Scottish Crop Research Institute

Annual Report 1990

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.

Price £15

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Governing Body

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Report of the Director

J.R. Hillman

Agricultural production on a world basis was generally satisfactory during 1990. Forecasts by the Foreign Agricultural Service of the United States Department of Agriculture indicate that world grain reserves of wheat and rice increased for the first time in four years, although the reserves of coarse grains were expected to decline. In most economically developed countries, the production of dairy products, sugar, wine and wool exceeded demand; output and consumption of oilseeds and their by-products appeared to be in balance. The Quarterly Bulletin of Statistics issued by the Food and Agriculture Organization of the United Nations reveals that the preliminary indices of total agricultural and food production rose for the second year in succession, but *per capita* food production remained the same as in 1989. War and civil strife exacerbated drought conditions or weak economies in parts of Africa, and emergency assistance was required to avert widespread famine. Bolivia, Haiti, Nicaragua and Peru also needed assistance.

The collapse in December of the multilateral trade negotiations led to urgent moves to rescue the Uruguay round. Political and economic pressures continue to be applied by the United States of America, the Cairns group of agricultural exporting nations and less-developed countries to the European Community to alter the operation of the Common Agricultural Policy with its associated import restrictions and export subsidy arrangements. Changes to international trade practices in agricultural commodities may have an immediate effect on the priorities and funding of agricultural research and

development programmes in both the public and private sectors.

In the food processing and marketing areas, there was increased emphasis on the health and safety aspects of food, food packaging and new products. Consumers favour innovation, freedom from residues and contaminants, pleasing appearance, detailed labelling, easy preparation and value for money. These expectations are transmitted directly to the grower, who is largely dependent on food-related products and unable to capitalise on added-value processes. Legitimate concerns about food irradiation, laboratory-based genetic engineering, and chemical methods to control weeds, pests and diseases can only be addressed by research, education and openness. The alternative is likely to be a ban on processes that could be crucial to meeting the complex requirements of various societies.

Throughout the year and relevant to two SCRI research projects, the predominant environmental issue continued to be the threat of climate change arising from the so-called 'greenhouse effect' and depletion of the stratospheric ozone layer. In many respects, international cooperation is good, with political commitments to fund research projects and introduce measures for environmental protection. Remarkably, an environmental agenda has emerged worldwide. This involves the creation, protection and management of wilderness areas, national parks and special ecosystems, in addition to greater care over waste disposal and xenobiotics, integration of control measures and the synthesis of national and international policies setting quality standards. Conflicts arise, however, between the needs of a rapidly expanding world population and the environment. Anthropocentric policies imperilling the existence of other species and sub-species horrify biologists and those sensitive to the natural world.

1990 was associated with tremendous concern over forestry activities. Bans, export taxes and adverse commentary on the environmental impact of harvesting hardwood and softwood timber in natural forests have in turn affected the supplies of wood and wood-products to the industrialised nations. In the arid and semi-arid areas of the third world, a shortage of wood for fuel adds to stressed lifestyles. There is an increasing role for agroforestry to create and maintain sustainable and environmentally sensitive agricultural systems. Research on woody plants - their genetics, breeding, pathology, physiology, propagation, cultivation, ecology and use - must become a priority in the life sciences. To this end, SCRI is already building on its investments and achievements in fruit crops and fibre biochemistry and biophysics.

One feature of the western world is a growing divide between stable or expanding urban and declining rural populations. In the UK, 91.5% of the population is urban, with relatively low mobility, a birth rate per 1000 population of half the world average, and a population doubling time in excess of 100 years. The overall population density is high (235 persons /km^2) revealing the extent of crowding in the urban areas. Perceptions of agriculture and horticulture by the urban population are complex: subsidies, "food mountains", high-technology methods, animal welfare issues, real and imaginary scares on food safety, and purported privileges for sections of rural communities contrast with appreciation of the countryside, poor financial returns, long hours of physical effort, dependence on the vagaries of the climate, lack of modern social facilities, and transport difficulties. Rational analysis of world population trends and all that implies, and realisation of the potential impact of climate change and the adaptability of pests and diseases, firmly point to a pivotal role for advanced agriculture to sustain urban mankind and continued development of social structures. Again, those concerned with rural affairs must publicise their meritorious activities and demonstrate their importance.

Links between SCRI and the Centres (Institutes) supported by the Consultative Group on International Agricultural Research (CGIAR) are critical to the development of the SCRI remit, aims and objectives. Established in 1971, CGIAR is an informal association of 40 public and private sector donors that in 1990 pledged $240 million to support an international network of 13 (now 16) Centres designated to investigate agricultural and food problems afflicting the world's disadvantaged peoples. The CGIAR system is one of the most successful international development initiatives in the postwar era, contributing to improved cultivars of wheat, rice, maize, sorghum, pearl millet, beans, cassava and potato, as well as introducing pest control systems, coordinated gene banks and germplasm collections, and assisting national programmes of developing countries on agricultural policies and research. In meeting the interwoven challenges arising from deteriorating environments, poverty and population growth, the Centres are beginning to focus on

sustainability, biotechnology, and technology transfer at the regional level. SCRI has a growing involvement in several of the Centres, notably Centro Internacional de la Papa, International Board for Plant Genetic Resources, Centro Internacional de Agricultura Tropical, Centro Internacional de Mejoramiento de Maiz y Trigo, International Center for Agricultural Research in the Dry Areas, International Crops Research Institute for the Semi-Arid Tropics and International Institute of Tropical Agriculture.

Domestically, the Institute thrives. Five techniques are permeating our research programmes *viz.* predictive modelling, mass spectrometry, nuclear magentic resonance, electron paramagnetic resonance and the polymerase chain reaction (PCR). Pioneering studies by Khorana, Mullis and colleagues that gave rise to PCR answered the needs of molecular biologists to study DNA, permitting specific DNA fragments to be copied repeatedly, resulting in enormous amplification of the starting material. Elaborations such as anchored, inverse and rapid PCR systems, especially in combination with Southern and Northern blotting and restriction fragment length polymorphism, are revolutionising research on heterogeneous and small DNA samples, detection and diagnosis of diseases, speciation, gene cloning, gene mapping and sequencing. Problems with false-positive signals from contamination, the accuracy of the type of DNA polymerase and statistics of the process are recognised and are being investigated.

Early in the New Year, Mr J. A. Inverarity OBE was appointed Chairman of the newly formed Scottish Agricultural College and stepped down as Chairman of the SCRI Governing Body. He retains membership of the Governing Body where his special expertise is invaluable. Mr James L. Millar CBE, Chairman and Chief Executive of Wm. Low & Company plc, replaced Mr Inverarity as the SCRI Chairman, emphasising the importance of matters financial and administrative in modern scientific organisations.

Senior staff appointments during the year reflected the scientific transformations taking place at Mylnefield. Dr H.V. Davies was appointed to UG6 Head of the Cellular & Environmental Physiology Department, Dr J.M. Duncan to UG6 Head of the Mycology & Bacteriology Department and Dr D.A. Perry to UG7 Head of the revamped Scientific Liaison & Information Services Department. Dr J.W.S. Brown, formerly Lecturer in Biological Sciences, Dundee University, was appointed UG7 Molecular Biologist in the Cell & Molecular Genetics Department, placing SCRI at the forefront of studies of plant gene splicing. Several junior appointments were also made of outstanding staff members, enhancing the scientific stature of the Institute. Supervision of research students is seen to be an integral part of the activities of a major research institute, and healthy growth in the numbers of students and participation in university activities was a feature of 1990.

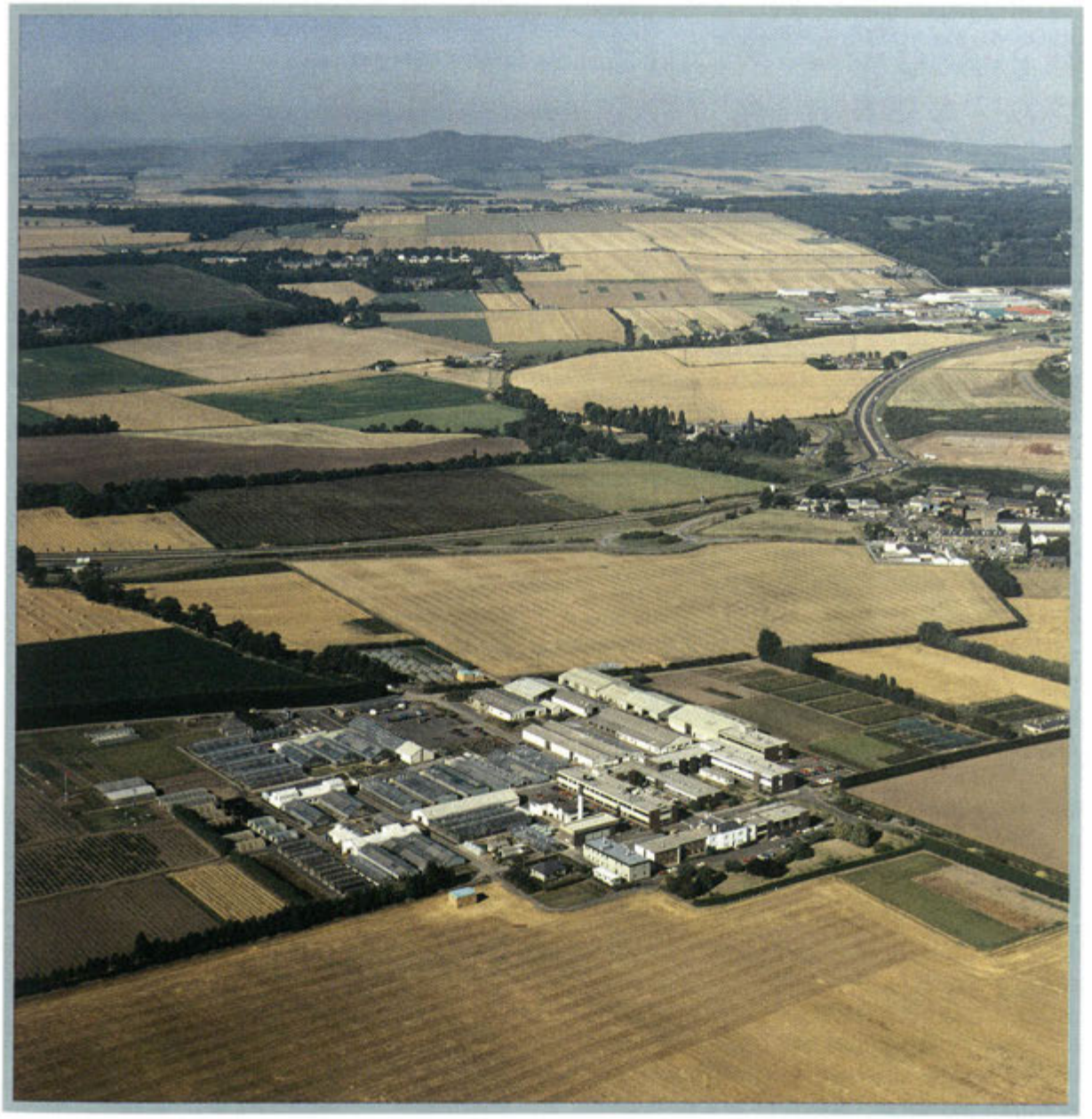
Implementation of the Control of Substances Hazardous to Health (COSHH) regulations and related legislation of health and safety in the workplace received particular attention during the year. In the Agricultural and Food Research Service, all Institutes followed the guidelines, adapting laboratory and field practice to conform to the rules and investing heavily in facilities and equipment. Cost and inconvenience are seen as the necessary price for a safe working environment.

Reorganisation of the Administration Department was essential to absorb changes in the research commissioning arrangements and to deal with the commercial interface. Mr S. L. Howie, Chartered Accountant, was appointed to manage the accounts. Implementation of the administrative computing system installed in Scottish Agricultural Research Institutes and the Scottish Agricultural College proceeded slowly as a result of modifications to the software supplied by McKeown's; this will prove to be a limiting factor in upgrading financial management in the Scottish system.

Through the generosity of Professor and Mrs R.B. Massalski, a Prize Fund was established in memory of their son, Dr Peter R. Massalski, who was a member of the Virology Department at the time of his death. The Fund which is administered by the Scottish Society for Crop Research provides the Peter Massalski Prize awarded biennially to the person under 36 years old who is considered to have done the most meritorious research while working at SCRI. Dr K.J. Oparka of the Cellular & Environmental Physiology Department was the first recipient of the Prize presented by Professor Massalski at a ceremony held on 19 June 1990.

