistribution from C_1 to C_6 and from C_6 to C_1 occurs both in glucose released from starch and in hexosyl moieties of sucrose (Fig. 3). The similar degree of isotopic redistribution in the molecules incorporated into starch and sucrose does not support the hypothesis that C3 compounds are transported into the amyloplast. The results obtained are more easily explained by the transport of C6 compounds (most likely hexose-phosphates). Data also indicates that **partial** interconversion between hexose-phosphates and triose-phosphates occurs in the cytosolic compartment. The results also

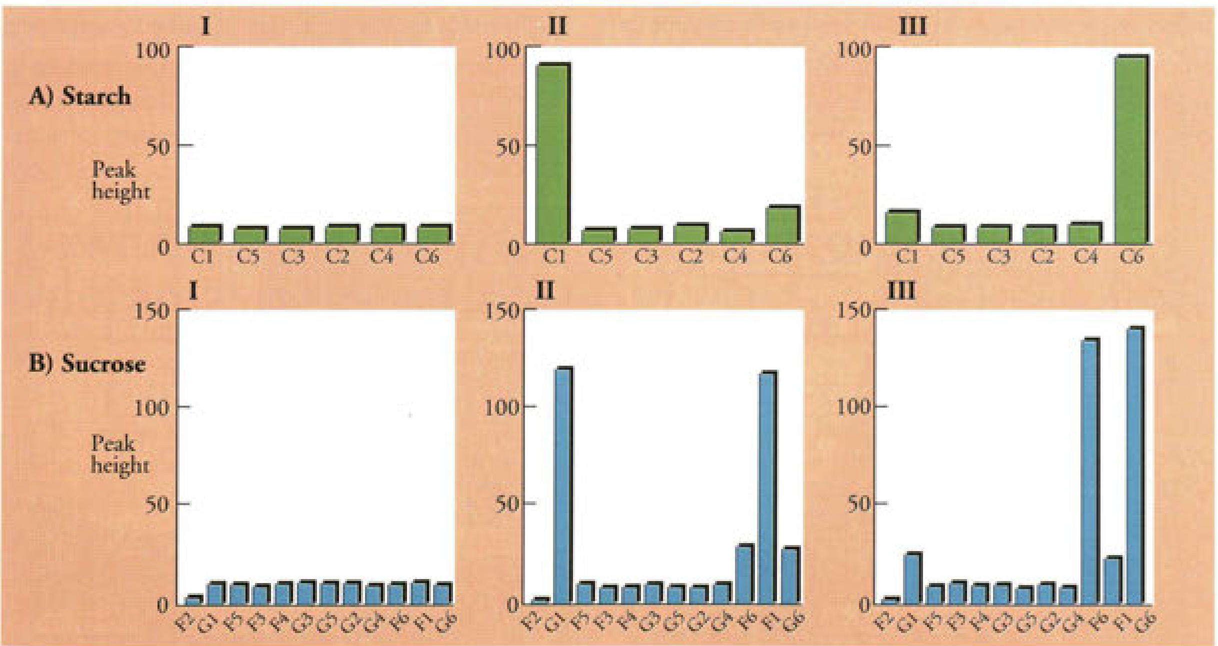


Figure 3 Schematic representation of ^{13}C-NMR spectra of glucose released from starch (A) and sucose (B) isolated from potato tuber discs incubated with 100mM glucose (I), [1-13C] glucose (II) or [6-^{13}C] glucose (III). Peak height of bars represents ^{13}C abundance of individual carbon atoms. (F = fructosyl, G = glucosyl varieties of sucrose).

indicate that the plastidic and cytosolic hexose-phosphate pools are in isotopic equilibrium and, consequently, that the direct precursors for starch biosynthesis are derived from the same pool of hexose-phosphates used as precursors for sucrose synthesis via the enzyme sucrose phosphate synthase. The results obtained with potato tubers and faba bean cotyledons[3] are similar to those obtained by others with wheat grain[4] and maize kernels[5]. Thus, the evidence indicates that transport of C6 compounds into the amyloplast is a general feature of non-photosynthetic starch-storing organs. Hexose-phosphate metabolism clearly represents the metabolic crossroad in carbon partitioning between ADPglucose (to support starch synthesis in the amyloplast) or UDPglucose (to support sucrose synthesis in the cytosol [Fig. 4]). This has important implications for the regulation of carbon partitioning during the sink to source transition in the potato tuber.

Pyrophosphate metabolism One of the factors known to affect hexose phosphate metabolism in plant cells is the concentration of inorganic pyrophosphate (PPi). The formation of ADPglucose and UDPglucose, in the amyloplast and cytosol respectively, is accompanied by the production of PPi (Fig. 4). Since conversion of glucose 1-phosphate into either ADPglucose or UDPglucose is readily reversible, net flux towards the synthesis of these compounds can be influenced by the potential of the tissue to remove the end product, PPi. Inorganic pyrophosphatase activity (PPase) appears to be very high in developing tubers which are actively synthesising starch. When PPase activity is inhibited by treatment with sodium fluoride, PPi concentration increases ten-fold. At the same time, fluoride almost completely inhibits the incorporation of ^{14}C metabolites into starch. This overall effect of fluoride on starch synthesis and PPi content is consistent with the inhibition of alkaline PPase in the amyloplast. Since, as the incorporation of the ^{14}C metabolites into sucrose is markedly stimulated by fluoride, it is unlikely that the removal of any PPi generated in the cytosol to support UDPglucose, and hence sucrose formation is catalysed by a fluoride-sensitive mechanism. It can be concluded that accumulation of PPi in tissue from developing tubers treated with fluoride occurs exclusively in the amyloplast due to the inhibition of plastidic PPase. These results provide evidence that PPase-mediated hydrolysis of PPi produced in the amyloplast is essential, thermodynamically, to drive starch biosynthesis.

Triose-phosphate recycling The observation that PPi removal from the cytosol is not regulated by a fluoride-sensitive mechanism is consistent with the hypothesis that PPases are absent from the cytosol in plant cells. The segregation of PPases away from this compartment permits the use of PPi as an energy donor for metabolic reactions. This has important implications for the regulation of carbon partitioning

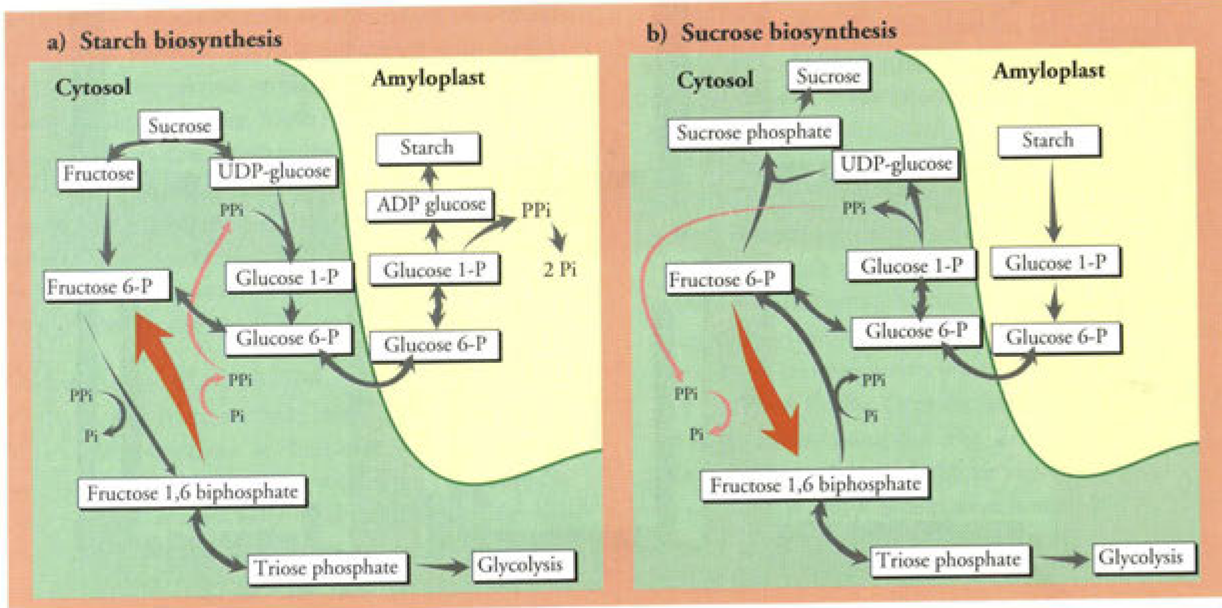


Figure 4 Schematic representation of hexose-P metabolism during starch (a) and sucrose (b) biosynthesis in the tuber cell. The hypothesis that triose phosphate recycling (red) is involved in the regulation of pyrophoshate metabolism is illustrated (the reaction catalysed by phosphofructokinase has been omitted for clarity).

in plant cells. It has been proposed that the triose-phosphate to hexose-phosphate (fructose 1,6-bisphosphate) interconversion referred to earlier represents one of the mechanisms involved in regulating the PPi concentration in the cytosol. This reaction can be catalysed by the enzyme fructose 6-phosphate,1 phosphotransferase (PFP) and, depending on the direction of a flux can result in net generation or consumption of PPi. Consequently, this could provide a mechanism for buffering the PPi concentration in the cytosol of plant cells. Thus, during starch biosynthesis, enhanced triose-phosphate recycling would lead to net production of PPi which is required to convert UDPglucose into glucose 1-phosphate in the cytosol. The glucose 1-phosphate generated can then be transported into the amyloplast directly or following its conversion into glucose 6-phosphate. Alternatively, during the conversion of starch into sucrose (e.g. in a sprouting tuber) triose-phosphates conversion into fructose 1,6-bisphosphate would remove PPi produced during sucrose formation (Fig. 4). The extent of triose phosphate recycling in plant tissues can be deduced from analyses of ^{13}C redistribution between the top and bottom half of hexosyl moieties of sucrose isolated following the supply of specifically labelled glucose. In agreement with the proposed hypothesis a lower degree of triose-phosphate recycling has been demonstrated in tissue from sprouting tubers (7-10%) compared to developing tubers (15-20%). However, it has also been shown that the apparent rate of triose-phosphate recycling in tissues containing little or no starch (etiolated potato sprouts, *Vicia* root tips, onion epiderm) is similar to that in developing tubers. This is not in agreement with the hypothesis that PFP-mediated triose-phosphate recycling generates the PPi required for driving sucrose conversion into starch in storage organs. The widespread occurrence of this phenomenon indicates that it may play an important role in primary plant cell metabolism.

Conclusions It has been shown that C6 compounds are transported into the amyloplasts of tuber cells to support starch synthesis and that plastidic PPase activity is required to drive carbon flux in the direction of starch deposition. Furthermore, the partitioning of hexose-phosphates plays a key role in controlling sucrose-starch interconversions. Hexose-phosphate metabolism is affected by PPi concentration and the latter may be regulated in the cytosol. The possibility that triose-phosphate recycling is involved in the regulation of cytosolic PPi concentration has also been investigated. Results show that recycling of triose-phosphates is a widespread phenomenon in plant tissues but it may not be directly responsible for the generation of cytosolic PPi required for the conversion of sucrose into starch in developing tubers.

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Towards an understanding of drought tolerance in potato

R.A. Jefferies & D.K.L. MacKerron

The potato is generally regarded as being sensitive to water-stress as yields are frequently constrained by drought in all the environments in which the crop is grown. In the UK more than 170 000 ha of potatoes are cultivated annually, of which ca. 30% could be irrigated. Limitations on the supply of water for irrigation will constrain further expansion of the area that may be irrigated and, indeed, the droughts of the past few years have resulted in regulatory restrictions on the amounts of water applied by current users of irrigation. Climate change may lead to more frequent and severe droughts and increase the constraints on water as a resource. Under these circumstances, the selection of drought tolerant genotypes would be useful, even in the UK, in conserving water resources by reducing the requirement for irrigation and might limit year-to-year fluctuations in yields in crops where irrigation is not available.

Selection of genotypes for drought tolerance is difficult for several reasons. First, the sensitivity of the plant to water stress changes as it develops and some developmental processes are more sensitive to water-

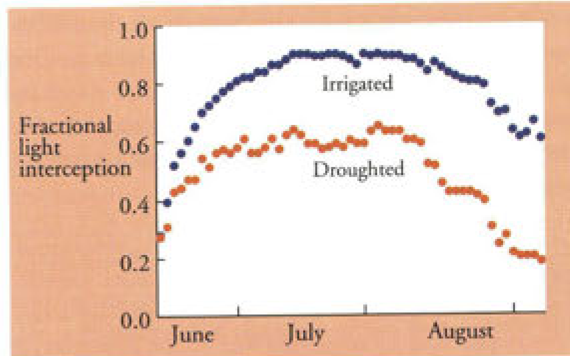


Figure 1 Fractional light interception in irrigated and droughted potato crops.

stress than others. Second, the degree of stress experienced by a plant depends on both soil moisture status and evaporative demand. Third, the nature of drought varies in both degree and timing between years. In addition, tolerance of drought may involve several different morphological and physiological characters, the relative importance of which may vary according to the type of drought experienced by the plant.

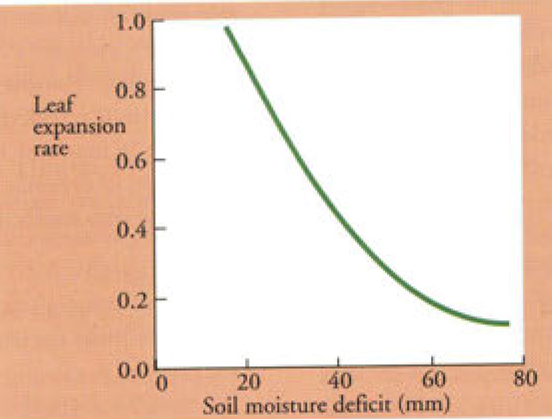


Figure 2 Relation between leaf expansion rate expressed as a proportion of that in the control, and soil moisture deficit.

In trying to understand the basis of drought tolerance in potato, we have combined physiological studies with simulation modeling. The growth of the potato crop can be divided into phases, each capable of detailed investigation, and the crop proceeds through them at rates that are determined by weather variables. Total growth is proportional to the amount of solar radiation intercepted by the crop across the several phases and so yield can be described by the following equation:-

$$Y = R \times L \times H/D$$

Where Y = yield, R = radiation intercepted by the crop, L = a coefficient for the conversion of light into dry matter, H = harvest index, and D = tuber dry matter concentration.

This approach has allowed us to identify the components of growth and yield most affected by water-stress and then to examine, in greater detail, the underlying physiological processes involved and the genetic control of them.

Physiological bases of drought tolerance. We have shown that the principal effects of drought are on the interception of radiation and on tuber dry matter concentration. In droughted crops the total amount of radiation intercepted is reduced as a consequence of slower and lesser canopy expansion, poorer light interception during the greater part of the season, and earlier canopy senescence (Fig. 1). Detailed analysis of leaf growth in cv. Maris Piper showed that the expansion rate of individual leaves was closely related to soil moisture deficit (SMD) and declined rapidly when the SMD was greater than 16 mm, reaching a minimum when the SMD was 77 mm (Fig. 2). In contrast, the coefficient for the conversion of intercepted radiation into dry matter was unaffected by SMD less than 47 mm (Fig. 3). It seems reasonable, therefore, to propose that those genotypes which are able to maintain their ability to expand leaves and to maintain light interception are going to achieve greater dry matter production and, possibly, higher yields in drought conditions. Such genotypes are likely to have improved relations between leaf expansion rate and SMD.

To test this hypothesis, the effect of drought on the expansion of individual leaves was examined in 24 different genotypes. Analysis of the relation between leaf expansion rate and SMD

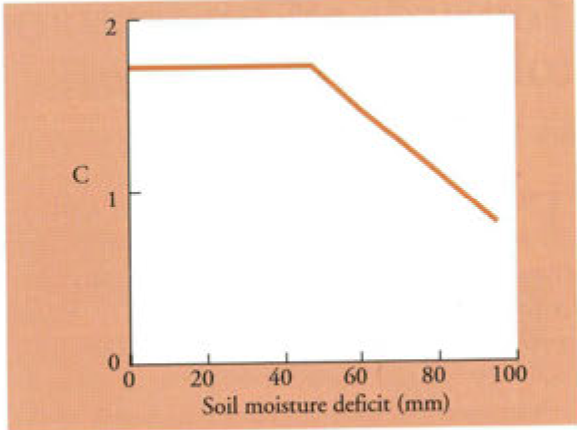


Figure 3 Relation between the coefficient for the conversion of intercepted radiation into dry matter (C) and soil moisture deficit.

	Threshold value	Reduced rate of expansion	
		50%	25%
Baillie	20	38	51
Cara	35	49	62
Désirée	11	42	58
Maris Piper	22	40	55
Pentland Crown	35	61	76
Record	27	47	56
Russet Burbank	34	52	61
Spunta	23	51	69

Table 1 Threshold values of soil moisture deficit (SMD) (mm) below which the rate of leaf expansion is unaffected and the SMD at which leaf expansion rate is reduced to 50% or 25%.

revealed that genotypes differed both in the threshold at which leaf expansion was constrained by soil moisture and in their ability to maintain expansion with increasing SMD (Table 1). Those genotypes considered to be drought susceptible (eg. cv. Baillie, Maris Piper, and Record), generally had low thresholds and were sensitive to increasing SMD. Genotypes considered to be drought tolerant (eg. cv. Cara, Pentland Crown, and Spunta) either had greater thresholds or were less sensitive to increasing SMD or both.

Tuber yields are strongly influenced by tuber dry matter concentration (DM) so that, for example, a reduction from 20 to 18% DM increases yield by 11% whereas a change from 20 to 22% DM reduces yield by 9%. We have found that, in an average genotype near to harvest time, each change of 10mm in SMD produces a change of 0.6 percentage points in DM. Any genotype showing less than average change in DM with SMD will be more tolerant of drought. In a simple comparison of 24 genotypes in wet and very dry conditions we found some suggestion they differed in the stability of DM with SMD. However, analysis of the effect of a range of drought levels on DM in seven genotypes found no differences in stability. Further work is required to determine both the degree to which stability of DM differs between genotypes and the physiological basis of any differences.

Differences in the relation between leaf expansion rate and SMD could have been the result of differences in capability for osmotic adjustment, that is the active accumulation of solutes within cells, lowering the solute potential so that turgor is maintained in the face of increasing water-stress. This phenomenon has been reported in several species as an important trait associated with drought tolerance, and may enable turgor-dependent processes, such as leaf expansion, to continue at lower leaf water potentials. However, we found that osmotic adjustment in potato is limited and not correlated with the ability to maintain leaf growth at increasing SMD.

Other possible causes of differences in the ability to maintain leaf expansion rate and tuber water content with increasing SMD are differences in rooting patterns and root functioning. Enhanced root growth would permit the extraction of more water from greater depths in the soil and increase the amount of water available to the plant by a given time so maintaining leaf turgor and allowing continued leaf expansion. Increases in root hydraulic conductivity might reduce stress initially at the cost of increased stress later but, conversely, reduced root hydraulic conductivity would restrict current water use and conserve some soil moisture over the season.

Experiments at SCRI, in which a range of potato genotypes were grown in 1.5m long plastic pipes and were either irrigated or droughted from the time of plant emergence, confirmed that they differed both in the amount of root produced and their depth of rooting and they differed in their response to the two watering regimes (Fig. 4). Moreover, in these experiments the quantity of root produced tended to match shoot production. However, this does not necessarily imply that capacity for root growth controlled the response of the plant to water-stress. It may be that differences in shoot characteristics such as stomatal control, carbohydrate assimilation and partitioning resulted in greater root growth in some genotypes.

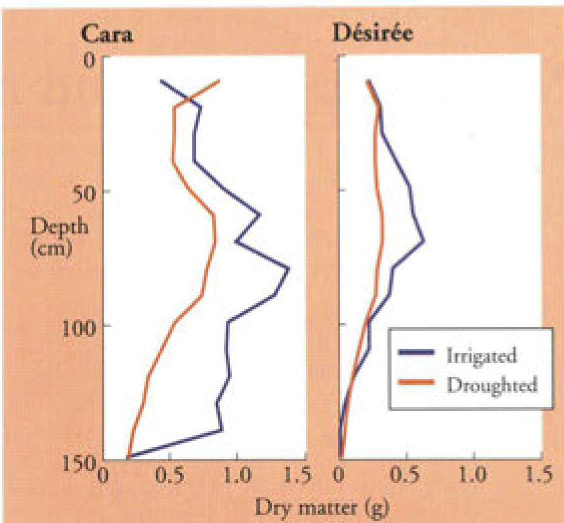


Figure 4 Profiles of root dry matter with soil depth in irrigated and droughted potatoes.

Detailed investigations are under way using reciprocal grafting to investigate the interaction between root and shoot in determining the response of plants to drought.

Use of simulation models to assess strategies of drought tolerance. The development of physiologically-based models simulating the growth and yield of crops, concurrently with physiological studies, provides the opportunity to examine potential strategies of drought tolerance. As already discussed, the response of a crop to drought may involve several different morphological and physiological traits, the relative importance of which may vary according to the type of drought experienced. Simulation models can be used to compare potential traits, and examine interactions between them and various drought patterns without reproducing the conditions experimentally. The results can then be used to assign priorities for further experimental studies.

We have used a simulation model of growth of potato crops constrained by water-stress developed at SCRI to compare different potential strategies of drought tolerance under Scottish environmental conditions. The results suggested that improving rooting depth or increasing water use efficiency would not increase yields compared with standard crops, except under severe drought cycles. Increasing the coefficient for conversion of intercepted radiation into dry matter (L) would improve yields under fully irrigated conditions, but the effect would diminish with increasing severity of drought. Over the whole range of drying cycles considered, improving the relation between L and soil moisture status would have no effect on yield compared with the standard. The greatest effect on tuber yield would be obtained by improving the relation between leaf expansion and soil moisture status which would give improvements in yield over the whole range of drought patterns simulated, with the greatest increases over the standard being found in conditions of moderate to severe drought (Fig. 5). Future work will examine and compare potential traits under climatic conditions different from those of Scotland. The development of other, more mechanistic models will allow the assessment of the relative importance of the underlying processes involved in determining the response of leaf growth to water-stress.

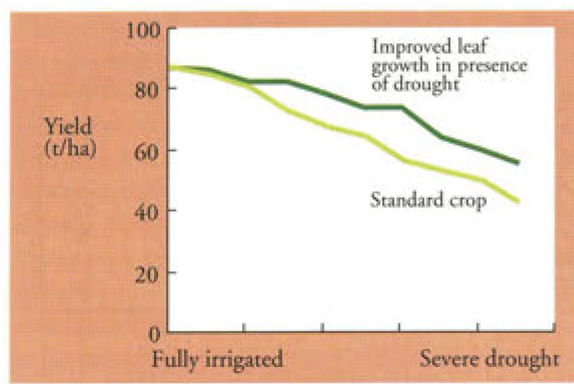


Figure 5 Simulated yields in relation to severity of drought.

Soil micro-fauna and nutrient cycling

B.S. Griffiths

Food chains are as much in evidence below ground as they are above ground, but instead of taking the form:

Plant → Herbivore → Carnivore 1 → Carnivore 2, the below ground decomposer food chain is usually:

Dead Organic Matter → Microorganism → Microbivore → Predator.

In agricultural soils, cultivation practices tend to discriminate against predators, such as mites, consequently the microbivores, nematodes and protozoa, predominate and constrain populations of microorganisms, composed mainly of bacteria and fungi.

Soil-dwelling nematodes and protozoa are essentially aquatic organisms adapted for life below ground and differ to such a degree that, although both compete for the available microorganisms, they coexist in the soil. Protozoa are unicellular organisms which in adversity survive as dormant structures called cysts (Fig. 1b), but rapidly excyst under favourable conditions and can double their populations in a matter of hours. Nematodes are multicellular organisms with a longer life-cycle of 7-10 days, but their ability to move rapidly over relatively large distances through soil confers an advantage (Fig. 1a).

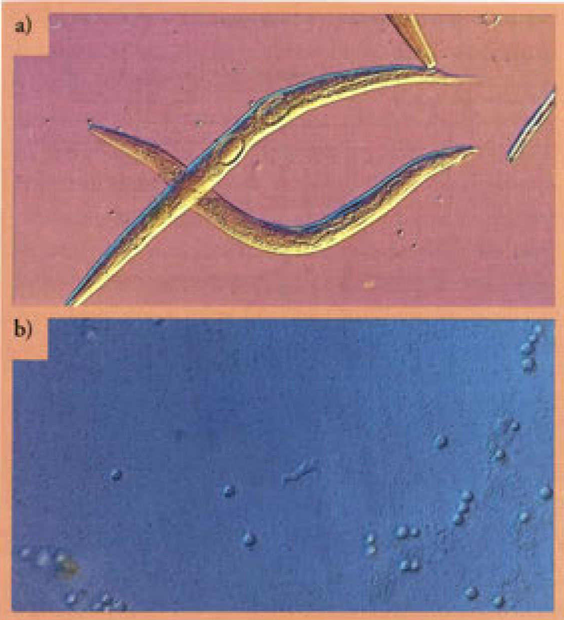


Figure 1 a) Male and female (with eggs) bacterial-feeding nematodes, *Caenorhabditis* spp.
b) Soil protozoa (amoebae) showing active stage (trophozooite) (T) and dormant cysts (C).

The role of nematodes and protozoa in nutrient cycling is twofold. First, they increase the mineralisation and availability of essential plant nutrients by consuming microorganisms. In a sterilised soil experimentally inoculated with a) bacteria alone, b) bacteria with protozoa or c) bacteria with nematodes, the presence of microfauna significantly increased the net mineralisation of nitrogen (N) (Fig. 2). Measurements made on plants grown in similarly treated soils clearly demonstrated that the extra N was readily available and of direct benefit to the plants (Fig. 2). Second, the presence of nematodes and protozoa stimulates the microorganisms on which they

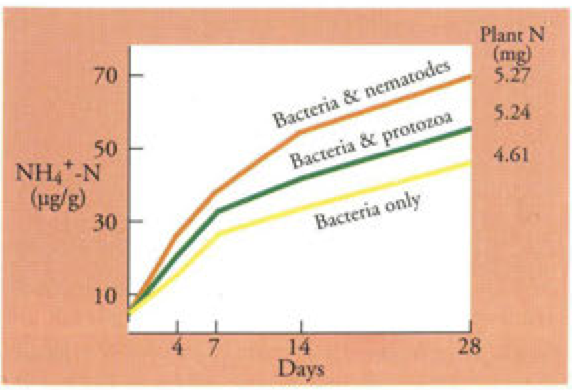


Figure 2 Release of ammonium in sterile soil, and the amount of N in ryegrass plants, increases with the addition of micro-fauna.

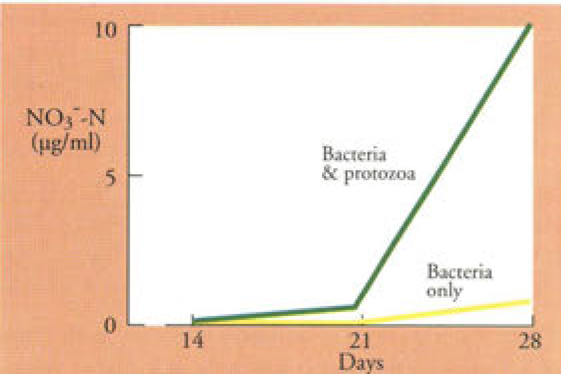


Figure 3 Rate of nitrification in liquid culture is stimulated by the addition of protozoa

feed into being much more productive. The activity of nitrifying bacteria, which convert ammonium to nitrate, was enhanced by the addition of bacterial-feeding protozoa (Fig. 3).

Nutrient cycling in the rhizosphere The rhizosphere is defined as soil in which the physical and biological properties are influenced by the presence of plant roots, and is usually taken to be soil within a radius of 1-2 mm from the root surface. The increased biological activity in the rhizosphere probably has a large effect on plant growth and a recent concept suggests that it is responsible for 'root-induced nitrogen mineralisation'. This hypothesis proposed that the release of carbon (C) from a growing root-tip stimulates the growth of microorganisms which were previously limited by a shortage of C, and that they also mineralise soil organic N to satisfy their N requirements for growth. Microfauna consume the microorganisms and release N which is then taken up by the plant (as in Fig. 2). If this hypothesis were correct plants would be able to make their own N available from the soil and it should be possible to breed plants able to utilise the large amounts of presently unavailable organic N in the soil. The validity of the concept of 'root induced N mineralisation' was tested by measuring populations of microfauna in the rhizosphere.

		Nematodes /g		Protozoa /g	
Soil	Fertiliser	no.	µg	no. x 10^{-3}	µg
Rhizosphere	-	63	1.9	49	0.3
	+	245	7.4	91	3.2
Bulk	-	19	0.6	21	0.3
	+	28	0.9	38	0.5

Table 1 Numbers (no.) and biomass (µg) of bacterial feeding nematodes and protozoa in the rhizosphere of barley. Fertilisation stimulates micro-faunal populations.

Crop plants	Biomass	Grass species	Biomass
Pea	8433	*Lolium perenne*	400
Barley	5378	*Festuca arundinacea*	350
Grass	4629	*Poa annua*	960
Turnip	1720	*Poa pratensis*	230

Table 2 Microfaunal biomass (ng) in the rhizospheres of different crop plants grown in field soil, or of different grass species grown in artificial soil (hence the lower biomass)

There was a positive rhizosphere effect for both types of bacterial grazer, that is a significantly higher population density in rhizosphere soil than non-rhizosphere (bulk) soil (Table 1). Bacterial feeding nematodes, however, had a larger biomass and increased relatively more in the rhizosphere than protozoa. Nematodes excrete more N per unit weight than protozoa, due to a lower assimilation efficiency, and would therefore, mineralise more N than protozoa in the rhizosphere. The addition of inorganic fertiliser to the soil significantly increased the micro-faunal population in the rhizosphere which indicates, as found by other workers, that the microorganisms in the rhizosphere are not solely limited by a shortage of C. The fact that additional **inorganic** N is required for greater microbial growth was an indication that the rhizosphere microbes were not effectively mineralising soil organic N. More detailed experiments looking at defined sections of root showed that nematodes would be able to supply only a small fraction of the N that the root would potentially be capable of absorbing (Fig. 4). Calculations from a mathematical model of rhizosphere C and N cycling (Fig. 5) demonstrated that less than 10% of the N required by a plant could come from 'root-induced N mineralisation' and it has to be concluded, therefore, that this pathway cannot supply plants with sufficient N even in natural systems with low N availability.

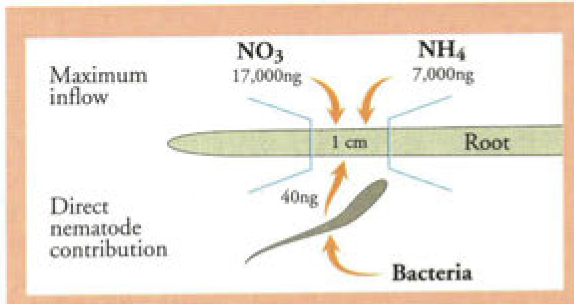


Figure 4 Comparison between the maximum potential daily influx of N to roots and the contribution of N from nematodes.

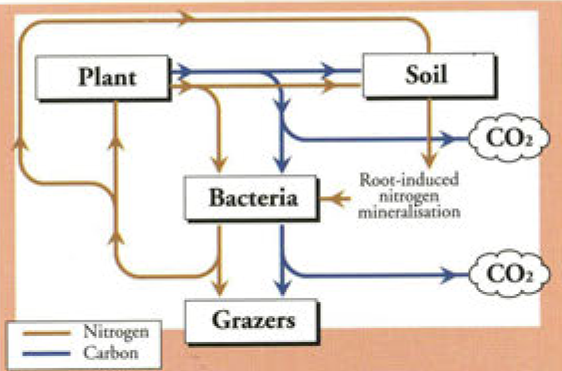


Figure 5 Rhizosphere model developed to calculate the flows of carbon and nitrogen between plant and soil.

Soil microfauna still have a vital role in the rhizosphere through regulating the activity and types of microorganism present. The manipulation of rhizosphere microorganisms, including the introduction of genetically manipulated bacteria to promote plant growth is a burgeoning field of research, and studies on the effects of nematodes and protozoa on their activity and survival will become important. Contrasting plant species can support different microfaunal populations (Table 2), and may be important when attempting to relate differences between plant species to their characteristic patterns of nutrient uptake.

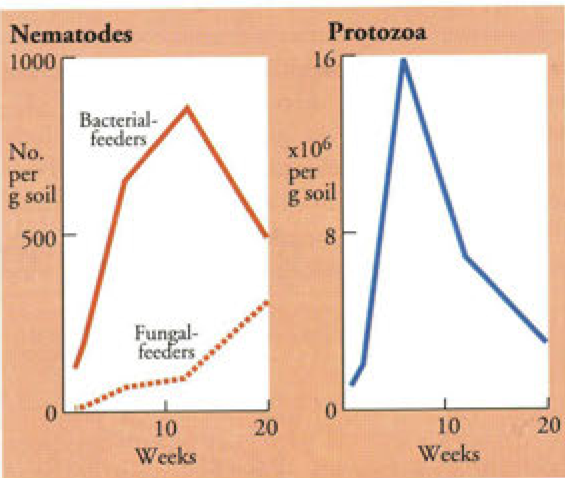


Figure 6 Numbers of nematodes and protozoa developing on decomposing barley roots.

Decomposition of organic matter If plants are unable to generate their own N within the rhizosphere, it follows that their main source of N must be from soil away from the roots. The N can come from added inorganic fertilizers, and the decomposition of either added organic material or the previous crop

residues. Microfauna are an essential part of the decomposition process and can build up large populations on organic matter (Fig. 6), that are much larger than those in the rhizosphere (c.f. Table 1). The characteristics of the faunal types can be used as an indicator of the decomposition process. For example, the peaks of populations of bacterial- and fungal-feeding nematodes shown in Figure 6 coincide with the periods of maximum bacterial and fungal activity. The large populations of microfauna imply a rapid rate of nutrient turnover and also a great selection pressure on the microorganisms active in decomposition. Future research will reveal the timing and pathways of nutrient release during decomposition, which is important for plant nutrient uptake. In addition, it will provide information about the release of pollutants into the environment such as the greenhouse gas nitrous oxide through denitrification, and the leaching of nitrate into ground water and water courses.

Exploiting the competition between vegetative and fruiting phases of growth in raspberry using cane desiccation

H.M. Lawson & J.S. Wiseman

Cane vigour control and biennial cropping are two management techniques which have been developed to aid the raspberry grower to achieve the right balance between fruiting and vegetative growth in his plantations. Vegetative canes in their first year and fruiting canes in their second year are intermingled in a conventionally grown plantation and compete for the available resources of light, water and mineral nutrients. In a good growing season, vigorous cultivars produce such an abundance of vegetative cane growth that fruiting is depressed, harvesting is made difficult and pickers or machines inflict damage on young canes (Fig. 1a). However, correctly managed, this vigour may be exploited so that the full yield potential can be realised. Research at SCRI during the 1970s established methods of achieving these objectives under UK conditions.

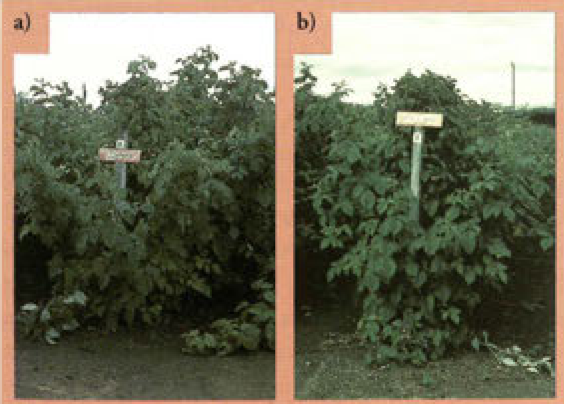


Figure 1 **a)** Without some control of cane vigour, prolific young cane growth can become unmanageable by harvest time.
b) The shorter canes of the second flush leave the fruit accessible at harvest.

Cane vigour control involves the removal of the first flush of vegetative canes when they reach a height of 10-20 cm in spring. Subsequently, a second, later flush of young canes is produced, which competes less with the fruiting canes. As a result, more and larger berries are produced, giving increases of up to 50% in yield per fruiting cane. The fruit is easier to see and to harvest without damaging vegetative canes, because the latter are still relatively short at the time of harvest (Fig. 1b). Nevertheless, the growing season is long enough to allow the second flush to reach a height adequate to carry the next year's crop. Cane vigour control can be repeated annually as long as the plantation continues to produce a more than adequate number of canes of sufficient height for fruit production in the following year. It is particularly suited to the management of vigorous cultivars such as Glen Clova and was used on 1350 ha of established plantations in Scotland alone in 1986.

Biennial cropping involves the complete separation of the fruiting and vegetative phases of growth. The removal of successive flushes of new canes in one year reduces competition with fruiting canes to a minimum, improving fruit size and numbers. There are

experimentally induced processes under examination. For the first time, we can study complex natural systems (agricultural and unmanaged) with some assurance of not measuring artifacts of the sampling method.

Until recently, it was not possible to routinely analyze statistically significant numbers of samples, and the usefulness of the natural abundances approach was limited in ecological and agricultural research where replication is especially important. SCRI has recently acquired state-of-the-art equipment, capable of routine natural abundance-level measurements on the isotope pairs $^{15/14}N$, $^{13/12}C$, and $^{18/16}O$. This, together with existing equipment and complementary instrumentation at the University of Dundee, now gives SCRI access to six modern mass spectrometers for routine analyses of natural abundance level samples. These include use of all of the stable isotopes mentioned above, except $^{34/32}S$. Mass spectrometry of sulfur isotopes presents technical difficulties generally unresolved. Because $^{34/32}S$ can be an excellent tracer in ecological studies and food web analyses, we have initiated development of $^{34/32}S$ instrumentation.

Although the natural abundances approach will permeate all of the natural sciences, we are initially emphasising this new method in seven areas: (1) ecology, (2) woody perennials, (3) crop selection, (4) metabolic physiology of plants, (5) plant pathogen-food web studies, (6) soil nitrogen and carbon cycling, and (7) continued isotopic methods development.

Recently, we were awarded a large EC-funded grant to study nitrogen cycling in savanna systems in Kenya and Spain using natural abundances of the stable isotopes of nitrogen, carbon and hydrogen. Previous collaborative work in Kenya showed the power of this method by identifying the occurrence and extent of symbiotic N_2-fixation in trees at 13 natural sites, the main source of their water supply, and their relative water cost of growth in this arid land. This study showed that amounts of N_2-fixation were much more variable than previously suspected and that one tree, which is very popular for plantation forestry (*Acacia brevispica*), fixes no atmospheric nitrogen in the field. It also revealed a number of interesting patterns of soil N and soil $^{15/14}N$ values, which have led to new hypotheses for further studies. A recent investigation at SCRI into the role of mycorrhizas in determining plant $^{15/14}N$ ratios in nature suggested that nitrogen metabolism by ectomycorrhizas is not an important source of isotopic variation but that VA mycorrhizas may dramatically change both the $\delta^{15}N$ value of inorganic N presented to the plant and the way in which the plant subsequently metabolises and allocates that N. In the newly funded EC project, a purpose-designed experimental plot, recently planted in Spain, will further our understanding of natural isotopic markers in a tree-grass-soil system.

We are also conducting experiments with the Forest Energy Project in Uppsala, Sweden to determine the water cost of growth in different willow clones to be used for biomass energy using $^{13/12}C$. These studies include testing the effects of nitrogen nutrition on plant $^{13/12}C$ ratios. The same $^{13/12}C$ technique is being used at SCRI for potato cultivars and other vegetable crops to ensure that seasonal rainfall (diminished in recent years) is used to best advantage for food production in the UK.

Natural abundance techniques will be combined with NMR to study the plant metabolism of nitrogen use and carbon allocation. Combinations of stable isotopes are being used *en suite* to elucidate the feeding patterns of the recently introduced New Zealand flatworm and to explain losses of nitrogen from soils, both gaseous emissions and nitrate leaching.

All of these studies are supported by a strong programme of innovative chemical methods development, keeping pace with research needs. One recent innovation is an improved technique for measuring the $^{2/1}H$ of water for plant studies of water use and competition for water in arid lands.

Chemistry

I.M.Morrison

This report is an overview of the progress made in the chemical interests of the Institute. As such, it is an amalgam of the work of the Chemistry Department, the Spectroscopy Group and the Fibres Group. Since a particular discipline cannot operate in isolation, the work described here is partly Chemistry-led, but integrating other disciplines, and partly support work for other Departments which require chemical expertise.

This report should have been the responsibility of Dr M.J. Allison, Head of the Chemistry Department until his untimely death in early 1992. A tribute to Dr Allison will appear in the 1992 Annual Report.

The primary objectives of the Chemistry Department are to develop and apply synthetic and analytical methodologies for investigating factors which affect crop quality and production. The peptide synthesiser has been used to prepare a range of conventional soluble peptides for application in specific ROAME's. Techniques have also been developed for the synthesis of peptides covalently linked to a phenylacetamidomethyl resin. This involves the use of both *t*-butyloxycarbonyl and 9-fluorenylmethoxycarbonyl protecting groups. These groups can be removed without cleavage of the covalent bond between the resin and the C-terminal amino acid of the peptide.

In a programme on resistance to the aphid *Amphorophora idaei*, the chemicals associated with the leaf surface of raspberry cultivars are being investigated by mass spectrometry. A wide range of compounds have been identified including acetyl esters of long chain alcohols, tocopherols and triterpenoids such as α- and β-amyrins. A comparison of the results from resistant and susceptible cultivars by linear discriminate analysis suggests that the non-volatile surface components have a rôle in resistance and further investigations are underway.

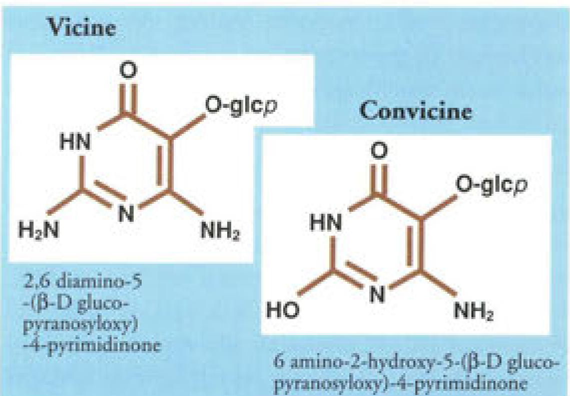


Figure 1 Structures of vicine and convicine.

Chemical ionisation mass spectrometry with ammonia as the reagent gas is being evaluated for the identification of de-sulphoglucosinolates from brassica pollen. A capillary GC method for the separation and quantification of petroselenic (18:1, *cis* 6) acid from its positional isomer oleic (18:1, *cis* 9) acid has been developed. It has been applied to coriander fruits to give both total oil content and composition.

The acquisition of a VG TRIO-1000 bench top mass spectrometer, which has been coupled to the thermal desorption unit, has greatly enhanced its capabilities to investigate volatile products from both plant and microbial sources. The facilities and their applications are described in a separate review.

Collaborative studies into the control of chlorogenic acid and glycoalkaloid concentrations in potatoes have been continued using colorimetric and HPLC techniques developed at the Institute. The distribution of vicine and convicine (Fig. 1), compounds implicated in the aetiology of favism (the haemolytic anaemia syndrome), have been investigated in the seeds of *Vicia faba*. Although the cotyledons contain the greatest amount of these glycosides in the seeds, the concentration in the radicle was greater than in the cotyledon by a factor of 10 and accounted for *ca.* 5% of the plumule dry matter. Investigations are continuing on the rôle of vicine and convicine in the defence mechanism of germinating seeds and seedlings.

Modifications have been made to the stable isotope mass spectrometer to test its potential for monitoring ^{15}N at natural abundance levels. By careful regulation of the sample size, a $\delta^{15}N$ precision of ±0.5% has been achieved.

The facilities of the Spectroscopy Group have been further expanded with the acquisition of a Bruker ESP300E X-band EPR spectrometer along with an ESP350 ENDOR attachment. This state-of-the-art equipment has been purchased as part of the SOAFD Flexible Fund initiative to develop procedures for the detection of irradiated foodstuffs of plant origin and to investigate the rôle of free radicals in plant senescence processes. It is envisaged that these facilities will feature extensively in other research initiatives concerned with the function of free radicals in a variety of biological processes. A review of the importance of free radicals in plant biology is presented as a separate article.

The NMR facility at Dundee University, which is shared with SCRI, has now been in operation for 2 years. Spectroscopic applications to the characterisation of plant fibres and fibre products is presented in a separate review. Other spectroscopic activities have been concerned with *in vivo* investigations of the distribution of major metabolites in plant parasitic nematodes (Fig. 2), elucidating metabolic pathways in both plants and nematodes using ^{13}C-labelled sugars and with identifying groups in the coat protein of pepper ringspot virus.

The major effort has been in the development of applications involving the micro-imaging facilities. These were reviewed in detail in the Annual Report for 1990. Advances have been achieved in extending

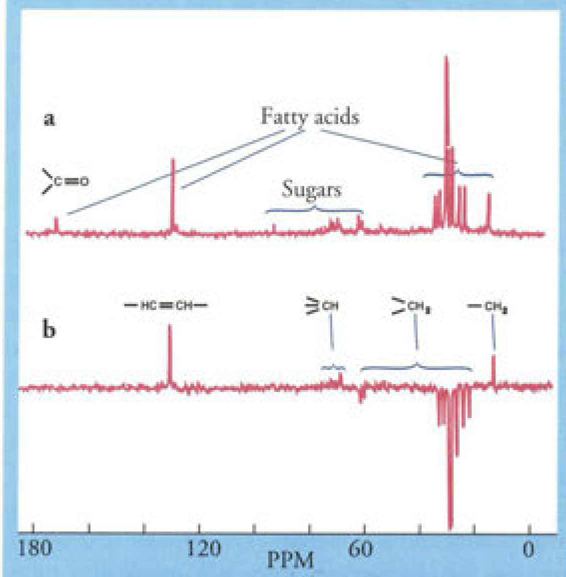


Figure 2 ^{13}C NMR spectra of the plant parasitic nematode *Longidorus elongatus.* a) broad band ^{1}H decoupled spectrum and b) DEPT spectrum, which inverts the peaks from ^{13}C atoms that are bound to an even number of ^{1}H atoms.

Figure 3 Delignification of barley straw with sodium peroxymonosulphate. Intact straw (left) and delignified straw (right).

Figure 4 Autofluorescence microscopy of lignin deposits around flax fibres. These deposits may "fix" the fibres to the surrounding cells of the stem.

the non-invasive histological investigations to a wide range of soft fruits and to further developing procedures for the identification of disease processes. It is confidently predicted that this technique will soon have an important rôle in non-invasive imaging of biological processes, especially if it can be combined with volume selective spectroscopy for the elucidation of various molecular changes.

Investigations have continued on the characterisation of cyclobutane-type dimers of phenolic acids with the aim of understanding their synthesis and function within the plant cell wall. Using capillary GC and mass spectrometry, a wide range of substituted cinnamic acids have been subjected to oxidative and photo-dimerising conditions and the products analysed for the presence and proportion of truxillic to truxinic type structures. The ability of the substituent at C-4 to hydrogen bond had a major effect on the structure while electronegative and steric effects had a lesser influence.

The efficacy of some novel non-chlorine based delignification reagents have been studied. The salts of peracids rapidly and efficiently oxidise the aromatic ring and the oxidised lignin can be removed under weakly alkaline conditions (Fig. 3). The most efficient is sodium peroxymonosulphate. The additional effect of H_2O_2 and metal ions is currently being investigated.

Lignification of plant cell walls (Fig. 4) is reported to involve two reactions which are catalysed by wall-associated peroxidases (*Ann. Rep. 1990, 61*). The first is the formation of extracellular hydrogen peroxide by the oxidation of reduced nicotinamide adenine dinucleotides (NADH) and the second is the oxidation of cinnamyl alcohols to phenoxy radicals via hydrogen peroxide. Cell wall peroxidases have been isolated from fibre-bearing tissue dissected from the first internode of flax at different stages of growth. Ionically-, covalently- and intrinsically-bound peroxidases were isolated and the activities of each class rose as lignification progressed. The ability of the covalently-bound peroxidases to oxidise NADH increased as the fibres became lignified. The presence of the intrinsically-bound fraction is of great interest and the mode of attachment is currently being investigated. Certain cationic and anionic peroxidases increase in abundance as lignification progresses. The presence of other key enzymes in the lignification process, such as cinnamyl alcohol dehydrogenase and phenylalanine-ammonia lyase, are also being assessed.

Non-cellulosic polysaccharides have been isolated from flax fibre and the xylose-rich polymers, xyloglucan and (glucurono)-xylan have been identified by the characteristic digestion products isoprimeverose and xylobiose respectively. A glucomannan was isolated by alkaline borate extraction and its structure shown to be similar to the glucomannans of softwoods from the oligosaccharides released on degradation using a β-(1->4)-endomannanase. Antisera have been raised to these oligosaccharides for the immunocytochemical localisation of these polymers in flax fibre.

Progress on the identification of plant fibres is reviewed separately.

Isolation and identification of plant fibres for industrial uses

I.M. Morrison & D. Stewart

Plant fibres encompass a wide range of natural products which have an even wider range of industrial uses. The only factor which is common to all plant fibres is that they are derived from the plant cell walls. Through the natural diversity of plants and therefore their walls, the properties of fibres from different sources are extremely varied and this is manifested in the potential applications. Even in areas which might be expected to have similarities, there are profound differences in the advantages of one fibre compared to another.

The most economically important plant fibres are cotton for textile applications and coniferous woods for pulp and paper use. Other plants, such as flax (*Linum usitatissimum*) from which linen is made, are used for textiles and may have specific advantages but cotton, the fibre isolated from the seed hair with low production costs, has no real rivals. On the other hand, many plants can be used for pulp production instead of gymnosperms and there are environmental as well as economic factors which favour an examination of this possibility. The product will determine the actual quality of fibre which can be used but, to a first approximation, the aim of most processes is to remove as much of the non-carbohydrate constituents as possible whilst causing the minimum degradation to the cellulosic fibres. A fine balance has to be achieved as well as finding ways to assess when the optimum properties have been attained.

Treatment	Cellulose	Non-cellulosic polysaccharide	Lignin
None	49.6	33.9	11.8
Permanganate	54.1	36.2	3.3
Chlorite	55.2	34.9	3.8
Acetic/Nitric	91.8	3.2	3.2
Hypochlorite	49.5	34.6	6.9
Peroxide	56.1	29.3	9.9
Peracetic	51.3	30.5	10.2
17.5% NaOH	78.3	8.3	9.9

Table 1 Composition of oat straw samples

Most of our pilot studies on fibre purification and properties have been carried out on cereal straws. Since there are abundant supplies available, the utilisation of this waste material would have undoubted benefits but it can also be seen as a "model" fibre source being high in non-carbohydrate constituents, such as lignin, whose removal is a prerequisite for any application. The effect of chemical and biological treatments on the colour of oat (*Avena sativa*) straw is seen in Figure 1 and can be equated with the changes in the major constituents of some of those products (Table 1). Treatments which give a white or pale product (and consumers prefer white paper) are those which reduce the lignin content (permanganate, chlorite and acetic/nitric), while lightening of the colour is also achieved with hypochlorite, peroxide and peracetic acid. The product becomes paler as more lignin is removed.

Direct chemical analyses suggest that there is very little loss of cellulose during the partial delignification reactions. However, these analyses only determine the amount of β-(1,4)-linked D-glucan present in the samples. Cellulosic fibres are far more complex. The association of individual polymer chains both laterally in the same plane and horizontally in the stacking of planes is the reason for the crystallinity of cellulose. On the other hand, the delignification reactions can remove most of the lignin but, since the reagents used are strongly oxidising, direct analysis cannot determine any oxidation of cellulose chains. To assist in the identification of these changes, a number of spectroscopic methods have been investigated.

Near Infra-red Reflectance Spectroscopy (NIRS). The NIRS spectra of the series of untreated and treated oat straws and modified flax (*Linum usitatissimum*) fibre and forage rape (*Brassica napus*) stems (Fig. 2) have been recorded and examined. It was not expected that any significant differences would be observed in the carbohydrate absorbances (1500 and 2100nm) since cellulose and non-cellulosic polysaccharides both absorb in the same region and all the samples con-

Figure 1 Photographs of treated oat straw samples.

tained ≥80% polysaccharide. Differences were expected in the lignin absorbancies (2300nm) since some of the delignifying reagents had removed *ca.* 80% of the lignin. This did not materialise and it was concluded that NIRS did not offer the resolution and definition required.

Fourier Transform Infra-red Spectroscopy (FTIR). Considerably more definition and structural information is obtained by this technique than from NIRS and the spectra (by courtesy of the Chemistry Department, University of Dundee) of some barley (*Hordeum vulgare*) straw samples are shown in Figure 3. This shows the intact straw with delignified, 1.0M NaOH and 1.0M HCl treated samples. As expected, little useful information could be obtained from the 4000-1900cm^{-1} region since this region is dominated by O-H and aliphatic C-H stretching frequencies. Such bonds are the major types present in polysaccharides and these samples, as noted previously, are all ≥80% polysaccharide.

The two regions of most significance are 1735 and 1510cm^{-1}, absorbances due to ester carbonyl and aryl-H vibrations respectively. The aryl-H vibrations mainly arise from lignin but other aromatic constituents, such as ferulic acid, will also contribute. The

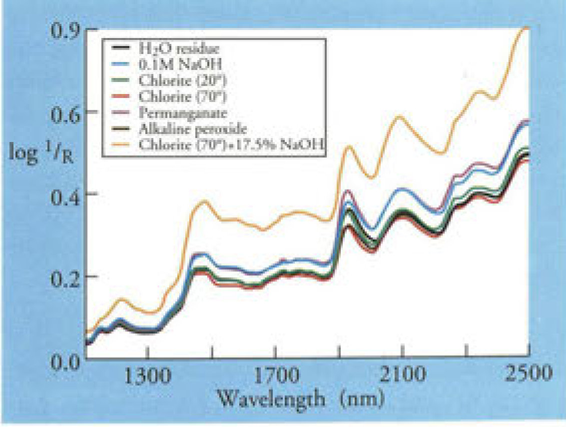


Figure 2 NIR spectra of brassica samples.

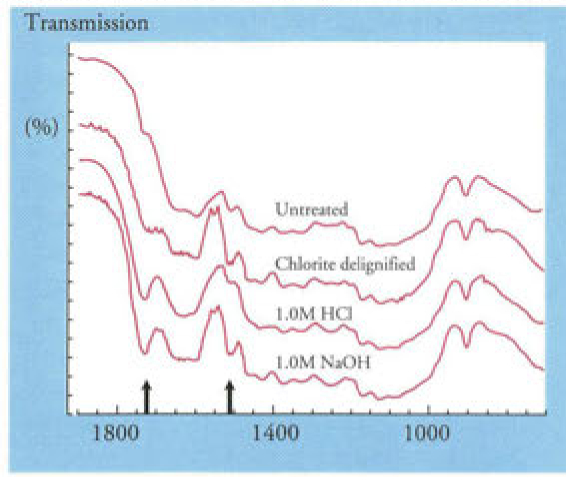


Figure 3 FTIR spectra of barley straw samples.

absorbance at $1735cm^{-1}$ is virtually unaltered on delignification showing that the ester bonds remain intact, while both the acid- and alkali-treated samples are almost devoid of this absorbance. However, a comparison of 0.1M acid and alkali treatments (not shown) do show that the acidic treatment has only a small effect while the equivalent alkali removes the absorbance. This may be due to the types of ester bonds present. Ester bonds are more stable under acidic than alkaline conditions hence the acetyl esters, linked to the D-xylopyranose residues in the main chain, remain intact on contact with acid. However, the phenolic acid esters are linked to L-arabinofuranose residues and the furanose bond is extremely acid labile. It is possible that the phenolic acid groups are removed from the fibre still linked to the arabinose residues.

There is loss of absorbance at $1510cm^{-1}$, the major absorbance of lignin, in each of the treated samples, but it is greatest in the delignified sample, less pronounced after the alkali-treatment and least in the acid-treated sample. This agrees with the known fact that lignin is partially soluble in alkali but more resistant to acidic conditions. It should also be noted that this absorbance is not completely removed from the so-called "delignified" sample. The treatment is a compromise between lignin removal and hemicellulose retention and is usually stopped before hemicellulose extraction is appreciable.

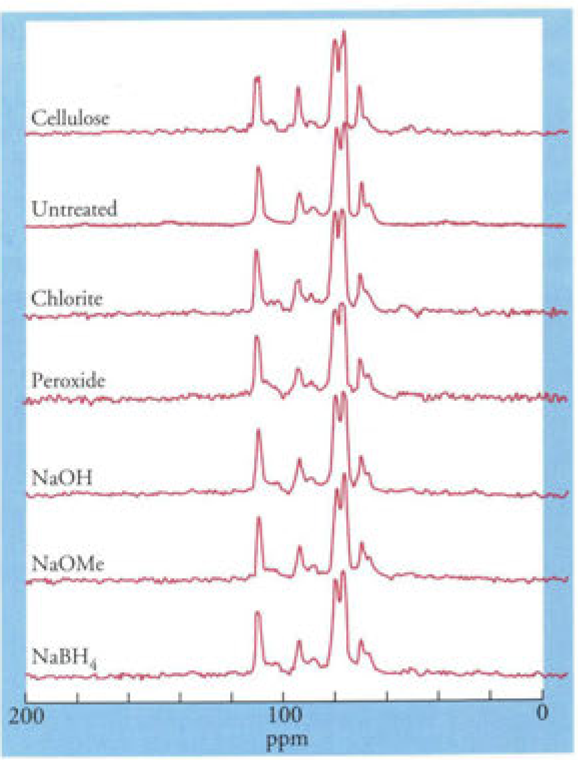


Figure 4 ^{13}C CP/MAS Spectra of treated flax samples.

Nuclear Magnetic Resonance Spectroscopy (NMR). To date, most effort has been made with the ^{13}C Cross Polarisation/Magic Angle Spinning (CP/MAS) accessory which allows the spectra of solid samples to be determined. The conditions selected were optimised for the carbohydrate signals. The signals for lignin are present but are not as well defined partly due to the nature of lignin whereby the number of different environments are great. The spectra of untreated and treated flax fibres (Fig. 4) are quite sharp and show the characteristic spectrum for cellulose (flax fibre is 75-80% cellulose). The signal for C-1 is a skew doublet at *ca.* 109.5 ppm while the signals for C-2, C-3 and C-5 are present in the doublet at 79.8-79.3 and 76.9-76.6 ppm. The more important signals are those for C-1 and C-4. The crystalline regions of flax cellulose show signals at *ca.* 93.5 and 69.9 ppm respectively. In addition, there are two broader signals downfield from each of these two which arise from the amorphous regions of the samples. The integrated ratios are, therefore, a measure of the degree of crystallinity.

The spectra of the carbohydrate region of oat straw samples are less well defined due to the presence of the non-cellulosic polysaccharides. A measure of the degree of crystallinity, however, can still be made. More recent results using cross polarisation and dipolar dephasing on flax fibre (in collaboration with the University of Strathclyde) indicates that the true lignin content is only 0.9% but considerable structural information is retained.

In connection with some investigations into purification and derivatisation of cellulose fibres, the NMR spectra of cellulose dissolved in trifluoroacetic acid and regenerated have been recorded. The ^{13}C solution spectrum has not yet been optimised due to the nature of the solvent and the low concentration achieved but the CP/MAS spectra were quite surprising in that around 20% of the crystallinity was regained. The derivatisation of cellulose as its acetate under special conditions has been monitored using solution state conditions and show that exceptionally high degrees of substitution can be obtained.

Pyrolysis Mass Spectrometry (PyMS). Although a degradative technique, PyMS only requires a minute amount of sample and, from the fragmentation pattern, considerable structural information is possible. In collaboration with Drs J J Boon and M Mülder,

FOM, Amsterdam, The Netherlands, spectra from several treated oat straw samples have been obtained. One example of the data obtained was the presence of ions of m/z 210 and 180 which are characteristic of the syringyl and guaiacyl groups in lignin. The ions were barely detectable in delignified samples while they were reduced in intensity in an alkali-treated sample. It is known that lignin is partially solubilised in an alkaline solution.

Conclusions. The use of spectroscopic techniques, especially FTIR, NMR and PyMS, have proved invaluable in characterising fibres and treated fibres. Their uses will be investigated further and the presence of the new electron paramagnetic resonance spectrometer will complement this work, especially in the area of radical-induced breakdown of fibres which cannot be assessed using NMR techniques.

Identification of plant volatiles by thermal desorption

G.W. Robertson & D.W. Griffiths

The concentration of compounds from the vapour phase by passing known volumes through tubes packed with a suitable adsorbent followed by some method of desorption is well established as the basis of many analytical procedures devised for monitoring the levels of various air-borne pollutants. The technique can be used to identify volatile compounds released from plant material and this article describes recent applications of the method at SCRI.

In early work, trapped compounds were eluted from the adsorbent by a suitable solvent such as dichloromethane, ether or carbon disulphide and, after re-concentration, the sample analysed by standard chromatographic techniques. Although these procedures have been successful in monitoring the levels of individual pollutants, the suitability of such an approach for the identification of the individual components of a complex volatile mixture such as that produced by biological material may be limited. In particular, the efficiency with which individual components of the volatile mixture are eluted from the adsorbent will depend on the solvent chosen and indeed contaminants may also be introduced by the solvent itself. More recently an alternative technique has been developed for the removal of adsorbed compounds. Broadly based on the principles of gas chromatography, the tube containing the adsorbent is heated in a temperature controlled oven to a preset value and the adsorbed compounds released into a stream of inert gas continually flowing through the tube. This process, known as thermal desorption, has been automated and successfully utilised for the identification of volatiles in both the food and drinks industries as well as in the identification of pesticides and in forensic investigations. The potential of this system for both the identification and quantification of volatiles released by various biological systems is currently being examined in several projects at SCRI. The instrumentation (Fig. 1) consists of three basic units, an automated thermal desorption system (Perkin Elmer ATD 50) linked to a capillary gas chro-

Figure 1 Automated thermal desorption and volatile analysis system.

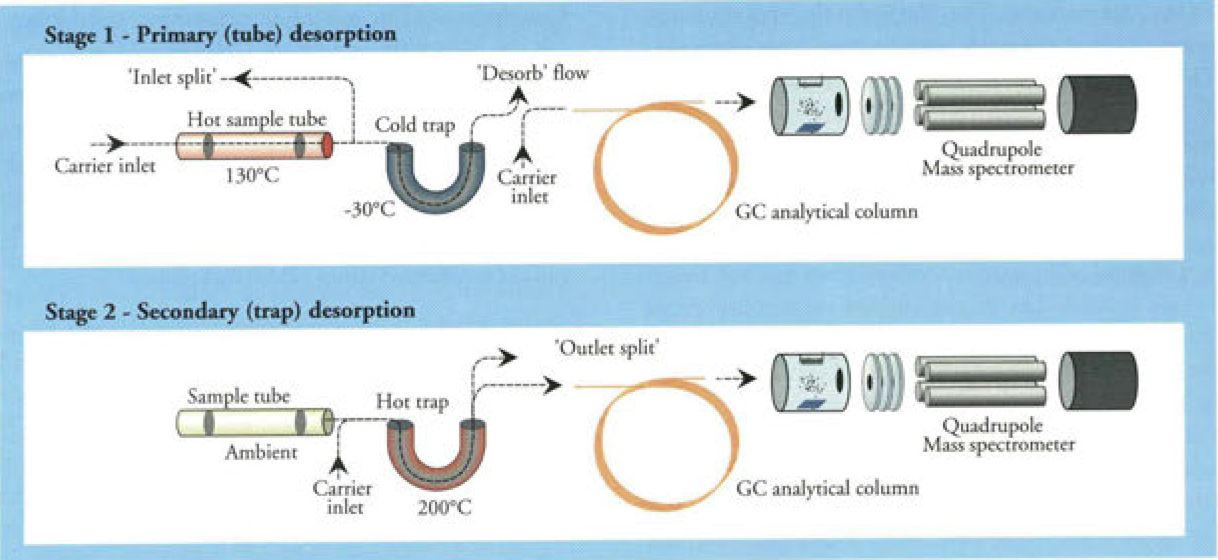


Figure 2 Schematic representation of the 2 stages of the thermal desorption process.

matograph (Hewlett Packard 5890) interfaced to a quadrupole bench top mass spectrometer (VG TRIO-1000). The desorption of each sample tube takes place in two stages (Fig. 2). During the first stage, the tube containing the adsorbed volatiles is selected from a 50 place carousel, checked for leaks and heated to a preset temperature for a specific time. In our studies on raspberry flower volatiles, the optimised conditions were 130°C for 20 min. The adsorbed volatiles are swept from the tube by a stream of helium gas and cryogenically focused in a cold trap held at -30°C. In the second stage, the sample tube is removed from the helium flow and the temperature of the cold trap rapidly increased to 200°C. This results in a concentrated band of volatiles being swept by the helium carrier gas onto the capillary gas chromatography column. Conventional temperature-programmed, high resolution gas chromatography is then used to separate the complex mixture of volatiles into its individual components, which then pass into the mass spectrometer.

The mass spectrometer can operate in two modes. In the first, it scans through a mass range of 20-450 amu every second and the mass spectrum of each components is recorded by a computerised data system. Thus the absolute identity of the compounds may be ascertained by comparison with published mass spectra. The information yielded in this mode is illustrated in Figure 3, which is part of a chromatographic trace of the volatiles entrained from flower of oil seed rape (cv. Bienvenu). A total of some 150 individual components appeared on the whole chromatogram and the identities of the major peaks such as dimethyl

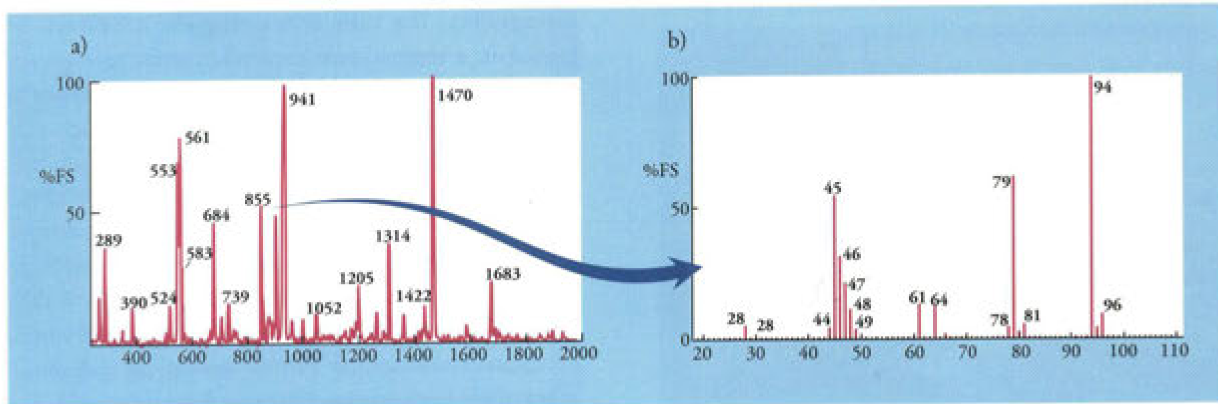


Figure 3 a) Capillary chromatographic trace of oilseed rape flower volatiles. b) Mass spectrum of peak with retention time of 855s (dimethyl disulphide)

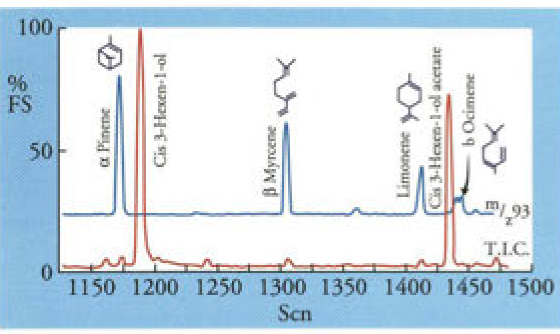


Figure 4 Total ion (TIC) and single ion (m/z 93) chromatograms of raspberry leaf volatiles.

disulphide were determined from their mass spectra. Alternatively, the mass spectrometer may be used in the single ion monitoring mode which allows for the detection of pre-determined compounds at extremely low concentrations. An example of a single ion chromatograph is shown in Figure 4 where both the total ion chromatogram and single ion chromatogram for m/z 93 are shown for the volatiles collected from raspberry leaves. The total ion chromatogram (TIC) is dominated by the two major green leaf volatile products *cis*-3-hexen-1-ol and *cis*-3-hexen-1-ol acetate. However, by examining the m/z 93 single ion chromatogram (a characteristic ion in the mass spectrum of monoterpenes) the presence of some four different monoterpenes at very low concentrations can be clearly demonstrated.

The results obtained using the technique outlined above are of course dependant on both the sampling procedures used and the selectivity of the adsorbent used during the process. A wide range of adsorbents are commercially available with their selectivity being related to the boiling range of the volatiles. We initially selected a divinyl benzene polymer which effectively adsorbed compounds with boiling points between 30°C-180°C and gave minimal background contamination under operational conditions.

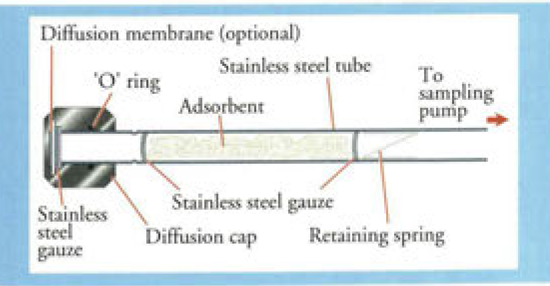


Figure 5 Cross section of sample entrainment tube.

However, further studies using alternative adsorbents will be undertaken as the applications for the technique are broadened. For both field and controlled environment studies the adsorbent is packed into commercially available stainless steel sampling tubes (Fig. 5).

Figure 6 Field sampling pump and manifold.

In preliminary studies, passive diffusive sampling techniques proved to be insufficiently sensitive to monitor short term changes in plant volatile production. Consequently, field sampling techniques employing small battery powered pumps and manifolds (Fig. 6) have been developed and evaluated. For controlled environment studies, where complete elimination of background contaminants are required, a laboratory entrainment system built of glass and PTFE and utilising a filtered air pumping system has also been constructed. These facilities are currently being used to study volatile semiochemicals from *Rubus* buds and flowers and their effects on the behaviour of the raspberry beetle and from leaves of cultivars resistant to the rasberry aphid (*Amphorophora ideae*). Field monitoring and controlled environment studies are being undertaken on flower volatiles from oilseed rape with the objective of elucidating any link between them and the allergic responses reported by some individuals in proximity to the crop. Modified entrainment systems have also been developed for the study of volatile products of microbial cultures. Furthermore, it is planned to expand this area of research to include the study of root volatiles and suitable apparatus is currently being designed. With improved entrainment facilities it would appear that thermal desorbtion has the ability to make an important contribution to our understanding of the role of plant volatiles at both the plant-animal and plant-insect interfaces.