Contributions to numerous scientific societies and journals by the staff are testimony to the rapidly expanding influence of the Institute in diverse areas of

the life sciences. These roles must be seen alongside the greatly enhanced output of published papers and patents as well as unprecedented levels of external grants and contracts. Such achievements are only possible by the commitment of highly competent staff in every department. We gratefully acknowledge the Scottish Office Agriculture and Fisheries Department and its core funding. Grants, contracts and donations from the Scottish Society for Crop Research, governmental agencies, grower levy boards, local authorities, commercial companies, farmers and individuals are also warmly appreciated.



Mylnefield and Gourdie Farm from the south.

Plant Genetics

G.R. Mackay & R.J. McNicol

Genetics is the science of heredity. The study of genetics in crop species is not an end in itself, but a means to an end. Whilst academic research into the genetics of 'laboratory friendly' model organisms may provide an insight into the fundamentals of heredity, they will not by themselves result in improved agricultural crop species required to feed a hungry world or enable moves towards more environmentally stable, sustainable agricultural systems, unless the results of fundamental research are translated into practically useful methods of understanding the genetic architecture of economically important traits of crop species. Plant breeding is a form of accelerated evolution and the evolution of modern cultivars has largely been achieved to date by phenotypic recurrent selection based on empirical methods. Modern plant breeders require the means to apply selection pressure increasingly efficiently and effectively in order to ensure that response to selection is maximised and due to true genetic gain, based on genotypic selection rather than phenotypic selection. It is therefore the objective of Crop and Soft Fruit Genetics research at SCRI to increase the efficiency and efficacy of selection methods in parallel with obtaining information on the underlying genetic architecture of economically important traits, in order that the enormous gains in agricultural productivity of the past few decades are maintained in the future. Studies on the genetics of economically important traits therefore go hand in glove with the development of techniques such as progeny tests and cross prediction to enable such studies to proceed. Some modern technologies offer potential alternatives to sexual hybridisation as sources of genetic variation. Genetic variation is a pre-requisite to the breeding of new cultivars and these emergent techniques require refinement and comparative studies with the more conventional approaches if they are to prove of practical benefit.

Studies on doubled haploid and single seed descent populations of barley are providing basic information on linkages between important genes. Recent research has demonstrated that the ml-o locus (conferring mildew resistance) on chromosome 4 of barley is associated with a number of quantitative trait loci (QTL's) including yield and malting quality. At the same time the SCRI bred spring barley cv. Tyne became one of the widest grown cultivars in Scotland in 1990 and two new spring barley selections combining good yield, pest and disease resistance characteristics with malting quality were submitted to National List Trials in 1991.

Substantial progress has been made towards creation of the first linkage map in *Vicia faba* using molecular, biochemical and morphological markers. Cytological studies on hairy roots of *Vicia faba*, following transformation with *Agrobacterium rhizogenes*, have revealed, for the first time, extensive changes in ploidy in such tissues. A rapid screening method for the antimetabolites vicine and covicine has been developed and used to identify an interesting mutant with low levels of these factors.

Research into the apparently simply inherited characteristic that restricts reproduction of the leafroll virus

(PLRV) in infected potatoes continues and PLRV titre data are now being compared with results from field exposure experiments. The production and use of parental clones possessing multiple copies, duplex, triplex and quadruplex, of genes conferring resistance to PVX and PVY continued and a study designed to evaluate seedling progeny tests for PLRV resistance completed.

The association between levels of PLRV resistance and quality traits has been examined and initial results suggest that there are no deleterious associations between enhanced resistance and quality.

The amalgamation of the Birmingham collection of wild *Solanum* spp. with the Commonwealth Potato Collection began in 1990. This four year task will practically double the size of the CPC, thus increasing its value as an international genebank, providing researchers in the UK and elsewhere with immediate access to valuable germplasm without the costly necessity of passage through quarantine.

Progress towards production of intrinsically disease and pest-resistant cultivars, essential to enable trends towards low input, sustainable agricultural systems, is exemplified by the production of advanced potato clones with very high levels of late blight resistance. Trials of these clones under organic farming systems, in collaboration with Elm Farm Research Centre and the Henry Doubleday Research Association, allied to SCRI experiments comparing their performance in the absence of fungicide with control plots with fungicide, have confirmed the substantial advantage that such clones offer growers who wish to reduce their reliance on chemical control measures. Several of these clones are now progressing forward as potential cultivars in association with SCRI's commercial partners and are currently in their final stages of statutory National List Trials.

Improved fruit quality, pest and disease resistance, consistency of cropping and extension of the season remain the major objectives of the applied breeding programmes for the range of *Rubus* and *Ribes* crops. Three raspberry seedlings (44C9, 15A12 and 14/106) continue to perform well in trials. The latter is particularly well adapted to mechanical harvesting and has been propagated and planted for large scale commercial evaluation. The late season dessert strawberry, Rhapsody, is now achieving widespread recognition for its production of high quality, attractive fruit with a good shelf-life and is being planted on an increasing scale.

Novel sources and increased levels of resistance to pests and diseases are always being urgently sought. The single gene transfer techniques that have been developed by Soft Fruit Genetics for all the soft fruit crops and our highly collaborative research projects at Institute, National and International level of seeking resistance to the raspberry root rot disease illustrate this. This disease is being tackled genetically on several fronts including conventional field screening on diseased land, glasshouse inoculation screening of clonal and seedling material and *in vitro* screening combined with genetic recombination. It is hoped that this combination of strategies, which brings together an array of expertise, will result in the fastest possible production of a resistant cultivar with acceptable agronomic characteristics.

Quality in potatoes

G.R. Mackay & M.F.B. Dale

There are many components of quality in potatoes. Tuber shape, depth of eyes, colour of flesh, colour of skin and skin finish are components of eye appeal which is an increasingly important criterion for the breeder of potatoes for the washed prepack trade. Texture, dry matter content and flavour are also important determinants of quality. However, in the UK fresh ware market, the majority of potatoes are sold unwashed and unless the surface of tubers is severely affected by diseases such as common scab, powdery scab or gangrene, the consumer will tolerate a degree of variation in skin finish. Furthermore, the peel is usually removed before consumption. Consequently, most of the effort in the selection programmes at SCRI is directed towards improving agronomically important traits such as yield and tolerance to stress, or enhancing disease and pest resistances. However, there are some aspects of quality which are at least as important as agronomic traits, and low temperature sweetening and after-cooking blackening are

Figure 1 Potato crisps, exhibiting different degrees of browning.

probably the most important in the context of processing and table use respectively (Fig. 1).

Low temperature sweetening Approximately 1.5M tonnes of the UK potato crop is processed into crisps and french fry products. The crisping component is valued at approximately £800M per year and is based almost exclusively on the single cultivar Record. Record, in common with other cultivated forms of the potato, exhibits a phenomenon known as low temperature sweetening. Potatoes harvested in autumn are stored throughout the winter and spring until fresh produce starts to become available again in summer to maintain continuity of supply to the factories. Tubers have to be stored between 2-4°C to maintain them in good condition, to prevent sprouting and minimise disease problems. However, at these temperatures starch reserves are mobilised and there is a rapid accumulation of sugars, particularly of reducing sugars (Fig. 2). When the tubers are fried, a complex reaction occurs between the reducing sugars and some amino acids, the Maillard reaction[1], which results in a dark bitter-tasting product. Consequently, the manufacturers have to store tubers at relatively high temperature (>6.5°C), thus adding to their costs and exacerbating disease problems. They also have to use chemical sprout suppressants which are increasingly being questioned because of concern about their residues. It is estimated that the "sugar problem" costs the industry in excess of £2.5M per year.

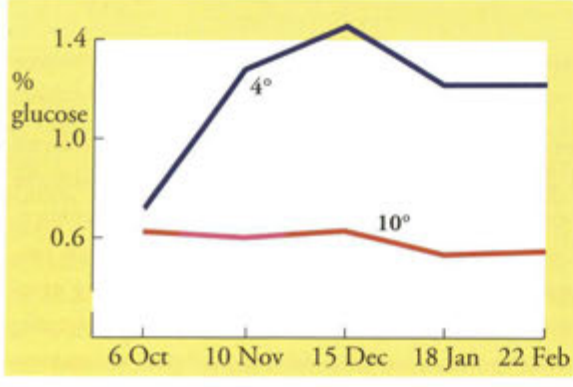


Figure 2 Mean glucose content of tubers at two storage temperatures.

Figure 3 Routine fry tests.

The underlying mechanisms of cold-induced sweetening are not fully understood and a number of enzymes have been implicated. Differences in amylolytic activity (α and β amylases) between tubers stored at high and low temperature and between clones with differing sugaring properties have been demonstrated in collaboration with the Edinburgh College of Agriculture[2]. Other views suggest that starch breakdown is primarily phosphorolytic and that some enzymes e.g. phosphofructokinase are cold labile[3]. Low temperature induced sweetening and the control of sugar levels in tubers is complex and possibly subject to control by many interacting genes.

The cultivated European potato, *Solanum tuberosum* ssp. *tuberosum*, is one of approximately 200 tuber-bearing species of the genus *Solanum*. Some wild species do not exhibit low temperature sweetening and introgression of this trait from them into *S. tuberosum* is being attempted[4]. This method will

Figure 4 Crisp colours after storage at 4° and 10° for a range of clones and cultivars.

require several generations of crossing, backcrossing and selection. Several wild species feature in the pedigrees of SCRI parental clones and cultivars and whilst they are primarily used as sources of disease and pest resistance, other traits may have been introgressed from species such as *Solanum phureja*, *S. vernei*, *S. demissum*, *S. acaule*, *S. stoloniferum* and *S. tuberosum* ssp. *andigena*. All clones are routinely subjected to frying tests in order to establish their suitability for crisping and chipping (Fig. 3). In 1982 tests on tubers stored at 4°C revealed a small but fairly constant proportion of clones in the early generations that produced pale coloured crisps that were superior to those from cv. Record when fried direct from storage at 4°C (Fig. 5). Analyses of tubers of a range of clones stored at 4 and 10°C showed clearly that the principal cause of the deterioration in crisp colour related to the rapid accumulation of glucose at 4°C but that the glucose levels of several SCRI clones did not change significantly over a 5 month storage period at either 4°C or 10°C (Fig. 5)[5]. This programme has culminated in the submission of two sugar stable clones to National List trials as potential varieties. One was admitted on to the UK National List as cultivar Brodick in 1990 and another named Eden will complete National List

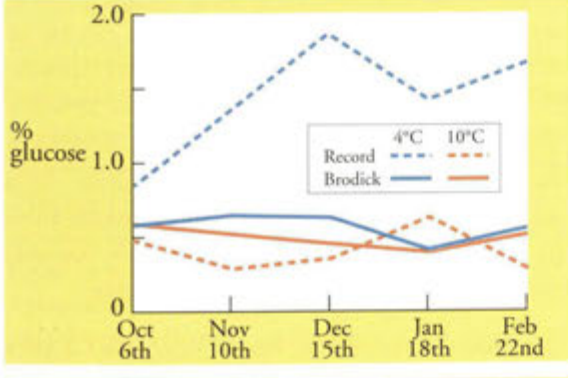


Figure 5 Glucose levels of tubers of Record and Brodick stored at 4° and 10°C.

Trials in 1991. In addition to their superiority to cv. Record in their suitability for crisping, Brodick possesses field resistance to late blight and the common viruses; and Eden is resistant to both species of potato cyst nematodes.

Having identified phenotypically low-temperature, sugar-stable clones, the genetic control of the phenomenon is being investigated. Progenies from the hybridisation of low temperature sugar-stable clones with conventional cultivars have been grown in the field, stored at 4 and 10°C, analysed for sugar content and subjected to frying tests. The results have shown that the low temperature sugar-stable trait has a heritable component, and several clones with superior low-temperature sugar stability to the better parents have been identified. The genetic control is complex and polygenic and the heterozygous, tetraploid nature of *S. tuberosum* renders interpretation difficult. Techniques such as the induction of dihaploids by induced parthenogenesis or production of monoploids by anther culture allied to the use of molecular techniques, including RFLP analyses may resolve the genetic architecture of this complex phenomenon. This selection programme has now produced more than 20 clones with superior sugar stability and fry colour characteristics after low temperature storage than Brodick but it will be some time before their agronomic potential is fully evaluated.