Research on free radicals

B.A. Goodman

Introduction Free radicals are molecules that contain one or more unpaired electrons. Many, but by no means all, have limited stability and participate readily in a range of chemical reactions. Several transition metal ions also have unpaired electrons (generally referred to as paramagnetic) and we include them here along with other inorganic and organic free radical species. Furthermore our definition of free radicals is extended to include any paramagnetic centres, transient or stable, that may be found in crystalline or polymeric matrices.

Generation of free radicals in biological systems Free radicals can be generated by either chemical or photolytic processes. One of the principal radicals in biology is the superoxide anion $O_2^{\cdot-}$. This is formed as a by-product of a number of processes including mitochondrial respiration, photosynthesis and enzymatic oxidations / reductions. Superoxide anion is simply an oxygen molecule with one extra electron. Oxygen itself can be considered a "diradical" with two unpaired electrons, superoxide has just one. Figure 1 illustrates the chemical reactions that give rise to other oxygen-derived free radicals from superoxide. The most reactive of these is the hydroxyl radical which can react with lipids, proteins, nucleic acids and carbohydrates. The radicals initiate chain reactions that only terminate when two radicals react to produce a diamagnetic species (i.e. containing no unpaired electrons).

$$O_2^{\dot{-}} + H^+ \longleftrightarrow H\dot{O}_2$$

$$2O_2^{\dot{-}} + 2H^+ \longrightarrow H_2O_2 + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^- + H\dot{O}$$

Proteins Lipids Carbohydrates

Figure 1 The principal oxygen-derived free radicals in biological systems.

Free radicals may also be generated photolytically. High energy UV radiation is able to eject an electron from certain types of molecule, leaving behind a free radical, whilst gamma-radiation is able to cleave chemical bonds, producing two free radicals in the process.

Involvement of free radicals in biological processes Free radicals are of fundamental importance to a wide range of biological processes, including respiration, metabolism and senescence. The reactive oxygen species shown in Figure 1 are of particular importance in many cellular processes and photosynthesis involves a series of free radical reactions (Fig.2). Free radical polymerisation reactions are common in nature (e.g. the formation of lignin and melanin) and often result in the trapping of stable unpaired electrons that are localized in macromolecular or polymeric matrices. Metalloproteins and enzymes are crucial to many essential catalytic processes in biological systems. Such species usually exist in more than one state, each differing by one electron. Consequently some are paramagnetic and fit our definition of free radicals.

Identification of free radical reactions The traditional approach that has been used for the identification of the existence of unstable free radicals in biochemical reactions has been to analyze specimens for the presence of likely end-products of free radical reactions or for increased production of known free radical scavengers such as superoxide dismutase (SOD). Although biological systems present difficulties because of the multiplicity of possible radical reaction pathways and the existence of a number of defence mechanisms against free radical damage, this approach has been very successful in developing our

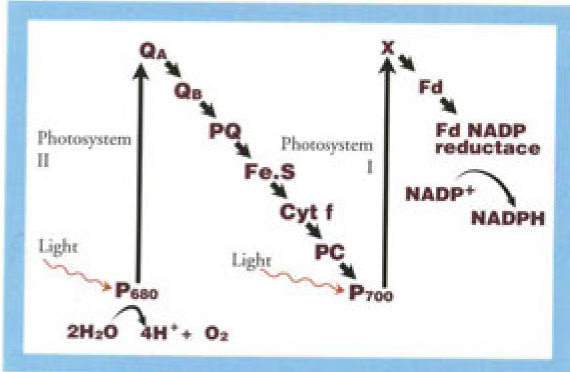
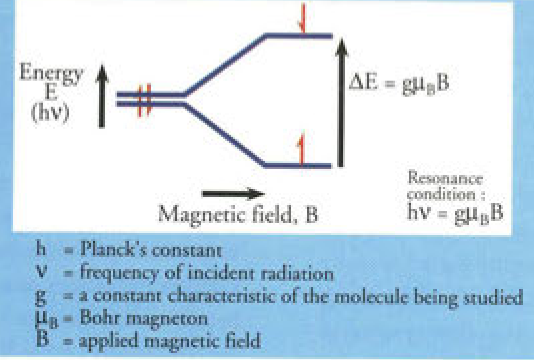


Figure 2 Photosynthesis reaction scheme.

Figure 3 The Bruker ESP300E X-band EPR spectrometer at SCRI.

basic understanding of the behaviour of free radicals in many different systems.

An example of a commonly used assay is the measurement of the oxidation of polyunsaturated fatty acids by means of the thiobarbituric acid test, which detects the aldehyde products of peroxidised lipids and carbohydrates.



h = Planck's constant
ν = frequency of incident radiation
g = a constant characteristic of the molecule being studied
μ_B = Bohr magneton
B = applied magnetic field

Figure 4 The resonance condition for the observation of electron paramagnetic resonance.

Spectroscopic techniques for radical characterisation
Free radicals can be studied directly by the technique of electron paramagnetic resonance (EPR) spectroscopy (Fig. 3). This technique makes use of the fact that an unpaired electron can exist in two quantum states which differ only in the sign of the electron spin. The energies of these states are not identical in the presence of a magnetic field and EPR spectroscopy is concerned with recording the discrete energies at which resonance between these states can be achieved (Fig. 4). In favourable circumstances the chemical nature of free radical species can be determined through identification of the atomic orbital characteristics of the molecular orbital containing the unpaired electron and the magnetic nuclei with which it interacts to produce spectral splittings known as hyperfine structure. Figure 5 shows this in the case of the paraquat radical.

Sometimes it is not possible to analyse EPR spectra unambiguously, especially when they are composed of a large number of overlapping peaks or contain hyperfine structure that is too small to be resolved. In such situations electron nuclear double resonance (ENDOR) spectroscopy may be of value. In ENDOR spectroscopy a nuclear resonance transition is observed simultaneously with the electron resonance; this results in a simplification of spectral detail and an increase in resolution of nuclear hyperfine structure.

During 1991 SCRI acquired a Bruker ESP300E EPR spectrometer, funding being provided by SOAFD as part of a Flexible Fund project for the development of research into methods for the detection of irradiated foodstuffs of plant origin and for the elucidation of the roles of free radicals in senescence-related processes in plants. The Institute also purchased a new state-of-the-art ENDOR ESP350 accessory, which is the only ENDOR facility in Scotland.

Characterisation of unstable radicals Unstable free radicals can be studied by EPR or ENDOR spectroscopy if procedures are used to trap them physically or chemically, or if they are generated *in situ* in the spectrometer cavity. Physical trapping of free radicals is most readily accomplished by use of a solid matrix, e.g. by freezing the sample or by incorporating it into an inert polymer. Chemical 'spin traps' are molecules that are able to react with unstable free rad-

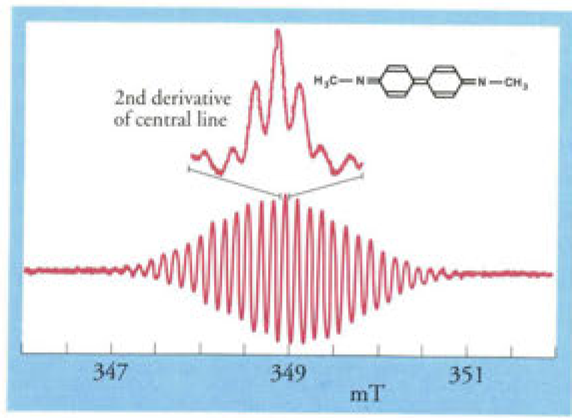


Figure 5 The EPR spectrum of the paraquat radical. The spectrum results from the interaction of the unpaired electron with nuclear spins, in this case, two nitrogens and eight ring protons. Further hyperfine structure arises from an interaction with six methyl protons.

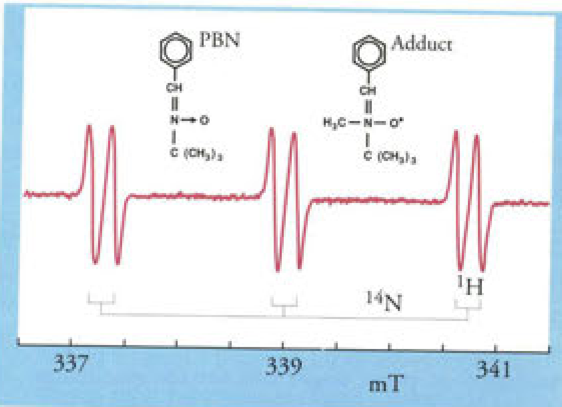


Figure 6 EPR spectrum of a spin-trapped radical. Phenylbutylnitrone (PBN) is dimagnetic and readily reacts with short-lived radicals to form a stable species in solution. The observed splittings from one nitrogen and the proton adjacent to the trapped methyl radical are indicated.

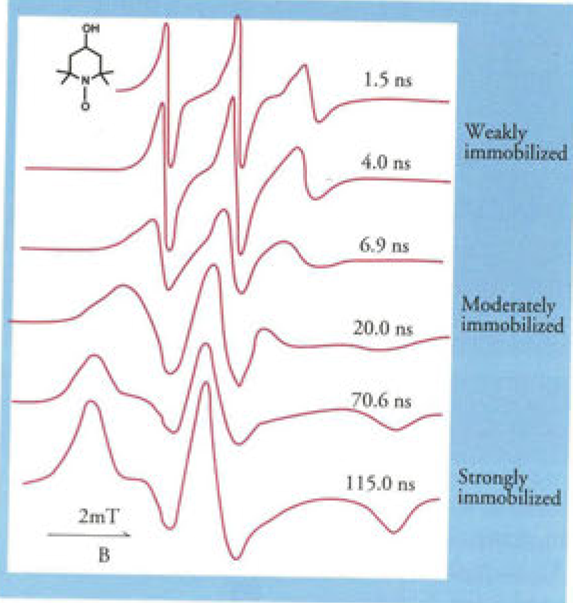


Figure 7 Variation of the EPR spectral lineshapes with rotational correlation times for a nitroxide spin label.

icals to produce new free radicals that are stable for long enough to allow their spectra to be recorded (Fig. 6). This is now a rapidly developing area of science with attempts being made to produce spin traps that are specific for different types of radical. Generation of free radicals *in situ* in the spectrometer may be achieved by irradiation of specimens with high energy radiation (e.g. UV), or by the use of a 'flow system' in which radicals are generated chemically outside the spectrometer and then flow through the cavity a short time later. This time can be adjusted physically by varying flow rate and distance that radicals travel before reaching the cavity. Thus kinetic information on the rates of decay of unstable free radicals can be obtained.

Use of free radicals as structural probes Stable free radicals, such as nitroxide-containing molecules (known as 'spin labels'), can be used as probes for the study of the dynamics of biological macromolecules. Line shapes in spectra obtained by EPR, ENDOR and some associated techniques are sensitive to the degree of mobility of the part of the molecule containing the unpaired electron, with each technique able to probe different time scales. The dependence of EPR spectral line shapes on motional correlation times of a nitroxide spin label is shown in Figure 7. For example, incorporation of spin label probe molecules into biological membranes at different depths would allow their fluidity to be measured as a function of distance from the surface. Similarly degrees of aggregation of macromolecules can be determined by analysing spectra of samples with spin labels bound to their surfaces.

Current research activities Several research projects, in which free radicals are expected to feature in an important role, are either underway or planned to commence in the near future. These are discussed briefly in this section.

1. Development of procedures for the identification of irradiated foodstuffs: During the preservation of foodstuffs by ionizing radiation free radicals are generated in large amounts as a result of homolytic cleavage of chemical bonds. Many of these free radicals, such as those illustrated in Figure 1, are very short-lived at ambient temperatures. However they may react with macromolecules or polymers to produce stable radical centres. Alternatively, stable free radical centres may be produced in macromolecules directly as a result of radiation damage. Indeed, the irradiation procedure is effective because it results in damage to the DNA of micro-organisms, which prevents them from multiplying. One of the objectives of this research project is to identify and characterise free radicals that are produced in plant materials as a result of ionizing radiation. The EPR spectrum of the free radical centre that is produced in cellulose is illustrated in Figure 8.

2. Identification of the roles of free radicals in senescence-related processes: Reactive oxygen radicals, such as those shown in the reactions illustrated in Figure 1, are thought to feature strongly in aging and senescence processes in a wide range of biological

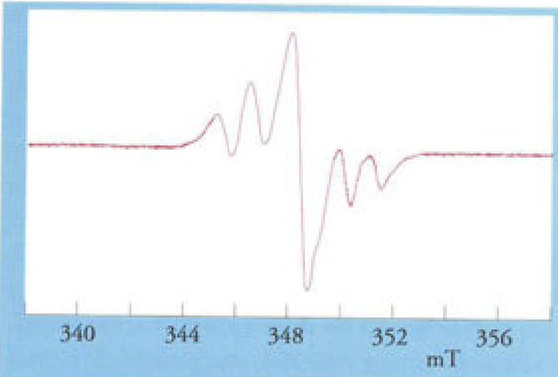


Figure 8 EPR spectrum of the free radical centre produced on gamma -irradiation of cellulose.

systems. The production of such radicals has also been proposed to occur as a response of plant tissues to pathogenic challenge. To date the evidence for this, rests on the simultaneous production of radical scavengers such as superoxide dismutase. We are now developing an EPR-based approach to this problem, which is being carried out at two different levels of sophistication. Firstly, searches are being made for the presence in senescent tissue of stable free radical products, that are absent from normal healthy tissue. So far these have been seen in necrotic tissue formed in a variety of plant types (e.g. in the Internal Rust Spot disorder of potatoes, where EPR shows the formation of a stable organic free radical and oxidation of the metal ions Fe^{2+} and Mn^{2+} to Fe^{3+} and Mn^{3+} or Mn^{4+}). The second phase of the work will be to attempt to 'trap' initiating and/or propagating free radicals by use of a chemical spin trap and also to identify the nature of trapped species by both EPR and ENDOR.

3. Determination of the levels of stable free radicals in various foodstuffs: Most foodstuffs contain stable free radicals that can be measured by EPR spectroscopy. Therefore, in conjunction with the work on irradiation-induced free radicals in plant-derived foodstuffs, the natural free radicals will also be studied in a range of food substances, including many that are unlikely to be the subjects of irradiation treatment. At present there is no information on either the amounts or the behaviour of stable free radicals in the human diet. They could be beneficial, deleterious or inert. They could conceivably react with beneficial free radical scavengers rendering them unavailable for their normal functions, or alternatively they could act as free radical scavengers in their own right. This project will commence the establishment of a data base for the free radical contents of some major food components and investigate their reactions with important free radical scavengers.

4. Elucidation of free radical processes in the photolytic degradation of xenobiotic molecules: The action of many xenobiotic molecules involves free radical intermediates (e.g. the paraquat radical illustrated in Fig. 5). Detoxification processes may also involve free radicals. This project aims to investigate the light-mediated decomposition of a number of xenobiotic molecules that contain one or more aromatic rings. Experimentally this will involve irradiation of solutions of xenobiotics with UV radiation, whilst simultaneously recording the EPR and/or ENDOR spectra. It is anticipated that the resulting free radicals will have sufficient stability as a result of electron delocalisation over the aromatic rings for measureable quantities to build up in the solutions

5. Characterisation of free radical intermediates in the biosynthesis and degradation of fibre cell walls in plants: Free radicals have been proposed to have essential functions in both the formation and degradation of plant cell wall materials. In common with the necrotic tissue formed as a result of the responses of plants to pathogenic challenges, plant cell walls invariably contain stable free radical centres, which readily yield EPR spectra. This project, however, will concentrate on investigations of unstable free radical intermediates in both the formation and decomposition of plant cell walls. The former experiments will make use of the same *in situ* irradiation facilities as the xenobiotic decomposition measurements, whereas the latter will concentrate on chemical degradation reactions.

6. Identification of paramagnetic species in fungi: Several species of fungi have a high tolerance to heavy metals. From preliminary EPR spectra it would appear that at least in part these are taken up and utilized by the organism. The EPR method is particularly good for characterizing complexes of the Cu^{2+} ion and we are now developing methods for identifying the coordination environments of Cu^{2+} in a variety of fungal species.

Plant Pathology

Plant Viruses

A.F. Murant

Research on plant viruses at SCRI is aimed at gaining as complete an understanding as possible of the molecular and biological properties of the viruses and of the ways in which they infect plants and cause disease. The investigations include studies of how the viruses spread, and of methods of disease prevention and control.

The year was marked by the retirement in June of B.D. Harrison, who had headed the Virology Department at SCRI for 25 years. Under his dedicated leadership SCRI has achieved international recognition for its research on plant viruses of all kinds, affecting not only temperate crop plants important in Scotland and other parts of the UK but also a number of tropical and sub-tropical plants essential to the economies of developing countries around the world. These studies have embraced all aspects of plant virology, including the aetiology, epidemiology, diagnosis and control of virus diseases, and the purification, characterisation, taxonomic affinities and basic molecular properties of the causal viruses. Particular emphasis has been placed on gaining an understanding of those molecular properties involved in virus replication, in the determination of host range (resistance and susceptibility) and in vector transmissibility. Although much of the research has been virus-oriented, special attention has been paid to viruses affecting certain crops, notably raspberry, potato and bulbous ornamentals. Viruses of bulbous ornamentals, especially daffodil and tulip, were a lifetime study of W.P. Mowat, who also retired during the year after 32 years' service to SCRI. His monument is the establishment in Scotland of the world's largest commercial stocks of virus-tested narcissus bulbs (*Ann. Rep. 1990*, 80). In recognition of his achievements for the industry, he received a special retirement presentation from the Scottish Nuclear Stock Association (Flower Bulbs) Ltd.

A knowledge of the genetic composition of plant viruses is essential for understanding how the viruses function and what affinities they have with others. This is especially important with viruses that belong to taxonomic groups that have been accorded little or no previous study. Two such viruses, which have been made the type members of newly constituted plant virus taxonomic groups or 'genera', are raspberry bushy dwarf virus (RBDV; idaeovirus group) and parsnip yellow fleck virus (PYFV; proposed group name 'sequivirus'). Each of these viruses was first described and characterised at SCRI. Determination of the genome nucleotide sequences of both viruses was completed during the year. The work on RBDV

is summarised in one of the accompanying research reviews. Work with PYFV has shown that it has a single genomic RNA, comprising 9871 nucleotides, most of which represents the sequence for a single large polyprotein of 3027 amino acids. This is probably cleaved enzymically to produce the functional proteins. Although the nucleotide sequence is different from that of any other plant virus so far described, comparison of the polyprotein sequence with those of several other viruses revealed similarities to the putative NTP-binding and RNA polymerase domains of cowpea mosaic comovirus, tomato black ring nepovirus and some picornaviruses of vertebrates. Evolutionarily, PYFV seems to occupy a position somewhere between the picornaviruses, which have one genome part, and the comoviruses and nepoviruses, which have two.

The satellite RNA associated with groundnut rosette virus (GRV) is another novel type of molecule. It is of interest because it is the main cause of the symptoms of groundnut rosette disease (different variants of the satellite being responsible for the different forms of the disease), and also because it is essential, along with a luteovirus, groundnut rosette assistor virus (GRAV), for the transmission of GRV by *Aphis craccivora*. The nucleotide sequences of 10 clones, representing five symptom variants of the satellite from Malawi and Nigeria, were determined. No significant similarities were detected with any other published sequences in computer databases. A stretch of 372 nucleotides, capable of coding for a protein of 124 amino acids, is conserved in size and position in all the variants sequenced so far. It is now important to know whether this protein, if expressed in infected plants, plays a part in the induction of rosette symptoms by the satellite and in its mediation of the aphid transmission of GRV.

Detailed examination can sometimes provide insights into the features of the nucleotide sequence that are responsible for the observed biological properties of a virus or virus isolate. One example of this was found in studies on potato leafroll luteovirus (PLRV), the full genome nucleotide sequence of which was published previously. In further studies, sequence comparisons were made of the genes for the particle proteins of an aphid-transmissible isolate and a poorly aphid-transmissible isolate. The results suggest that only one or two amino acid changes in the read through protein account for the difference in the aphid-transmissibility of the isolates.

Another example showing how features of the nucleotide sequence can explain the biological properties of a virus was found in work with strain I6 of tobacco rattle virus (TRV). This strain is anomalous because although it behaves as part of the TRV gene pool it is serologically related to pea early-browning virus (PEBV). Studies at SCRI have shown that, whereas the sequence of RNA-1 of I6 is similar to that of typical TRV strains, RNA-2 is a naturally occurring recombinant molecule, most of whose sequence resembles that of PEBV except that the 5' end contains sequence derived from the 5' end of TRV RNA-2. This explains how the RNA-2 of strain I6 can be recognised by the replicase enzyme encoded by TRV RNA-1. This is one of the few examples known of natural recombination among RNA viruses; it confirms what has long been suspected that viruses and other organisms might have acquired pieces of their genetic information from others.

Studies of another type are enabling us to understand more about the molecular basis of immunological reactions. Analysis of polyclonal antisera to TRV strain PLB with synthetic overlapping peptides (Pepscan) showed that the immunodominant regions of the coat protein were an amino acid sequence near the C-terminus and an internal sequence that may be part of a loop in the tertiary structure. Few antibodies were detected against the N-terminal region. The immunodominant regions are in parts of the protein that vary between strains, and explain their antigenic diversity. Five monoclonal antibodies (MAbs) were obtained to strain PLB. Two of these were serotype-specific, two reacted with only some representatives of the serotype to which PLB belongs, and the fifth reacted with members of the two serotypes against which it was selected but not with others. Thus different MAbs have different specificities and can be used for different diagnostic purposes. Mabs with a similar range of properties are being sought against a number of viruses, including black raspberry necrosis virus, GRAV, PYFV and a South American isolate of PLRV.

Examples of the uses to which such a range of MAbs can be put are given in the accompanying articles on potato mop-top virus and the whitefly-transmitted geminivirus (WTG), okra leaf curl. Another example is their use in the investigation of the ecology and epidemiology of WTGs. A panel of 27 MAbs raised previously to particles of African cassava mosaic virus (ACMV) or Indian cassava mosaic virus (ICMV) show that different WTGs have characteristic epitope

profiles. These MAbs were used to detect and type WTGs in wild plant species growing in or near cultivated fields in tropical countries. The results show that wild plants are probably epidemiologically important sources of Indian tomato leaf curl virus and Indian isolates of tobacco leaf curl virus. Several WTGs found in wild plants had epitope profiles different from those found so far in cultivated plants. Some of these WTGs may have the potential to become crop pathogens following the introduction into an area of new crops or crop cultivars, or of biotypes of the vector, *Bemisia tabaci*, with altered host preferences.

The same panels of MAbs to ACMV and ICMV were used to compare three WTGs that occur in Europe: abutilon mosaic, tobacco leaf curl and tomato yellow leaf curl. Each virus was found to have a distinct epitope profile, and MAb-based diagnostic tests were designed for each virus.

Development and application of sensitive diagnostic techniques is an important part of the work at SCRI, and MAbs form the basis of many such tests. Others are based on nucleic acid hybridisation. For example, the molecular work on the GRV satellite RNA has enabled the development of a cDNA probe that will detect all forms of the satellite, including forms that induce few or no symptoms in groundnut. Another line of approach that shows great promise is the use of the polymerase chain reaction (PCR) technique, by which specific nucleotide sequences can be sought out and amplified many times. In principle, the PCR technique is capable of detecting a single molecule of a previously known sequence. During the year it was used to detect PLRV in plant tissues, protoplasts and vector aphids, potato virus Y in potato shoot and tuber tissue, raspberry ringspot virus in vector nematodes, and several WTGs in plant tissue and vector whiteflies. A PCR test based on the RNA-1 of TRV was used to detect the full range of serological variants of TRV, as well as NM isolates that lack RNA-2 and therefore lack the coat protein gene; it also distinguished TRV variants, including strain I6, from PEBV and pepper ringspot virus.

Such approaches are not always possible with viruses that are difficult to study and characterise, but the accurate diagnosis of such viruses is nevertheless necessary in plant quarantine. As part of a programme for germplasm enhancement in *Ribes*, more than 100 *Ribes* species and selections were imported under SOAFD licence from Poland, Scandinavia and countries of the former USSR. All the material was tested by graft-inoculation to indicators of known *Ribes* viruses and virus-like agents, and by manual inoculation of sap to herbaceous test plants. No manually transmissible virus was detected, but 13 cultivars or selections of blackcurrant were found to be infected with the agent of reversion disease, in most instances with that causing the severe (Russian) form of the disease, and three *Ribes* species were infected with gooseberry veinbanding virus. Interception of these diseased plants indicates the importance of the voluntary quarantine scheme adopted at SCRI.

The most successful and environmentally acceptable way of controlling plant virus diseases is by planting resistant or immune varieties, and assessment of the resistance of new cultivars is an important and continuous part of the work at SCRI. In recent years, however, molecular biology has provided a new approach, following the finding that introduction into the plant genome of genetic material derived from virus genes can sometimes confer resistance to the viruses. The production of clones of two potato cultivars carrying the PLRV coat protein gene was described previously (*Ann. Rep. 1990*, 78), and further work in potato and other crops is in progress. However, it is important to understand how these newer forms of resistance operate and whether they differ fundamentally from natural forms of resistance already known. Previous studies have shown that multiplication of PLRV is restricted in potato plants that have been transformed with the coat protein gene of PLRV. This form of resistance in transgenic plants resembles a form of host gene-mediated resistance found in some potato clones: both are expressed as a restriction of virus multiplication in external phloem bundles but not in the internal phloem bundles. Aphids were shown to transmit PLRV much less efficiently from naturally resistant plants than from susceptible plants, but tests to show whether the same is true of plants with transgenic resistance have not yet been possible. Work has begun to combine the two forms of resistance.

The programme of work outlined here illustrates the numerous ways in which molecular and traditional approaches to virology can come together in a broadly based programme, to provide new scientific insights and to bring about improvements in the control of plant viruses and virus diseases.

Diversity of tobraviruses and transmissibility by nematodes

D.J.Robinson, A.T.Ploeg, D.J.F.Brown, & F.J.Legorburu

The tobravirus group of plant viruses includes three members. Tobacco rattle virus (TRV) has been found in many countries of Europe, and in North America, Japan and New Zealand. It infects an exceptionally wide range of plants, including weed and crop species. As its name suggests, it causes a characteristic disease in tobacco, in which the leaves become necrotic, dry out and rattle in the wind.

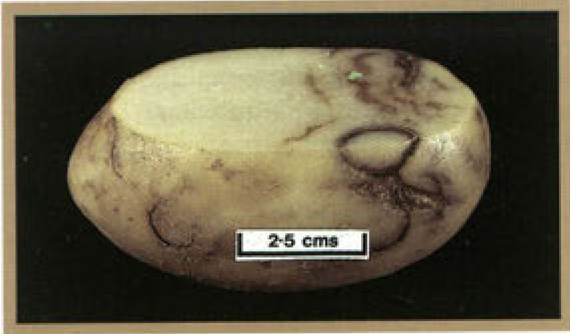


Figure 1. Symptoms of spraing disease in a potato tuber cv. Pentland Crown.

In the UK, TRV is best known as the causal agent of one form of spraing disease of potato, in which broken arcs of brown corky tissue form in the tuber flesh (Fig.1), and which, if it occurs in a significant proportion of a ware crop, can make it unsaleable. The second virus that causes spraing symptoms, potato mop-top virus, is described in another article on p. 80 of this Report. TRV also causes diseases in several ornamental plants, including tulip, hyacinth, narcissus, gladiolus, lily and crocus. The leaves of most of these become mottled, and those of gladiolus may develop tears at intervals ('notched leaf'). Hyacinth bulbs may develop necrotic spots ('malaria') and tulip petals may have streaks of darker colour (Fig. 2).

Pea early-browning virus (PEBV) is known to occur only in Western Europe and North Africa, and causes diseases in peas, beans (both *Phaseolus vulgaris* and *Vicia faba*) and lucerne. Pepper ringspot virus (PRV) is confined to South America, where it infects pepper, tomato and artichoke.

Figure 2. Dark streaks in the petals of tulip cv. Apeldoorn caused by infection with TRV.

An unusual feature of the tobraviruses is the extent to which isolates of the same virus differ from one another. This diversity is manifested in several ways. Serological tests divide isolates into groups, called serotypes, members of which are closely related to one another but only distantly related to members of other serotypes. So far, at least eight serotypes of TRV and three of PEBV have been recognized. Isolates also differ widely in the symptoms they cause in test plants

Figure 3. Symptoms caused in *Nicotiana clevelandii* by NM-type isolates (left) are often more severe than those caused by particle-producing isolates (right).

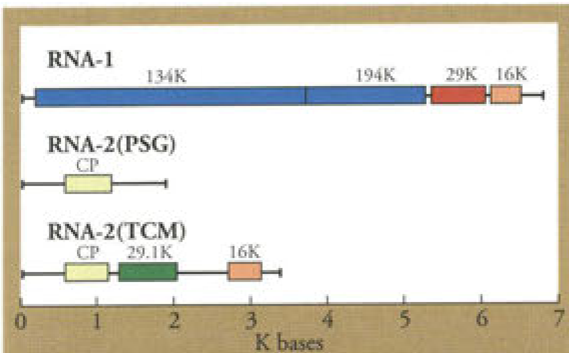


Figure 4 Genetic maps of typical tobravirus genome RNA.
RNA-1 (top) contains genes for RNA replication (134K and 194K), for cell-to-cell movement (29K), and another whose function is unknown (16K).
RNA-2 of strain PSG (middle) contains the particle protein gene (CP).
RNA-2 of some isolates, e.g. strain TCM (bottom), contains, in addition to the particle protein gene, a duplicate copy of a gene from RNA-1 (16K) and another gene of unknown function (29.1K).

in the glasshouse, but this is not correlated with the serological variation. A major focus of work at SCRI has been to determine the molecular basis of tobravirus diversity, and to investigate some of its consequences.

Another kind of variability that is unique to tobraviruses is the existence of so-called NM-type isolates. The genome of tobraviruses consists of two pieces of single-stranded RNA, RNA-1 and RNA-2, which are separately contained in long and short rod-shaped virus particles, respectively. Inoculation of plants with RNA-1 or long particles alone leads to infections that can spread systemically throughout the plant, but in which no virus particles are produced. Symptoms are usually slower to develop and more necrotic (Fig. 3) and persistent than those of particle-producing isolates. These NM-type isolates are common in nature, particularly in potatoes. The explanation for the occurrence of NM-type isolates lies in the distribution of genes between the two genome parts (Fig. 4). RNA-1 contains genes for RNA replication and for cell-to-cell movement, as well as another whose function is unknown. RNA-2 contains the gene for the virus particle protein. Thus, RNA-1 can multiply and spread through plants on its own. RNA-2 is dependent on RNA-1 for these functions, but is required for the production of virus particles.

The nucleotide sequence of RNA-1 is strongly conserved among isolates of each of the viruses, and most of the genetic variability is in RNA-2. Thus in some isolates RNA-2 contains, in addition to the particle protein gene, duplicate copies of one or two of the genes from RNA-1, and/or an additional gene of unknown function (Fig. 4). Moreover, although RNA-2 always contains the particle protein gene, the sequence of this gene, and hence that of the protein for which it codes, is highly variable. Some sporadic TRV isolates resemble PEBV in their serological properties, although in other respects they are typical of TRV. RNA-2 of these isolates consists largely of PEBV-like sequences, whereas RNA-1 and the ends of RNA-2 are TRV-like. These isolates have presumably arisen by RNA recombination and were one of the first examples of the natural occurrence of such a process among plant viruses.

More detailed comparison of the particle protein sequences of tobravirus isolates showed that some regions, including both ends and some internal segments, were highly variable, whereas other regions were conserved. To ascertain which of these regions are important for serological reactivity, all possible octapeptide fragments of the protein of TRV strain PLB were synthesized and tested for their ability to react with an antiserum against particles of strain PLB. Two regions proved to be immunodominant, a sequence of 25 residues at the C-terminus and an internal segment of 12 residues. Both are regions that vary between different isolates. Comparisons with other viruses whose particles have similar architecture suggest that the C-terminal sequence may be exposed along the sides of the rod-shaped tobravirus particles and the internal loop at one end.

Figure 5. Head of *Paratrichodorus pachydermus.*

Tobraviruses are transmitted by species of *Trichodorus* and *Paratrichodorus* nematodes (trichodorids; Fig. 5). These are relatively small nematodes, up to about 2mm long . The virus particles become attached to the inner surface of the nematode's oesophagus and are thought to be ejected subsequently with saliva to

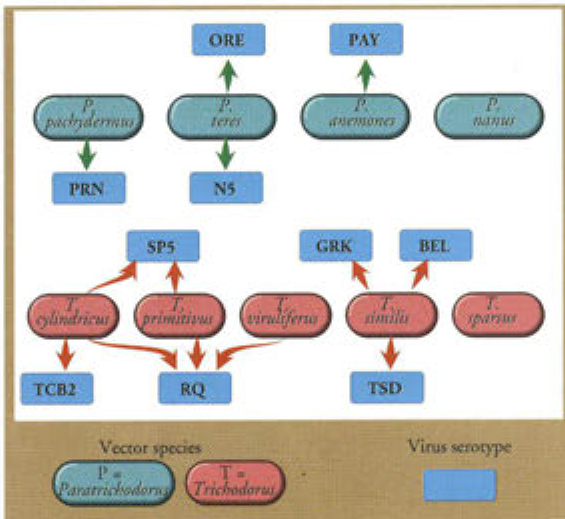


Figure 6 Associations between trichodorid species and TRV serotypes.

infect root cells that are punctured by the stylet. However, at least for TRV, each vector species is able to transmit only certain virus isolates. Tests with single, naturally viruliferous nematodes showed that, for example, virus transmitted by *Paratrichodorus pachydermus* almost invariably belonged to the PRN serotype, and isolates of this serotype were never transmitted by other trichodorid species. Instead, other vector species each transmitted members of one or a few particular serotypes of TRV. Conversely, each TRV serotype was recovered only from transmissions by one or a few particular trichodorid species. Figure 6 shows in diagrammatic form the associations that have been identified. Thus, there is clearly a correlation between virus serotype and specific vector species, but not a simple one-to-one correspondence. Presumably, both antibodies and nematodes recognize features on the surface of the virus particles, but the features that they recognize are not exactly the same.

In a further series of tests, *P. pachydermus* from virus-free populations were allowed to feed on plants infected with selected TRV isolates and then tested for their ability to transmit. As expected, only isolates of the PRN serotype were transmitted, but among such isolates there were differences in the efficiency with which they were acquired and transmitted. Populations of *P. pachydermus* from Scotland and from the Netherlands both transmitted a Scottish virus isolate more efficiently than a Dutch isolate, suggesting that efficiency, which might be of acquisition by the vector or of subsequent steps in transmission, is a property of the virus isolate rather than of the nematode population. Several isolates, although members of the PRN serotype, were not transmitted in these tests. These isolates were all originally obtained from potato plants and had been maintained in glasshouse plants by mechanical transmission for periods varying from a few to many years. Thus these isolates appear to have lost the ability to be transmitted by vector nematodes.

The vector-transmissible isolate PPK20 and the vector non-transmissible isolate PLB were used to prepare pseudo-recombinant isolates in which RNA-1 was derived from one parent and RNA-2 from the other. The combination of RNA-1 from PLB with RNA-2 from PPK20 was transmitted by *P. pachydermus*, whereas the isolate comprising RNA-1 from PPK20 and RNA-2 from PLB was not. Therefore, the ability to be transmitted by *P. pachydermus* was controlled by RNA-2, in agreement with the idea that it is a function of the particle protein.

In addition to variations in transmissibility within a serotype, there are also serological differences. Among a panel of monoclonal antibodies (MAbs) prepared to TRV strain PLB, a member of the PRN serotype, were two that reacted only with some members of this serotype and not with others. Unfortunately, the antigenic differences recognized by these MAbs were not correlated with differences in vector transmissibility. There are yet further levels of diversity in the ability of isolates to cause symptoms in infected plants. For example, a series of 13 isolates obtained from individual *P. teres* from a site in the Netherlands all belonged to the same serotype, but induced symptoms in *Nicotiana glutinosa* that ranged from barely discernible or scattered necrotic ringspots to severe systemic necrosis (Fig. 7).

Figure 7. Symptoms in *Nicotiana glutinosa* induced by three serologically indistinguishable isolates of TRV, each obtained from an individual *P.teres* from the same Dutch population.

The diversity of tobraviruses has several practical implications. The antigenic diversity causes problems of detection and diagnosis. Serological methods such as ELISA, which are widely used for other plant viruses, are unsatisfactory because it is impossible to design a test that will detect the whole range of variants. Tests based on nucleic acid hybridization or on the polymerase chain reaction have been developed to overcome this problem.

The diversity in nematode transmissibility has implications for the ecology of the viruses. TRV, in particular, is difficult to eradicate from a site once it becomes established in association with its vector. The diversity of symptom production in test plants suggests that there is probably also variation in the ability to cause diseases in crop plants. Although the extent of this variation is not known, there are some isolates that cause spraing in potato cultivars that are normally resistant. Resistant cultivars offer the most effective measure for disease control, but variation in the virus should be taken into account when screening for resistance in breeding programmes.

Detection and diagnosis of potato mop-top virus

L. Torrance, G.H. Cowan, K.P. Scott, L.G. Pereira, I.M. Roberts, B. Reavy & B.D. Harrison.

Although many of the viruses that infect British potato crops are transmitted from plant to plant by aphids, a few have soil-inhabiting vectors. Potato mop-top virus (PMTV) is a furovirus, a group of plant viruses which have root-infecting fungal vectors and rod-shaped particles. In the last decade, viruses with soil-borne fungal vectors have caused major disease epidemics in sugar beet, e.g. rhizomania, and barley, e.g. barley yellow mosaic virus, in Europe and elsewhere. Serious outbreaks of PMTV have occurred in crops of potato cultivars used for crisp making in the Nordic countries, especially Denmark and Finland causing problems for the potato processing industry.

The main effect of PMTV on potatoes is on tuber quality. In sensitive cultivars, it causes spraing symptoms consisting of brown arcs and lines in the tuber flesh, and the lines may also be visible on the tuber surface (Fig. 1). Similar symptoms are caused by

Figure 1. Tuber symptoms (surface lines and internal brown arcs or spraing) of PMTV in cv. Arran Pilot.

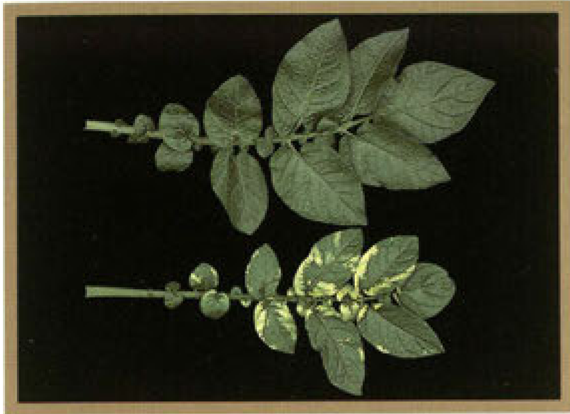


Figure 2. Yellow leaf markings in potato cv. Maris Piper caused by PMTV.

tobacco rattle virus (TRV) described on p 77 of this Report. Yellow markings (Fig. 2) are produced on leaves of plants grown from infected tubers and the haulms of some cultivars become stunted, producing the mop-top symptom (Fig. 3). The severity of symptoms induced by PMTV varies greatly with the potato cultivar and environmental conditions. PMTV does not infect all the tubers produced by infected mother plants and so is passed on to a variable proportion of the progeny plants in the following year. It is transmitted by the motile zoospores of the fungus *Spongospora subterranea*, which causes powdery scab of potatoes.

Figure 3. Potato cv. Alpha infected with PMTV: infected plants have short internodes resulting in the mop-top symptom (healthy plant on left).

The spraing symptoms caused by PMTV can be confused with those induced by the nematode-transmitted TRV but, because PMTV can survive for years in soil in association with dormant fungal resting spores, control measures for the two viruses are very different. It is therefore important to have a rapid diagnostic procedure to detect PMTV and distinguish it from TRV.

A method was devised to purify the particles of a Scottish isolate (T) of PMTV from herbaceous experimental host plants, and the resulting preparations were used to produce polyclonal and monoclonal antibodies (MAbs). Three of these MAbs (code-named SCR 68, 69 and 70) were selected for use in virus assays. In triple antibody sandwich (TAS) ELISA, polyclonal antibodies were used to coat microtitre plates and thus to trap virus from the sample onto the plate. The MAbs were used as the second antibody, and each of them readily detected the trapped virus. This system successfully detected PMTV isolates from Northern Ireland, Finland, Sweden, Norway, Denmark and Japan.

When PMTV particles were stained with gold-labelled MAb and the preparations examined in the electron microscope (Fig. 4), MAb SCR 68 was found to react with one end of the rod-shaped particles (Fig. 4a), whereas MAb SCR 69 was found to bind to the surface along the sides of the particles (Fig. 4b). Using this information, tests based solely on MAbs were devised. MAb SCR 68 was used to coat microtitre plates and to trap virus particles, and MAb SCR 69 labelled with either alkaline phosphatase or with biotin was used to detect them. Biotin-labelled antibody was detected with a streptavidin-alkaline phosphatase conjugate. More than 20 virus isolates from the UK and the Nordic countries were readily detected by these tests.

The usefulness of TAS ELISA (using MAb 68 as the second antibody) was assessed by comparing it with double antibody sandwich (DAS) ELISA in which polyclonal antibody was used both for coating the plates and, when conjugated with alkaline phosphatase, for detecting trapped virus. The

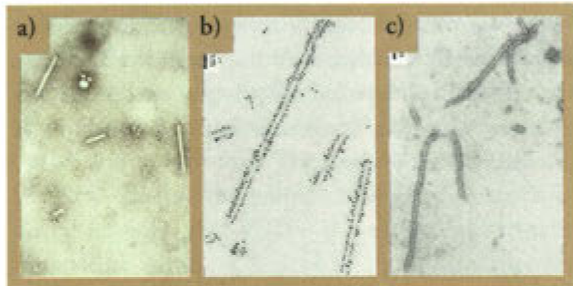


Figure 4. Reactions of gold-labelled monoclonal antibodies with particles of PMTV. The immunogold reactions are visible as small black spots:
a) MAb SCR 68, b) MAb SCR 69, c) unlabelled particles

Plant	Detection of PMTV in samples of Leaf*	Tuber*
1	2/6†	5/8
2	1/4	7/8
3	4/7	0/9
4	1/6	12/13

* Samples were taken from different leaves or tubers.
† Number of samples in which PMTV was detected/total number tested.

Table 1 Erratic distribution of PMTV in infected potato plants as indicated by ELISA.

samples consisted of extracts of leaves and tubers from field grown potato plants of cultivars Désirée and Maris Piper which had yellow leaf markings typical of infection with PMTV. Both TAS ELISA and DAS ELISA detected PMTV in the same extracts. In addition, the results supported other evidence that the virus was distributed erratically in individual plants (Table 1). For example, it was detected in some leaflets from a compound leaf but not in others. Similarly, it was detected in some extracts but not in others from different parts of the same tuber. TAS ELISA proved to be suitable for detecting PMTV in tuber extracts and gave negligible non-specific background reactions.

Although serological tests effectively detected PMTV, an alternative approach, based on nucleic acid hybridisation tests was examined. RNA was extracted from purified PMTV-T particles and used to produce a cDNA library in the expression vector λZAPII. Hybridisation studies with four of the resulting cDNA probes showed that the PMTV-T genome consists of three distinct RNA molecules, RNA-1, RNA-2 and RNA-3 (Fig. 5). The probes detected PMTV in the leaf and tuber samples that were taken from the field grown plants for the serological tests. Total RNA was extracted from the plant tissues, separated by electrophoresis in agarose gel, transferred to nitrocellulose membrane by Northern blotting and hybridised to the radioactively-labelled cDNA probes. PMTV was detected readily in leaf tissue but only weakly and unreliably in tuber tissue.

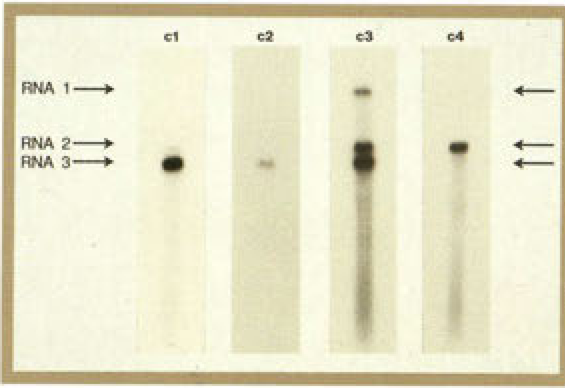


Figure 5. Northern blots of total RNA from *Nicotiana debneyi* leaves infected with PMTV isolate T and hybridised with ^{32}P-labelled cDNA probes C1-C4.

Hybridization studies with these probes also showed that the sizes of the RNA species varied in different isolates, which could be divided into three groups. The first comprised two Scottish isolates T and R, which had RNA species of 6.4 kb, 3.0 kb and 2.5 kb. The second group contained four isolates from Denmark, Norway and Japan which had RNA species of 6.4 kb, 3.0 kb, and 2.9 kb. A third group, represented by two isolates from Sweden, had RNA species of 6.4 kb, 3.1 kb, 2.9 kb and 2.8 kb. Both the 3.1 kb and the 2.8 kb molecules hybridised with the probe for RNA-2. Further work is needed to ascertain the reasons for these size differences.

The main economic importance of PMTV derives from its effect on tuber quality, but it also poses problems in potato certification schemes because, although tests are available for other potato viruses, there are no reliable widely available serological tests for PMTV and no agreed European standards for tests or diagnostic reagents. Of the tests that have now been examined, TAS ELISA seems likely to meet the need and to facilitate the rapid large-scale indexing that could increase the export potential of Scottish seed potatoes.

Acknowledgements. We thank M Sandgren, K Ryden, T Munthe, P Mills, A Kurppa and S Toriyama for supplying virus isolates.

Molecular studies on raspberry bushy dwarf virus

M.A. Mayo, A.F. Murant, A. Ziegler & T. Natsuaki

Raspberry bushy dwarf virus (RBDV) is an important pathogen of raspberry and loganberry in the UK and in other parts of the world. It was so named because of its association with bushy dwarf or symptomless decline disease of the raspberry cv. Lloyd George, though it now seems probable that this disease is caused primarily by black raspberry necrosis virus and is merely exacerbated by coinfection with RBDV.

RBDV induces yellows (Fig. 1) in some raspberry cultivars, such as Lloyd George, Norfolk Giant and Autumn Bliss, and is one of the causes of crumbly fruit (Fig. 2) in which some druplets abort and others become overlarge, resulting in distorted fruit that collapse and crumble when picked. Cv. Glen Prosen is especially prone to show crumbly fruit symptoms following RBDV infection.

RBDV has been studied intensively at SCRI since it was first described in 1961 by C.H. Cadman. It spreads in association with pollen from infected plants and can infect both the plant being pollinated (horizontal transmission) and the progeny of the fertilization (vertical transmission). Therefore, the only practical method of control is to plant resistant cultivars. Resistance to RBDV, which is governed by a single dominant gene, has been incorporated into several recently released cultivars. However, isolates of the virus that can overcome resistance have appeared and are spreading and causing disease in continental Europe. In the hope of learning more about the nature of the resistance-breaking character, and of finding ways to overcome it, studies were commenced on the molecular biology of the virus.

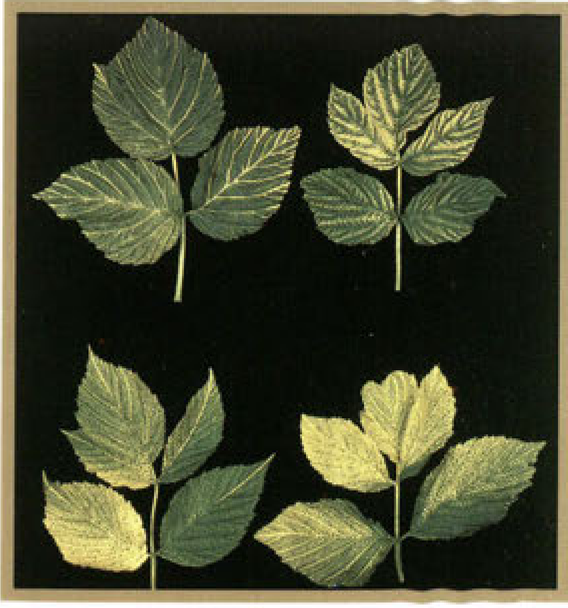


Figure 1 Leaves of Norfolk Giant raspberry infected with RBDV, showing (upper left to lower right) progressive steps in the development of the yellows symptom.

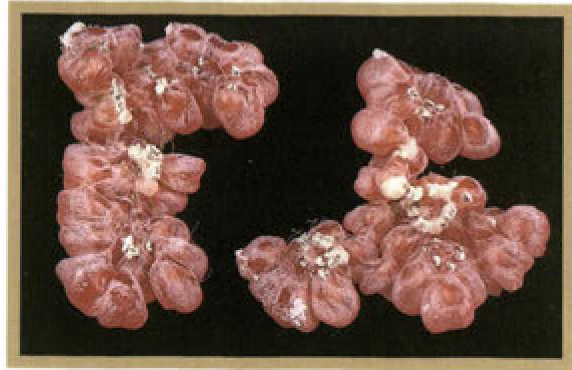


Figure 2 RBDV is one of several causes of crumbly fruit, a condition in which the fruits are distorted and crumble when picked.

RBDV has an unusual combination of properties which distinguish it from all other viruses examined. The virus particles are isometric, about 33 nm in diameter, but are easily deformed on electron microscope grids (Fig. 3). The particles have a single species of coat protein with a mol. wt of 30K and three species of single-stranded RNA of about 6 kb, 2.5 kb and 1 kb in size. The 1 kb RNA is the messenger RNA for the coat protein. Although these properties suggested that RBDV is taxonomically distinct from other viruses, it was necessary to determine the nucleotide sequence of the RBDV RNA molecules to test the hypothesis and also to understand how the genome is expressed.

The nucleotide sequence of RNA-1 comprises 5449 nucleotides and encodes one large 190K protein which corresponds in size to the largest product of *in*

Figure 3 Particles of RDBV in uranyl formate. Bar represents 100 nm.

vitro translation. RNA-2 consists of 2231 nucleotides and the 3'-terminal 946 nucleotides correspond exactly to the sequence of RNA-3, i.e. RNA-3 is a subgenomic RNA derived from RNA-2. Thus the genome of RBDV is bi-partite comprising RNA-1 and RNA-2; the arrangement of genes is shown in Fig. 4. RNA-2 contains two genes but only the 5'-most is expressed *in vitro* and it encodes a 39K protein which may be involved in cell-to-cell movement of the virus. There is no double-stranded form of RNA-3 in infected plants and presumably *in vivo* the coat protein is produced by translation of RNA-3 molecules that arise by partial transcription of RNA-2. This arrangement of genes in RBDV RNA-2 and some features of the amino acid sequences of the encoded proteins resemble those of RNA-3 molecules of viruses in the family Bromoviridae, namely the ilarvirus, cucumovirus, bromovirus and alfamovirus taxonomic groups. However, RBDV differs from all these viruses in having a bipartite rather than a tripartite genome. Nevertheless the 190K protein encoded by RNA-1 has regions of sequence which are similar to parts of the 125K and 90K proteins encoded by RNA-1 and RNA-2 of alfalfa mosaic virus (AlMV) and, to a lesser extent, with parts of the 183K protein encoded by tobacco mosaic virus RNA (Fig. 4). A lesser degree of sequence similarity exists with proteins of viruses in several other families that have been grouped together in a supergroup (Sindbis-like viruses). Comparison of RBDV with several viruses of the Sindbis-like supergroup for similarities in amino acid sequence among the proteins corresponding to that encoded by AlMV RNA-1 yielded the pattern of relationships shown in Figure 5. A similar pattern was obtained from comparisons among proteins corresponding to the protein encoded by AlMV RNA-2. The result illustrates the similarity of RBDV to viruses with tripartite genomes in the family Bromoviridae and a lesser similarity with tobamoviruses and tobraviruses. As a result of this work, RBDV has been placed in a new taxonomic group for which the name idaeovirus (from *Rubus idaeus*) has been coined. Our results also suggest that, despite some distinctive features, RBDV could be classified in the family Bromoviridae.

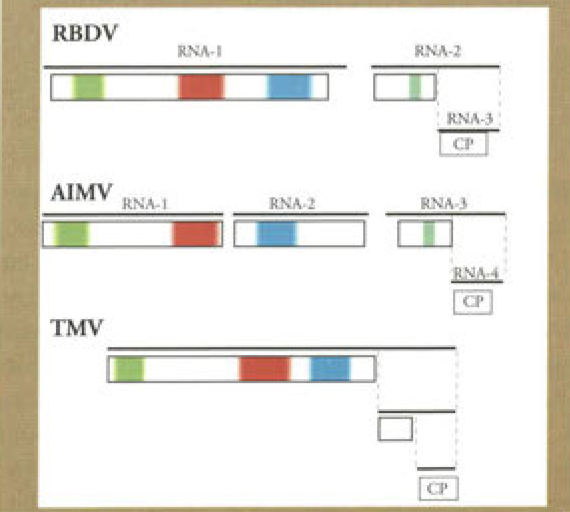


Figure 4 Arrangement of the genes in the RNA of RBDV, in comparison with those of alfalfa mosaic virus (AlMV) and tobacco mosaic virus (TMV).
Solid lines represent RNA molecules; dashed lines indicate derivation of sub-genomic RNA; boxes indicate the positions of encoded genes. Colours indicate regions of similar amino acid sequence. CP = Coat protein.

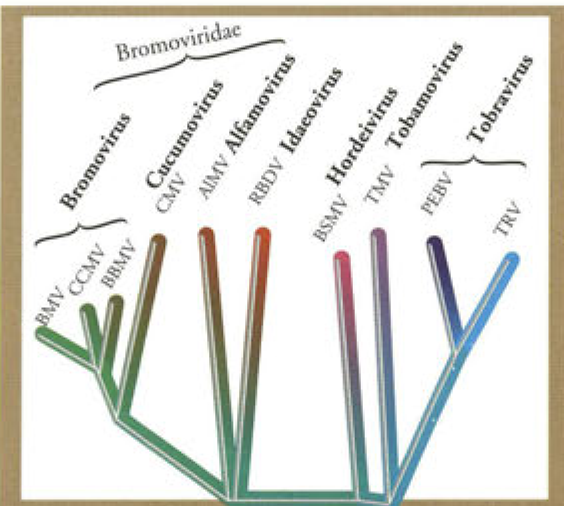


Figure 5 Diagram illustrating the relationship of RBDV to other plant virus groups inferred from comparisons of RBDV RNA-1-encoded protein with analagous proteins of other viruses.

Okra leaf curl virus: a whitefly-transmitted geminivirus from West Africa

P.F. McGrath, M.M. Swanson, K.P. N'Guessan, D. Deng, G.H. Duncan, D.J. Robinson & B.D. Harrison*

Leaf curl is the most serious virus disease affecting okra (*Abelmoschus esculentus*) crops in West Africa and we have recorded it in samples from Burkina Faso, Chad, Ghana, Ivory Coast and Nigeria. The disease causes upward or downward curling of the leaves (Fig. 1), distortion and thickening of the leaf veins, stunting of the plants and substantial loss of crop yield, depending on the okra variety grown and the age of the plants at the time of infection. The causal agent is a geminivirus, okra leaf curl virus (OLCV), which is transmitted by the whitefly, *Bemisia tabaci*. Recent externally-funded research at SCRI has provided further information on the properties, antigenic relationships and vector relations of this little-studied virus, and has enabled diagnostic reagents to be produced and tested.

Figure 1 Okra seedling infected with okra leaf curl virus (left); non-infected plant (right).

OLCV cannot be transmitted experimentally by inoculating leaves with sap, so its host range was examined by exposing test plants to virus-carrying *B. tabaci*. The virus infected several malvaceous species, such as *Malva crispa*, although not cotton (*Gossypium hirsutum*). It was also transmitted sporadically to non-malvaceous species such as *Nicotiana tabacum* and *N. benthamiana*, in both of which it caused typical leaf curl symptoms. Initial attempts to purify OLCV particles from okra leaves were hampered by the mucilaginous nature of leaf extracts. However, further attempts were greatly aided by the availability of a monoclonal antibody (MAb), which had been prepared to particles of African cassava mosaic virus (ACMV) and was found to react with OLCV particles. By using this MAb in serological assays (ELISA) to check the recovery of OLCV antigen at each step in purification, a procedure was devised that gave small yields of purified virus particles, which have the size and shape typical for geminiviruses (Fig. 2).

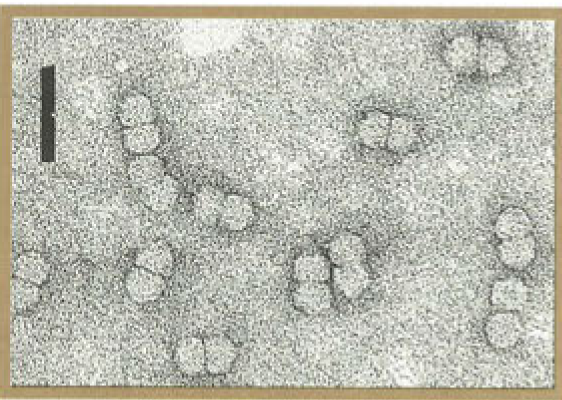


Figure 2 Purified particles of OLCV, negatively stained with uranyl acetate for electron microscopy. Bar represents 50 nm.

The purified virus particles were used to prepare a panel of MAbs. These anti-OLCV MAbs, and the panel of anti-ACMV MAbs produced previously, were used to examine the detectability of, and extent of antigenic variation among, OLCV isolates from different countries. Figure 4 shows examples of the variation detected in epitope profiles (relative reactivity with each of the MAbs) of different isolates. Comparatively little variation was found among OLCV isolates from West Africa including Chad, all the isolates reacting with many of the anti-ACMV and most of the anti-OLCV MAbs. An isolate from

*University of Dundee

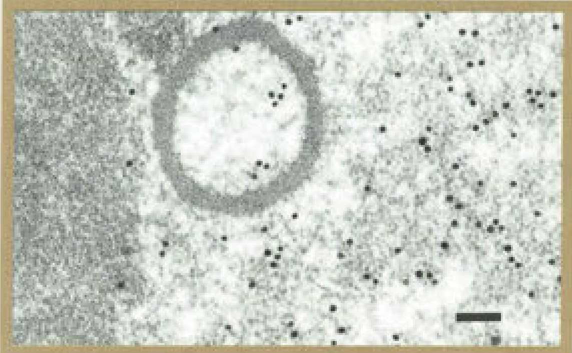


Figure 3 Electron micrograph illustrating detection of OLCV antigen in a nucleus by immunogold labelling (black dots). Note the occurrence of label inside a fibrillar ring. Bar represents 100 nm.

Oman was slightly less closely related to the other isolates than they were to one another. In contrast, a geminivirus which causes a distinct disease of okra in India, okra (bhendi) yellow vein mosaic virus (OYVMV), reacted with only a few anti-OLCV or anti-ACMV MAbs and is antigenically very different from OLCV. Several of the OLCV MAbs detected all the OLCV isolates but not those of OYVMV, and therefore are suitable for routine detection of OLCV and for distinguishing it from OYVMV.

Further tests with the anti-OLCV MAbs have provided additional evidence of antigenic relationships among different whitefly-transmitted geminiviruses. Several of the MAbs reacted with strains of ACMV from East or West Africa, and some reacted with Indian cassava mosaic virus, with African forms of tomato yellow leaf curl virus and/or with other well characterized geminiviruses, such as bean golden mosaic virus. The closest relationships are those with West African isolates of ACMV.

OLCV antigen was detected in leaf cells by electron microscopy of sections that were stained with an anti-ACMV MAb, which in turn was detected with an anti-globulin antibody coupled to 15 nm diam. gold particles. The viral antigen was confined largely to nuclei of the phloem cells. Several of the virus-containing nuclei also contained fibrillar rings (Fig. 3) resembling those found in nuclei of plants infected with a range of other geminiviruses. Examination of serial sections of these structures showed that they were spherical shells. Virus particle antigen was detected inside some of the shells, suggesting that they had formed after virus particles started to accumulate in the nucleus. The apparent restriction of viral antigen, and probably of OLCV infection, to vascular cells may explain some of the symptoms, such as vein distortion and the differential decrease in growth of vein tissue that causes the leaf laminae to curl.

When used in triple antibody sandwich (TAS)-ELISA, an anti-ACMV MAb (SCR 23) was found to be capable of detecting OLCV in viruliferous *B. tabaci*. For best results, alkaline phosphatase was replaced by penicillinase, which gave lower background reactions with extracts of virus-free *B. tabaci*. This penicillinase-based ELISA detected OLCV in single whiteflies which, however, contained very different amounts of virus. Assays on groups of *B. tabaci*, collected at intervals after they had fed on OLCV-infected okra, showed that the virus could still be detected for at least 10 days after the insects were transferred to cotton (immune to OLCV), and that their virus content fell gradually during this period. No evidence was obtained that OLCV replicates in *B. tabaci*.

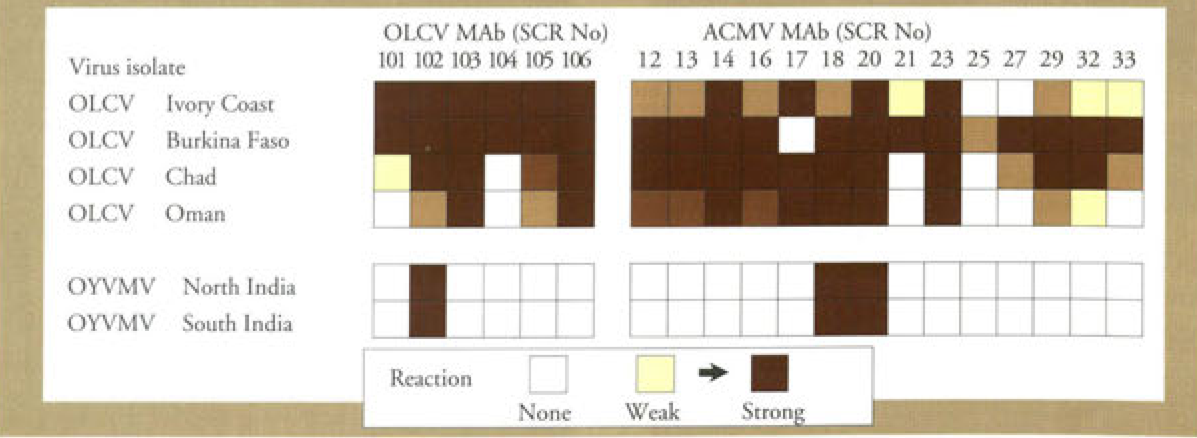


Figure 4 Reactivity of monoclonal antibodies, raised to particles of OLCV or ACMV, with isolates of OLCV and okra yellow vein mosaic virus (OYVMV) from different geographical areas.

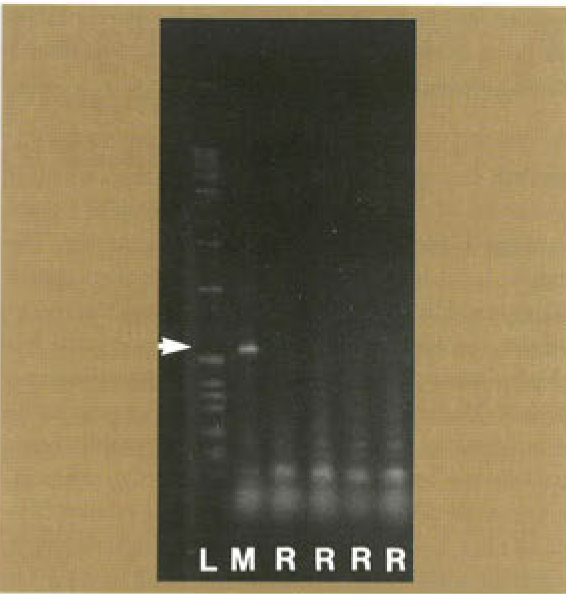


Figure 5 Detection of OLCV in *Bemisia tabaci* by PCR. Left(L) track, size markers; middle(M) and right(R) tracks, PCR products derived from extracts of OLCV-carrying and control *B. tabaci*, respectively. Arrow shows virus-specific band.

Penicillinase-based ELISA was at the limits of its sensitivity when detecting OLCV in *B. tabaci* that had been kept on infected plants for only 1 day. The polymerase chain reaction (PCR), a much more sensitive detection technique, was therefore tried. For this purpose, degenerate primers were designed which bind to regions of the viral DNA that are conserved in those whitefly-transmitted geminiviruses for which sequence data are available. The resulting PCR proved to be capable of detecting OLCV in infected plants and in single viruliferous *B. tabaci* (Fig. 5).

The methods devised for use with OLCV are suitable for diagnostic tests on field-grown plants, for quantitative assays of OLCV concentration when screening cultivars for virus resistance, for assessing antigenic variability among isolates, and in epidemiological studies for identifying reservoir hosts of OLCV and for assessing the occurrence of viruliferous *B. tabaci*. Furthermore, we have found that these techniques can also be applied successfully to other whitefly-transmitted geminiviruses.

The work was supported by funding from the Natural Resources Institute (Project X0060) and the European Community STD2 programme (Project TS2A 0137C(CD)).

Fungal & Bacterial Diseases

J.M. Duncan

The relationships between different groups of pathogens has always been of interest to plant pathologists, whether looking at families of fungi, a single genus or species, or even races within a forma specialis. *This interest has been boosted recently by the availability of powerful molecular techniques which free the researcher from the restriction of markers which are reflections of some aspect of pathogenicity, e.g. race patterns. All hierarchical levels of taxa have been investigated but studies at the generic and sub-generic levels have been most common.*

Pathologists place much emphasis on the affinities among pathogens because the limits of variation in a pathogen or related groups are of importance in devising appropriate control strategies. Knowledge of variation in a group and of affinities between groups of pathogens can help explain the emergence and adaptability of pathogens to new hosts and new cultivars of existing hosts.

New genes for host resistance to plant disease have been repeatedly overcome within a few years of their deployment by the emergence of new races of the pathogen and durable resistance must depend therefore in part on the variation and adaptability of the pathogen.

Barley mildew is a classic example of a disease in which the breeder has always been faced with the continual erosion of existing sources of resistance by the emergence of races with previously unknown virulences. The disease fits the classic 'boom and bust' cycle with the area of a new cultivar initially expanding because the cultivar is resistant to disease, only for it to decline with the emergence of new races which can overcome the resistance. Molecular marker studies in barley mildew have shown that new races are often nothing more than giant clones. Once the appropriate genetic event resulting in the emergence of a new race has taken place, spread is often very rapid eventually affecting very large areas e.g. Europe. Analysis of the pathogen population reveals the vulnerability of a control strategy based on 'breakable' major resistance genes. A possible exception might be the *ml-o* gene.

This continual struggle against new races has forced pathologists and breeders to examine alternative strategies. The use of mixtures of cultivars, alike in as many respects as possible but different in their spectrum of resistance (multilines), is now well known but unfortunately not yet widely exploited. More recently attention has focused on partial resistance, i.e. incomplete resistance based on polygenes. This is promising but still at a relatively early stage and as yet the various ways in which this form of resistance could be exploited have not been fully examined. Its successful deployment would be a major advance for resistance breeding. A complete account of breeding for barley resistance is given on p20 of this Annual Report.

Some parallels exist between barley mildew and potato late blight caused by *Phytophthora infestans*. The pathogen exists as series of races, each of which can attack a different spectrum of cultivars possessing one or several R-genes. The nature of the interrelationship between pathogen and host was analyzed in the 1950s but it is only now that molecular techniques are being applied to the pathogen to look at its affinities with other *Phytophthora* species and among races within the species. RFLPs in mitochondrial and genomic DNA are being exploited in studies of field populations and mating systems.

In late blight, the search for durable resistance has resulted in a movement away from R-genes, which is less useful in a vegetatively propagated crop like potato than in one grown from seed such as barley. The emphasis is now on polygenically controlled partial resistance. The latter form of resistance and the mechanisms involved in it are still poorly understood but this has not prevented its successful incorporation into potato. However, some forms of it do appear to be dependant on the environment. For example, some cultivars are resistant at low but not at high temperatures, while others are insensitive to temperature. The same may apply to light intensity and exposure period. The effect of environment on this form of resistance is discussed in some detail in an article by Wastie and Harrison (p. 16).

Phytophthora spp. are important causes of root rots of perennial crops such as tree and soft fruits. In many such diseases a number of species may be involved, for example at least eight species have been implicated in raspberry root rot. Identifying and discriminating among several species is often difficult due to a shortage of distinctive morphological characters. Molecular and biochemical markers, e.g. protein patterns or RFLPs of mitochondrial and genomic DNA are being used increasingly to distinguish both at the inter- and intra- specific level. In raspberry root rot, one species appears to be the cause of most of the serious outbreaks of disease. Combining classical morphological criteria with molecular markers has made it possible to determine the relationship of this species with others. The pathogen is a variety of another long recognised species but distinct enough to warrant its own epithet at the variety level. The implications of this work on variation of the raspberry root pathogen for control of the disease are discussed on p. 89.

Each of the articles mentioned above highlights in its own way the continuing importance of studying genetic (genomic or otherwise) variation within plant pathogens. With the increasing application of the powerful techniques of molecular biology, such work will be of even greater relevance and importance to strategic studies of plant disease.

Raspberry root rot: A summary of recent progress

J.M. Duncan & D.M. Kennedy

Raspberry root rot is now the most serious threat to raspberry production in Europe. It is especially damaging in the cooler, wetter parts of N.W. Europe but it is not restricted either in its importance or location to that part of the Continent, having been reported from at least ten European countries. It is also probably present but as yet unreported in many others.

Figure 1 A serious outbreak of raspberry root rot seen in early July.

In the last five years there have been considerable advances in clarifying the identity of the principal pathogens involved in root rot, the etiology and epidemiology of the disease, and in the search for resistant cultivars. This article reports on some of these advances and on the impact which they are likely to have on future research efforts and the prospects of practical control.

Causal Organisms A number of *Phytophthora* spp. have been isolated from raspberries affected by root rot. Most are pathogenic when inoculated onto plants, particularly if the infected root systems are kept waterlogged after inoculation (Table 1), but not all cause serious outbreaks of root rot in the field.

P. citricola is an important cause of root rot and has been recorded in many parts of the world. It is probably most serious in warmer areas of raspberry production e.g. California in N. America, and S. Germany in Europe, although it has also been found in Scotland and elsewhere. Pot tests have confirmed its pathogenicity although symptoms were no more severe when the tests were done under warm conditions.

In pot tests, *P. cambivora* caused moderate to severe root rot which was exacerbated considerably by flooding. It has been isolated occasionally from diseased samples in UK, although they rarely show the severe symptoms of root rot.