After-cooking blackening Discoloration of the flesh of potato tubers after boiling is due to the formation of complex compounds of chlorogenic acid and iron which, on exposure to air, oxidise to form bluish-grey coloured compounds. Several factors such as iron content, amount of orthodiphenols, presence of organic acids and pH are involved. For example, high concentrations of citric acid may reduce the extent of after-cooking blackening (ACB) by acting as an alternative substrate for iron present in the tuber. ACB is more common in tubers grown on heavy, organic or peaty soils, and application of fertilisers with a high N:K ratio tend to increase the tendency to darken. There is also a relationship between degree of darkening and temperature during the growing season and large tubers have a greater tendency to darken than small tubers of the same cultivar. ACB has no known effect on flavour or nutritive value but it greatly detracts from the appearance of cooked potatoes and, whilst primarily a problem in potatoes boiled for home consumption, it is also a problem in canned potatoes, oil-blanched french fries and even reconstituted dehydrated products.

Potato tubers of different cultivars with similar concentrations of chlorogenic acid do not blacken to the same degree and the tendency to discolour after cooking is known to have a high heritability. All clones in the SCRI selection programme are assessed for their tendency to ACB in routine cooking tests. However, testing for ACB by routine cooking is subject to the environmental variations mentioned above, and presents problems in the early stages of clonal selection when all the clones are grown in a single environment.

Chemical analysis of the chlorogenic acid content of tubers offers a more reliable and objective method of predicting the likely extent of ACB in the selection programmes. Several methods of analysing chlorogenic acid in tubers have been investigated at SCRI including HPLC, colorimetric and chromatographic assays. A rapid quantitative method to determine chlorogenic acid based on sodium nitrite has been developed and compared with other established colorimetric assays using sodium ethoxide and sodium molybdate, and with the HPLC Folin-Denis assays on material with a range of chlorogenic acid contents.

All three colorimetric methods were highly correlated with each other and with the HPLC method. The reproducibility of the sodium nitrite method was similar to that of the other two colorimetric assays and better than that of the HPLC method. Thus it is now possible to effectively screen large numbers of clones and expedite research into this important character. The accumulation and distribution of chlorogenic acid, citric acid and iron levels in tubers of different genotypes is being examined throughout the growing season and the inheritance of the principal factors affecting ACB levels and their interaction with various environmental factors which influence the expression of the trait will be determined.

Conclusion Quality is becoming an increasingly important characteristic with respect to consumers' expectations of potatoes and although disease and pest resistance, yield and agronomic performance, storability and appearance remain primary objectives of research at SCRI, the chances of a new cultivar succeeding will be negligible if it fails in an important quality characteristic. Public demand for high quality food, allied to demands to reduce reliance on prophylactic chemicals and pressure for more environmentally benign, sustainable agricultural systems require increasing, multi-disciplinary research into the genetics and breeding of potatoes. Only this can ensure that future cultivars will combine the agriculturally important characteristics required by growers with the quality traits demanded by consumers.

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Anti-nutritional factors in faba beans, forage brassicas and potatoes

J.E. Bradshaw, M.F.B. Dale, D.W. Griffiths, W.H. Macfarlane Smith & G. Ramsay.

Anti-nutritional factors are plant substances which are potentially harmful to the digestive systems of humans and domesticated livestock. Breeding cultivars with lower levels of these natural toxicants would allow crops such as faba beans and forage brassicas to be used as feedstuffs more widely and in greater quantities than at present. In the potato, anti-nutritional factors have a very bitter taste and were virtually removed by selection during domestication. It is important, however, to ensure that unacceptable levels are not re-introduced when plant breeders use wild species as sources of disease and pest resistance.

Faba beans Faba or field beans (*Vicia faba* L.) are a major protein source for animal feed compounders in Europe, and are also a staple human food in parts of the Middle East. Several anti-nutritional factors

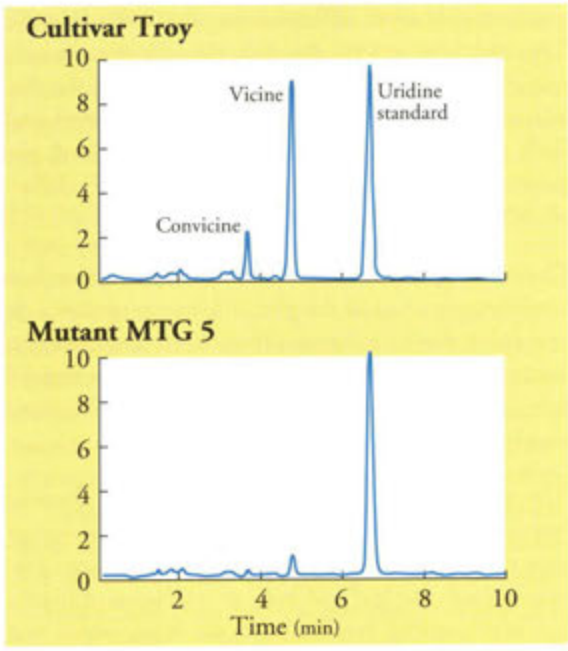

Figure 1 HPLC traces of glucopyranosides of faba beans.

detract from their food value, particularly tannins and glucopyranosides. The tannin problem has now been resolved by the development of white-flowered, tannin-free bean cultivars.

Vicine and convicine The glucopyranosides, vicine and convicine, present a different problem. They account for around 1% of the dry weight of the seed in most current cultivars. After ingestion they are hydrolysed to divicine and isouramil, two compounds which create oxidative stress and can cause rupture of red blood cells. Laying hens are particularly sensitive to these compounds, and in genetically susceptible human populations, the haemolytic anaemia syndrome favism can result.

A recent success in the legume research programme at SCRI has been the creation and identification of mutants containing much lower amounts of vicine and convicine than the wild types. Seeds of cv. Troy containing 1.2% glucopyranosides (dry weight basis) were treated with a chemical mutagen, sodium azide. The surviving plants were self-pollinated in insect-screened conditions and the resulting seed sown in the field to generate a population of 7,000 plants. Extracts were made by crushing one developing green seed from each plant, and screened for glucopyranoside content with a rapid paper chromatography method developed specially for the purpose. Two plants with low glucopyranoside contents, one with 0.5% and another with 0.1%, were identified and their contents verified by HPLC. In other respects the mutants appeared normal. Studies are under way to verify the mode of inheritance of glucopyranoside content and determine the expression in different plants. The selections can then be used for breeding cultivars with low glucopyranoside content.

Forage brassicas Brassicas are grown for feeding to cattle and sheep during the autumn and winter in the UK and in other countries with a similar climate. Whilst root crops such as swede (*Brassica napus* L.) and turnip (*B. campestris* L. or *B. rapa* L.) have proved a safe feed, it has been known for some time that leafy crops such as kale, cabbage (*B. oleracea* L.) and rape (*B. napus* L.) can cause both goitre and haemolytic anaemia when used to excess.

SMCO Haemolytic anaemia was first reported during the 1940's when dairy cattle were fed too much kale, and the phenomenon became known as kale poisoning. In 1974, work at the Rowett Research Institute traced the cause to an unusual amino acid, S-methylcysteine sulphoxide (SMCO), which is broken down in the rumen to dimethyl disulphide, a substance which is absorbed into the blood stream and destroys red blood cells. Haemolytic anaemia has been associated with poor live-weight gains in lambs grazing kale, but the extent of the problem with rape and other brassicas is less clear.

In 1979, an automated method for SMCO analysis was developed at the former Welsh Plant Breeding Station (now part of IGER) and adapted at SCRI. It involved extracting plant material, separating SMCO from other amino acids on a chromatographic column and estimating their contents colorimetrically.

Since 1979, information has been accumulated at SCRI on the SMCO content of forage brassicas over the period of utilisation from September to March. Peaks of SMCO concentration of 1.71%, 1.21% and 0.99% dry matter in cabbage heads, whole kale plants and swede bulbs (swollen hypocotyl), respectively, were found in field trials between the beginning of December and mid January. Despite being considered a safe feed, swedes may therefore contain enough SMCO under some growing conditions to harm ruminants. In contrast, two peaks of SMCO concentration were found in a glasshouse study of three forage rapes, where the highest concentration of 0.8% occurred in cv. Hobson.

Although genetical variation in SMCO content has

Figure 2 Dairy cattle eating kale.

been found in the SCRI forage brassica selection programmes, progress in achieving lower concentrations has been slow. Differences between cultivars and between breeding lines and families in some trials have not always been found in other trials and in subsequent generations. Furthermore, it is still not clear if SMCO has an essential role in the sulphur metabolism of cruciferous plants. Research in this area could lead to new approaches to selecting for lower SMCO content, or at least show that there are biochemical reasons why a major reduction is unlikely to be achieved.

Thiocyanate content of kale The thiocyanate ion (SCN^-) is released in crushed or macerated kale foliage by hydrolysis of indole glycosinolates. It can cause goitre when dietary iodine is inadequate, and experiments with rats suggest that it is the major goitrogen in kale. Goitre has often been reported in new-born lambs from ewes grazing thousand-head kale, and is more serious than the enlarged thyroids of lambs grazing kale. However, some of the effects of indole glucosinolates and their hydrolysis products may be beneficial. For example, indole compounds have been implicated in the anti-carcinogenic activities of brassica vegetables.

When genetical variation for SCN^- was found at SCRI in 1978 in a kale population being selected for improved digestible organic-matter yield, sub-populations for high and low SCN^- contents were selected. Families of each sub-population were produced in a polythene tunnel using blowflies as pollinators and the SCN^- content of 12 young leaves harvested from each family during September and October assayed using an automated colorimetric method developed at the Welsh Plant Breeding Station.

Four generations of selection resulted in populations with almost a two-fold difference in their SCN^- content and only small differences in other traits (Fig. 3). These populations are therefore valuable for research into the possible effects of SCN^- content on the disease and pest resistance of kale, and on the growth and milk production of animals fed kale. If required, the populations could be selected for even larger differences in SCN^- content.

Goitrin content of rape and swede Goitrin is a hydrolysis product of the glucosinolate progoitrin. It is a more potent goitrogen than the thiocyanate ion because it interferes with the synthesis of the thyroid hormone thyroxine, and its effects cannot be overcome by larger amounts of iodine in the diet.

The glucosinolate content of resynthesised *Brassica napus* was compared with that of traditional forage rape cultivars. Progoitrin was the most abundant glucosinolate in the leaf and stem of traditional cultivars, but was lower in two resynthesised rapes which had complex pedigrees including thousand-head kale (*B. oleracea* var. *fruticosa*), curly kale (*B. oleracea* var. *fimbriata*) and an oriental salad vegetable (*B. campestris* ssp. *nipposinica*), as well as traditional forage rape cultivars. One of the new rape selections has been released as cv. Bonar. A glasshouse study revealed complicated variations in the concentrations of individual glucosinolates in cv. Bonar, a second resynthesised *B. napus*, and cv. Hobson with time and in various parts of the plants that are at present unexplained. There are however good prospects for producing a zero-progoitrin form of *B. napus* because

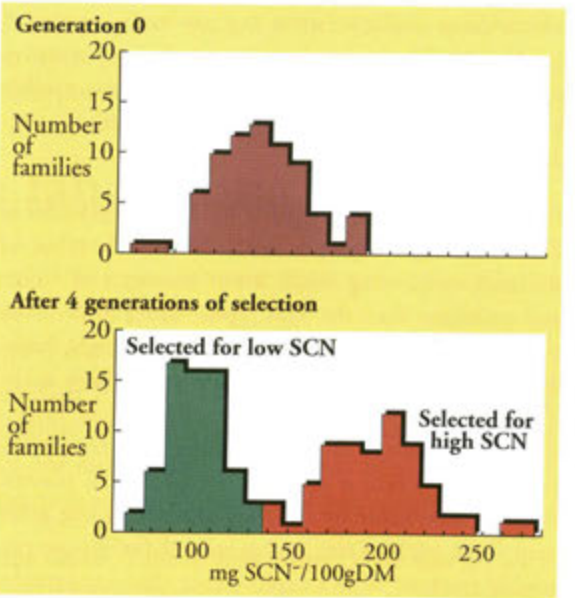

Figure 3 Selection of kale for high and low SCN^-.

cultivars of *B. campestris* and *B. oleracea* have been identified in Canada and the USA which lack progoitrin and it should be possible to synthesise a zero-progoitrin *B. napus* from them and backcross the trait into cultivars of forage rape.