P. cactorum causes little root rot in pathogenicity tests, but some atypical isolates have been found almost ubiquitously in high grade planting material from a wide range of sources. Isolates of the atypical form are very slow growing and have oospores and sporangia towards the upper end of the size range for *P. cactorum*. They are also unable to grow on defined media with nitrate as the sole nitrogen source, unlike more typical isolates of this species (Fig. 2). Electrophoresis (SDS) of whole proteins give patterns which are close but not identical to those obtained for typical isolates from other hosts, but RFLPs of typical and atypical

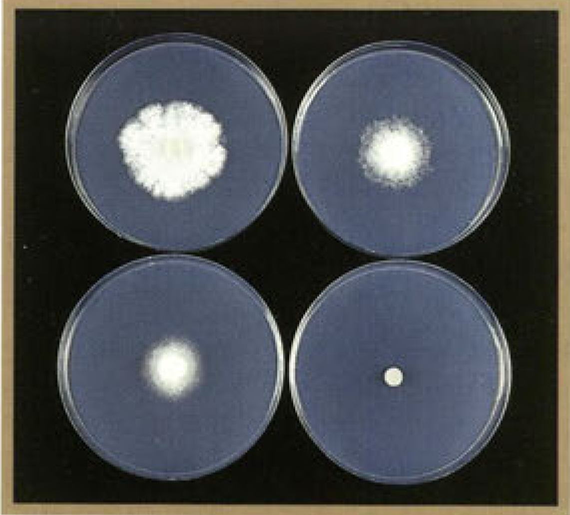
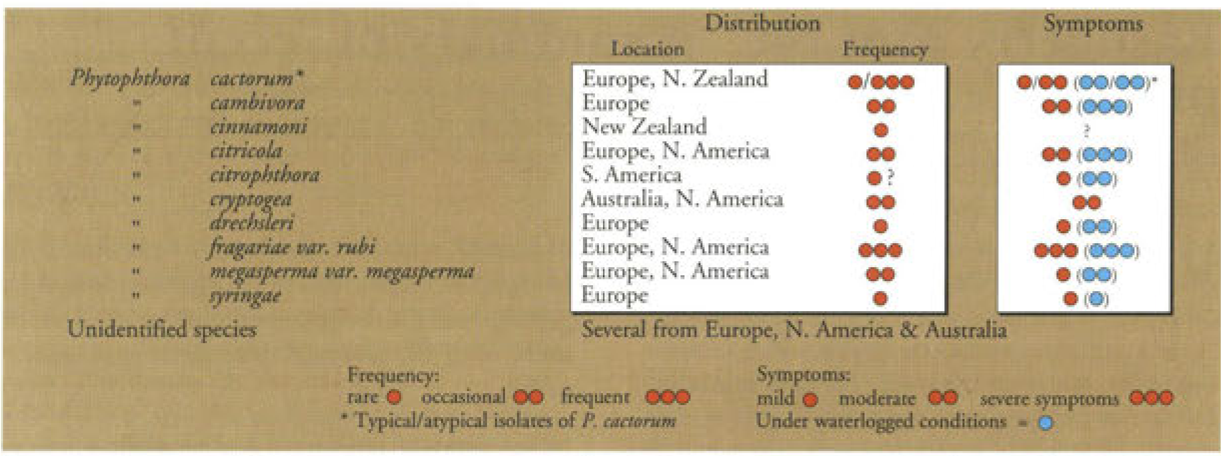


Figure 2 Growth of a typical and an atypical isolate of *Phytophthora cactorum* (both from raspberry) on a defined medium with either L-asparagine or sodium nitrate as a nitrogen source. Top- typical isolate; Bottom - atypical isolate; Left -L-asparagine; Right-sodium nitrate.



		Distribution		Symptoms
		Location	Frequency	
Phytophthora	*cactorum**	Europe, N. Zealand	●/●●●	●/●● (○○/○○)*
"	*cambivora*	Europe	●●	●● (○○○)
"	*cinnamoni*	New Zealand	●	?
"	*citricola*	Europe, N. America	●●	●● (○○○)
"	*citrophthora*	S. America	● ?	● (○○)
"	*cryptogea*	Australia, N. America	●●	●●
"	*drechsleri*	Europe	●	● (○○)
"	*fragariae var. rubi*	Europe, N. America	●●●	●●● (○○○)
"	*megasperma var. megasperma*	Europe, N. America	●●	● (○○)
"	*syringae*	Europe	●	● (○)
Unidentified species		Several from Europe, N. America & Australia		

Frequency: rare ● occasional ●● frequent ●●●
* Typical/atypical isolates of *P. cactorum*

Symptoms: mild ● moderate ●● severe symptoms ●●●
Under waterlogged conditions = ○

Table 1 *Phytophthora* species associated with raspberry root rot world wide and their relative importance in the disease.

isolates are identical[1]. Although more pathogenic to raspberry than more typical isolates from other hosts, the atypical isolates do not appear to cause serious problems in established raspberry plantations, although they may be involved in problems of establishment in newly planted areas. However, these problems have been quickly overcome by fungicide treatments applied to control the pathogenic *Phytophthora* spp. involved in root rot.

The importance of the atypical form of *P.cactorum* lies in its ubuiquitous distribution and production of oospores in infected roots which complicates the detection of pathogenically more important species in root material.

P. fragariae var. *rubi* is the most important cause of root rot in the Northern Hemisphere. It has been isolated from more than 80% of all outbreaks in the UK, and similar frequncies have been recorded from other parts of Europe and N. America. The pathogen has only recently been recognised as a variety of *P. fragariae* and the new name replaces *P. erythroseptica, P. megasperma* and *P. fragariae,* all of which have been applied in the past to this most serious pathogen.

Extensive morphological, cultural and molecular comparisons show that the same fungus has caused root rot throughout N. America and W. Europe and that it is much more closely related to *P. fragariae,* the fungus which causes red core disease of strawberry, than to any other species in the genus. Nevertheless, there are differences in morphology, cultural characteristics, molecular biology and pathology that are sufficient to warrant the separation of the two forms at the varietal level within *P. fragariae.* The raspberry pathogen has therefore been named *P. fragariae* var. *rubi*[2] and the strawberry pathogen has become *P. fragariae* var. *fragariae.*

Recent studies of RFLPs of mitochondrial DNA at the University of California have not only confimed that the varieties are different, though closely related, but have shown that each is very homogeneous. These results have been confirmed by collaborative studies of mitochondrial and genomic RFLPs carried out Germany and at SCRI[1]. Our work has also shown that the nearest species to *P. fragariae* is probably *P. cambivora,* a species which also has a protein profile very similar to that of *P. fragariae.* The two species can readily be distinguished from one another by Southern blotting using probes selected for specificity towards *P. fragariae* var. *rubi,* but dot blots with the same probes did not distinguish between them. No other *Phytophthora* species reacted with the probes in the dot blot tests.

The possibility of comparing and distinguishing *Phytophthora* spp. using polymerase chain reaction (PCR) technology with primers based on conserved sequences from zinc finger protein regulatory genes was examined with support from the SOAFD Flexible Fund. The technique consistently distinguished between different species and varieties of *Phytophthora* and, in particular, the two varieties of *P. fragariae* and *P. cambivora* (Fig. 3). Future work will determine the suitability of the method for detecting the species and varieties in infected plant material.

Epidemiology Both varieties of *P. fragariae* may have come originally from the Pacific rim of N. America, where suitable hosts of both species (members of the tribe Potentilleae in the family Rosaceae) are fairly abundant components of the flora[3]. Interestingly, N.

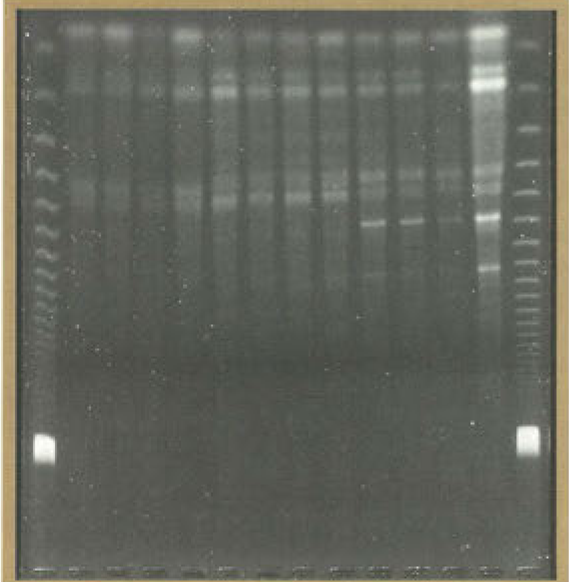


Figure 3 Patterns obtained after agarose gel electrophoresis of PCR products obtained with zinc finger protein primers and whole DNA from *P. fragariae* var. *fragariae*, *P. fragariae* var. *rubi*, and *P. cambivora*; four isolates of each grouped together from left to right respectively.

American isolates of both varieties are slightly more heterogeneous in most characters, including molecular, than European isolates.

Nevertheless, there is so much homogeneity within the two varieties of *P. fragariae* that both varieties may be regarded as no more than giant clones. Such high levels of homogeneity are usually indicative of rapid spread from one or few foci. Rapid spread is certainly true for *P. fragariae* var. *rubi*, which was first recorded less than thirty years ago in N.W. America[4], and in the last 10 years it has been recorded in areas as widely spread as the states of Missouri, Ohio and New York and in at least ten European countries. The spread of the raspberry pathogen is similar to the spread of *P. fragariae* var. *fragariae* in the first half of this century, when it appeared in parts of Europe, N. America and Australasia in the space of less than 20 years, and a second wave occurred in continental Europe in the 1970s affecting countries as far apart as Sweden and Bulgaria.

There is considerable evidence that in both cases the pathogen was distributed mainly in infected nursery plants. Red core has not yet been found in Norway which bans imports of strawberry runners while it has been very serious in neighbouring Sweden which does not. Likewise, raspberry root rot has not occurred in Sweden, which after its experience with red core, operates very stringent inspection and control on imports of raspberry plants. However, it has appeared in Norway shortly after the importation of some new raspberry cultivars.

There is an urgent need for techniques which can readily detect both the raspberry and strawberry varieties of *P. fragariae* in infected nursery plants. An effective bait test, similar to the one developed a decade ago for strawberry red core, has been devised for raspberry root rot but it is time-consuming and takes 5-10 weeks to complete. There is clearly a need for something faster and equally sensitive and a sensitive immunoassay based on monoclonal antibodies is being developed at ASS, Edinburgh in collaboration with SCRI. The PCR technology could also provide practical, sensitive and discriminatory tests to eliminate root rot from nursery stocks of raspberry. It can distinguish between *P. fragariae* var. *rubi* from other species found on roots and, in particular, the atypical form of *P. cactorum*.

Host resistance All of the present raspberry cultivars are highly susceptible to infection, but potential hosts related to raspberry, for example the blackberry (also *Rubus* sp.), are more resistant than raspberry, and the resistance extends to hybrids between blackberry and raspberry, such as Tayberry. Breeding raspberry cultivars resistant to root rot is the best long term solution to the disease. However, the degree of variation within *P. fragariae* must influence attempts to produce resistant raspberry cultivars. The narrowness of the genetic base within the fungus is matched by its restricted host range; a more variable pathogen could be expected to have a wider range of hosts than the red raspberry. Nevertheless, the worldwide expansion of *P. fragariae* may lead to increasing genetic diversity through genetic recombination, mutation and strong selection pressures, and perhaps the fungus will become more adapted to other hosts related to raspberry. Already the fungus has twice been isolated from blackberry and Tayberry, although the symptoms of disease were generally light and there is as yet no evidence that these isolates have shifted host range.

Shifts in virulence between strawberry cultivars but not in overall host range have been observed in *P. fragariae* var. *rubi*. Early isolates of this fungus belonged to very simple races but with the advent of resistant cultivars new races soon emerged which could overcome their resistance. No pathogenic races have been detected in *P. fragariae* var. *rubi*, probably because there are no commercial cultivars with any useful levels of resistance.

Despite the potential for shifts in pathogenicity, either at the level of host range or, more likely virulence, the search for resistance must still have a high priority. Techniques have been developed for screening seedlings and vegetatively propagated material for resistance, and already several potential sources have been identified. All blackberries and most hybrids with blackberry, have been shown to be resistant to disease. Joint work with HRI, East Malling, has shown that autumn-fruiting material derived from *R. spectabilis* is significantly more resistant to root rot than summer fruiting cultivars. Other wild raspberry species such as *R. crataegifolius* and *R. pileatus* also have useful levels of resistance.

Within summer-fruiting red raspberries, several North American cultivars have intermediate levels of resistance while Latham from N. America and Winkler's Sämling from Germany have high levels of resistance. Both of the latter cultivars are old and not agronomically adapted to British conditions, but hopefully their resistance can be transmitted to hybrids. Crosses with Latham have been made at SCRI and families of seedlings have been planted on a root rot site. Differences between families have been observed and in some a high proportion of seedlings have survived, apparently healthy, for nearly two seasons. Work in Germany with Winkler's Sämling is proving equally promising.

New isolates of the pathogen must be examined for variation in pathogenicity and other characters which might threaten the breeders' efforts. Also it will be important to see if raspberry cultivars resistant to *P. fragariae* var. *rubi* are also resistant to other *Phytophthora* species, in particular to *P. citricola* which has proved a serious problem elsewhere.

Propects for control Fungicides have been used to control root rot in the short term. and several useful materials, mainly mixtures of phenylamides and contact fungicides such as mancozeb, have been identified One of them, a mixture of metalaxyl and mancozeb, has received MAFF Off-label Approval and the search for other effective materials continues with the support of the H.D.C.

It is possible that the fungus may develop strains resistant to phenylamides, as has already happened with *P. fragariae* var. *fragariae* on strawberry in which resistant strains have been isolated in Europe and Canada. The prohibition of the use of phenylamide fungicides on nursery stocks of raspberry should reduce the risk of the same development with raspberry root rot. Fungicide-resistant strains spread in nursery stocks would present a most serious problem to growers since no other suitable materials for control are available, a different situation from strawberry red core where there are alternatives.

Some form of integrated control with reduced fungicide inputs seems feasible. Researchers in Norway are experimenting with combinations of low levels of fungicides, ridges, mulches, trickle irrigation and cultivars with intermediate levels of resistance and early results are very promising.

Raspberry root rot has cast a pall over the UK and European raspberry industries in the past ten years. The future now appears to be brightening as potential control strategies emerge from the efforts of pathologists and breeders.

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Nematode and insect pests

D.L. Trudgill

The more basic elements of our research increasingly find common ground with other disciplines or require their specialist skills. Consequently, a substantial part of our work is collaborative. This usually involves the formation of teams with a common purpose and these have evolved naturally in several areas. In addition, some advanced molecular techniques such as RFLP analysis of nematode and insect genomes are so basic to our long term objectives that they are becoming routine. Even so, their development has been in collaboration with appropriate specialists in other disciplines and the wide range of interacting expertise is a major strength of the SCRI.

Much modern, basic research crosses traditional boundaries between disciplines and has wide relevance. Hence work reported here which started as a study using proteins called lectins to label the glycoproteins on nematode cuticles developed into a collaborative study on human HIV with a group of German biochemists.

We are internationally recognised for our studies on European virus vector nematodes and recently initiated collaborative work involving three North America specialists to investigate virus vector associations in the *Xiphinema americanum* group. We investigated four species distinguished by molecular criteria, but all of which have three rather than the more usual four juvenile stages. One species transmitted only one of the three North American nepoviruses we tested, whereas the others transmitted all three. Such an apparent lack of specificity contrasts with our earlier results with European *Xiphinema* vectors of viruses (Fig. 1), which were shown to have a high and complex degree of specificity between virus isolate and vector population. Detailed studies with the (Para)Trichodorid vectors of tobacco rattle tobravirus (TRV) have also revealed a similar high degree of specificity between some, but not all, virus serotypes and their vectors. This investigation required team work between virologists and nematologists at SCRI and was based on the development of transmission systems which took considerable time and skill. These systems were also used successfully, in collaboration with Dutch scientists, to identify the lack of resistance to infection with TRV carried by nematodes in genetically transformed plants expressing the TRV coat protein gene.

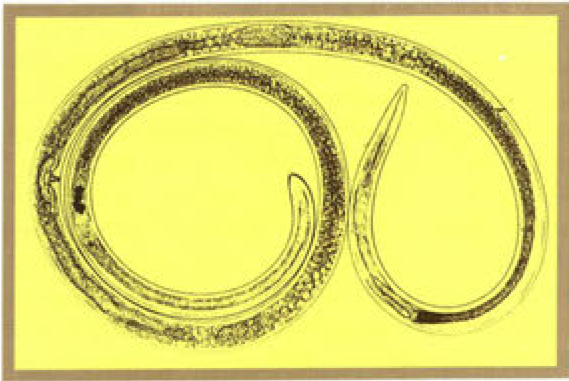


Figure 1 *Xiphinema diversicaudatum* female - the natural nematode vector of arabis mosaic and strawberry latent ringspot nepoviruses.

Our studies on potato cyst nematodes (PCN) form a major and complex area of interaction. Foremost in these has been our support for colleagues in Crop Genetics in their efforts to combine high levels of resistance and tolerance to *Globodera pallida* with resistance to other pathogens and desirable agronomic characters. Understanding the genetics and mechanisms of the nematode/plant interactions and how to model their effects on yield loss and nematode multiplication has been a primary objective. However, interactions with environmental factors are a major complicating factor, especially in relation to the assessment of partial resistance. In collaboration with colleagues in Europe, we undertook a series of studies, sponsored by the European Plant Protection Organisation, designed to establish a scientific basis for classifying the degree of partial resistance of potato cultivars submitted for statutory testing and for classifying pathotypes of PCN. The results showed that resistance should be classified on a continuous scale with standard genotypes as reference points. It was also recognised that the pathotype scheme represented an arbitrary and over-simplified view of the range of variation in virulence in PCN and focused attention on how to identify representative populations to be used in assessing levels of partial resistance. To identify the range of virulence in European populations molecular techniques are being used to identify the distinct introductions of PCN, their virulence characteristics and how these have been modified during spread within Europe. One interesting result is the identification of a population of *Globodera pallida* from Scotland which appears to be a distinct introduction and which is highly virulent on partially resistant cv. Morag.

Virus vector aphids are another major interest and their ability to reproduce rapidly by an asexual cloning process makes them important crop pests. Root-knot nematodes, *Meloidogyne* spp., are also major crop pests which reproduce asexually. Both groups of organisms share the ability to produce virulent populations which can reproduce on resistant crops. A further similarity is that, within each group, virulent and avirulent individuals are morphologically identical. The genetic causes of differences in ameiotic insects or nematodes that are distinguishable only on the basis of their response to particular host plants or environmental stimuli have long interested taxonomists. The problem of deciding whether "biotypes" should be regarded as distinct species rather than host-adapted races is not only of theoretical interest. Understanding the mechanisms that produce and maintain variability in these important crop pests helps us to predict the extent to which new biotypes will arise and prevents resources being wasted by developing crops that are resistant to only a narrow range of biotypes. Molecular probes have distinguished virulent from avirulent populations of *Meloidogyne* from the Ivory Coast and of the virus vector aphid, *Amphorophora idaei*, collected from raspberry plantations in Britain. By comparing the genetic heterogeneity in *Meloidogyne* from different areas, it was concluded that the virulent populations from the Ivory Coast constituted a distinct species, *M. mayaguensis*.

Seed potato production provides the main economic focus for our research on virus vector aphids. The epidemic of potato leafroll virus (PLRV) in the 1970s was the stimulus for collaborative work with virologists and entomologists from SAC that demonstrated the importance of controlling early infestations of aphid vectors. The spread of PLRV is well controlled in most seasons by the removal of plants growing from tubers infected the previous year, but not after mild winters when aphids colonise potato crops unusually early. The potential for early flights can be predicted in March from air temperatures in January and February. This aphid forecast, used together with information on the proportion of PLRV-infected tubers in the planted crop, provides the basis for a model to advise on the use of granular insecticides. Our current interest concentrates on predicting the extent to which PLRV infections will increase in potato crops, using data on the rate at which aphid populations develop on the growing crop.

Integrated Pest Management, the watchword for research to find environmentally safe methods of managing insect pests, requires the integration of many scientific disciplines. Powerful new methods are now available at SCRI to collect and analyse chemicals that help insects to find their host plants or discriminate between susceptible or resistant plant cultivars. Collaboration between chemists, entomologists and plant geneticists is required to understand these behavioural mechanisms and develop effective plant breeding strategies. Following our initial discovery that brassica-feeding root flies laid fewer eggs on certain brassica genotypes, and that these differences were also observed when the flies were presented with model leaves sprayed with surface extracts of these genotypes, links were established with researchers in Switzerland and London to isolate the surface extract chemicals and assess their neurophysiological and behavioural effects on root flies.

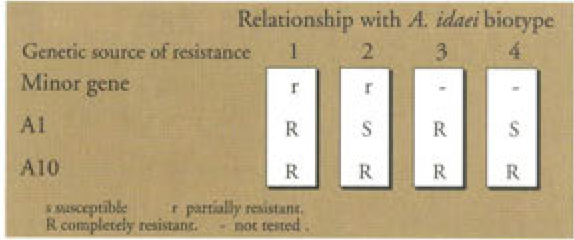
The use of molecular markers to identify aphid and nematode biotypes

A.N.E. Birch, M. Fargette, B. Harrower, G. Malloch, A.T. Jones, M.S. Phillips & M.A. Catley

Most crop pests are adaptable and respond to control measures by developing biotypes able to overcome those measures. Such selection may be from within existing variation, or be based on specific mutations but whatever the basis, it threatens the effectiveness of control strategies. Molecular techniques enable us to investigate the degree of genetic variation (heterogeneity), relationships between pest populations and the basis of biotypic differences. Using such techniques we are investigating variation in several pests, including the large raspberry aphid, *Amphorophora idaei* (Fig. 1) potato cyst nematode, *Globodera pallida*, and populations of four species of root-knot nematode, *Meloidogyne* spp.

Figure 1 Large raspberry aphid (*Amphorophora idaei*) feeding on leaf petiole.

Root-knot nematodes are major pests of tropical and subtropical crops with a world-wide distribution. Three species, *M. incognita, M. javanica* and *M. arenaria,* are particularly important, and several populations of each were examined. These species reproduce by mitotic parthenogenesis (a form of clonal reproduction) and previous research on their isozyme phenotypes revealed little variation within each species. An analysis of their restriction fragment length polymorphisms (RFLPs) using a range of restriction enzymes to cut their DNA at specific base-pair sequences and to label the products using low-copy homologous probes supported the isozyme results. Almost no variation was observed between 10 populations of *M. incognita* from around the world, even though three different resistance-breaking biotypes



Genetic source of resistance	Relationship with *A. idaei* biotype 1	2	3	4
Minor gene	r	r	-	-
A1	R	S	R	S
A10	R	R	R	R

s susceptible r partially resistant.
R completely resistant. - not tested.

Table 1 Relationship between *A. idaei* biotypes and resistance genes in red raspberry.

were represented. There were no differences within the four populations of *M. javanica* analysed, but these populations shared several restriction sites with the *M. arenaria,* suggesting that they may be related. However, the six populations of *M. arenaria* formed two groups and cytological investigations suggested that these groups possess different numbers of chromosomes.

The aphid, *A. idaei,* also reproduces by mitotic parthenogenesis for much of the year, but in the autumn there is a sexual stage which produces the over-wintering eggs. *A. idaei* is of interest because not only is it the main vector of several raspberry viruses, but biotypes have developed that can overcome resistance genes recently incorporated into raspberry (Table 1). Analysis of the RFLPs based on a ribosomal probe of different clones and populations showed that within a clone all aphids were the same (Fig. 2)

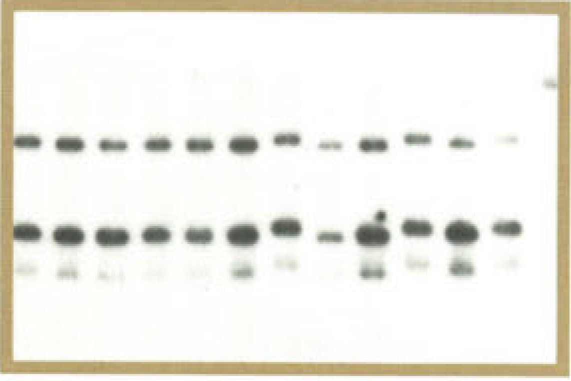


Figure 2 RFLP patterns of individual aphids taken from a clonal population of *A. idaei* biotype 1.

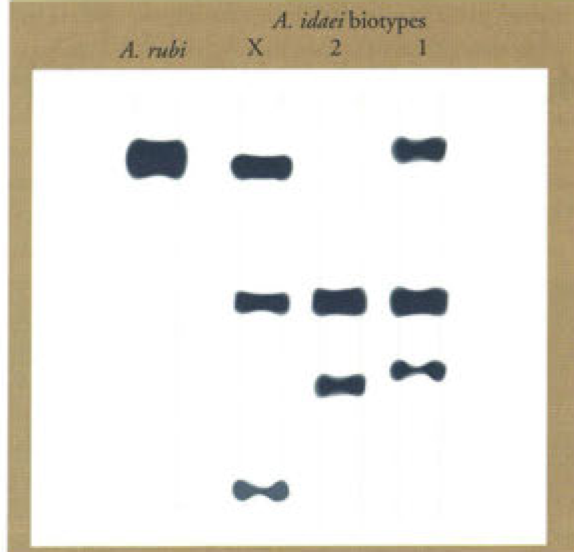


Figure 3 RFLP patterns of *Bgl* II digested DNA from *Amphorophora rubi* and three biotypes of *A. idaei*, after probing with a ^{32}P labelled flax ribosomal DNA probe.

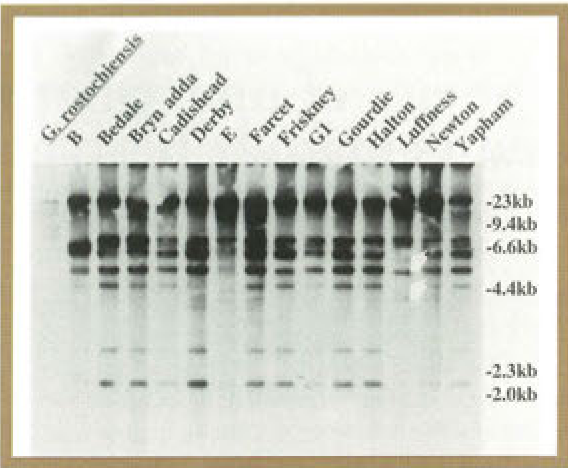


Figure 4 Autoradiograph of DNA from populations of potato cyst nematode probed with mtDNA from the Derby population. The major hybridising band of >23Kb mol. wt. is probably the result of traces of genomic DNA in the hybridisation probe. The bands absent in only the Luffness population are highlighted.

but that those coming from raspberry hosts with different genes for aphid resistance tended to have distinct patterns (Fig. 3). The RFLP patterns of *A. idaei* populations from different raspberry growing regions in the UK indicate that virulent biotype 2 is now widespread, due to adaptation to resistance gene A1. These results indicate either reproductive isolation between biotypes, or a degree of chromosomal linkage between the fragments labelled and the sites of the virulence genes. However, a small proportion of aphids had RFLP's characteristic of hybrids between biotypes, suggesting a limited degree of cross breeding.

G. pallida is a major pest of potato and its incidence is increasing following the widespread growing of potatoes with resistance to the yellow species of potato cyst nematode, *G. rostochiensis*. It reproduces sexually and is naturally very heterogenous. However, within the UK the degree of heterogeneity has been restricted to that present in the original introductions from S. America, and it has been further restricted during spread within the UK. Our purpose was to use molecular techniques to determine the degree of heterogeneity of populations, the groupings present and their potential relationship to the original introductions, and linkage with virulence differences on resistant potatoes. RFLP's were generated for 18 populations using homologous probes and a series of restriction enzymes. Although considerable heterogeity was present, three groups were identified; one comprised a limited group of populations from Northern Ireland and parts of Scotland classified as pathotype Pa1(B), a second was represented by a single population from south east Scotland (Luffness), while the third comprised the largest group of pathotype Pa2 and Pa3 populations from England and Scotland. These results therefore confirmed the distinction of Pa1 and the lack of difference between pathotypes Pa2 and Pa3. To unequivocally demonstrate that Pa1 and Pa2/3 are separate groups derived from different introductions, a mitochondrial probe was prepared (mitochondrial DNA is maternally inherited and reproductively isolated populations tend to develop distinctive differences). This revealed small differences between Pa1 and Pa2/3 populations and a much larger difference between these and the Luffness population (Fig. 4). As Pa1 is distinct from Pa2/3 because it lacks virulence on potatoes with the H2 gene for resistance derived from *Solanum multidissectum*, and Luffness is distinct because it is extremely virulent on certain potato cultivars with resistance from *S. vernei*, our results support the hypothesis that large differences in virulence between populations often derive from separate introductions. They also suggest that pathotype Pa2 and Pa3 populations from the UK are related and probably derive from the same introduction, confirming our view that the small and variable differences in virulence between populations on certain *S. vernei* clones are not a satisfactory basis for separating them into pathotypes.

The perception of flower odours by the raspberry beetle

J.A.T. Woodford, A.N.E. Birch, G.W. Robertson & D.W. Griffiths

Most phytophagous insects, including some major pest species, are specialised feeders, adapted to exploit a narrow range of taxonomically related plants. The mechanisms by which they select their host plants involve responses to a variety of chemical, visual and mechanical stimuli. How insects find their host plants is one of the least understood aspects of this process, but increasingly sensitive analytical, physiological and behavioural assays indicate that they can perceive and respond from a distance to olfactory signals in plant odours. If we could identify the volatile compounds that cue these responses, it might be possible to lure pests away from crops or mask the olfactory signals they produce.

We selected the raspberry beetle (*Byturus tomentosus*) as a model insect to investigate this approach. At present, insecticides applied close to harvest before the larvae damage the developing fruits are the only effective means of controlling this major pest of raspberries. The adults (Fig. 1) are frequently found in raspberry flowers in early summer where they congregate to feed and mate soon after the blossoms open. They usually start to emerge from the soil in mid-May and, in warm weather, migrate to feed in the flowers of hawthorn (*Crataegus* spp.) and other Rosaceous plants until raspberries begin to flower.

Figure 1 Raspberry beetle feeding on a raspberry flower.

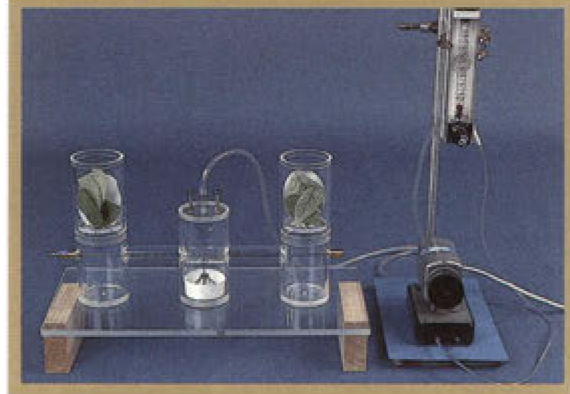


Figure 2 'Linear track' olfactometer.

Olfactometer tests provided clear evidence that volatile compounds in the odours produced by flowers of raspberry and other Rosaceous plants are attractive to raspberry beetles. In the 'linear track' olfactometer (Fig. 2), beetles placed in the central chamber climb up the vertical wire towards an overhead light source. Air is drawn from the two lateral chambers and leaves the horizontal tube through a hole directly beneath the central T-junction. Smoke tests indicated that there is a rather sharp boundary between air from the two chambers at the T-junction. Thus the number of beetles choosing to move towards either chamber provides a useful assay of the beetles' ability to discriminate volatile compounds. Given a choice between flowers or humidified air (blank control), beetles showed a clear preference for the chamber containing flowers of raspberry (host plant), or hawthorn, but not for flowers of a non-host plant (oilseed rape) (Table 1). In choice tests with flowers in both lateral cham-

Plant species	Flowers	Control	No. of tests
A. Raspberry, cv. Glen Prosen	250	42	24
B. Wild hawthorn	179	26	10
C. Oilseed rape, cv. Pasha	174	122	22

Table 1 Number of raspberry beetles selecting flower odours or humidified air (control) in olfactometer choice tests.

Plant species	No. of beetles selecting	No. of tests
A. Raspberry/	49	8
Wild hawthorn	179	
B. Raspberry/	24	3
Oilseed rape, cv. Pasha	3	

Table 2 Response of raspberry beetles to flower volatiles from raspberry, hawthorn and oilseed rape in olfactometer choice tests.

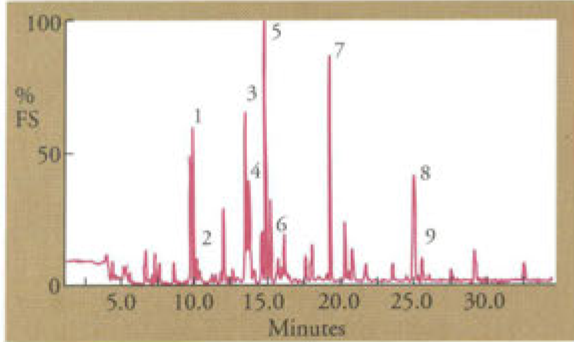


Figure 3 Chromatographic trace of volatile chemicals from flowers of raspberry, cv. Glen Prosen.

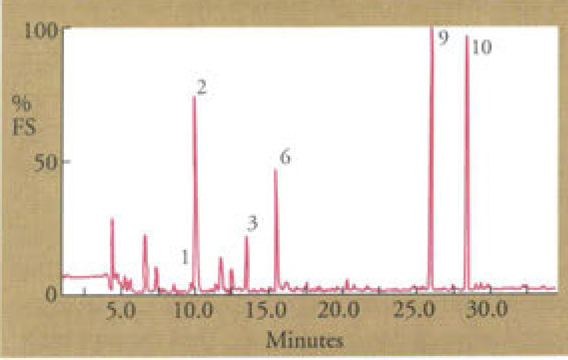


Figure 4 Chromatographic trace of volatile chemicals from hawthorn flowers.

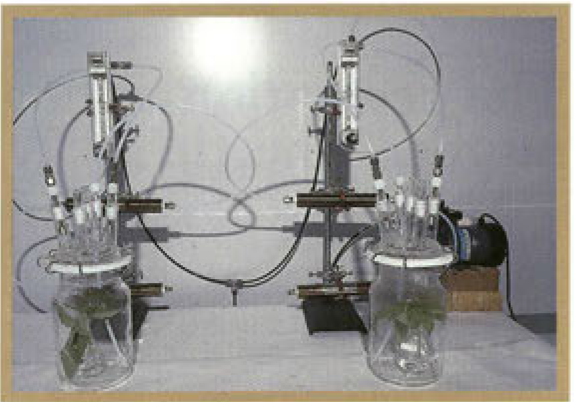


Figure 5 Entrainment apparatus for collecting plant volatile chemicals.

bers (Table 2), beetles preferred hawthorn flower odour to that of raspberry flowers, but the tests were done early in the season when hawthorn was in full flower but raspberry flowers were just opening. Raspberry flower odour was more attractive than that from oilseed rape flowers.

Flower odours comprise complex blends of volatile chemicals. The approach taken at SCRI for the collection and analysis of the individual components is to trap them on to suitable adsorbents and subsequently release them by thermal desorption for analysis by gas chromatography mass spectrometry (GC-MS). Figures 3 and 4 illustrate the range of volatiles collected on Haysep Q adsorbent, using an entrainment system (Fig. 5) over a 24 h period, from flowers of raspberry cv. Glen Prosen and wild hawthorn respectively. Up to 180 compounds have been detected in each profile, and 10 of the components identified using GC-MS are detailed in Table 3. Although several of the components are found in the volatile profiles of both host plants, their concentrations relative to the other components are different. Indeed, several

Peak No.	Compound	RT Min	Mol. Wt.	Formula	Relative abundance cv. Glen Prosen	Relative abundance Wild hawthorn
1	2,3-Butane Dione	10.08	86	$CH_3CO.COCH_3$	+++	+
2	2-Butanone	10.23	72	$C_2H_5COCH_3$	+	+++
3	3-Pentanone	13.63	88	$C_2H_5.CO.C_2H_5$	+++	++
4	3-Pentanol	13.83	88	$C_2H_5.COH.C_2H_5$	++	nd
5	C6-Ketoxime*	14.98	115	$C_5H_{12}CNOH$	+++	nd
6	3-Methyl-1-Butanol	15.67	88	$(CH_3)_2CH(CH_2)_2OH$	+	+++
7	2,3-Butane Diol	19.32	88	$CH_3CH_2OHCH_2OHCH_3$	+++	nd
8	cis-β-ocimine	25.07	136	$C_{10}H_{16}$	++	nd
9	Benzaldehyde	25.97	106	C_6H_5CHO	tr	+++
10	4-Pyridine Carboxaldehyde*	28.50	107	$(C_5H_4N)CHO$	nd	+++

+++ Major component   + Minor Component   nd Not detected
++ Present in quantity   tr Trace   * Tentative identification

Table 3 Volatile components of raspberry and hawthorn.