Although swede has proved a safe feed for ruminants, a zero-progoitrin swede is desirable for human consumption. Concern about increased levels of natural toxicants in new cultivars prompted surveys of glucosinolates in brassica vegetables, including swedes. These confirmed that progoitrin is a major glucosinolate in swede, and as goitrin has a bitter taste, its absence would remove a potential toxicant without impairing flavour. The progoitrin content of swede cv. Melfort was found to be only 29% that of cv. Angus although they were both selected at SCRI for high dry matter yield and content as well as winter hardiness, and they had cv. Bangholm Wilby as a common parent. Therefore, differences can arise by chance whilst selecting for other traits and hence the progoitrin content of new cultivars should be checked. Furthermore, selection for lower levels should be possible in existing swede breeding programmes. As with the high and low SCN^- kales, rapes like cv. Bonar and swedes like cv. Melfort with altered glucosinolate contents can be used to determine the possible effects of

Figure 4 *Solanum vernei.*

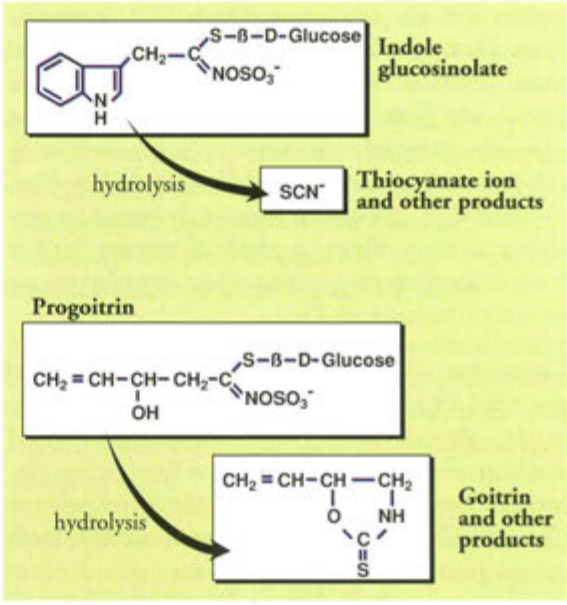

Figure 5 Goitrogens produced from glucosinolates in forage brassicas.

glucosinolates on disease and pest resistance, as well as their effects on the animals which feed on brassicas.

Potatoes -tuber glycoalkaloids The tubers of many wild species of potato contain potentially toxic amounts of steroidal alkaloids which have a bitter taste. During the evolution of the potato to a major food crop, less bitter and hence largely alkaloid-free types were selected which could be safely eaten in quantity.

Today the cultivated potato (*Solanum tuberosum*) contains only small quantities of tuber glycoalkaloids (TGA) with two solanidine glycosides, α-chaconine and α-solanine, accounting for over 95% of the total. There has, however, been some concern at SCRI that when *S. vernei* was used as a source of resistance to potato cyst nematode, unacceptable levels of TGA might inadvertently be introduced. A rapid and reliable method of measuring TGA content was therefore sought to ensure that potential cultivars did not exceed the accepted safe maximum level of 20 mg TGA per 100 g fresh weight of tuber. An HPLC method was chosen in preference to both an ELISA test and a dye-binding method. The results from all three methods were highly correlated, but HPLC was the quickest, the most reliable, and the least labour intensive. It was also able to estimate α-solanine and α-chaconine levels separately.

The inheritance of TGA content is being determined in glasshouse- and field-grown progenies derived from

parents with low, medium and high TGA concentrations. In the glasshouse-grown material, levels ranged from 5.3 to 78.7 mg TGA/100 g fresh weight in the parents and from 4.3 to 83.0 in the offspring, and generally exhibited continuous variation, indicating polygenic inheritance. When levels have also been determined in field-grown material, it should be possible to develop efficient methods of selecting for low levels in breeding programmes where some parents are known to contain high TGA.

Conclusion High performance liquid chromatography (HPLC) has proved a valuable method for accurately determining the concentrations of anti-nutritional factors in faba beans (pyrimidine glycosides), forage brassicas (glucosinolates) and potatoes (glycoalkaloids). It has been used to check that levels are acceptable in new cultivars; for example, potatoes with resistance to potato cyst nematode derived from the wild species *S. vernei*. It has also been used to confirm the success of selecting for lower levels using simpler and hence faster methods. Recent successes at SCRI have been the production of a faba bean line with a very low glycoside concentration using a chemical mutagen sodium azide; and forage brassicas with lower concentrations of those glucosinolates which hydrolyse to substances which can cause goitre. Attempts to produce forage brassicas with low concentrations of SMCO, an amino-acid which can cause haemolytic anaemia in cattle and sheep, have been less successful. Research is required into the role of SMCO in the sulphur metabolism of cruciferous plants.

Malting quality of barley

J-P. Camm, R.P. Ellis, J.S. Swanston& W.T.B. Thomas

Barley is the most widely grown arable crop in Scotland: over 340,000 ha were sown in 1990 to produce nearly 2 million tonnes of grain. Maltsters purchase a large amount of this to use in breweries and distilleries.

Figure 1 Ripening spring barley plots in breeders trials.

Malting exploits the changes which take place in germinating barley grains. Most of the grain consists of the endosperm which is the food store for the developing embryo when growth starts. The endosperm cells contain starch granules embedded in a protein matrix and, during germination, enzymes are produced which break down the cell walls and the protein matrix. This modification process exposes the starch granules to more enzymes which convert the insoluble starch to sugars which the growing embryo consumes. Temperature and water supply can affect the germination processes so the maltster must regulate such factors to encourage the grain to break down rapidly without using too much of the starch reserves of the endosperm.

The enzymes which break down starch remain active during extraction of the milled malt with hot water with the result that most of the starch is converted into simple sugars. Fermentation with yeast in breweries and distilleries produces alcohol from the sugars in the hot water extract. A high hot water extract, i.e. one with a high sugar concentration, is, therefore, the most important characteristic of 'good' malting quality.

As maltsters pay a premium for varieties with the capacity to produce high hot water extracts, these varieties comprise the vast majority of the spring barley crop. Environmental conditions affect even the best malting barleys, so maltsters test each consignment and may reject those with high nitrogen levels or poor germination as they will not produce the best extracts. Measurement of the hot water extract of each sample is impossible due to the number of samples involved and the time required to malt the grain and then analyse the extract.

Barley breeders face a similar problem in choosing from the very large numbers of new lines which they produce (Fig. 1). Malting quality is a major objective of most spring barley breeding programmes and many winter barley ones. Thus there is a demand from the grain trade and plant breeders for a rapid and effective method of predicting hot water extract.

The very best malting barleys possess a soft, mealy endosperm which requires less energy to mill than feeding types. At SCRI, we have developed the milling energy test, which determines the mechanical energy used in milling a 5g grain sample with a Comparamill. This is a modified hammer mill with an integral micro-processor which calculates milling energy (Joules) from the deceleration of a flywheel, linked to the hammers, when a grain sample enters the mill chamber.

Milling energy is inversely related to hot water extract so that no varieties with high milling energy produce high levels of hot water extract (Fig. 2). The milling energy test is very rapid as up to 150 weighed samples can be measured in 1h. While the conditions under which the crop grows influence milling energy, the genetic control of the character is much greater than environmental, so milling energy is a highly heritable and repeatable character. The test therefore fulfils the requirements of plant breeders who need a rapid and repeatable means of predicting malting quality.

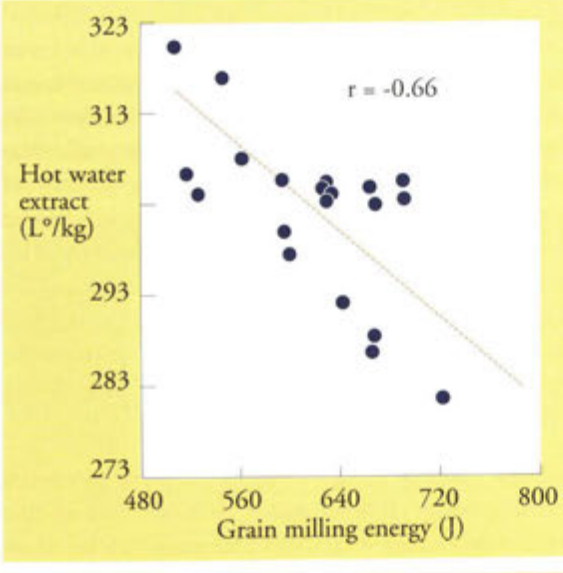

Figure 2 Relationship between milling energy of grain and the hot water extract of malt from the same samples.

Further research showed that the test distinguished between 'good' and 'poor' varieties when carried out on laboratory-dried grain from an unripe crop. This enables breeders to discard poor malting quality lines before harvest.

Each year barley breeders make many hybridisations, or crosses, and select lines with good malting quality and high yield from their progeny. Some crosses prove better than others but this generally becomes apparent in the later stages of a breeding programme. If breeders could predict crosses most likely to yield useful lines, they could concentrate on these, greatly improving efficiency.

Figure 3 shows the joint distribution of the progenies of three barley crosses for yield and milling energy. The mean milling energy and yield of the progeny is at the centre of the innermost ellipse and, as the ellipses increase in size they enclose more of the distribution. The difference between individuals (variation) within the cross and the relationship, or correlation, of the two characters determines the shape of the ellipses around the mean. A greater variation results in a greater difference between the innermost and outermost ellipses. A positive correlation elongates the ellipses into the bottom left and top right-hand corners (Fig. 3b) whereas a negative correlation produces the opposite effect (Fig. 3a). Although the mean yield of the three progenies is the same, the mean milling energy of B87-90 is much higher and none of the distribution falls into the area of high yield and low milling energy (Fig. 3c). At SCRI, we use lines from the early generations of a cross to highlight such relationships and discard crosses such as that identified in Figure 3c and concentrate resources upon crosses like that in Figure 3a.

Milling energy as a test is not confined to selecting breeding lines however. As malting progresses the milling energy of the malt decreases; the decreased value compared to that of the dry grain provides an accurate measure of the extent of modification and

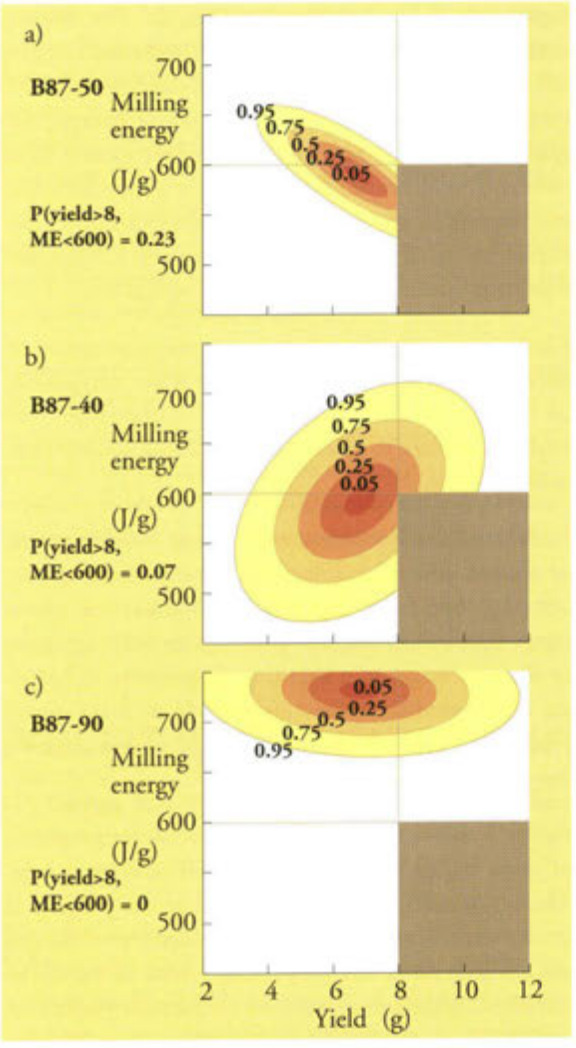

Figure 3 Probability contours for the joint distribution of yield and milling energy. The ellipses indicate the probability that lines from a cross lie within them.

could be used by the maltster to determine when to halt germination and dry the malted grain in kilns.

The milling energy of the dry grain and the decline in milling energy as malting progresses both determine whether or not a variety has 'good' malting quality. We are studying these factors in order to understand more fully the physical and biochemical factors controlling malting quality. The aleurone and sub-aleurone are the outer layers of the barley starchy endosperm. The former is important in malting as it is where enzymes used in the modification of the endosperm and breakdown of starch are synthesised. The husk is the outermost layer and protects the grain from damage. The milling energy of each layer decreases from the husk to the endosperm (Fig. 4).

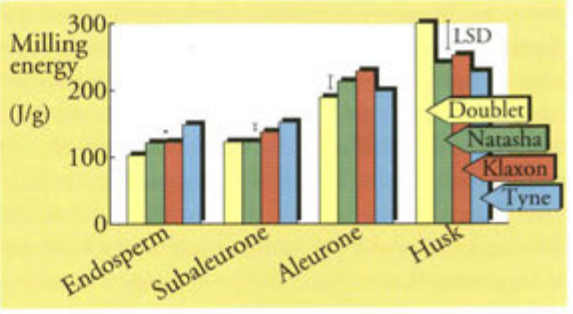

Figure 4 Milling energy per gram of tissue.

The relative milling energies of varieties differ according to the properties of these layers. Doublet has the softest endosperm but the hardest husk (Fig. 4). The endosperm comprises some 80% of the weight of the whole grain, however, and will largely determine both milling energy and malting quality.

Electron microscopy enables us to observe in some detail the changes which take place during the modification of the endosperm. The endosperm cell walls and the protein matrix, into which small starch granules are deeply embedded, are clearly visible in the early stages of malting (Fig. 5c). In the later stages of malting the protein matrix is much reduced and the starch granules become more visible (Fig. 5d). Over several days, these changes lead to both increased hot water extract and reduced milling energy. Cultivars of diverse malting quality may show the same relationship between decrease in milling energy and increase in hot water extract. Thus a unit decrease in milling energy produces the same increase in hot water extract for the three cultivars shown in Figure 6.

If the decrease in milling energy during malting is plotted against cell wall breakdown, however, the 'good' malting cultivar Triumph demonstrates clear differences from the other two (Fig. 7). The rate of cell wall breakdown and the accompanying loss of milling energy appears to vary between cultivars. This makes precise definition of malting quality, and the use of tests which measure a single aspect e.g. cell wall breakdown, less useful. It does, however, reinforce the value of milling energy as a means of monitoring changes during malting.

Several genes or groups of genes are likely to control milling energy. It is important to locate them so that their relationship with other characters can be determined and the control of milling energy at the molecular level can eventually be revealed. The location of such genes can be determined by observing linkage

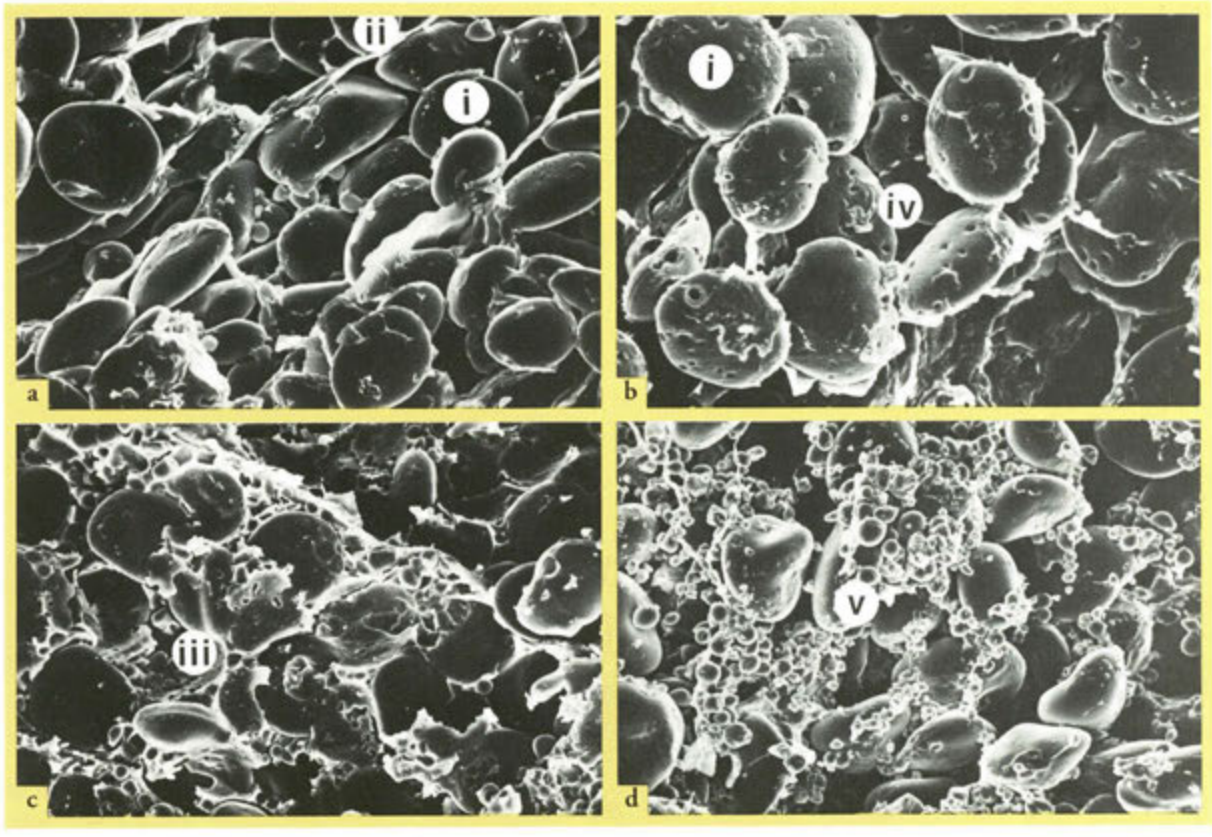

Figure 5 Changes in cell wall, protein and starch near the embryo as seen in scanning electron micrographs during malting. Triumph (a) day 1, (b) day 7; Koru (c) day 1, (d) day 7.

(i) Starch A granules.

(ii) Cell walls visible initially but absent after 7 days.

(iii) Protein matrix with embedded starch B granules in the poor quality cultivar early in the malting process.

(iv) Endosperm of the good quality cultivar at the end of malting. Most of the A granules are pitted due to enzymic action.

(v) Protein matrix of the poor quality cultivar mainly removed revealing B granules at the end of malting. A granules are still relatively obscured.

relationships with marker genes on established chromosome maps. So far, we have identified factors on two barley chromosomes that appear to be involved in controlling milling energy. One is located on the same chromosome arm as a gene controlling winter habit.

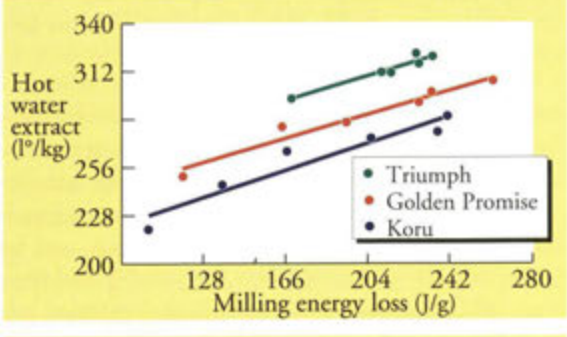

Figure 6 Loss of milling energy during malting as a predictor of hot water extract.

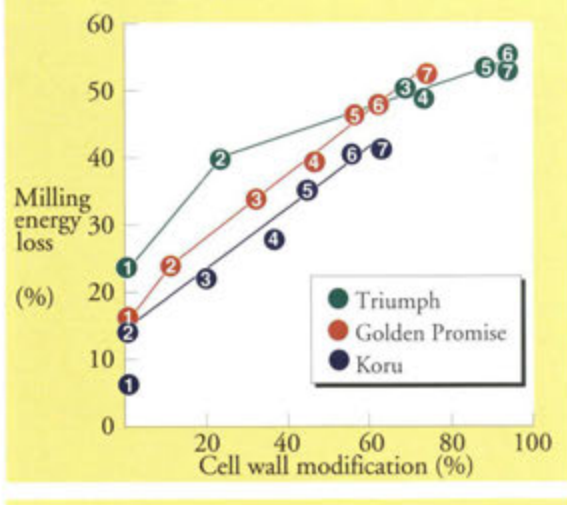

Figure 7 Relationship between loss of milling energy and cell wall modification for 1-7 days of malting.

With few exceptions, winter barleys have a higher milling energy than spring barleys. Although this may be caused in part by higher levels of nitrogen, it may also be due to genes linked to those controlling winter habit.

In addition, higher levels of milling energy have been found to be associated with the *'erectoides'* dwarfing gene found in cultivars such as Golden Promise and Tyne, although subsequent analyses showed it to be caused by a reduction in grain size. Milling energy is clearly a character of considerable interest to the barley breeder and the Comparamill will remain an important selection and research tool.

Low temperature hardiness and avoidance of frost damage in woody perennials

R.M. Brennan

Low temperature injury is a major cause of crop losses in many temperate genera of fruit and other woody perennials[1]. Consequently, low temperature tolerance is a major objective in most germplasm enhancement programmes, including those at SCRI, with particular emphasis on the programme for *Ribes* fruits.

Blackcurrant (*Ribes nigrum* L.) is the major *Ribes* fruit grown in northern Europe, and there were *ca.* 3 kha grown in the UK in 1988. The efforts of breeders and geneticists to improve the available cultivars has progressed to encompass interspecific crossing, the incorporation of foreign germplasm and the utilisation of cellular and molecular techniques. These efforts have been directed particularly towards the production of cultivars with improved tolerance of low temperatures.

Figure 1 Effects of frost on flowers of cv. Baldwin (left) and cv. Ben More (right).

The most widely-grown cultivar in the UK in the past 100 years was Baldwin, a seedling of unknown parentage which combined good yield potential with desirable fruit quality characteristics. Baldwin still occupies a significant proportion of the UK hectarage, but although the cropping potential of Baldwin and its derivatives is high, they often suffer serious annual fluctuations in yield caused by low temperature damage at flowering time (Fig. 1). In severe conditions, yields can be reduced to practically zero, especially in the absence of frost-protection irrigation. Damage to flowers by spring frosts is the most serious factor limiting consistent cropping of blackcurrants.

Attempts to overcome this problem at SCRI have involve utilising germplasm from northern regions in the *Ribes* breeding programme. *Ribes* spp. are distributed throughout most northern temperate regions, including Scandinavia and the Soviet Union, and by incorporating environmentally-adapted germplasm from these northern centres of diversity, cultivars and breeding lines have been produced with greatly improved tolerance of spring frosts and improved cropping stability. Spring frost tolerance is transmit-

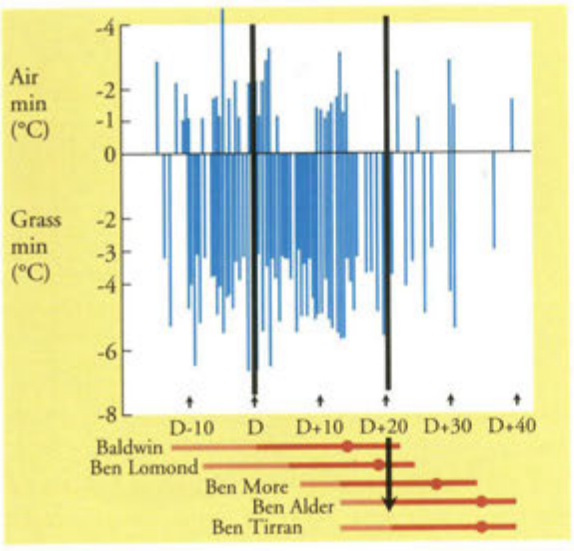

Figure 2 Relationship between flowering date of blackcurrant cultivars and occurence of spring frosts.

ted to hybrid progeny, but the mode of inheritance is not clear. Furthermore, the relationship between genes controlling spring frost tolerance and winter hardiness has not been examined. Genotypes adapted to extremely severe winter conditions can often suffer serious frost injury at flowering in the UK, since the relatively mild winters lead to earlier bud-break and flowering, rendering the flowers vulnerable to frost damage at the more sensitive later stages.

Figure 3 SCRI Blackcurrant cv. Ben Tirran.

In general, low temperature tolerance decreases as flowering progresses and the plants deacclimate, but the duration of tolerance shows considerable intercultivar variation. Reasonable levels of tolerance are retained throughout flowering in some Soviet cultivars, and clearly the relative rates of acclimation and deacclimation of different genotypes are key factors in determining spring frost tolerance.