	No. of beetles selecting	No. of tests
A. 100 µl crude extract/ blank control	23 4	2
B. 50 µl distilled extract/ blank control	14 3	2
B. 50 µl distilled extract/ raspberry flowers	18 11	2

Table 4 Response of raspberry beetles to ether extracts of raspberry flowers (cv. Glen Prosen) in olfactometer choice tests.

of the major components are unique to each plant species and large differences are apparent when the two chromatograms are compared.

Extracts containing flower volatiles, made by dipping raspberry flowers into ether, were also attractive to adult beetles in olfactometer tests (Table 4). Collaborative work with IACR, Rothamsted has given a clue to the identity of some of the physiologically-active compounds that raspberry beetles perceived in these extracts. Components in the volatile flower extracts were separated by GC and passed simultaneously over a raspberry beetle antenna. Electrophysiological recordings from the whole antenna (an electroantennogram), and from individual olfactory receptors, (Fig. 6) coupled with simultaneous gc analysis, identified a number of active components in ether extracts of raspberry and hawthorn flower volatiles. Studies are now in progress to determine which are the key volatile components in the complex odour profile that the beetle detects with its antennal receptors, and how these compounds influence insect behaviour during feeding, mating and egg laying.

Figure 6 Scanning electron micrograph of part of a raspberry beetle antenna. White bar = 50 microns.

Acknowledgement: We thank Dr L.J. Wadhams (IACR) for permission to refer to unpublished observations.

New uses for lectins from plants

J.M.S. Forrest, D. Stewart & W.E.G. Müller*

Lectins are proteins or glycoproteins which are found both in plants such as *Narcissus pseudonarcissus* (daffodils), *Galanthus nivalis* (snowdrop) as well as in animals. They have the power to combine specifically with branching chains of sugar molecules (oligosaccharides) attached to proteins on the surface of cells. As most of them have multiple binding sites, they can bridge large numbers of cells, causing them to agglutinate or clump together. Because the binding of individual lectins is specific for one or a few sugars, they can be used to study the sugar composition of cell surface glycoproteins. For example, classical studies showed that human red blood cells can be divided into their groups A, B and O by their reaction with different lectins. Using other lectins, it is possible to

Figure 1 Daffodils

*University of Mainz, Germany

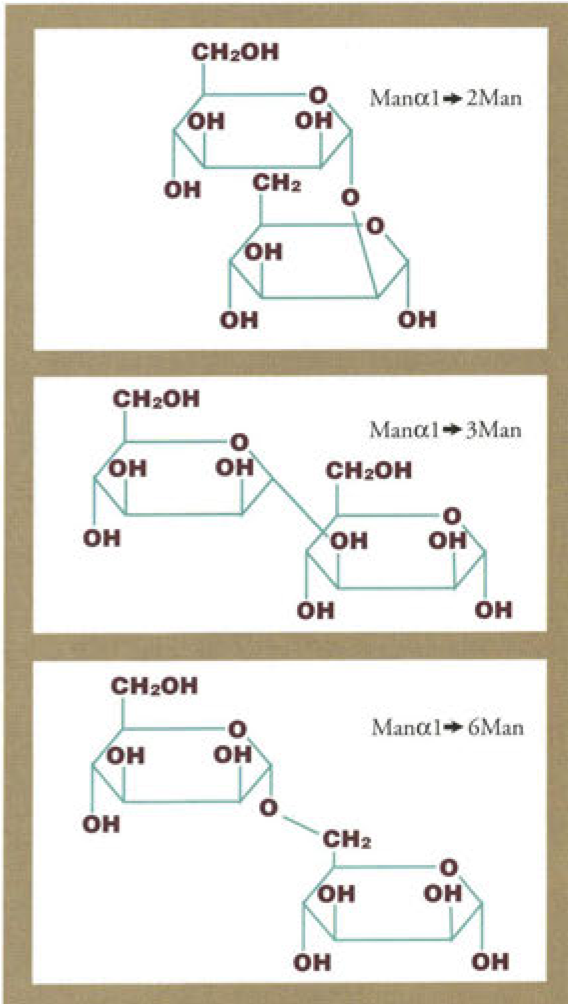


Figure 2 Structure of mannose and illustration of α1-2, α1-3, α1-6 linkages.

distinguish between normal and malignant cells in culture which differ in the sugar composition of only a few surface glycoproteins.

Most commercially available lectins are derived from plants, but only a tiny fraction of the total plant species has been extracted let alone studied and the potential value of lectins for studying glycoproteins of importance in agriculture and medicine is many times greater than it was when they were discovered just over 100 years ago.

Since the early 1980s, lectins have been used at SCRI to study saccharides on the surface of plant parasitic nematodes where they are capable of accurately locating very small quantities of oligosaccharides.

Because of the proposed role of the sugar mannose (Fig. 2) in cell surface recognition between parasites and their hosts, we developed an interest in lectins from the plant family *Amaryllidaceae*, especially daffodil lectin (NPA) which was known to be specific for this sugar. The protein was extracted from daffodil bulbs, conjugated to a fluorochrome, and used unequivocally to demonstrate by fluorescence microscopy the presence of mannose on the exudate from the amphids, paired chemosensory organs which occupy a small area on the head of juveniles of potato cyst nematode. About this time we became aware of the work of Professor Müller's group at the University of Mainz, where a lectin from the anthozoan (coral) *Gerardia savaglia*, also specific for mannose, had been shown to inhibit the binding of human immunodeficiency virus (HIV) to human cell lines *in vitro*. We exchanged lectins and it was shown that *Gerardia* lectin bound to the amphids of potato cyst nematode juveniles, whereas NPA bound to HIV 1 and 2 and prevented *in vitro* infection of human cell lines. Both lectins are proteins which bind specifically to mannose, but there are small differences in their specificities which depend on how the sugars are linked together (Fig. 2) and their positions in the side chain. Although the differences are small, their effects on the properties of the lectins may be profound. Thus *Gerardia* lectin agglutinates human red blood cells of all groups, but NPA has no effect.

Other lectins from plants of the same family, such as snowdrop (GNA), differ slightly in their composition, structure and specificity. Lectins from the *Amaryllidaceae* exist in many different forms which are distributed throughout the plant and their concentration varies with developmental stage and organ examined. Until recently their role in nature was uncertain

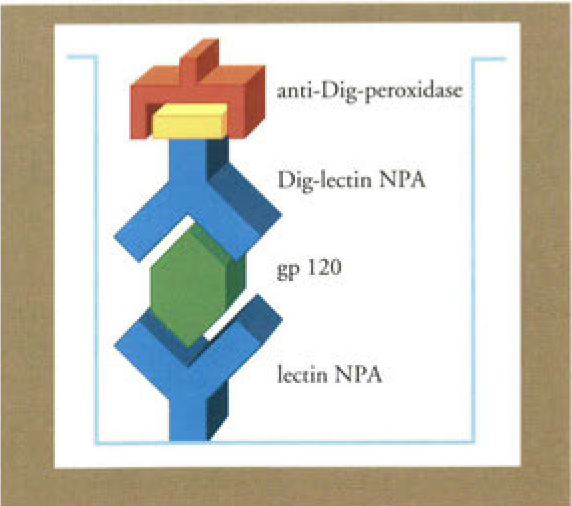


Figure 3 Diagrammatic representation of a lectin ELISA conducted in the well of an ELISA plate.

but now it has been shown that snowdrop lectin acts as an antifeedant for sap-sucking insects. Research workers at the University of Durham have proposed that the genes coding for the lectin should be inserted into crop plants to control insect pests.

Whether the lectin has anti-viral properties *in planta* is not known, but when tested at the University of Leuven against 19 human and animal viruses it only inhibited infection of cell lines by the human viruses HIV 1, 2, and cytomegalovirus (CMV), and simian immunodeficiency virus (SIV), which causes a disease in monkeys similar to AIDS. The component of HIV recognised by the lectins, is the envelope or external glycoprotein gp120. Work at the University of Mainz has progressed with the successful development of a 'lectin ELISA' using NPA extracted at SCRI. Because NPA and GNA recognise and bind to gp120 in an 'antibody-like' manner, they can be used in place of antibodies to detect and quantify the virus by capturing the glycoprotein.

Lectin (either NPA or GNA) is bound to the base of a well in a plastic ELISA plate (Fig. 3). After washing, the liquid medium from a culture of HIV-infected cells is added, incubation is followed by repeated washing and the addition of either lectin labelled with digoxigenin (another plant product). Any gp120 present is trapped in the 'sandwich' and the second lectin is quantified by a labelled antibody which binds to digoxigenin. This assay is currently being developed for estimating the presence of HIV 1 and 2 in human serum.

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.

In June 1991, SASS received its first Visiting Group which was appointed by AFRC to review the Unit's activities over the past four years and comment on its future direction. The Group's report was highly favourable and endorsed the SASS remit and organisational structure. The Visiting Group were particularly impressed by the high quality of consultancy and training provided for scientists and the arrangements to stimulate research activity. Considerable importance was placed on the role of a strong programme of innovative research within SASS. The Group supported the development of distinctive research programmes in three areas of application, environmental modelling, image analysis, and molecular biology and genetics, and an increase in emphasis on relevant areas of applied mathematics.

Following acceptance of the Report, four research programmes are being established with additional SOAFD funding. The four topics are: image analysis and spatial processes; modelling spatial distribution in the context of Geographic Information Systems (with MLURI); genetic linkage and chromosome mapping with special reference to quantitative traits (with SCRI); and mathematical modelling in animal and plant epidemiology (with MRI and SCRI). The greater emphasis on mathematical aspects of our remit is illustrated by our contribution to animal epidemiology programme centred in Edinburgh and land use modelling in Aberdeen. In addition, SASS is a member of the Dundee Centre for Non-Linear Dynamics, an interdisciplinary centre linking SCRI, SASS and the Department of Mathematics and Computer Science in the University of Dundee which fosters the development and application of novel mathematical and statistical techniques in biology, particularly in the area of plant-soil dynamics. Strong links are also being forged between SASS and the Department of

Statistics and Modelling Science at the University of Strathclyde.

In addition to these research topics, SASS has expanded its work in food science and nutrition with funding from EC and FAO. In food, work with HRI has concentrated on sensory studies, investigating the design and analysis of experiments with taste panels and relating panel results to instrumental and chemical measurements using multivariate techniques. There is good collaboration with the Norwegian Food Research Institute, MATFORSK, and a number of other European Institutes. At RRI, investigations covered both human and animal nutrition including the doubly-labelled water technique for assessing energy expenditure in free-living individuals. Studies on the body-mass index of third-world populations were carried out for FAO and the International Institute of Nutrition, Rome.

Research into improved methods for field evaluation of plant cultivars continued. Much of this work is directed at the UK official variety testing programmes with funding from MAFF, SOAFD and HGCA. A two-year MAFF Open-contract was awarded to SASS and NIAB to determine the extent of interplot interference in small plot variety trials and develop statistical methods for correcting the resulting bias to variety means. SASS is collaborating with CIMMYT and Michigan State University in developing a data base management programme for plant breeders, and staff visited Czechoslovakia during the year to advise plant breeding institutes and variety testing authorities.

Our training programme continued with over 30 courses given in the SARIs and SAC during the year. In addition to the range of statistics courses, a new course on DNA sequence analysis was prepared and presented to molecular biologists.

Simulating the power of the brain with artificial neural networks

G. J. Gibson

A historical perspective. Why are humans so much smarter than computers? How can we, with hundreds of our brain cells dying each second, continue to function while a computer program is killed by a single mis-typed character? Can computers be built that will emulate the human brain? The design and study of artificial neural networks attempts to provide answers to these questions.

For the past decade, researchers in scientific disciplines from neurophysiology to theoretical physics have devoted much effort to the study of neural networks and their application to practical problems. Indeed, this field of research has become so widespread that it is not easy to formulate a comprehensive definition of the term. For the purposes of this article an artificial neural network (ANN) will be defined as any computational device, whether it be in the form of a computer programme, hardware component, or an idea in the mind of a researcher, which reflects some aspect of the human brain. The similarity may be in the form of a structural analogy as, for example, in the multilayer perceptron (MLP), discussed later, which is composed of a number of interconnected units (connectionist architecture), just as the brain consists of neurones linked together. The link with the brain may also be functional, with the ANN showing some ability to learn, adapt or remember. A common feature of almost all ANNs is the use of non-linear processing, a phenomenon found in the brain. In short, if an architecture is connectionist, adaptive and non-linear, we can safely describe it as an ANN.

The study of ANNs has its roots, not surprisingly, in the attempts of neurophysiologists and cognitive scientists to understand the brain's function. As long ago as the 1940s, McCulloch and Pitts, and Hebb were investigating the possibility of modelling and understanding neural processes mathematically. Researchers in the emerging field of electronics were also active, seeking to emulate the learning capability of the brain in electronic circuits for applications in signal processing and pattern recognition. Widrow's ADALINE

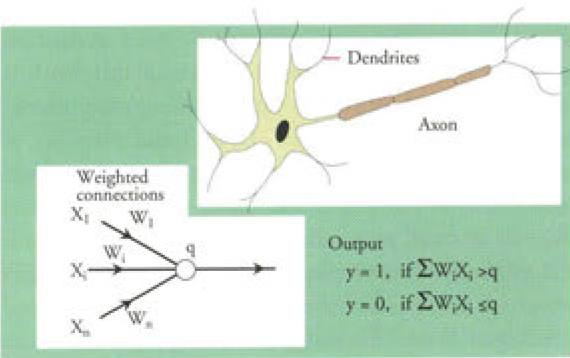


Figure 1 A nerve cell and an ADALINE unit.

(ADAptive LINEar) and MADALINE (Multilayered ADALINE) networks were the forerunners to the MLP, which is most commonly applied today. The ADALINE unit functioned by taking a weighted sum of its inputs and producing an output of 0 or 1 depending on whether some threshold is exceeded. It could be considered to be an idealised version of a nerve cell which "fires" if the total activity of connected neurones reaches a certain level. Figure 1 depicts an ADALINE unit and its analogy with a nerve cell. Widrow's main contribution to ANNs was the realisation that simple ADALINE units can be assembled in layers to build a larger architecture, a MADALINE network, which can produce complicated functions. A key feature of the MADALINE net was the use of parallelism in the architecture, and its ability to "learn" functions from sets of training examples, using a simple learning procedure. Figure 2 illustrates how ADALINE units can be arranged in a MADALINE net to realise the exclusive OR logic function, defined in Figure 2. The accompanying table shows the output produced by nodes A, B, and C for each input combination. Notice how the 1st-layer nodes, A and B, reconfigure the problem as one which can be solved by the single output node, C.

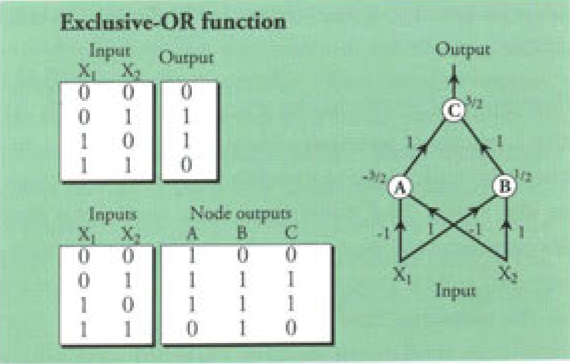


Figure 2 MADALINE network creating Exclusive -OR.

Despite these advances, however, interest in the subject receded in the seventies, in response to a celebrated book by Minsky and Papert which highlighted the inability of these early ANNs to learn functions beyond a certain complexity. For example, they could not be trained to learn the case illustrated in Figure 2. The subject was largely dormant until the early eighties when knowledge of the back-propagation (BP) algorithm became widespread. This algorithm, whose origins are variously attributed, was a generalisation of Widrow's earlier learning rule which allowed all layers in a network to be adaptive, thereby overcoming the deficiencies cited by Minsky and Papert. This discovery prompted an explosion in neural network research which has continued to this day. Thanks to work by Hopfield, Kohonen, Grossburg and others, the variety of ANNs and the range of situations in which they are applied are wider than ever before. Examples are diverse and include the application of neural networks to classify sounds from spectral data in artificial speech recognition, the diagnoses of medical conditions from patients' symptoms, and the automatic detection of edges in digitised images. Their use has even spread into the world of commerce and finance. For example, in the USA neural networks are used by credit companies to assess the credit-worthiness of loan applicants and can match the performance of human decision-makers. Figure 3 shows the range of scientific disciplines to which the impact of ANNs has spread.

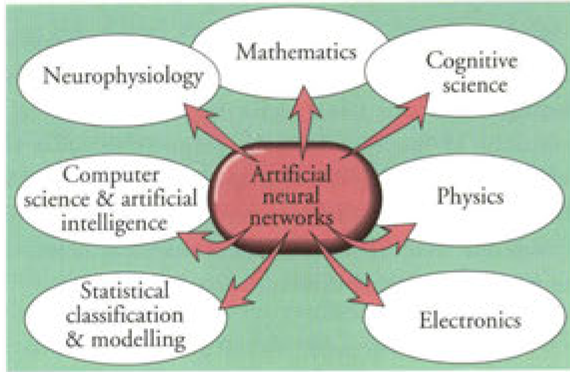


Figure 3 Artificial neural networks in the context of science today.

Non-linear modelling and classification with ANNs. While ANNs are of interest to biologists by virtue of their analogy with the brain, their relevance to the work of SASS lies in their capacity as non-linear statistical classifiers, and their application in the modelling of non-linear systems. Many commonly applied statistical techniques assume that a linear model underlies the system of interest. Such simplicity is the exception

rather than the rule in nature. For example, suppose that we wish to model the suitability of a potential habitat for colonisation by a given species, in terms of variates such as temperature, humidity, or altitude. The fact that extreme heat or cold is undesirable forces the dependence on temperature to be non-linear in a well-designed model. We might incorporate this non-linearity into the model by adding additional variates corresponding to products and powers of the original variates, perhaps with a term corresponding to the square of temperature. This approach is often adopted, but becomes computationally intensive if too many variates, or large powers are considered.

By using a particular ANN, the multilayer perceptron (MLP), to formulate non-linear models, some of these computational difficulties can be avoided. The MLP is a development of the MADALINE net which uses a smooth activation function for each unit in place of the discontinuous thresholding function of the original. Like the MADALINE network, it has a layered structure with the outputs from the nodes in one layer forming the inputs to nodes in the subsequent layer, from input to output as illustrated in Figure 4. Although apparently complex, the MLP exploits its highly parallel architecture to give computational efficiency. Since the output of a unit is not affected by the outputs of the other units in the same layer, all outputs from a layer can be calculated simultaneously on a parallel computer. With suitable hardware, the time to compute the output from an MLP is determined only by the number of layers in the network as opposed to the number of units. In practical applications three layers are most commonly used. This choice, although based on heuristic reasoning, appears to offer sufficient flexibility for the network to tackle a wide range of problems.

Fitting an MLP model to a data set is not straightforward. With weights and thresholds to be specified for every unit in the architecture the model's parameter space is clearly of high dimension. The problem is further complicated by the fact that the output of the MLP is not a linear function of its parameters and techniques such as least-squares regression cannot be used to identify optimal parameters. However, the MLP can be trained to "learn" the appropriate model from a data set using the BP algorithm described below. Suppose we are supplied with a data set consisting of input/output data (x,d(x)). Here x might represent a vector quantifying a set of physical features of an organism, and d(x) might denote its species, in which case the net is learning to classify. Equally, d(x)

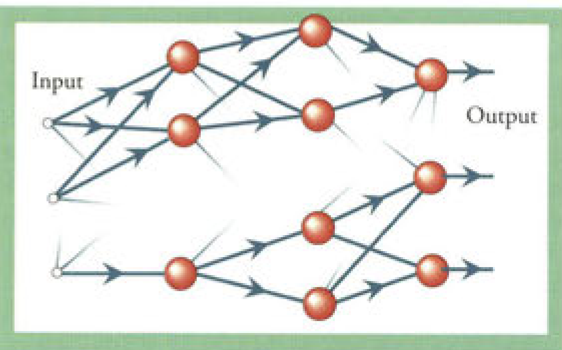


Figure 4 Multilayer perceptron architecture.

may itself be a continuous variable and the problem might be one of system modelling. Such an instance would arise if x quantified environmental and chemical factors and d(x) the growth-rate of an organism in response to them. In every case the algorithm's mode of operation is the same. Data are presented individually to the network. For each pair (x,d(x)) the MLP calculates its output y = g(x), say, and compares this quantity with the desired output d(x). All the network's parameters are then adjusted to decrease the size of the error between d(x) and g(x). This process is repeated, presenting the data many times if necessary, until the parameter values converge, and the desired function is learned. Figure 5 depicts this BP learning process schematically.

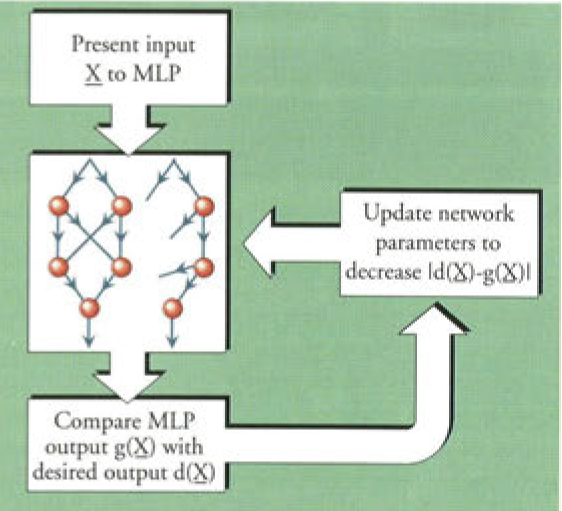


Figure 5 Back propagation learning algorithm.

An example. The learning process is illustrated by the following example of a 2-dimensional classification problem. Suppose we wish to train a 3-layer network to recognise whether or not inputs lie inside or outside the square

$S = \{(x,y) \mid -1 \leq x \leq 1 \ , -1 \leq y \leq 1 \}.$

We therefore generate training data in the form of input/output pairs (x,d(x)), where d(x) = 1, if x lies in S, and d(x)=0, otherwise. These are presented to the network which updates its parameters after each datum using the BP algorithm. The dynamics of the learning process are illustrated by plotting the region which the network "thinks" represents the set S at different points during the training. If training is successful then this region should eventually conform to the square described above. Figure 6 shows this region in comparison with the boundary of S after 6000, 16000, and 60000 training samples. After 6000 samples, the neural network has "realised" that the region which it should classify as being in S (i.e. its output should be 1) is a patch located near the origin. However, the exact nature of the boundary has not been determined. As training proceeds, the network adjusts the boundary of this region which begins to resemble a square. Finally after 60000 samples, the network has learned to recognise inputs coming from S with a high degree of accuracy. Practical examples of classification problems tackled by neural networks are rarely as simple as this but the approach is the same.

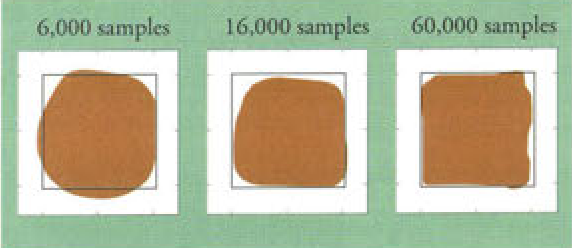


Figure 6 Development of decision region during training

Some pitfalls and unresolved problems. It would be wrong to portray ANNs as a set of techniques whose modes of application have been thoroughly established and accepted. There are still many practical and theoretical problems which must be solved before neural networks can be readily applied by the naive user, as many statistical techniques are today. One source of difficulty concerns the choice of network architecture which governs the complexity of the mappings which the network can produce. In the above example, the architecture chosen proved capable of learning the appropriate mapping. However, if a network with only two first layer units had been chosen this would not have been the case. In practice, experimentation is often required to identify a network architecture which is capable of modelling the data, but which avoids excessive computational complexity.

Further difficulties are associated with the learning algorithm itself. In the first place the BP algorithm is slow. While the above example requires only a few seconds effort by a SUN workstation to learn the appropriate classification function, more complicated examples may take hours of computer time. However, speed of convergence is not the only failing of the BP algorithm. Often the user will find that repeated attempts to identify a model by the algorithm using different initial parameters will result in a range of solutions, which differ markedly in terms of how well they fit the data. This is the problem of "local minima" and it has been encountered to some extent by virtually every researcher who has applied the MLP. Usually, it is tackled by *ad hoc* methods, such as increasing the number of units within the network or retraining many times with a range of initial conditions and selecting the best solution which results. These methods are time-consuming, and still do not guarantee that the global optimum will be found.

The difficulties cited above mean that the use of neural networks has remained something of a "black art", where the user applies experience, judgement and intuition rather than any hard and fast guidelines. Until the whole area is placed on a surer theoretical footing, this is likely to remain the case. SASS is currently devoting effort to studying mathematical aspects of neural networks, with particular emphasis on eliciting the relationship between the architecture of a network and its capabilities, and the development of reliable training methods for multilayer perceptrons.

Applications within SASS. As with any emerging technique, it is important that it should not be applied to problems in isolation, but in comparison with alternative established techniques. Practical examples where SASS are considering the use of neural networks include modelling the sensory attributes of supermarket cheeses in terms of their chemical composition. Linear modelling techniques have already been applied to this problem within SASS and have yielded results which are highly encouraging. However there is good reason to think that a non-linear model might offer some advantages. The perception of a sensory attribute such as "acidity" is likely to exhibit the phenomenon of saturation because different degrees of acidity above a certain level cannot be distinguished by an observer. The sigmoid non-linearities commonly used in the MLP lend themselves naturally to modelling functions with this behaviour making it a sensible choice as a candidate non-linear

model but, at present, this work is in its infancy. However, it should lead to a greater understanding of the capabilities of neural networks as statistical classifiers and elicit their value in comparison with traditional methods.

The future of ANNs. There is no doubt that neural networks have already made a major impact on scientific thinking, but this has come about largely because of their innovative nature and the beliefs of scientists regarding their potential. The future development of the subject will be dependent on some of this potential being realised in the form of established techniques which can be applied with confidence, few of which exist at the moment. Some scientists have complete confidence that ANNs will develop into powerful thinking machines to rival the human brain. Indeed, a survey of American researchers revealed in 1988 that 9% of those polled thought it possible that an ANN would exhibit psychokinesis within the next 25 years! The view of this author is perhaps not so optimistic (or pessimistic). For one thing, the analogy between ANNs and the genuine article has grown more tenuous with time as researchers do not believe that the BP algorithm is any reflection of human learning. A more likely scenario is that some of the current avenues of research in ANNs will end, while others go on to produce established, valuable methodologies which will pass into common use. SASS research is directed towards these more realistic goals.

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Plot interference in field experiments

R. A. Kempton, J. W. McNicol, A. D. Milner and M. Talbot

Good statistical design and analysis is essential if new developments arising from agricultural research are to be properly assessed under field conditions. Statistical work has traditionally focused on the need to take account of experimental variability in order to improve the precision with which treatment effects are estimated. However, less attention has been paid to studying situations in which treatment estimates from experiments may be systematically biased.

Bias will occur when the experimental conditions under which treatments are assessed do not reflect commercial farming practice. The resulting distortions to treatment means will then tend to recur from trial to trial and, unlike other sources of error, will not be removed by averaging across replicates, sites or seasons. For example, the measured yield of an early maturing cultivar, harvested at the same time as conventional cultivars in trial may consistently underestimate the cultivar's true potential yield because of premature shedding of grain. But the example we consider here is more fundamental and pervasive for field experimentation. It arises because the need to minimise resources (land, labour, seed or treatment) and maintain experimental precision means that treatments are tested within a mosaic of small plots. This contrasts with the uniform conditions found in UK agriculture and may itself lead to experimental bias due to interference between plots.

Interference occurs when plot response is affected, not only by its own treatment, but also by the treatments applied to neighbouring plots. Figure 1 shows some

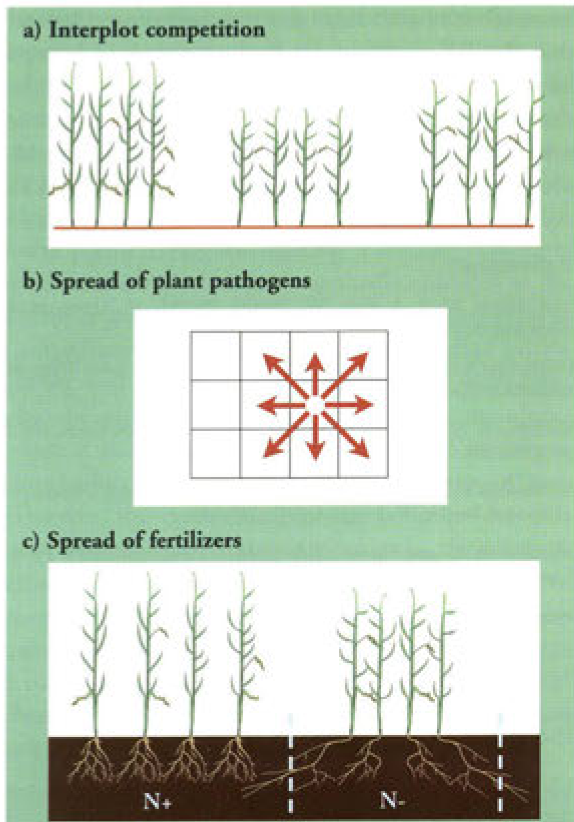

Figure 1 Causes of interference between plots. a) Interplot competition: Plots of more vigorous varieties depress yields of neighbours. b) Spread of plant pathogens: susceptible varieties act as foci of infection. c) Spread of fertilizers: plants on unfertilized plots poach nutrients from treated neighbours.

ways in which this may occur. In a cultivar yield trial, for example, interplot competition may lead to the yields of shorter cultivars, with poor competitive ability, being depressed relative to their taller, more aggressive neighbours (Fig. 1a). In disease screening trials, a highly susceptible cultivar may act as a secondary source of infection for other cultivars in the trial (Fig. 1b), so that the effectiveness of a resistant cultivar, or a chemical treatment, is underestimated; conversely, pest damage to a susceptible cultivar may be increased when surrounded by resistant cultivars. In fertilizer trials, the roots of plants in a low treatment plot may poach nutrients from neighbouring plots (Fig. 1c), or, when trials are laid out on a slope, nutrients may leach between plots.

The bias to treatment estimates that can result from interference is illustrated for two cultivar trials (Fig. 2). Figure 2a gives the mean blight scores for four potato cultivars grown in pure and in mixed stands. Although the overall mean score and ordering of cultivars is the same in both cases, the difference between susceptible and resistant cultivars is much reduced in mixtures. Thus, when screening plants for disease in the early stages of a breeding programme, interference may lead to the effects of resistant genotypes being underestimated. Figure 2b compares the yields of six cultivars of field beans of differing height in a 1-row plot trial with their yields in the centre two rows of a 4-row plot trial. The ranking of cultivars in the two trials was very different: in the 1-row plot trial, yields of taller cultivars were enhanced and shorter cultivars reduced as a result of interference effects from neighbouring plots. Yield measured under the conditions of inter-cultivar competition experienced in small plot trials may thus provide a poor indicator of the relative performance of the cultivars in monoculture. These two examples illustrate the importance of taking account of interference effects when testing cultivars in small plots.

Careful attention to the design and layout of an experiment may reduce interference or avoid it altogether. For example, plots are sometimes separated by alleys or ditches, or by strips of crop (guards) outwith the

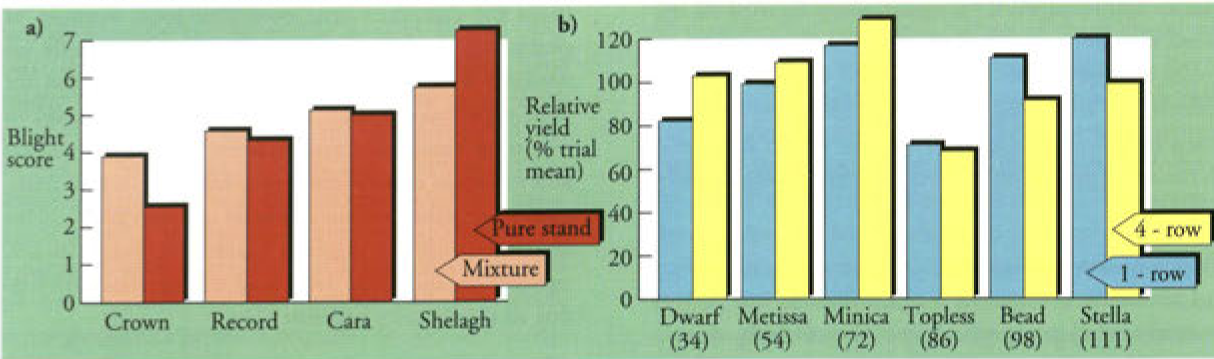

Figure 2 a) Blight score for four potato cultivars in pure and mixed stands. b) Relative yields of six field bean cultivars in unguarded 1-row and centre rows of 4-row plot. Figures in brackets are the effective variety heights.

a)

Rep I (GUARDS)

8	10	7	9	6
2	1	3	4	5
19	16	20	17	18
14	15	11	12	13
23	25	24	22	21

GUARDS

Rep II

19	17	16	18	20
23	25	24	21	22
14	12	15	13	12
4	2	5	3	1
6	8	9	10	7

GUARDS

Rep III

25	24	21	23	22
15	11	13	12	14
6	7	9	10	8
18	16	19	20	17
1	4	2	3	5

GUARDS

b)

Rep I	GUARDS	1 2 3 4 5	6 7 8 9 10	11 12 13 14 15	16 17 18 19 20	21 22 23 24 25
RepII		1 6 11 16 21	22 17 12 7 2	3 8 13 18 23	24 19 14 9 4	5 10 15 20 25
Rep III		1 7 13 19 25	21 20 14 8 2	3 9 15 16 22	23 17 11 10 4	5 6 12 19 24

GUARDS

Figure 3 Two designs for minimising the effect of interference between plots in one dimension (row). The 25 treatments are allocated to five ordered interference groups of five treatments which exhibit minimum interference, viz (1...5), (6...10), (11...15), (16...20), (21...25). a) Split-plot design where interference group are allocated to blocks (main plots). b) Lattice design with randomisation restricted so that adjacent pairs of cultivars appear in the same or a consecutive interference group. Note, using a linear arrangement of plots for each replicate reduces the number of guard plots.

experiment. However, this requires extra resources and may in itself introduce additional experimental variation. Another approach is to design the experiment so that treatments which significantly interfere with each other do not appear in neighbouring plots. In a cultivar trial, for example, cultivars might be grouped by height or disease susceptibility, so that the interference between treatments within groups is small. Treatments from different interference groups might then be allocated to separate experiments, or a split-plot design used with the groups allocated to separate blocks (Fig. 3a). However, this approach has the disadvantage that it is often difficult to make a proper statistical comparison between treatments in different interference groups. A better approach would use conventional incomplete block designs, but restrict randomisation of treatments within the blocks so that adjacent pairs of treatments come from the same or a similar interference group. This will reduce the variation in precision of treatment comparisons. An example based on a three-replicate 5x5 lattice design is shown in Figure 3b.