The low temperature tolerance mechanism that has so far been used most successfully to date is frost avoidance through delayed flowering. Most of the SCRI Ben series and other cultivars base their tolerance partially or entirely on this character, since a delay of 2-3 weeks in flowering in northern Europe avoids the majority of damaging frosts. The occurrence of spring frosts during the period 1960-1987 and the flowering dates (first open flower stage) of UK cultivars is illustrated in Figure 2. The frost-escaping character of the later-flowering cultivars such as Ben Alder and Ben Tirran is shown in the greatly reduced number of frosts that coincide with the most susceptible stages of flowering. The value of selecting for late flowering is therefore clear, and the latest-flowering SCRI cultivar to date is Ben Tirran (Fig. 3). However, inevitably as flowering is progressively delayed, a point is reached beyond which yield declines because the growing season is shortened. Consequently, physiological hardiness, possibly allied to late flowering, is therefore preferred, particularly for the production of early-ripening cultivars with consistently high yields.

Controlled-environment frost cabinets are used at SCRI to assess the ability of genotypes to tolerate low temperatures at flowering, because the natural occurrence of frost is unpredictable (Fig. 5). Genotypes can be examined at similar stages of development and the results obtained are closely related to equivalent field data. Typical results of testing for frost tolerance at different flowering stages are presented in Figure 6, and show clearly the variation in responses of several cultivars. Annual assessments are made of enhanced germplasm and experimental material, and relative rankings of tolerance are the same from year to year, irrespective of variations in pre-test conditions[2].

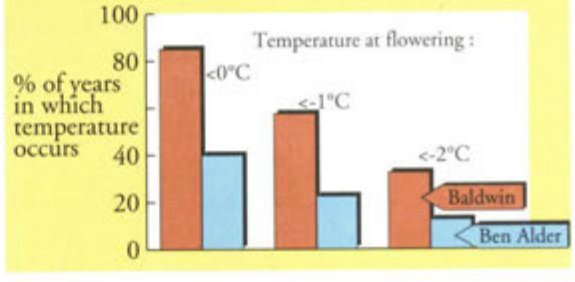

Figure 4 Statistical likelihood of the avoidance of sub-zero temperatures by late flowering in cv. Ben Alder.

Figure 5 Frost simulation chamber at SCRI with test plants.

The use of whole plants inevitably puts constraints on the number of genotypes that can be assessed, in terms of time, space and cost, and an alternative rapid, sensitive and non-destructive test method to assess cold/frost tolerance has been developed[3]. The measurement of chlorophyll fluorescence in leaves is a well-known indicator of stress such as frost damage, particularly in *Solanum* spp[4]. Although winter injury can occur within the vegetative parts of *R. nigrum*, the primary site of injury is the flowers. Nevertheless, the use of chlorophyll fluorescence has been developed as a physiological marker in breeding for spring low temperature tolerance in blackcurrants. The results from different genotypes showed good correlation with visual assessment of damage in both field appraisals and frost chamber studies using potted plants. Whilst it appears unlikely from our work to date that the basic chlorophyll fluorescence studies used so far offer a definitive quantitative assessment of the hardiness of a genotype, it does provide a method for the qualitative assessment of genotypes in the genetic improvement programme. Recently, assessments have been made on small leaf discs, so that *in vitro* plant material could be used, enabling accelerated progeny screening to take place.

Studies of the mechanisms of low temperature tolerance are becoming increasingly important to incorporate physiological hardiness rather than frost

Genotype	Origin	Date of first open flower	Reduction of % survival		
			Grape stage	First open flower stage	Full flower stage
Baldwin	UK	Apr 6	46.4	48.9	59.8
Ben Alder	UK	Apr 26	16.1	10.3	6.2
Ben Lomond	UK	Apr 14	6.0	19.0	7.8
Ben More	UK	Apr 26	8.6	3.4	42.1
Ben Tirran	UK	Apr 26	19.1	6.0	1.0
Stor Klas	Sweden	Apr 14	17.9	7.5	9.9
Öjebyn	Sweden	Apr 6	12.0	7.9	5.0

Figure 6 Reduction in percentage survival of flower buds due to simulated frost.

avoidance. The tolerance of overwintering *Ribes* buds was examined in a collaborative study between SCRI and the University of Birmingham, using differential thermal analyses (DTA) and microscopic observations[5]. These indicated that water freezes first in the pith, whilst the primordia and meristem remain unfrozen and undergo freeze dehydration followed by supercooling. In this way, the overwintering buds can survive UK winter temperatures, but comparable studies of buds during the spring have not been made.

Molecular techniques are being used in low temperature research in soft fruit species at SCRI, and joint research with Hatfield Polytechnic has begun to examine nucleic acid changes in cold-tolerant plants and synthesis and accumulation of proteins associated with cold acclimation. The long-term objectives of this approach are the identification of the genes involved in low temperature tolerance in *Ribes* and other woody perennial plants, and improved understanding of the inheritance and expression of cold tolerance.

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New potato cultivar

Figure 1 Brodick potato tubers

Brodick is a new potato cultivar which was placed on the National List and was granted Plant Variety Rights in 1990. It is a high-yielding cultivar with an excellent spectrum of resistances to most fungal diseases, especially late blight and to the common viruses PVY and PLRV. Brodick produces an extremely attractive sample of uniformly-shaped, regular oval tubers admirably suited to the prepack trade but its table quality is marred by a tendency to after-cooking blackening when grown at some sites. Brodick is a special purpose crisping variety with a dry matter content comparable to, and a yield potential higher than, cv. Record. It also possesses a unique capacity to store at low temperature, 4°C, for up to 5 months under controlled conditions without developing low temperature sweetening and it can be reconditioned if it "sugars". Brodick provides the crisping industry with a new cultivar which has superior agronomic and disease resistance characteristics compared to cv. Record, and which is capable of being stored for extended periods at low temperature without the need for sprout suppressant chemicals. SCRI tests indicate that Brodick is reasonably resistant to tuber soft rot.

Origin 7683A12 x 8898AC14

Year of cross 1978

Maturity class Early maincrop, similar to cv. Désirée

Foliage Medium-tall, semi-erect, bushy dense; leaf narrow, moderately open, mid-green colour; flowers numerous, petals mauve with white tips.

Tubers Oval, usually bold sample, regular and uniform with shallow eyes; parti-coloured, white skin with pink eyes; flesh pale cream/lemon.

Cooking quality Fairly floury texture, high dry matter, tendency to after-cooking blackening but no off flavours and low level of enzymic browning.

Processing High dry matter and fry colours equal or superior to cv. Record under similar conditions of storage, allied to an ability to store at low temperature (c. 4°C) without suffering from low temperature sweetening, confirm excellent processing (crisping) potential.

Wart	Field immune to common European race 1
Late Blight - foliage	8
- tuber	7
Gangrene	8
Dry rot	7
Skin spot	5
Comon scab	5
Virus PVA	Field immune
PVY	8
PLRV (leafroll)	5
PVX	Susceptible
Tobacco rattle virus (spraing)	Susceptible
PCN	Susceptible

Figure 2 Disease resistance of potato cultivar Brodick

Numerical scores on 1-9 comparable to those of NIAB where 1 = complete susceptibility and 9 = complete resistance = immunity.

Molecular Biology and Tissue Culture

W. Powell

The Cell and Molecular Genetics Department at SCRI is making significant contributions in the areas of genetic markers, development of tissue culture and plant transformation technology, and the study of gene expression. The integration of research in these areas underpins much of our current success and future research goals. The expertise in these areas is being applied to fundamental aspects of plant biology and is being exploited in addressing problems in industry and agriculture. The technology available in the Department has led to collaborations not only within the Institute but also internationally as reflected by the sources of external funding.

Genetic markers Methods for the identification, characterisation and quantification of genetic variability are essential to many basic and applied research programmes. New methods (RFLPs and RAPDs) for the detection of polymorphisms and their exploitation represents one of the most significant recent developments in plant biology. In particular, technology based on the polymerase chain reaction (PCR) has allowed the development of user friendly methods for detecting variability. Linkage of genetic markers to loci of economic importance, for example, spring or winter habit in barley, will allow crop improvement programmes to proceed with greater speed and precision. In addition, molecular methods may be used to improve our understanding of genome organisation, evolution and gene synteny in related organisms. The construction of genetic linkage maps and the analysis of genetic diversity and inheritance are goals which are being addressed not only in traditional temperate crops such as potato, barley and field bean, but also in tropical crops, e.g. cocoa, groundnut and coffee, and in nematode and aphid pests. The application of genetic markers is highlighted in the following topic reports.

Cell and tissue culture Genetic transformation and tissue culture systems have been developed and established for a range of species (e.g. potato and brassicas) and protocols modified to allow regeneration of recalcitrant genotypes within a species. For example, a wide spectrum of potato cultivars can

now be incorporated into genetic transformation programmes. This achievement is particularly important for our programmes on gene expression and carbohydrate metabolism. The role of cell and tissue culture in the transfer of alien genomes into adapted potato germplasm is highlighted in the report describing the production of somatic hybrids. Protoplast fusion techniques have successfully created somatic hybrids between *Solanum brevidens* and dihaploid *S. tuberosum* genotypes. In addition, intraspecific *S. tuberosum* fusion hybrids have been generated and characterised at the molecular level.

Gene expression Research into basic mechanisms of gene expression is vital to our understanding of many areas of plant physiology and biochemistry. For example, the recent cloning of the first plant snRNP protein gene is not only of significance to plant pre-mRNA processing and gene expression, but also to RNA-protein interactions which are of fundamental importance to numerous cellular processes. The application of molecular biology to carbohydrate metabolism offers many new possibilities for analysing the physiology and biochemistry of potato tuber formation and low temperature sweeting. These objectives are being pursued in conjunction with the Cellular and Environmental Physiology Department.

In addition to the research topics, the Cell and Molecular Genetics Department has a strong commitment to training students, visiting workers and Institute Staff.

Genetic markers

W. Powell, B.P. Forster & R. Waugh

The concept of utilising markers in plant breeding as an indirect method of selecting desirable recombinant genotypes is well established. Until relatively recently, the markers employed were morphological characters which are limited in number and often agronomically undesirable. As a means of increasing the number of markers, different strategies have been developed based on existing natural variation present in a plant's genome. The significance of this variation is that a large number of genetic markers can be assembled in a single cross. Furthermore, these markers are inherited in a Mendelian, and hence, predictable manner. The availability of such markers provides new opportunities to improve the speed and precision of gene transfer in crop improvement. Some of our more recent research is described.

Using specially constructed genetic stocks we have located two genes on the long arm of chromosome 4H of barley. These genes code for the endosperm proteins β-amylase and water-soluble protein, both of which can be easily extracted from small sections of single barley seed and visualised in iso-electric focussing gels. Further genetic studies in collaboration with the Crop Genetics Department (R.P. Ellis) showed that these two genes lie very close to the vernalisation requirement gene (Vrn-1) which is the major determinant of spring or winter habit in barley. β-amylase and water-soluble protein are therefore ideal markers for spring or winter habit and can be exploited in breeding programmes. Single seed from a cross which is segregating for habit can be divided in half (Fig. 1), the embryo-less half analysed for the two endosperm proteins, and from the results, the spring or winter genotype of the embryo can be predicted. Spring or winter barleys can therefore be selected before even germinating the seed. This strategy can save valuable time, space and expense in growing unwanted plants which would otherwise require growing through an entire season before spring or winter growth habit could be determined.

The emergence and general accessibility of molecular biological techniques has allowed the relatively extensive variation which occurs in the DNA sequence of a given organism to be exploited. The standard method by which this is achieved relies on the ability of certain bacterial enzymes, restriction endonucleases, to recognise and cleave specific DNA sequences within the extremely long DNA molecules which comprise a plant's genome. Cleavage results in the

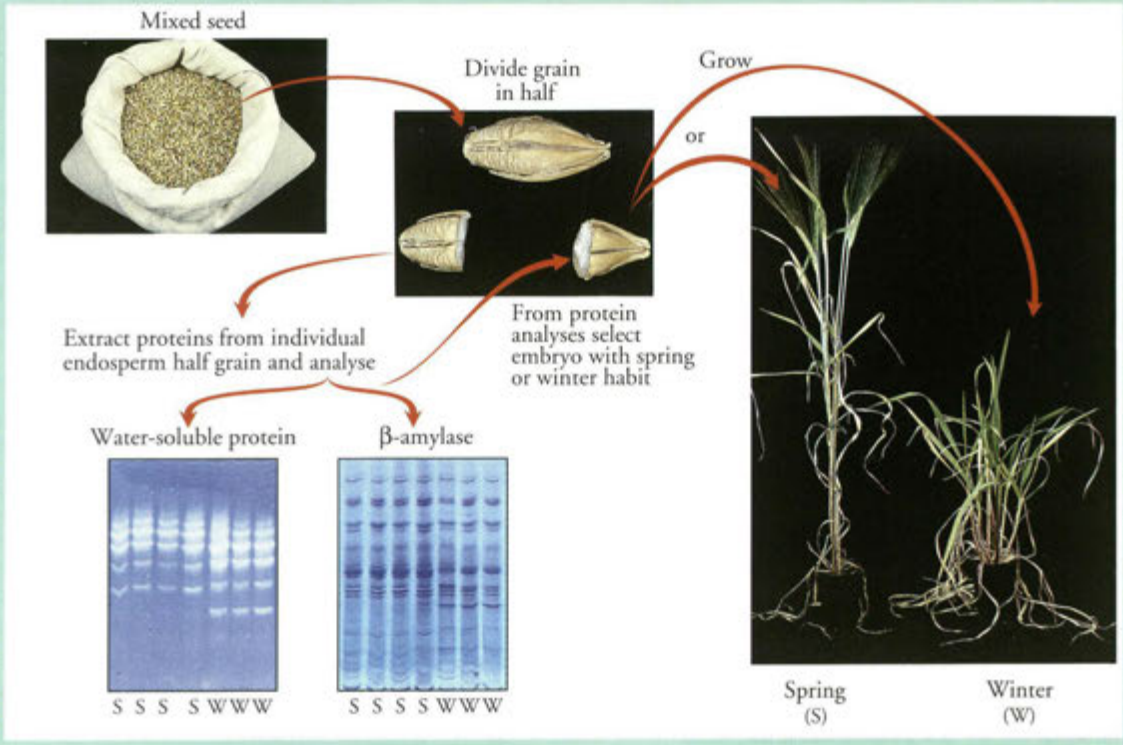

Figure 1 Scheme of determining spring/winter habit in barley seed. Single seed are sampled from a mixed population, the seed is cut in half, the embryo end is retained whilst the protein 'fingerprint' of the endosperm end is determined. From the protein results the growth habit of the embryo can be predicted and the desired spring or winter types selected.

generation of a set of restriction fragments of differing lengths which reflect restriction site changes within a given individual. After electrophoretic separation of the fragments according to their length and transfer to a solid membrane, identification of restriction fragments is achieved by Southern DNA:DNA hybridisation with a radioactively labelled cloned DNA probe and visualised by exposure of the hybridisation membrane to photographic film. The variations which occur have been termed restriction fragment length polymorphisms or RFLPs. Since the mid-1980s, RFLPs have been used extensively for the construction of high density genetic linkage maps, and RFLPs linked to many desirable characters have been identified. RFLPs are also useful for other applications including cultivar identification, evaluating germplasm resources, identifying distantly related parents for inclusion in a breeding programme and for phylogenetic studies.

We have exploited RFLP technology to unequivocally fingerprint a range of 27 important potato cultivars and both nuclear and cytoplasmic RFLPs have been used to assess the level of genetic variation within the cultivated European potato gene pool. RFLPs have also been used in conjunction with cytological observations to demonstrate that *Solanum phureja* induced dihaploid potato clones were aneusomatic and contained DNA from *S. phureja*. Potato dihaploids generated in this way cannot therefore have developed via parthenogenesis as previously proposed. RFLPs from the nuclear and mitochondrial genomes have also been used to study the genetic relationships between field bean, *Vicia faba*, and approximately 50 accessions of wild *Vicia* species (Fig. 2). These examples serve to illustrate the power of RFLP procedures to characterise germplasm resources and investigate mechanisms of ploidy manipulation in crop plants.

Mapping the components of polygenic systems is an important objective in both basic and applied genetic research programmes. The identification of specific regions of the genome which enhance the expression of quantitatively controlled characters may allow the development of more efficient plant breeding strategies. The availability of a greater spectrum of genetic markers has provided considerable impetus for the mapping of quantitative traits in crop plants. Our approach has been to use doubled haploids (DH) of

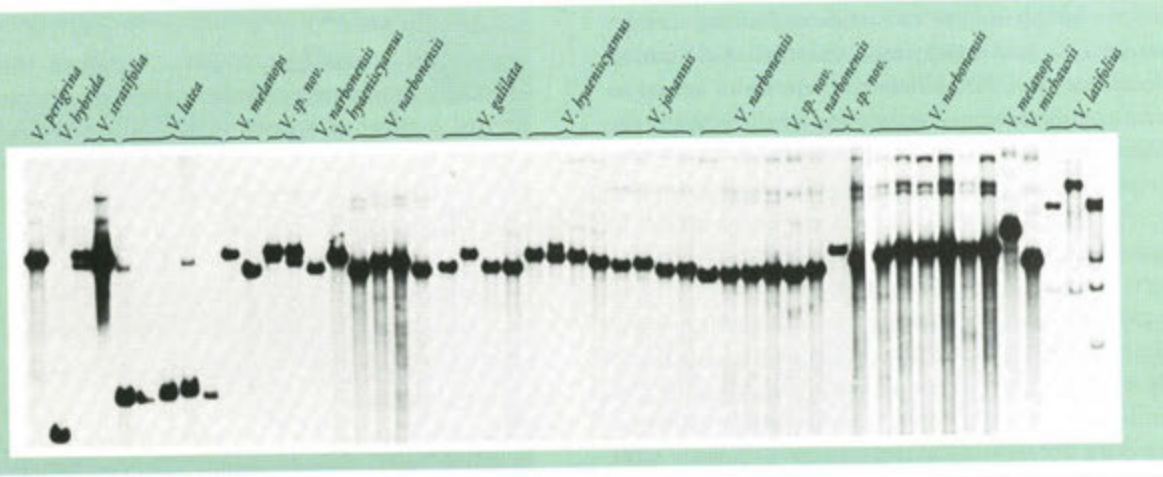

Figure 2 DNA from a wide range of *Vicia* species was digested, blotted and probed with an anonymous genomic DNA clone. The lengths of the hybridising fragments are clearly different and can be diagnostic of distinct species or a given individual. By performing a similar type of analysis with a large number of probes and scoring the number of shared fragments, the relationships between individual species can be estimated.

barley in conjunction with isozyme and RFLP markers to locate genes controlling quantitative traits to specific regions of the genome. The segregation of alleles at the *Nor-H3* locus on chromosome 5H has been monitored using an rDNA probe in DH progenies extracted from the F1 of a barley cross between Blenheim and E224/3. Allelic variation at the *Nor-H3* locus was found to be significantly associated with genes controlling the milling energy requirement of the grain. Over 28% of the genetic variation for milling energy is associated with the *Nor-H3* locus on the short arm of chromosome 5H. This is illustrated graphically in Figure 3. There are two distinct frequency distributions and selection of progeny possessing the *Nor-H3* allele from E224/3 would result in a population with a significantly lower milling energy. Ribosomal DNA specific probes may therefore be used as genetic markers for grain endosperm texture in barley.

A major drawback of RFLPs is that their application is technically difficult and in the majority of laboratories the detection method relies on the use of short-lived radioisotopes. In many cases, these features may inhibit the routine application of RFLPs in plant breeding. However, the advent of polymerase chain reaction (PCR) technology has brought the potential of DNA based marker systems one step closer to being routinely and reliably applied. PCR technology hinges on the availability of a DNA polymerase (*Taq* polymerase) from the thermophyllic bacterium *Thermus aquaticus* which retains activity even after prolonged incubation at temperatures which denature double stranded DNA templates. Defined segments of minute quantities of target DNA can be specifically amplified by supplying *Taq* polymerase, excess nucleotides and oligonucleotide primers (which are

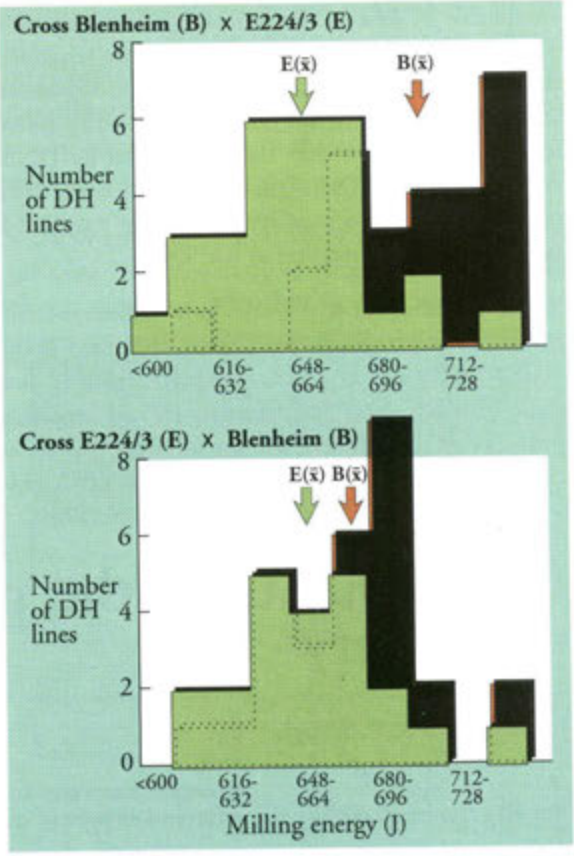

Figure 3 Frequency distributions for milling energy in a doubled haploid population of barley.

exactly complementary to sequences flanking a target sequence), and repeating a thermal cycle which denatures the DNA, allows the primers to anneal to their complementary sequence and finally activates the *DNA* polymerase. *Taq* Polymerase will copy the single stranded target DNA undirectionally from the annealed primer. Any sequence up to a size of approximately 4000 nucleotides, which is flanked by two primer binding sites, can be amplified exponentially by repeating the thermal cycle up to 45 times. The specificity of the amplification is determined by the nucleotide sequence of the individual primers. After amplification, sufficient product is produced to be visualised directly after electrophoresis by ethidium bromide staining and illumination by UV light.

A modification of the PCR technique which requires no prior knowledge of nucleotide sequence is becoming increasingly attractive as a DNA based marker system. The approach is based on the probability that in the genome of the organism under study a given single nucleotide sequence will occur in inverse orientation within a distance that is amplifiable by PCR. The primers used are generally only 10 nucleotides in length with their sequence determined arbitrarily. Differences in the sequences amplified from related individuals are caused by either mutations in the primer binding site or by DNA rearrangements. Differences detected using this technique have been called randomly amplified polymorphic DNA markers or RAPDs.

We have evaluated RAPDs in both temperate and tropical crop plants. In both potato and field bean, RAPD based polymorphisms have been identified and their inheritance monitored in segregating populations. RAPDs have also been used to demonstrate the introgression of *S. phureja* DNA into potato dihaploids (Fig. 4) and to 'fingerprint' both potato and cocoa genotypes. In cocoa this is especially important since there are currently only a limited number of systems capable of discriminating between accessions and very often these can only be scored when the plants reach maturity. Extracting cocoa DNA of sufficient quality and quantity for RFLP studies is also difficult and requires a large amount of fresh material. The RAPD approach for this crop and other long lived, woody perennials is therefore particularly attractive.

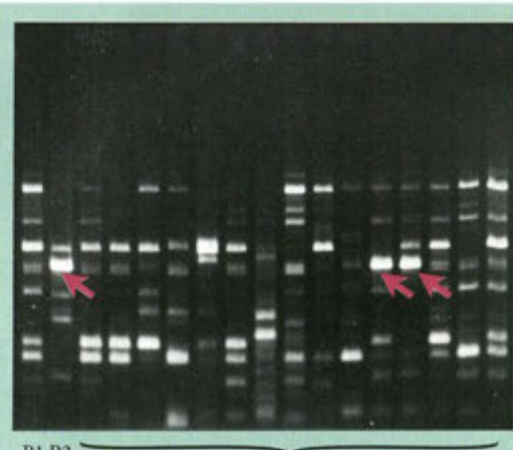

Figure 4 Dihaploids (PDH) were extracted from the tetraploid potato cultivar Pentland Crown by pollination with the dihaploid inducer clone *Solanum phureja* IVP48. DNA isolated from a range of PDH clones was used as the template for amplification with a 10-mer oligonucleotide using the RAPD approach. Introgression of DNA from the inducer clone is demonstrated by the presence of amplified bands which are not present in the Pentland Crown derived products but are present when *S. phureja* DNA is used as the template (arrowed bands). P1 = Pentland Crown, P2 = *S. phureja*.