The designs proposed above assume that the experimental treatments can be classified on a single measure of interference. This is often impracticable. In cultivar trials, for example, interference may be related to a number of plant characters e.g. plant height, root size and susceptibility to several diseases. Furthermore, when screening new cultivars, the appropriate character scores are not always known in advance. In these cases, it is necessary to take account of interference effects in the subsequent experimental analysis.

Interference effects may be studied most easily using triple plots in which every treatment appears bordered by every other treatment. For three treatments A,B,C the nine triplets are

AAA BAB CAC ABA BBB CBB ACA BCB CCC.

The yields of the centre plots, for a hypothetical competition trial with five cultivars following this design, are shown in Table 1. The yield of cultivar A with cultivar B as neighbour may be partitioned as

$$\text{Yield A with B} = \text{Grand mean} + \text{Direct effect A} + \text{Interference effect B} + \text{Residual.}$$

The direct effects and interference effects are given by the margins in Table 1, while the residuals are given in the body of the table. In this example, the cultivars are ordered by their direct effects A,B,C,D,E. Two cultivars, B and E, have large interference effects. This means that yield is decreased on average by 15 units when a neighbour of B, but increased by 25 when a neighbour of E. The yields of the cultivars in pure stands (self-neighbours) are represented by the diagonals of the original table. The above equation can be rewritten as

$$\text{Yield A with B} = \text{Grand mean} + \text{Pure stand effect A} + \text{Interference effect B} - \text{Interference effect A} + \text{Residual}$$

which shows that the bias in yield of A with neighbour B, compared with its pure stand yield, depends on the difference in their interference effects. The residual effects in Table 1 comprise random effects and possible interactions between the direct and interference effects. If these interactions can be ignored, then fitting the above equation to plot data with dif-

Cultivar	Neighbour A	Neighbour B	Neighbour C	Neighbour D	Neighbour E	Cultivar (direct) effect
A	**273**/+1	**251**/-4	**268**/+6	**263**/-3	**295**/0	70
B	**259**/+2	**243**/+3	**247** / 0	**253**/+2	**273**/-7	55
C	**191**/-1	**176**/+1	**183**/+1	**183**/-3	**217**/+2	-10
D	**171**/-1	**157**/+2	**158**/-4	**168**/+2	**196**/+1	-30
E	**116**/-1	**98**/-2	**104**/-3	**113**/+2	**144**/+4	-85
Neighbour (interference) effect	+2	-15	-8	-4	+25	Grand mean 200

Table 1 Components of yield for competition diallel experiment. The plot yields (in bold figures) are partitioned into the grand mean, a direct effect, an interference effect and a residual (see text).

ferent neighbours allows cultivar means to be corrected for interference effects.

In Figure 4 the mean yields of the six field bean cultivars from the 1-row trial, before and after correction for interference, have been plotted against the centre row means for the 4-row trial. The interference model only goes part of the way to explaining the difference between the two trials. Other factors may be important, for example, the 4-row trial was overall higher yielding which might benefit the shorter cultivars.

Neighbour-balanced designs in which centre plots also act as neighbours are particularly efficient for investigating interference. Figure 5 shows designs in one and two dimensions for three replicates of seven treatments. In both designs, each treatment has every other treatment as a neighbour the same number of times (once for Design A, twice for Design B). This allows the direct and interference effects to be estimated independently. More extensive designs allow different interference effects for row and column neighbours, for left and right-hand neighbours, or for interactions between direct effects and interference effects.

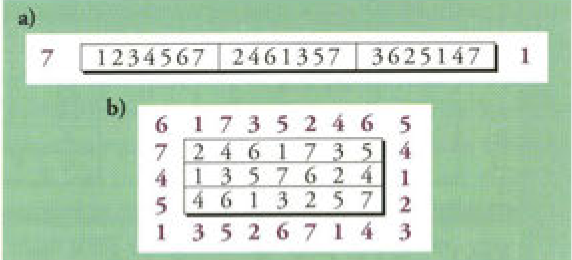

Figure 5 Designs for three replicates of seven treatments balanced for nearest neighbours. a) in one dimension. b) in two dimensions. Note the large number of guard plots for design b.

In standard trials with limited replication it is often not feasible to estimate separate interference effects for all treatments. Several studies in SASS are therefore aimed at relating interference effects to particular plant characters. In cultivar yield trials with cereals and field beans, interference effects have been found most frequently to be related to cultivar height, although susceptibility to mildew or yellow rust are also sometimes important determining factors; while for root crops (eg sugar beet, swede and potato), root or tuber yield has been shown to be affected by the yield in neighbouring plots. A plant character that has been associated with plot interference may be used as a covariate to adjust plot yields for interference bias. This is illustrated for a tall and dwarf cultivar of field bean in Figure 6, where the covariate is the mean height difference of two neighbours. Here the appar-

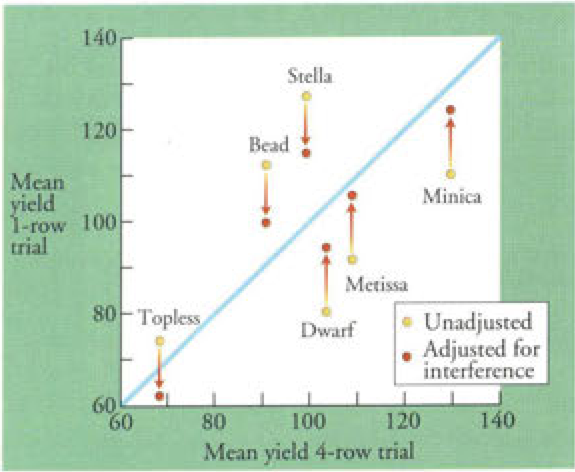

Figure 4 Relative yields (% trial mean) of six field bean cultivars in 1-row and 4-row plots.

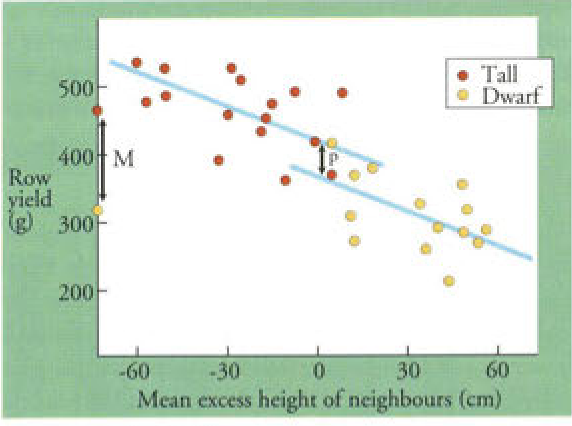

Figure 6 Yield of single-row plots of a tall and dwarf bean cultivar when grown adjacent to cultivars of differing height. M is the observed difference in mean yields, P is the difference after adjustment for interference from neighbours.

ent yield superiority of the tall cultivar becomes insignificant when the yields are adjusted for interference from neighbours. This is supported by the observed yields in guarded plots (Fig. 2).

Bias due to interplot interference represents a significant curb on the efficiency with which good new cultivars are brought into agricultural production. Studies of wheat breeding trials have indicated that there is a 1% yield advantage to a cultivar for every 10cms it is taller than a standard. A consequence is that selection pressures will tend to produce cultivars which are taller than necessary with all of the cost implications to farmers of growth regulators and other measures for lodging control. While it will not be possible to eliminate bias completely, nevertheless there are good prospects of developing statistical design and analysis methods which can minimize the effects of inter-plot interference in cultivar trials.

Research Services

Data Processing

R.J. Clark

The SCRINet local area network, with Sun workstations at its centre, continued to increase in popularity as scientists were retrained on the new system. 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. Over one hundred PCs are currently installed and this number is increasing.

Laser printers, graphplotters and lineprinters are attached to SCRINet as shared output devices, that is, any user can produce high quality printed documents or coloured graphs from any PC attached to the network. 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.

User training on the Unix operating system and network software occupied DP Unit staff over much of the year. The large open-plan user area with up to eight terminals is used for training, so that a small group of up to sixteen scientists can gain 'hands on' experience. Smaller rooms can be used for informal training and the seminar room adjacent to the DP Unit affords facilities for an efficient teaching mix of lecture and practical demonstration. DP staff also prepare user-notes as training materials.

A training session on SCRINet.

Services on the network are primarily for access to statistical analysis packages like Genstat and Minitab. The CHIP package was adapted to run on SCRINet. CHIP is a crop geneticists package for managing genetic databases, generating experiment plans and labels, performing statistical analysis, and selecting progenies. Other services, such as the relational database package Oracle and the Uniras graphics package are available.

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

The personal computer is regarded as another tool by the many scientists who have one on their desk. Science departments consult the DP Unit regarding computer equipment and software. Bulk purchasing reduces equipment costs, and standardising on selected hardware and software enables DP staff to develop expertise for supporting and training users. Microsoft Works is the standard integrated package for applications ranging from papers to graphs and databases. WordPerfect is the standard word processing package. More complex applications for displays and posters, using Supercalc and Harvard Graphics, are produced in co-operation with Information Services.

Scientific Liaison and Information Services

D.A. Perry

The Library contains a stock of *c.* 5,000 text books covering a wide range of subject matter relevant to the work of the Institute. It also subscribes to *c.* 700 scientific periodicals and newsletters and has collections of leaflets, maps and miscellaneous reports. A large collection of material pertaining especially to potato and soft fruit crops is maintained. The library catalogue is currently being transferred from a written card index to a computer-mounted system on the Institute's computer network.

The CAB International database from 1984 onwards has been acquired on CD-ROM and access to other on-line databases, e.g. BIOSIS, CAB International and Food Science and Technology Abstracts is available via DIALOG. University library catalogues are available for scrutiny through JANET.

Inter-library loans are available from the British Library Document Supply Centre and essential translations of foreign papers can be undertaken locally.

The Visual Aids section provides a photographic and graphics service to the staff. All forms of photography of biological specimens from field plot scale down to macro and microscope level can be undertaken with a range of specialised camera equipment using monochrome, colour, UV and infra-red sensitive film. During 1991 some 3,000 separate photographic assignments were undertaken. All of the monochrome film is processed on site and printed using an automated processor. A library of colour transparencies and monochrome negatives of all photographs taken for staff is maintained. Increasing use is being made of video recording within the Institute, particularly for time-lapse photomicrography, and facilities and expertise in this area are expanding.

Graphics are produced to illustrate scientific publications, posters and exhibitions. An Apple Macintosh computer system has greatly enhanced the capacity to produce high quality graphics, leaflets and internal newsletters. It can also be used as a desk top publish-

SCRI exhibit at Scotgrow, 1991.

ing system to design pages of publications such as this report before being printed commercially.

The Scientific Liaison section makes arrangements for many individual and groups of visitors and organises exhibitions of the work of the Institute. It is responsible for the editing and production of the Annual Report and other occasional publications. Liaison is maintained with bodies such as the European Commission, international agencies, levy boards and commercial organisations to promote contract research.

Estate, Glasshouse and Field Experiments Department

W.I.A. Jack

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

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

Hand planting potato trials.

New glasshouses for the Potato Genetics Group, formally opened in 1991.

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

Land is divided into packages of 10-12 ha providing areas for arable crop trials with an 8-year break

Part of the range of controlled environment cabinets at SCRI.

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

The Department maintains the UK virus-free nuclear stocks of *Ribes, Rubus* and *Narcissus* used for first-stage commercial production of planting stocks. Six research glasshouse complexes cover 8000m^2 and have fully automatic control of heating and supplementary lighting. Some automatic or semi-automatic irrigation and liquid feed systems are installed on new aluminium benches. Each complex has an integral header-house which provides some laboratory accommodation, conditioning rooms and a rough working area. The individual glasshouse cubicles range in size from 12m^2 to 350m^2 and provide modern facilities that satisfy the demand for out-of-season plant production and flexibility of use. Some cubicles are designed for specific research objectives, including quarantine and isolation, and feature double-door access, sealed glass and filtered air conditioning. 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. This allows for a diverse range of environments to be created allowing both temperate and sub-tropical plants to be grown.

Engineering and Maintenance Department

S. Petrie

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

The Department is divided into sections that specialise in a variety of engineering disciplines such as electrical, electronic, refrigeration, heating and mechanical engineering. It provides an engineering design and maintenance service to cover scientific and ancillary equipment and building services including heating, ventilation and air conditioning. There is also a garage section providing maintenance facilities for a substantial fleet of road vehicles, tractors and

Routine maintenance operations

Engineering workshop facilities.

agricultural machinery. The Department provides a general stores facility and a cleaning and security service. The workshops are generally well equipped to deal with the maintenance tasks assigned to them.

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

Legislative demands appropriate to the Department (such as pressure vessel and lifting tackle regulations, electricity regulations, building regulations, Health and Safety at Work Act etc) have been met, but recent regulations have imposed additional demands on resources and steps are being taken to cope with them.

Major works completed during the year included the re-wiring of the laboratory area encompassing the Virology and Zoology Departments, an extension to the automatic fire alarm system and the installation of an intruder alarm system.

Mylnefield Research Services Ltd.

R.J. Killick

Mylnefield Research Services Limited is a wholly owned subsidiary company of the Scottish Crop Research Institute. It exists primarily to protect the Institute's charitable status and to expand the funding base. It does this by marketing the Institute's scientific expertise and undertaking contract work with commercial and other non-SOAFD sponsors. MRS Ltd. then subcontracts the work to SCRI on a full cost basis. Any profit remaining with MRS Ltd. at the end of its financial year is then available for transfer to the Institute via one of two tax efficient routes.

Although MRS Ltd. was incorporated in November 1989 it did not begin to trade until April 1991. It would be premature to project a profit figure for the first full financial year. However, it seems clear after 9 months trading that the company is proving a success. We look forward to acquiring premises and the first permanent staff of the company during 1992.

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. The Committee of Management met on two occasions (3 April and 6 November).

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

£300 to Mr M.S. Phillips, Zoology Department, to Landerneau, France.

£150 to Dr J.M.S. Forrest, Zoology Department, to Sweden.

£156 to Dr R.A. Jefferies, Cellular and Environmental Physiology Department, to France.

£500 to Dr I.M. Young, Cellular and Environmental Physiology Department, to Japan.

From the Thyne Bequest:-

£325 to Dr J.E. Bradshaw, Crop Genetics Department, to Czechoslovakia.

£500 to Mr G.R. Mackay, Crop Genetics Department, to France.

Other donations made during the year were £130 for financial assistance for the production of an Occasional Paper for the Soft Fruit Genetics Department.

A Soft Fruit Walk was held on 23 July when the heavens opened and almost washed away the 40 or so members who heard about replacement for dinoseb-in-oil, blackcurrant breeding objectives, raspberry breeding objectives, grey mould and fungicides, detection and control of raspberry root rot and the raspberry cane midge.

Chairman and speakers at the SSCR meeting, Prospects for Cereals, 20th November, 1991.

There was a Potato Walk on 22 August where the breeding programme was examined with emphasis on resistance to late blight and blackleg. Fertiliser trials and use of split applications of fertilisers were also discussed.

Prospects for Cereals was the title of the meeting held on the 20 November and delegates heard from Mr Barnes, Under Secretary of MAFF: Arable Crops and Alcoholic Drinks Group, Mr Patterson, General Manager of Dalgety Grain, Mr Morgan, Managing Director of Moray Firth Maltings plc, Dr Jordan, Head of the LIFE research project at the Institute of Arable Crops Research. Dr Powell, Head of the Cellular and Molecular Genetics Department, SCRI, Dr Cranstoun, Cereal Specialist in the Crop Husbandry Department, SAC and Dr Ellis of the Barley Genetics Group, SCRI.

A bulletin will be issued of the proceedings of this Conference.

The Field & Glasshouse Staff Prize was awarded to Mr David Simpson in April when he retired after 37 years' service and a Scholarship was awarded to Senor J.A. Goñzales from the Canary Islands to enable him to complete his Ph.D. studies on Potato Cyst Nematode at the Institute.

Membership of the Society was 320 on 31 December 1991.

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Extracts from the Accounts

Income and expenditure account for the year ended 31 March 1991

Income	£
Grants from SOAFD	6,246,850
Research, contracts and consultancy	811,629
Trading activities	112,078
SASS income	128,709
Bank interest receivable	25,090
Depreciation contribution from reserve	401,368
Other income	28,676
	7,754,400

Expenditure	
Staff costs	5,056,317
Supplies and services	1,412,405
Other expenditure	695,030
Depreciation	517,753
	7,681,505

Surplus for year	72,895
Balance from previous year	899,678
Transfer from revaluation reserve	21,650
Balance carried forward	994,223

Balance sheet as at 31 March 1991

	£	£
Fixed assets		
Tangible fixed assets		11,209,661
Current assets		
Stocks	106,021	
Debtors	368,681	
	474,702	
Current liabilities		
Creditors		
Amounts falling due within one year	382,027	
Net current assets		92,675
Total assets less current liabilities		11,302,336
Represented by:		
Capital and reserves		
Capital reserves		10,308,113
Income and Expenditure Account		994,223
		11,302,336

Staff list as at 31 December 1991

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)

Name	Grade
Head : G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol.[5]	UG6
J.E. Bradshaw, M.A., M.Sc., Ph.D.	UG7
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 (Prom. Apr)
R.L. Wastie, M.A., Ph.D., F.I.S.P.	UG7
I. Chapman, B.Sc.	SSO
M.F.B. Dale, B.Sc., Ph.D.	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
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.	SO
G.R. Young	SO
Eva Bennett	ASO
A. Booth, O.N.C.	ASO
W. Craig, B.Sc.	ASO
P. Davie, O.N.C.	ASO
Ann Donnelly, H.N.C.	ASO
Norma Dow	ASO
Michelle Fleming, H.N.D., B.Sc.	ASO
Frances Gourlay, H.N.C.	ASO
R. Keith	ASO
Karen 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	EWII
J.D. Fuller	EWII
Patricia Lawrence	EWII
S. McDonald, B.Sc.	EWII (Appt. Oct)
A. Margaret McInroy	EWII
Moira Myles	EWII
Joyce I. Young	EWII

Soft Fruit Genetics Department (SFG)

Name	Grade
Head : R.J. McNicol, B.Sc.	UG7
R.M. Brennan, B.Sc., Ph.D.	SSO (Prom. Apr)
Julie Graham, B.Sc., Ph.D.	HSO
Sandra L. Gordon, H.N.C.	ASO
Kay Greig, Dip. H.E.	ASO (Appt. Jan)
Amanda J. Thomson, H.N.D.	ASO

Cell & Molecular Genetics Department (CMG)

Name	Grade
Head : W. Powell, B.Sc., M.Sc., Ph.D.[5,6]	UG6 (IMP) (Prom. July)
J.W.S. Brown, B.Sc., Ph.D.[6]	UG7
B.P. Forster, B.Sc., Ph.D.	UG7 (Prom. Apr)
A. Kumar, B.Sc., Ph.D.	SSO
R. Waugh, B.Sc., Ph.D.[6]	SSO
S. Millam, B.Sc., Ph.D.	HSO
C.G. Simpson, B.Sc.	HSO (Appt. Dec)
E. Baird, H.N.C., B.A.	SO
Gillian Clark, HNC	SO (Appt. Dec)
B. Harrower, H.N.D., B.Sc.	SO
Jackien Jyon	SO
Diane Davidson	ASO
Nicky Duncan, H.N.C.	ASO
M. Macaulay, H.N.C.	ASO

Cellular & Environmental Physiology Department (CEP)

Name	Grade
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 (Prom. Apr)
K. Ritz, B.Sc., Ph.D.	SSO
D. Robinson, B.Sc., Ph.D.[6]	SSO
R.E. Wheatley, B.Sc.	SSO
A.G. Bengough, B.Sc., Ph.D.	HSO
G. Goleniewski, B.Sc., Ph.D.	HSO (Appt. Jan)
D.C. Gordon, H.N.C.	HSO
Heather A. Ross, H.N.C., C.Biol., M.I. Biol.	HSO
M. Taylor, B.Sc., Ph.D.	HSO
R. Viola, B.Sc., Ph.D.	HSO (Appt. Nov)
J.S. Wiseman, S.D.H.	HSO
Katherine M. Wright, B.A., Ph.D.	HSO
I. Young, B.Sc., Ph.D.	HSO
Sandra Caul, H.N.C.	SO
A. Gardner, B.Sc.	SO
Sandra E. Millar, O.N.C., H.N.C.	SO (Tr. from M&B Apr)
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
C. McKenzie	ASO
Diane McRae	ASO
Lesley Scobie	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)

Name	Grade
Head : M.J. Allison, B.Sc., Ph.D.	UG7
D.W. Griffiths, M.A., Ph.D.	SSO
G.W. Robertson, B.Sc.	SSO
H. Bain, H.N.C., L.R.S.C.	HSO
W. Matheson, B.Sc.	HSO
T. Shepherd, B.Sc., Ph.D.	HSO (Tr. from CEP Nov)
Winifred M. Stein, H.N.C.	HSO
K. Taylor, H.N.C.	SO
Fiona Falconer, H.N.C.	ASO
Anne Morrice, S.N.C., H.N.C.	ASO
Jean Wilkie	EWII

Director's Group (DG)

Name	Grade
I.M. Morrison, B.Sc., Ph.D.	UG7
B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C.[6]	UG7
G. J. McDougall, B.Sc., Ph.D.	SSO
D. Stewart, B.Sc.	HSO

Mycology and Bacteriology Department (M & B)

Name	Grade
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 (Appt. Oct)
Jacqueline Heilbronn, H.N.C.	SO
D.J. Johnston, B.Sc.	SO
Naomi A.. Williams, H.N.C.	SO
D.C. Guy	EWII (Appt. Nov)
Evelyn Warden	EWII

Virology Department (Vir)

Name	Grade
Head : 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)
A.T. Jones, B.Sc., Ph.D.	UG6 (IMP) (Prom. Jul)
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
Maud M. Swanson, B.Sc.	HSO (Appt. Apr)
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
Rena Reid	EWIII

Zoology Department (Zoo)

Name	Grade
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 (Prom. Apr)
J.M.S. Forrest, B.Sc., Ph.D.	UG7
M.S. Phillips, B.Sc.	UG7
W.M. Robertson, N.H.C., F.L.S.	UG7
J.A.T. Woodford, M.A., Ph.D.[6]	UG7
A.N.E. Birch, B.Sc., Ph.D., C.Biol., M.I.Biol.	SSO
S.C. Gordon, H.N.C.	SSO
R. Neilson, H.N.C.	SO
Ailsa Smith, B.Sc.	SO (Tr. from CEP Feb)
Anne M. Holt	ASO
Sheena Lamond	ASO
Gaynor Malloch, B.Sc.	ASO

Data Processing Unit (DP)

Name	Grade
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 (Prom. Apr)
Jennifer Gorrod, H.N.C.	ASO

Scientific Liaison & Information Services Department (SLIS)

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

Administration Department (Admin)

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

Engineering & Maintenance Department (EM)

Institute Engineer : S. Petrie, B.Sc.	HP&TO	E. Lawrence	Craftsman
R. Macdonald	HP&TO	R. Pugh	Craftsman
D. Gray	P&TO	T. Purves	Caretaker
A. Low	TGI	J. Rowe	Caretaker
K. Low	TGI	C. Conejo	EWIII (Appt. May)
R. White	TGI	J. Oldershaw	EWIII (Appt Jul)
J. Anderson	Craftsman	Janice McDonald	AO (P/T)
D. Byrne	Craftsman	G.C. Roberts	Craftsman
N. Craigie	Craftsman (Appt. May)	I.M. Scrimgeour	Craftsman
J. Duguid	Craftsman (Appt. May)	J.Flight	Storeman II

Estate, Glasshouse & Field Experiments Department (EGF)

Head :W.I.A. Jack	SSO		
G. Wood, B.Sc., Ph.D., F.E.T.C.	HSO	J.K. Wilde	EWI (Prom. Jan)
P.A. Gill, H.N.D.	HSO	J.T. Bennett	EWII (Prom. Jun)
W.D.J. Jack, B.Sc.	P&GS(D) (Prom. Jan)	Gillian Pugh	EWII
D.S. Petrie	P&GS(D)	D.L.K. Robertson	EWII (Prom. Jul)
R.W. Reid	P&GS(D)	Angela M. Thain	EWII
C.C. Carrie	P&GS(E)	G. Dow	EWIII
A.W. Mills	P&GS(E)	B. Fleming	EWIII (Prom. Mar)
R. Ogg	P&GS(E)	I. Fleming	EWIII (Prom. Aug)
D.G. Pugh	P&GS(E)	M.J. Soutar	EWIII (Appt. Apr Prom. Oct)
B.D. Robertson	P&GS(E)	C. Conacher	EWIV
J.R.K. Bennett	EWI (Prom. Mar)	A.C.Fuller	EWIV
C.R. Dalrymple	EWI	T.A. Mason	EWIV (Appt. Oct)
E.A.M. Gardiner	EWI	R. Murray	EWIV (Appt. Oct)
J.P.T. Grant	EWI	J.G. Sim	EWIV (Appt. Feb)
N. McInroy	EWI	Carol Taylor	EWIV
L.A. McNicoll	EWI (Prom. May)	Lorna Doig	AO (P/T)
J. Mason	EWI		

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 (Prom. Dec)
G.J. Gibson, B.Sc., Ph.D.	SSO (Appt. Jun)
G.W. Horgan, B.A., M.Sc.	SSO
M. Talbot, F.I.S., M.Phil. [8]	SSO
A.D. Mann, B.Sc.	HSO
F.G. Wright, B.Sc., M.Sc., Ph.D.	HSO
Muriel A.M. Kirkwood, D.A.	ASO
Irene M.S. Terris	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 (Appt. Jan)
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 (Prom. Apr)
Karen L. Cattanach, M.A., M.Sc.	HSO
Elizabeth I. Duff, B.Sc.	SO
I.M. Nevison, M.A.	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
Joanne E. Hall, B.Sc.	SO

Short Term Contracts

SOAFD Increased Flexibility Schemes

Cell and Molecular Genetics	
J.F. Guerineau, B.Sc., Ph.D.	HSO
P. Whitty, B.Sc.	HSO
Cellular and Environmental Physiology	
J.A.C. Smart, B.Sc.	HSO
Elizabeth A. Murant, B.Sc.	HSO
Director's Group	
Karen Brierley, B.Sc., M.Phil.	HSO
Mycology and Bacteriology	
Anne Wallace, B.Sc., Ph.D.	HSO
Lisa Fyffe	ASO
Virology	
A.D. Turnbull-Ross, B.Sc., Ph.D.	HSO
Zoology	
S.F. Howard	SO

SOAFD Flexible Funding

Cellular and Environmental Physiology	
Yiqun Gu, B.Sc.	HSO (Appt.Mar)
Susan Smith, B.Sc., Ph.D.	HSO
Ramanee Peiris, B.Sc.	SO
Sheena J. Rodger	ASO
Crop Genetics / Soft Fruit Genetics	
Aileen Timmons, B.Sc., Ph.D.	HSO (Appt. Nov)
Sharon Dubbels	ASO (Tr. from Zoo Dec)
SASS	
D. Hitchcock, B.A.	HSO (Appt. Oct)

SOAFD Other Funding

Zoology	
Lisa Palmer, B.Sc.	SO (Appt. Jul)

AFRC / Dundee University

Cell and Molecular Genetics	
G. Simpson, B.Sc., Ph.D.	Res. Asst. 1A
David Leader, B.Sc.	Res. Asst.
Petra Vaux, B.Sc., Ph.D.	Res. Asst.

BTG

Zoology	
Irene E. Geoghegan	SO

EEC / ECSA ECLAIR

Cell and Molecular Genetics	
G. Machray, B.Sc., Ph.D.	SSO
P. Hedley, B.Sc.	SO
Cellular and Environmental Physiology	
L.R. Burch, B.Sc., M.Sc., Ph.D.	SSO
R. Viola, Doct. Agr. Sci.	HSO
Edna Cuthbert, S.N.C., H.N.D.	ASO

EEC

Cell and Molecular Genetics	
K.J. Chalmers, B.Sc., Ph.D.	HSO (Appt. Dec)
Virology	
Sybil M. Macintosh, B.Sc.	SO

H-GCA

Cellular and Environmental Physiology	
Allison Cooper, H.N.D.	ASO (Appt. Oct)
Mycology and Bacteriology	
A. Reglinski, B.Sc., Ph.D.	HSO
SASS	
Alison V. Wheelwright, B.Sc.	HSO (Appt. Dec)

MAFF

Cell and Molecular Genetics	
A.L. March	SO (Appt. Dec)
Cellular and Environmental Physiology	
Elizabeth A.Robertson	ASO
SASS	
A.D. Milner, B.Sc., Ph.D.	HSO (Appt. Jul)

ODA

Cell and Molecular Genetics	
Stephanie Cooper-Bland, B.Sc., Ph.D.	HSO
P. Lanham, B.Sc., Ph.D.	HSO
Sarah Fennel, B.Sc.	SO
Jennifer Watters, H.N.D.	ASO
Virology	
Vivian Blok, B.Sc., M.Sc., Ph.D.	HSO
P.M. Derrick, B.Sc., Ph.D.	HSO
Mary-Jo Farmer, B.Sc., Ph.D.	HSO
P. F. McGrath, B.Sc., Ph.D.	HSO

ORSTOM

Virology	
Michelle S. Leslie	ASO

PMB

Cellular and Environmental Physiology	
M. Young, H.N.D.	SO
SigrunHoldhus, Cand.mag	EWV (Appt. Jun)
Zoology	
Jane Roberts	EWV (Appt. Mar)

United Biscuits

Cell and Molecular Genetics	
I. Morrison	ASO

Resignations

Name	Dept.	Grade	Month
H.K. Brown	SASS	HSO	November
Marion Burnett	M&B	SO	July
Susan E. Burrows	DG	SO	October 1991
Mary Coleman	CG	HSO	November
D. Diduca	EM	Craftsman	April
A. Lorimer	CG	ASO	October
Gina MacPhail	CEP	SO	December
Shona McIntosh	CEP	ASO	September
Hazel Thomson	M&B	ASO	April
H. Wallace	SASS	AA	April
Lesley Wilkinson	Admin	AO	February
P.W. Yeaman	EGF	EWII	August
Karen Young	SFG	ASO	January

Deaths

Name	Dept.	Grade	Month
M.R. Cormack	SFG	SSO	February
G.W. Pollock	EM	PTO	December

Staff Retirements

Name	Dept.	Grade	Month
J.R. Caithness	EM	Craftsman	September
Isobel Christie	CEP	Research Assistant	September
B.D. Harrison	Vir	UG5	June
J. Heeney	EM	Storeman II	December
T. Hopton	EM	SP&TO	September
D. Hutcheson	EM	Craftsman	August
W.W. Killoh	EGF	EWI	December
W.P. Mowat	Vir	UG7	September
D.S. Simpson	EGF	EWI	April

Redundancies, Voluntary and Flexible Retirements

Name	Dept.	Grade	Month
J.M. Cooper	SASS	SSO	July
I.A. Cowe	Chem	SSO	October
D.C. Cuthbertson	Chem	SO	October
A.D. Lindsay	EGF	P&GS(E)	March
J.H. Raschké	Vir	HSO	April
J. Taylor	Chem	SO	October

Longer-term visitors and Research Fellows

Name	Country of origin	Dept.	Month/yr of arrival	Length of stay
Jill Ellis	UK	CG	Jan 91	1 years
D. Fargette	France	Virology	Jan 91	3 years
Mireille Fargette	France	Zoo	Jan 88	4 years
Linda L. Handley	UK/USA	CEP	Jun 91	5 years
Dorothy Spencer	UK	CG	Aug 89	3 years
W.T.G. van de Ven	The Netherlands	CMG	Oct 88	3 years
Angelika Ziegler	Germany	Vir	Jan 91	2 years

Short term workers and visitors

Name	Country of Origin	Dept.	Month/yr of Arrival	Length of stay
J. Allainguillaume	France	CG	Apr 91	6 months
Z. Allingham	UK	CMG	Jul 91	1 year
R. Anderson	UK	CMG	Aug 91	4 months
Leena Andreeva	Estonia	Vir	Aug 91	2 months
A. Barrett	UK	M&B	Nov 91	1 year
J. Bennett	UK	SASS	Aug 91	2 months
J. Bol	The Netherlands	Zoo	Jun 91	3 months
L. Catalano	Italy	Zoo	Nov 91	6 weeks
J. Cussick	UK	CMG	Apr 91	6 months
D. Deng	China	Vir	Oct 90	1 year
Marie Christine Dubs	France	Vir	Jul 91	1 month
N. Ebblewhite	UK	CMG	Mar 91	1 year
J. Ellis	UK	CG	July 91	10 weeks
B. Farkas	UK	SASS	Oct 91	3 months
Karen Ferguson	UK	CEP	Jun 91	4 months
S. Fryer	UK	CMG	Aug 91	2 weeks
Vivienne Gepp	Uruguay	Vir	Aug 91	3 months
Sandra Hay	UK	CEP	Aug 91	3 months
G. Hill	UK	CEP	Aug 91	3 months
F. Hosein	Trinidad & Tobago	CMG	Nov 91	3 months
A. Homes	UK	CMG	Aug 91	4 months
Emily Hoover	USA	SFG	Mar 90	11 months
E.C.K. Igwegbe	Nigeria	Vir	Aug 91	1 week
E. Johnson	Trinidad & Tobago	CMG	May 91	1 month
E. Johnson	Trinidad & Tobago	CMG	Nov 91	4 months
S. Kashiwazaki	Japan	Vir	Oct 91	1 year
R. Knight	UK	Zoo	Jul 91	6 months
Margo de Kort	Holland	CEP	Jan 91	6 months
S. Korie	Nigeria	SASS	Oct 91	6 weeks
P. Kunz	Switzerland	Zoo	Aug 91	2 weeks
R. Legg	UK	Zoo	Aug 91	1 year
N. Leitch	USA	Zoo	Nov 91	3 months
J Luby	USA	SFG	Mar 90	11 months
J. Mann	USA	Zoo	June 91	3 months
A. Maxwell	UK	CG	Jul 91	10 weeks
C.B. McConnell	USA	CEP	May 91	2 months
P. McNicol	UK	CMG	May 91	1 year
P. Milne	UK	CG	Jul 91	10 weeks
J. Milne	UK	CMG	Jun 91	1 year
J.P. Moss	India	CMG	Feb 91	6 months
Ivana Obradodic	Yugoslavia	M&B	Jul 91	3 months
F. Ogbe	Nigeria	Vir	Sep 91	4 months
A. Orcheson	UK	CG	Oct 91	5 months
J. Passioura	Australia	CEP	Apr 91	5 months
Helle Frost Pederson	Denmark	CEP	Jan 91	4 months
S. Rakhit	UK	Zoo	Sep 91	1 year
J. Reilly	UK	CMG	Apr 91	2 months
A. Reutenauer	France	Vir	Nov 91	1 month
Alison Roberts	UK	CEP	Jun 91	4 months
E. Rojancovski	Romania	Zoo	Sep 91	1 week
S. Ross	UK	CEP	Jun 91	3 months
G. Rossi	France	M&B	Jul 91	6 weeks
T. Sangster	UK	CEP	Nov 91	10 months
T. Sangster	UK	CEP	Jun 91	3 months
F. Scott	UK	CG	Jul 91	10 weeks
I.M. Al Shahwan	Saudi Arabia	Vir	Sep 91	3 months
D. Vyas	UK	Zoo	Jul 91	1 year
F. Van Waes	The Netherlands	SFG	May 91	3 months
A. Watson	UK	Zoo	Jan 91	6 months
S. Varsha Wesley	India	Vir	Jul 91	1 year
E. Witter	Sweden	CEP	Apr 91	2 weeks
Elizabeth Wojciechowski	U.K.	SLIS	Apr 91	3 months
Gilly Zimand	Israel	M&B	Mar 91	4 months

Postgraduate Students

Name	Dept.	Subject
I. Abdalla	SASS	Automatic detection of tissue boundaries in ultrasound scans of pedigree sheep.
R. Anderson	CMG	Retrotransposons in plants.
Siti A. Mad Arif	CMG	Plant genetic transformation and gene expression.
Karen Backett	Zoo	A novel screen for PCN.
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.
Karen Brierley	DG	NMR spectroscopy for characterisation of the coat protein of pepper ringspot virus.
Debbie Cawston	CMG	Quantitative trait loci and genetic markers in barley.
K. Chalmers	CMG	Molecular genetics of barley.
F.A. Comerford	CMG	Lamins in the plant nuclear membrane.
T. Connolly	SASS	Interplot competition in variety trials.
Sarah Fennel	CMG	Biochemical and molecular markers of *Arachis.*
R.S. Forrest	M&B	Phytoalexin eliciting cell wall fragments in *E. carotovora.*
Shirley Friar	CMG	Transformation methods in *Brassica napus.*
A. Gardner	CEP	Purification and properties of potato tuber hexokinases.
A. Gleadle	CMG	Somatic hybridisation in potato.
J. Gonzalez	Zoo	Biochemical and molecular identification of PCN.
Mary Gray	CEP/SFG	Regulation of anthocyanin gene expression in blackcurrant.
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.
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.
J.D. Madulu	Zoo	Alternatives to chemical control of *Meloidogyne* in Tanzania.
I. Manoussopoulos	Vir	Mechanisms of aphid transmission of potyviruses.
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.
A.J. Nisbet	Zoo	Prevention of plant virus transmission by antifeedant compounds.
N.E. Nyange	SFG	Breeding for resistance to coffee berry disease and coffee rust.
L.G. Pereira	Vir	Monoclonal antibodies to potato mop-top virus.
J. Phelpstead	CMG	Cell biology of potato.
A.T. Ploeg	Zoo	Transmission of tobraviruses by trichodorid nematodes.
Jennifer Robb	Zoo	Nematode gland cell secretions.
M.R. Roberts	CMG	Transposon mutagenesis in flax.
Heather A. Ross*	CEP	Investigation of the control of sugar breakdown.
Karen Scott	Vir	Genome structure of potato mop-top virus.
J. Seraphin	SASS	Variance-distance relationships in agricultural field plot experiments.
J. Shaw	SASS	Techniques for discrimination of seed types using imaging measurements.
C. Simpson	CMG	Transposable elements from maize.
J.A.C. Smart	CEP	Use of artificial intelligence in crop modelling.
Joanne Smith	M&B	DNA polymorphisms as genetic markers for rust fungi.
D. Stewart*	DG	Physico-chemical studies of plant fibres.
Maud Swanson	Vir	Study of two new viruses in cassava.
J.S. Swanston *	CG	Malting and brewing properties of novel barley starch combinations.
I.K. Toth	M&B	The isolation of novel *Erwinia* phages.
W.T.G. Van De Ven	CMG	Construction of a genetic linkage map in *Vicia faba.*
R. Viola	CEP	Biochemistry of starch-sugar interconvertions.
Wendy Wallis	M&B	Downy mildew of *Rubus* cane fruits.
A. Ward	CMG	Application of protoplast technology in potato improvements.
Susan Wharam	M&B	Molecular genetics of *Erwinia* pathogenicity.
A.V. Wheelwright	SASS	Estimation of edges in medical images.
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

Visits Abroad

Name	Country Visited	Month of visit	Duration of visit
A.G. Bengough	The Netherlands	April	5 days
	Austria	September	9 days
B. Boag	France	June	1 week
	New Zealand	July	6 weeks
J.E. Bradshaw	Czechoslovakia	July	6 days
R.M. Brennan	Sweden	July	5 days
	USA	September	3 weeks
D.J.F. Brown	Germany/Belgium	March	2 weeks
J.W.S. Brown	USA	May	8 days
	Bulgaria	May	5 days
	USA	October	1 week
	Germany/Italy	July	3 weeks
	Portugal	September	10 days
	The Netherlands	October	5 days
L. Burch	The Netherlands	November	3 days
S.T. Buckland	Iceland	February	1 week
	Iceland	May	11 days
	USA/Canada	Nov/Dec	4 weeks
S. Cooper-Bland	USA	October	2 weeks
J. Crawford	The Netherlands	April	1 week
	The Netherlands	November	1 week
H.V. Davies	Belgium	May	3 days
	The Netherlands	November	3 days
	USA	August	1 week
	The Netherlands	December	3 days
	Italy	May	4 days
	France	July	4 days
P.M. Derrick	Latvia	May	6 days
J.M. Duncan	France & Germany	May	1 week
	Norway	September	3 days
R.P. Ellis	Sweden	July	1 week
	Spain	October	1 week
D.A. Elston	Germany	March	4 days
S.R. Fennell	USA	October	2 weeks
B.P. Forster	Sweden	July	1 week
	Denmark	August	1 week
	Australia	Sept/Oct	5 weeks
M.F. Franklin	Germany	March	5 days
	Canada	Aug/Dec	4 months
	Italy	June	5 days
C.A. Glasbey	Italy	January	2 days
	France	May	2 days
	Australia	July	3 weeks
B.A. Goodman	France	February	3 days
	USA	April	6 days
J. Graham	France	September	5 days
B.S. Griffiths	Denmark	March	3 weeks
J.F. Guerineau	USA	October	10 days
B.D. Harrison	Latvia	May	1 week
L.L. Handley	Spain	August	10 days
	Portugal	October	10 days
P.E. Hedley	USA	October	10 days
	The Netherlands	November	2 days
J.R. Hillman	Belgium	February	1 day
	Belgium	August	1 day
D.J. Hirst	France	June	1 week
	Norway	September	2 weeks
G.W. Horgan	Netherlands	September	1 week
E.A. Hunter	France	June	1 week
	Finland	August	1 week
	Greece	November	5 days
L.J. Hyman	Italy	June	5 days
N L Innes	Burundi, Kenya, Uganda	February	10 days
	Peru	April	5 days
	Italy	June	1 week
	USA	October	8 days
	Peru	December	5 days
R.A. Jefferies	France	June	1 week
C.A. Jolly	France	February	1 month
A.T. Jones	Austria	July	5 days
	Germany	July/August	4 days
R.A. Kempton	Czechoslovakia	January	1 week
	Czechoslovakia	July	1 week
	Spain	September	2 weeks
	Finland	August	3 days
	Zimbabwe	November	1 week
D.M. Kennedy	France & Germany	May	1 week
	France	October	3 days
A. Kumar	USA	October	2 weeks
P.G. Lanham	India	January	6 weeks
G.D. Lyon	Holland	September	1 week
W.H. MacFarlane-Smith	Belgium	June	2 days
G.C. Machray	Germany	July	3 days
	The Netherlands	November	2 days
	USA	October	10 days
G.R. Mackay	USA	March	5 days
	China	August	1 week
	Spain	October	4 days
D.K.L. MacKerron	France	June	1 week
	USA	October	1 week
	The Netherlands	December	3 days
B. Marshall	Czechloslovakia	May	2 weeks
	The Netherlands	December	5 days
M.A. Mayo	Belgium	January	2 days
	Philippines	September	6 days
	Austria	November	6 days
	Latvia	May	8 days
	USA	April	5 days
	France	November	8 days
	France	February	5 days
P.F. McGrath	Nigeria	January	2 weeks
R.J. McNicol	France	October	6 days
S. Millam	USSR	May	2 weeks
I.M. Morrison	USA	December	1 week
A.C. Newton	Sweden	July	1 week
K.J. Oparka	The Netherlands	January	1 week
	Germany	December	1 week
	USA	July/August	6 weeks
R.Peiris	The Netherlands	April	1 week
M.C.M. Pérombelon	Italy	June	5 days
	The Netherlands	Sept/Oct	8 days
W. Powell	The Netherlands	February	3 days
K. Ritz	USA	July	1 month
	Sweden	October	1 week
	The Netherlands	November	1 week
D. Robinson	The Netherlands	March	1 week
W.M. Robertson	Germany	October	4 days
	USA	October	1 week
J.R. Russell	USA	October	2 weeks
S.B. Smith	USA	October	1 week
J.S. Swanston	Germany	June	1 week
M. Talbot	USA	June	1 week
	Germany	November	3 days
	Spain	September	4 days
	Germany	June	2 days
W.T.B. Thomas	Sweden	July	1 week
L. Torrance	The Netherlands	September	6 days
	Austria	November	5 days
D.L. Trudgill	Tanzania	March	12 days
R. Viola	Italy	November	5 days
	Germany	December	3 days
	The Netherlands	December	1 day
R. Waugh	Denmark	January	2 days
	Austria	November	3 days
	The Netherlands	December	3 days
	Germany	July	3 days
R.L. Wastie	The Netherlands	February	2 days
M.J. Wilkinson	USA	August	1 week
J.A.T. Woodford	Indonesia	January	4 days
	The Netherlands	September	6 days
F.G. Wright	USA	April	4 days
I.M. Young	Japan	May	10 days

Service on External Committees or Organisations

Name	Position	Committee or Organisation
H. Barker	Member	AAB Virology Group Committee
A.G. Bengough	Member	Scottish Soils Discussion Group Committee
A.N.E. Birch	Member	AAB Entomology Group Committee
R.M. Brennan	Member	NFT Blackcurrant and Bush Fruit Panel
D.J.F. Brown	Secretary and Treasurer	European Society of Nematologists
S.T. Buckland	Member	Working Group on Integrated Population Monitoring, British Trust for Ornithology
	Member	Review Group to the National Countryside Monitoring Scheme, Nature Conservancy Council for Scotland
	UK delegate	Study Group on Pilot Whales, International Council for the Exploration of the Seas
	Member	Advisory Panel to the Southern African Bird Atlas Project
	Invited Expert	Research Review on monitoring of porpoise stocks in the eastern tropical Pacific, United States
	Member	Scientific Committee of the International Whaling Commission
H.V. Davies	Organiser	2nd International Potato Molecular Biology Symposium
S.M.S. Dawson	Member	Scottish Nuclear Stock Association (Flower Bulbs) Ltd, Bulb Technical Committee
J.M. Dickson	Member	International Federation of Classification Societies
J.M. Duncan	Membership Secretary	Council of British Society Plant Pathology
	Translator	Potato Research
G.H. Duncan	Member	Scottish Microscopy Symposium Planning Committee
R.P. Ellis	Tech. Secretary	SSCR Cereals Group
	Member	BSPB Cereal Crop Group
	BSPB Representative	Scottish Colleges Cereals Recommended List Advisory Committee.
B.P. Forster	Co-ordinator	International Committee of Barley Chromosome (4) Genetic Mapping
J.M.S. Forrest	Member	AAB Nematology Group Committee
M.F. Franklin	Member	Biometric Society, British Region Committee
B.D. Harrison	Member	Advisory Committee, Advances in Virus Research
	Member	Advisory Committee for the NERC Institute of Virology and Environmental Microbiology
	Member	Research Grants Board F, Royal Society
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
	Member	Publications Committee, Journal of Horticultural Science
	Member	Royal Society of Edinburgh (Section Committee B)
	Chairman	SCRI/ASS/COSAC Liaison Group
	Member	SNSA Adviser to Committee
	Member	Strategic Quintet (ADAS/AFRC/SAC/SARI/SOAFD)
	Chairman	Crop Production Quartet
	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	Visiting Group, Royal Botanic Gardens, Edinburgh
E.A. Hunter	Member	EC Flair Concerted Action No 2 - 'Relating Sensory,Instrumental and Consumer Choice Studies'
N L Innes	Chairman	Governing Board & Executive Committee, CIP, Peru
	Convener	Royal Society of Edinburgh, Section Committee, Biology (I)
	Member	SCRI/ASS/COSAC Liaison Group
	Member	University of Dundee Botanic Garden Committee
	Vice-President	Association of Applied Biologists
	Member	UK Genetic Resources Committee
	Member	SARIC International Steering Committee
A.T. Jones	Chairman	ISHS Working Group on Virus Diseases of Small Fruits
R.A. Kempton	Council member	International Biometric Society
	Member	Environmental Change Network Statistics Working Group
H.M. Lawson	Chairman	U.K. Weed Liaison Group
	Member	BCPC R&D Sub-committee - Weeds
	Chairman	Scottish Weed Group Experimental Committee
G.D. Lyon	Member	'Course Validation Group' for the HND in Biological Sciences at Dundee Institute of Technology
W.H. MacFarlane Smith	Member	BSPB Oilseed & Industrial Crop Group
	Member	AFRS Safety Officers Group
	Member	SCRI/ASS/COSAC Liaison Group
	Chairman	SCRI/ASS/COSAC Brassica Working Group
	Member	NPTC Plant Variety Development Panel
	Technical Secretary	Sectoral Quartet on Crop Production
D.K.L. MacKerron	Chairman	Working Group on Water Relations in Potato Production, EAPR Physiology and Agronomy Sections

Name	Position	Committee or Organisation
G.R. Mackay	Chairman	Eucarpia Potato Section
	Member	SARI/SAC/ASS Potato Working Group
	Secretary	SSCR Potato Crop Sub-committee
	Coordinator	SOAFD Climate Change Research Group
	Secretary	SARI/SAC/ASS Potato Working Group
	Member	BSPB Potato Genetics Group
	Member	Interdepartmental Committee for post-entry quarantine of potato material
B. Marshall	Coordinator	Soil-Plant Microbial Interactions, AFRC Soil Science Steering Group
M.A. Mayo	Executive Committee	International Committee on Taxonomy of Viruses
	Member	ACIAR review of a project on virus and virus-like diseases of coconuts
R.J. McNicol	Member	NFT Raspberry Panel
	Member	NFT Strawberry Panel
	Secretary	NFT Scottish Soft Fruit Panel
	Member	SNSA Adviser to Committee
W.P. Mowat	Convener	Scottish Nuclear Stock Association (Flower Bulbs) Ltd, Bulb Technical Committee
A.F. Murant	Member	Plant Virus Sub-Committee, International Committee on Taxonomy of Viruses
I.M. Nevison	Chairman	North East Scotland Operational Research Group
A.C. Newton	Member	UK Cereal Pathogen Virulence Survey
	Council Member	British Society for Plant Pathology
D.A. Perry	Treasurer	Association for Crop Protection in Northern Britain
M.C.M. Pérombelon	Committee Member	COST 88 Bacteriology Committee
	Member	SARIC International Steering Committee
W. Powell	Technical Secretary	Royal Society of Edinburgh Symposium on Opportunities and Problems in Plant Biotechnology
	Adviser/Referee	International Foundation for Science, Stockholm, Sweden
B. Reavy	Member	AFRC Protein Engineering Liaison Group
K. Ritz	Member	Advisory Committee, Soil Biology of the Winter Period Programme, Swedish University of Agricultural Sciences
Member	AFRC	Soil Plant Microbial Interactions Working Party
D. Robinson	Member	AAB Plant Physiology Group
I.M. Roberts	Chairman	AFRC Electron Microscope Advisory Group
	Safety Representative	Royal Microscopical Society
D.J. Robinson	Member	Society for General Microbiology Virus Group Committee
	Member	Advisory Committee on Releases to the Environment
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 Group
L. Torrance	Member	COST 88 Managerial Committee
D.L. Trudgill	Convener and Chairman	EPPO *ad hoc* Committee on Potato Cyst Nematodes
R.E. Wheatley	Member	Group reviewing research on Nutrient dynamics in soils, reporting to AFRC Soil Science Steering Group
	Member	Group reviewing research on gaseous exchange mechanisms in soils reporting to AFRC Soil Science Steering Group
	Member	Soils Sub-Group of D of E review of the Impact of nitrogen deposition in the terrestrial environment (INDITE)
M. Wilkinson	Member	Interdepartmental Committee for post-entry quarantine of potato material
	Member	Association for potato intergenebank collaboration, USA.
J.A.T. Woodford	Regional Hon. Sec.	Royal Entomological Society
	Member	SEQNET/CCP11 User Documentation Group
F.G. Wright	Member	AFRC Protein Engineering Liaison Group
I.M. Young	Member	SAC Soil Compaction Study Group
	Chairman/Member	AFRC Soil Physics Working Party

Editorial Duties

Name	Position	Journal Title
H. Barker	Editorial Board	*Annals of Applied Biology.*
B. Boag	Editorial Board	*Annals of Applied Biology*
D.J.F. Brown	Editorial Board	*Nematologia Mediterranea*
J.M. Duncan	Associate Editor	*Journal Horticultural Science*
	Associate Editor	*Mycological Research*
G.J. Gibson	Guest editor	*IEE Proceedings Part F- RADAR in Signal Processing. Special Issue on Adaptive Filters: Theory & Practics*
C.A. Glasbey	Associate editor	*Applied Statistics*
B.D. Harrison	Editor	*AAB Descriptions of Plant Viruses*
	Editorial Board	*Proceedings B, Royal Society of Edinburgh*
T.D. Heilbronn	Editor	*SCRI Annual Report*
	Editor	*SSCR Newsletter*
J.R. Hillman	Managing Editor	*Crop Research*
	Publication Committee	*Journal of HorticulturalScience*
	Editorial Board	*Agricultural Systems*
N L Innes	Editorial Board	*AgBiotech News & Information*
	Editorial Board	*Crop Research*
H.M. Lawson	Associate Editor	*Journal of Horticultural Science*
M.A. Mayo	Editorial Board	*Journal of General Virology*
D.K.L. MacKerron	Associate Editor	*Journal of Horticultural Science*
	Editorial Board	*Euphytica*
J.W. McNicol	Statistical editor	*Annals of Applied Biology*
I.M. Morrison	Management Committee	*Journal of the Science of Food and Agriculture*
	Series Editor	*Advances in Plant Cell Biochemistry and Biotechnology*
A.F. Murant	Editorial Board	*Virus Research*
	Editor	*AAB Descriptions of Plant Viruses*
I.M. Nevison	Statistical editor	*British Journal of Nutrition*
D.A. Perry	Editorial Board	*Crop Research*
	Editor	*SSCR Bulletin*
	Editor	*COST-88*
	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*
	Consulting Editor	*Plant and Soil*
	Editorial Board	*Revue de Nematologie*
R.L. Wastie	Editorial Board	*Annals of Applied Biology*
	Editorial Board	*Potato Research*
B. Williamson	Editor	*Annals of Applied Biology*
J.A.T. Woodford	Editorial Board	*Annals of Applied Biology*
I.M. Young	Editor	*British Soil Science Society Newsletter*

Awards and Distinctions

Name	Dept.	Degree/Award/Distinction/Appointment
R. Anderson	CMG	M.Sc. Biotechnology, University of Bristol
E. Baird	CMG	B.A., Open University
J.R.K. Bennett	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
J.T. Bennett	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
B. Boag	Zoo	Honorary Lecturer, University of Dundee
C.C. Carrie	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
K.J. Chalmers	CMG	Ph.D., University of St Andrews
W. Craig	CG	B.Sc. Life Sciences
C.R. Dalrymple	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
N. Duncan	CMG	H.N.C, Dundee Institute of Technology
S.J. Finnie	CMG	Ph.D., University of Edinburgh
B. Fleming	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
M. Fleming	CG	B.Sc. Life Sciences
R.S. Forrest	M&B	Ph.D., University of Dundee
J. Graham	SFG	Ph.D., University of St. Andrews
B.E. Harrower	CMG	B.Sc., Napier Polytechnic
A. Holmes	CMG	M.Sc. Biotechnology
W.D.J. Jack	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
A. Lorimer	CG	H.N.C., Dundee Institute of Technology
M. Macaulay	CMG	H.N.C., Dundee Institute of Technology
L.A. MacCulloch	Zoo	Ph.D., Aberdeen University
Jane McNicoll	CG	H.N.C., Dundee Institute of Technology
Karen McIlravey	CG	H.N.C., Dundee Institute of Technology
L.A. McNicoll	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
A.W. Mills	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
Sandra Millar	CEP	H.N.C. Computer Studies
R. Ogg	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
D.S. Petrie	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
G. Pugh	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
D.G. Pugh	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
L.D. Ramsay	CG	Ph.D., University of Birmingham
B.D. Robertson	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
D.L.K. Robertson	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
J.G. Sim	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
A.M. Thain	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks
D.M. Thompson	CMG	Ph.D., University of Reading
R. Viola	CEP	Ph.D., University of Dundee
J.K. Wilde	EGF	ATB Scotland Certificate: Basic & Specific training in the Operation of Fork Lift Trucks

Research Projects

Note: All projects are SOAFD core funded except where indicated after the title by specified alternative funding eg. [MAFF], or unspecified [ext.]. [Project Leaders in brackets]

PU01 Develop enhanced germplasm in potato and more effective methods of genetic manipulation and breeding

(a) 040 Genetic architecture of tetraploid potatoes and production of enhanced germplasm. [*Bradshaw J.E.*]
(b) 041 Genetic architecture of traits of strategic importance to the UK seed potato industry. [*Mackay G.R.*]
(e) 045 Develop, improve and use screening methods for resistance to diseases and pests of the potato. [*Wastie R.L.*]
(f) 046 Develop and use screening tests for biochemical compounds in potatoes. [*Dale M.F.B.*]
(h) 047 Maintenance, improvement and evaluation of the Commonwealth Potato Collection. [*Wilkinson M.J.*]
(i) 187 Investigate the genetics and biochemistry of the low temperature sugar stability characteristics of potatoes for use in fried food products. [*Mackay G.R.*]
(j) 263 Incorporation of the Birmingham Potato Collection into the Commonwealth Potato Collection. [*Wilkinson M.J.*]
(k) 264 Correlating glasshouse and field performance of true (botanic) potato seed populations. [*Clulow S.A.*]
(l) 265 Development and evaluation of methods for specific applications of high-technology instrumentation for the SCRI research programme. [*Allison M.J.*]
(m) 297 Development and exploitation of tissue culture techniques, in particular microspore culture technology in *Solanum tuberosum L.* [*Coleman M.C.*]
(n) 298 Genetic characterisation of unreduced male gametes from diploid potatoes. [*Clulow S.A.*]
(o) 299 Exploitation of protoplast technology in the development of new material and in the introduction of new genes into existing material. [*Coleman M.C.*]
(p) 300 The mechanism of dihaploid formation following pollination of tetraploid potatoes with dihaploid inducer clones. [*Wilkinson M.J.*]
(q) 301 The production of dihaploids and their use in improving the efficiency of germplasm enhancement and the genetical study of *Solanum tuberosum*. [*De,Maine M.J.*]
(r) 302 The production of hybrids between dihaploids of *Solanum tuberosum* and wild *Solanum* species as a means of producing novel sources of material for germplasm enhancement and genetical studies at the diploid level. [*De,Maine M.J.*]
(s) 303 Inheritance of resistance to potato virus diseases and production of resistant enhanced potato germplasm. [*Solomon-Blackburn R.M.*]
(t) 304 The production, maintenance, distribution and associated management of facilities to produce disease-free tubers of genetic stocks of potato clones. [*Chapman I.M.*]

PU03 Develop enhanced germplasm in soft fruit and more effective means of genetic and vegetative manipulation

(f) 189 Develop methods of using *Agrobacterium spp.* as vectors for introducing DNA into soft fruit germplasm. [IFS] [*McNicol R.J.*]
(k) 261 Introduction of exogenous DNA into *Rubus, Ribes, Fragaria,* and other soft fruit genera using *Agrobacterium tumefaciens.* [*McNicol R.J.*]
(l) 288 Devise techniques for modifying the competitive relationship between fruiting and vegetative phases in raspberry. [*Lawson H.M.*]
(m) 289 The collection, evaluation, and conservation of genetic resources of perennial soft fruit genera. [*McNicol R.J.*]
(n) 290 The development of molecular and biochemical markers in woody perennial fruit crops. [*Brennan R.M.*]
(o) 291 Investigations of the genetics and mechanisms of pest and disease resistance in *Ribes, Rubus* and other soft fruit genera. [*McNicol R.J.*]
(p) 292 Investigation of mechanisms and genetic control of low temperature tolerance in perennial fruit crop genera. [*Brennan R.M.*]
(q) 293 The floral biology of perennial soft fruits. [*McNicol R.J.*]
(r) 294 Gene flow from cultivated to feral populations of soft fruit species and its implications for the release of genetically engineered plants. [*McNicol R.J.*]
(s) 343 The selection of improved genotypes of *Rubus.* [*McNicol R.J.*]
(t) 5082 The genetic control of Anthocyanin production in Blackcurrant. [SmithKline Beechams] [*McNicol R.J.*]
(u) 5086 Raspberry and blackberry: The breeding and selection of improved cultivars. [ext.] [*McNicol R.J.*]

PU09 The biology and control of diseases and pests of soft fruit crops in Northern Britain

(a) 070 Epidemiology and pathogenesis of fungal pathogens of soft fruit. [*Williamson B.*]
(b) 076 Prediction and assessment of damage caused by pests of cane and bush fruits. [*Gordon S.C.*]
(j) 5200 Epidemiology and pathogenesis of fungal pathogens of soft fruit. [HDC] [*Williamson B.*]
(k) 295 Study the properties, relationships and resistance mechanisms to viruses and virus-like diseases of soft fruit crops (*Rubus, Ribes* and *Fragaria*). [*Jones A.T.*]
(l) 296 Produce and maintain virus-tested stocks, assess resistance and index British and imported *Ribes* and *Rubus* for virus infection. [*Jones A.T.*]

PU11 Characterisation, effects and control of viruses of ornamentals

(a) 135 Determine properties, relationships and detection of previously undescribed viruses from narcissus. [*Mowat W.P.*]
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(b) 5141 Groundnut rosette diseases: aetiology, diagnosis and resistance. [ODA] [*Murant A.F.*]
(c) 5203 Detection and properties of West African whitefly-transmitted geminiviruses. [EEC] [*Robinson D.J.*]
(d) 5335 European whitefly-transmitted geminiviruses in Europe. [EEC] [*Robinson D.J.*]
(e) 5336 Variation in important potato viruses in the Andean region in relation to virus detection, transmission by vectors and durability of resistance. [EEC] [*Torrance L.*]
(f) 5337 Mechanisms of PLRV resistance in potatoes. [ODA] [*Barker H.*]
(g) 5338 Detection and properties of African geminiviruses from tomato. [ORSTOM] [*Fargette D.*]
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(b) 147 Exploitation of protoplasts and microspore systems in crop improvements. [*Millam S.*]
(g) 175 Genome organisation and structure at the nucleic acid level. [*Waugh R.*]
(h) 152 Identification and exploitation of genetic markers in crop improvement. [*Forster B.P.*]
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(o) 259 Transposon mutagenesis - a strategy for the isolation and cloning of important genes in potato. [IFS] [*Kumar A.*]
(p) 260 UsnRNA-based transformation vectors for the delivery of antisense RNAs to plant cell nuclei. [IFS] [*Waugh R.*]
(q) 305 Genetic studies within the family *Brassicaceae*, as model systems for the study of cytotaxonomy, polymorphism and gene introgression. [*Millam S.*]
(r) 306 The development basis of stable plant regeneration systems. [*Millam S.*]
(s) 307 Somatic hybridisation in the genus *Solanum*: its application for gene introgression, gene mapping and organelle transfer. [*Kumar A.*]
(t) 308 Use of genetic transformation methods for studying genetic stability and expression of foreign and chimaeric genes in transgenic dicot and monocot plants. [*Kumar A.*]
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(d) 166 Statistical research and consultancy for HRI. [*Hirst D.J.*]
(e) 167 Statistical research and consultancy for MLURI. [*Buckland S.T.*]
(f) 168 Statistical research and consultancy for MRI. [*Wright F.G.*]
(g) 169 Statistical research and consultancy for SCRI. [*McNicol J.W.*]
(h) 170 Statistical research and consultancy for RRI. [*Franklin M.F.*]
(i) 171 Statistical research and consultancy for SAC. [*Hunter E.A.*]
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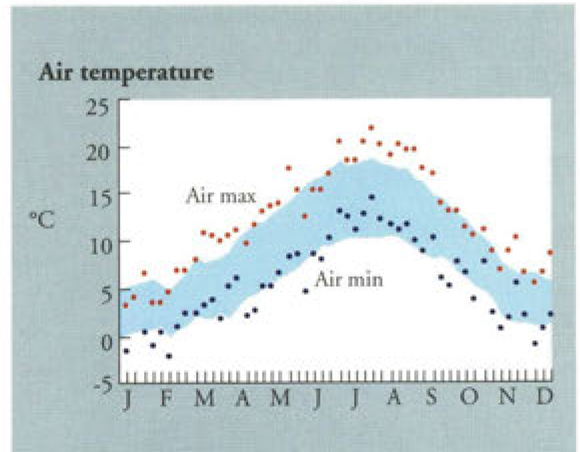
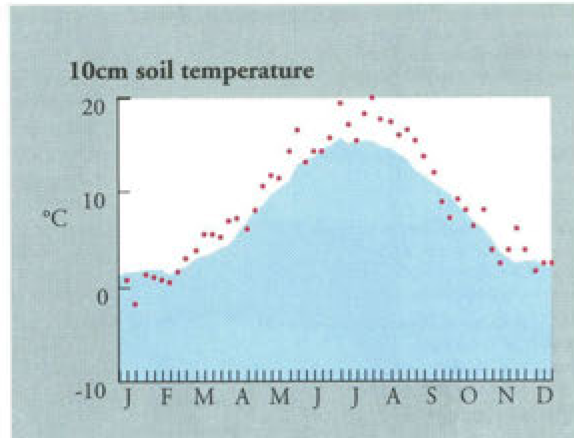
(e) 5085 Development of molecular and biochemical markers for coffee germplasm in Central America. [EEC] [*Powell W.*]

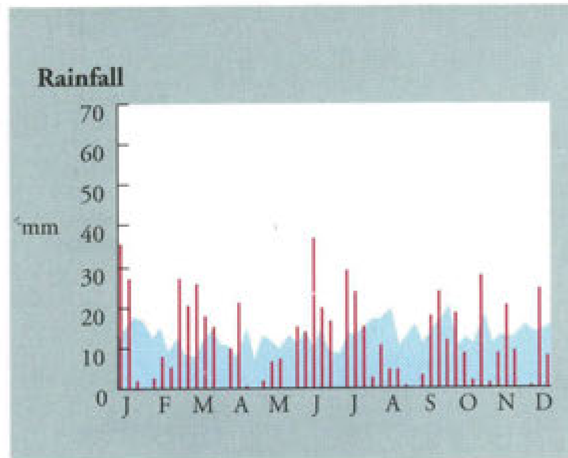
Meteorological Records

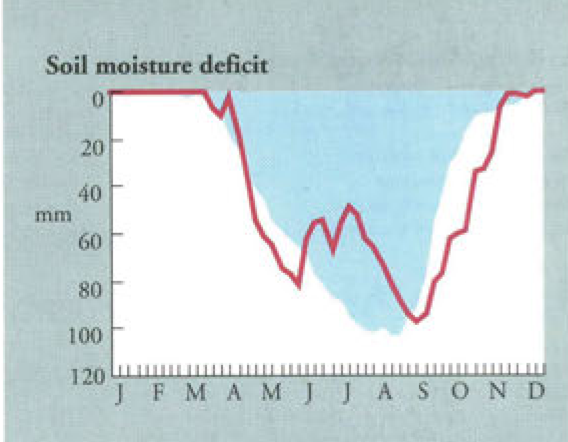
D.K.L. MacKerron

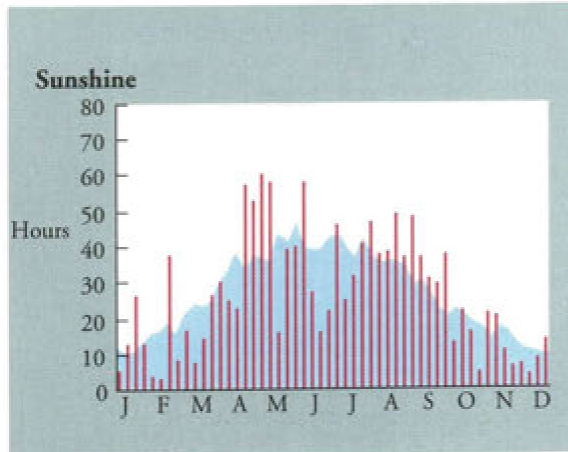
Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 1991 and the long term average for 1961-1990( )

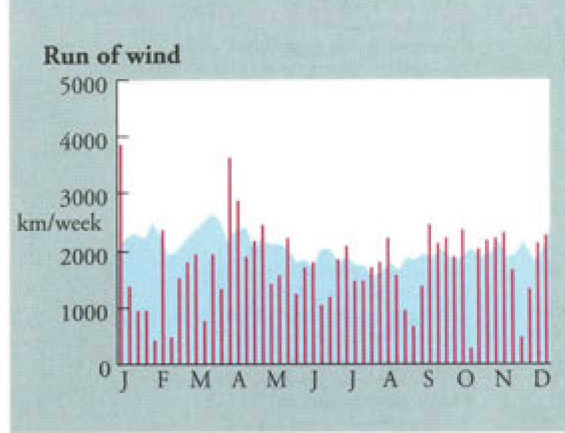












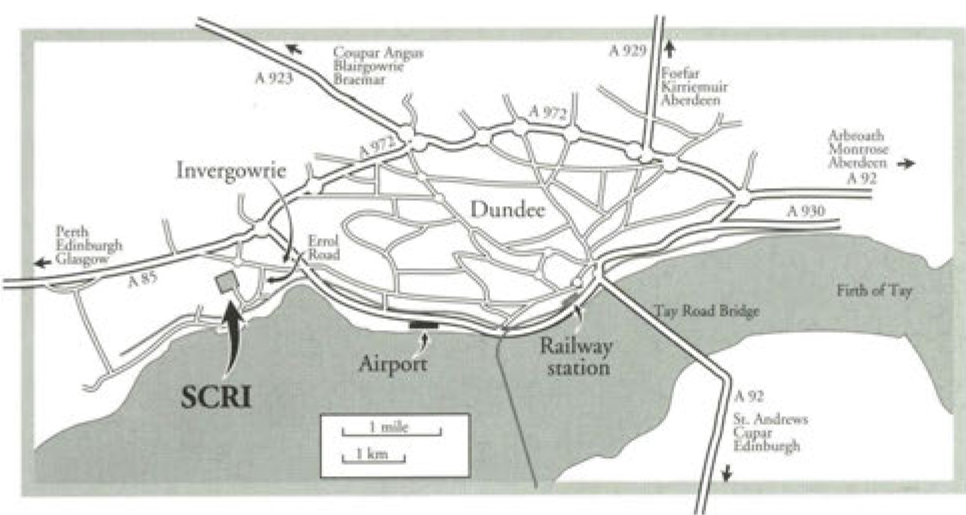
Agricultural and Food Research Service Institutes

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
Houghton Laboratory	Houghton, Huntingdon, Cambridgeshire PE17 2DA	0480-64101
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 Institute of Animal Physiology and Genetic Research	Babraham Hall, Babraham, Cambridge CB2 4AT	0223-832312
Cambridge Research Station	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
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	09473-307
AFRC Institute of Engineering Research	Wrest Park, Silsoe, Bedford MK45 4HS	0525-60000
AFRC Institute of Food Research	Earley Gate, Whiteknights Rd, Reading RG6 2EF	0734-357055
Norwich Laboratory	Colney Lane, Norwich NR4 7UA	0603-56122
Reading Laboratory	Shinfield, Reading RG2 9AT	0734-883103
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 Centre	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
	Pentlandfield, Roslin, Midlothian EH25 9RF	031-445-3401
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	031-650-1000

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
IFS	Increased Flexibility Scheme
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 Inra-Red
NMR	Nuclear Magnetic Resonance
NPTC	National Proficiency Test Council
ODA	Overseas Development Administration
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
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
WSC	The West of Scotland College

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