Components of the plant pre-mRNA splicing machinery

J.W.S. Brown & R. Waugh

In all organisms, reading the information present in the genes and the subsequent decoding of that information into a functional product - a protein - is performed by a multi-step and multi-component pathway. The primary event occurs in the nucleus and involves transcribing (or copying) the DNA sequence of a gene into a precursor-messenger RNA (pre-mRNA). Still in the nucleus, the pre-mRNA undergoes a series of modification and processing events which result in the formation of a mature

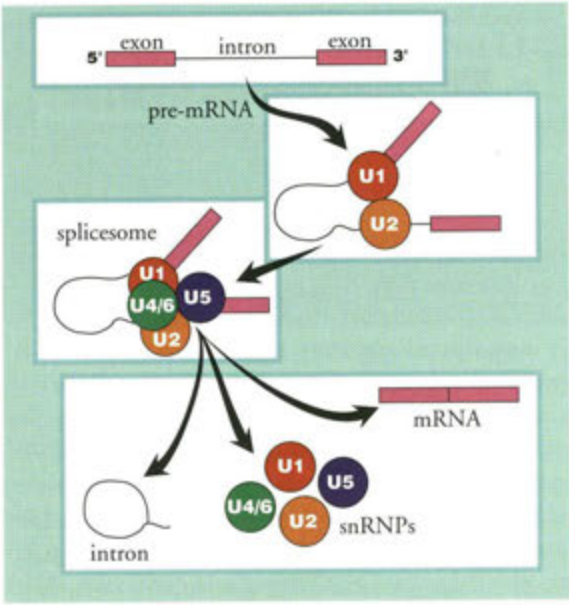

Figure 1 Splicing of pre-mRNA: Intron sequences are recognised by U1 and U2snRNPs before association of the U5 and U4/6snRNPs and other proteins to form the spliceosome. The intron is excised, the exons are joined together and the complex is disassembled.

messenger RNA (mRNA) which is transported out of the nucleus into the cytoplasm where translation into a protein can occur. Our interests lie in one particular processing event - known as splicing - which occurs in the nucleus after transcription.

Splicing is a fundamental process of eukaryotic gene expression and is one level at which gene expression may be regulated. When a gene is transcribed, the non-coding intron sequences are removed from the pre-mRNA and the coding sequences (exons) are rejoined to form the mRNA. Mature mRNA is transported into the cytoplasm where it can be translated into protein. The excision of intron sequences from pre-mRNA transcripts must be highly accurate and occurs via the assembly of a large ribonucleoprotein complex called the spliceosome (Fig. 1). The main constituents of the spliceosome are small nuclear ribonucleoprotein particles (snRNPs) which are in themselves complexes of RNA molecules (small nuclear RNAs - snRNAs) and proteins.

The study of splicing in plants is of primary importance in gaining an understanding of the biochemistry and molecular biology of plant gene expression, which underpins strategies for biotechnological plant improvement. In particular, the difference in splicing between monocotyledonous and dicotyledonous plants, the increased levels of gene expression due to the presence of certain introns and the role of alternative splicing in regulating gene expression make an understanding of this process essential. An analysis of splicing and the factors involved in splicing in plants will also provide information on the evolution of this process by comparing with animals and yeast, which have been extensively studied due to the availability of *in vitro* splicing systems.

Most progress on the study of the components of splicing in plants has been made in the characterisation of snRNAs. The spliceosomal

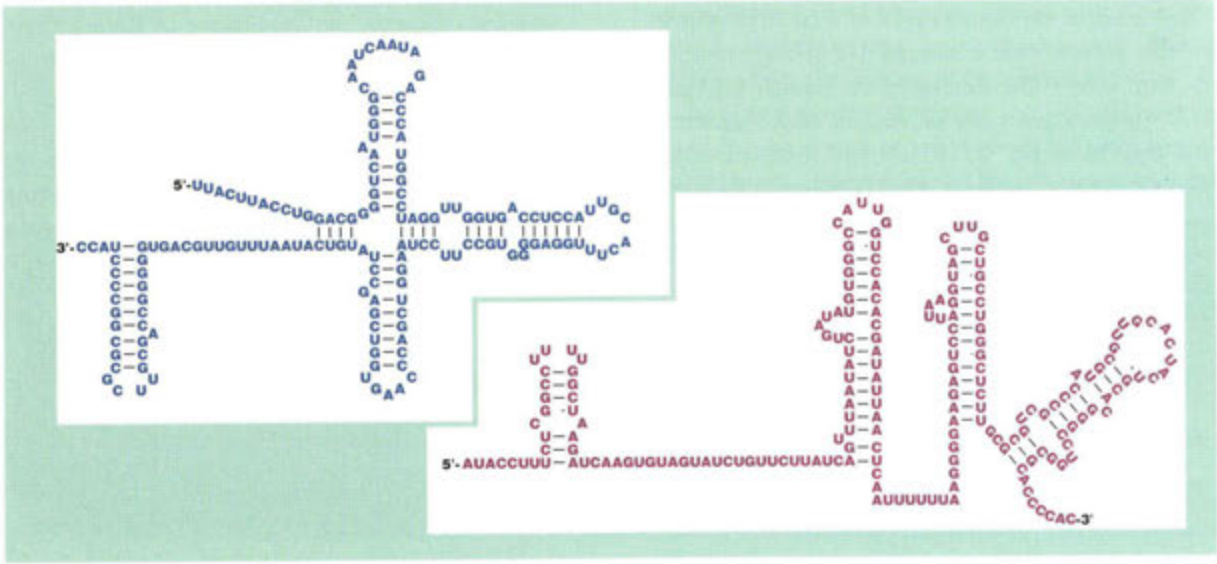

Figure 2 Sequence and secondary structure of potato U1and U2snRNAs.

snRNAs, U1, U2, U4, U5 and U6 are integral parts of snRNPs which mediate splicing. To date, approximately 50 plant snRNA genes have been cloned and sequenced, mainly from *Arabidopsis* by W. Filipowicz and colleagues at the Friedrich Miescher Institute, Basel, Switzerland, and from potato and maize at the SCRI. Analysis of these snRNA sequences has shown a degree of sequence variation in the coding regions previously unseen in animal systems. In many cases, sequence changes are complimentary in nature conserving the important secondary structure of the UsnRNAs (Fig. 2). The isolation of maize UsnRNA genes at SCRI also allows direct comparison between monocotyledonous and dicotyledonous UsnRNA sequences. The function of UsnRNA variation in plants remains to be elucidated. However, such variation may give rise to variant UsnRNPs with altered affinities for particular introns which can, in turn, regulate gene expression.

In the cell, snRNAs are complexed with proteins to form snRNPs which, during splicing, interact with other spliceosomal components such as the pre-mRNA, other snRNPs and transiently associated spliceosomal proteins or splice-specific proteins. The interaction between UsnRNAs and snRNP-specific proteins provides a system to study RNA-protein interactions in plants. The most abundant snRNPs, U1 and U2, have received particular attention in animal systems because two proteins, U1A and U2B", which are specific components of the U1snRNP and U2snRNP respectively, bind to very similar single-stranded regions of the U1snRNA and U2snRNA. U1A binds to the stem-loop II of U1snRNA while U2B" binds to the stem-loop IV of U2snRNA (Fig. 2) in the presence of a second U2snRNP-specific protein, U2A'. The cloning of the human U1A and U2B" protein genes has allowed the identification of the regions of the UsnRNAs and proteins which directly interact. In plants, the U2 sequence lacks one of the conserved sequence determinants such that the plant U1 and U2 loop sequences differ by only a single nucleotide pointing to a difference in these proteins between animals and plants.

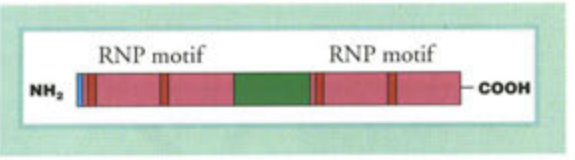

Figure 3 Schematic representation of U2B" protein. The U2B" protein contains two regions which can interact with RNA (RNP motifs), each of which contains RNA binding consensus domains.

A monoclonal antibody against the human U2B" protein has been used to isolate a full length cDNA clone from a potato expression library. This is the first plant snRNP protein gene and only the second U2B" gene to be cloned from any organism. The potato U2B" protein is 231 amino acids long and is 52% identical to that of man. Its overall structure is similar to the human U2B" consisting of two RNA recognition sequences in the N-terminal and C-terminal halves of the protein, each containing RNA binding motifs conserved in a range of proteins known to interact with RNA (Figure 3). Phylogenetically conserved protein domains often reflect conservation of important protein functions and the cloning of plant UsnRNP proteins is of importance in the identification of such regions. Polyclonal and monoclonal antibodies raised against other human snRNP proteins and affinity-purified antibodies raised against yeast PRP fusion proteins are being used to look for analogous proteins in plant nuclear extracts.

snRNP assembly and function clearly is dependent upon interactions between snRNA moieties and proteins, reflecting the importance of RNA-protein interactions in many cellular processes, such as, polyadenylation, transport and storage of mRNAs, and mRNA translation. The cloning of UsnRNA genes and UsnRNP protein genes now provide the tools to analyse the structure of RNA-binding proteins and their specific RNA-protein and protein interactions in snRNP and spliceosome assembly.

Somatic hybridisation of potato by protoplast fusion

S. Cooper-Bland, E. Baird, A. Kumar, M. De, Maine & W. Powell

The ability to fuse somatic plant protoplasts (a plant cell lacking the cell wall) and regenerate somatic hybrids provides an opportunity to overcome evolution imposed barriers to gene exchange. The technique also provides a novel means of combining cytoplasmic genomes (i.e. chloroplast and mitochondrial genomes) in the somatic hybrid products which is difficult to achieve through sexual hybridisation. The technique of somatic protoplast fusion has special significance for potato. The tetraploid and heterozygous nature of potato (*Solanum tuberosum* L. ssp. *tuberosum*, 2n = 4x = 48) coupled with its tetrasomic inheritance, means that conventional genetical analysis is difficult. However, the production of dihaploid potato lines (2n = 2x = 24) provides an opportunity to simplify the identification of useful genotypes. Unfortunately, dihaploid lines are invariably sterile and somatic hybridisation via protoplast fusion is a potentially useful technique for the potato breeder since it offers a way of circumventing any sexual incompatibility between dihaploid lines. It also offers a way of precisely combining genetically well characterised dihaploid genomes and avoiding the meiotic disruption of complementary agronomic traits. The procedure thus brings greater precision to the resynthesis of tetraploid genotypes from previously selected donor dihaploid genotypes. In addition to achieving intraspecific somatic hybridisation of dihaploid lines, the technique can be used for interspecific somatic hybridisation of dihaploid lines with wild *Solanum* spp. possessing useful agronomic and disease resistance traits. Such somatic hybrid plants provide a means of introducing novel sources of genetic variability into the cultivated potato gene pool.

Our research sought to optimise the conditions for regeneration of plants from potato protoplasts and exploit protoplast technology to achieve both inter- and intraspecific somatic hybridisation. A diploid, non-tuber bearing wild potato species, *S. brevidens* (CPC 33brd 2451) possessing resistance to potato leafroll virus (PLRV) and cold tolerance was selected to hybridise with a *S. tuberosum* dihaploid line (PDH 417) possessing resistance to potato cyst nematode (PCN, *Globodera rostochiensis*). For intraspecific hybridisation, a range of dihaploid potato genotypes were identified with a complementary range of agronomic and disease resistance characters. These included resistance to PCN and foliage blight (*Phytophthora infestans*).

Before somatic hybridisation experiments could commence, the conditions necessary to yield uniform protoplasts and regenerate plants required to be defined. It has been shown that if one of the parent lines is a source of regenerable protoplasts (i.e. totipotent) then the character will be expressed as a dominant trait in somatic hybrid cell lines and somatic hybrid plants can be regenerated. Factors which affect the success of protoplast isolation and culture and plant regeneration include the medium used for the maintenance of shoot cultures, enzyme formulation, protoplast purification procedures and

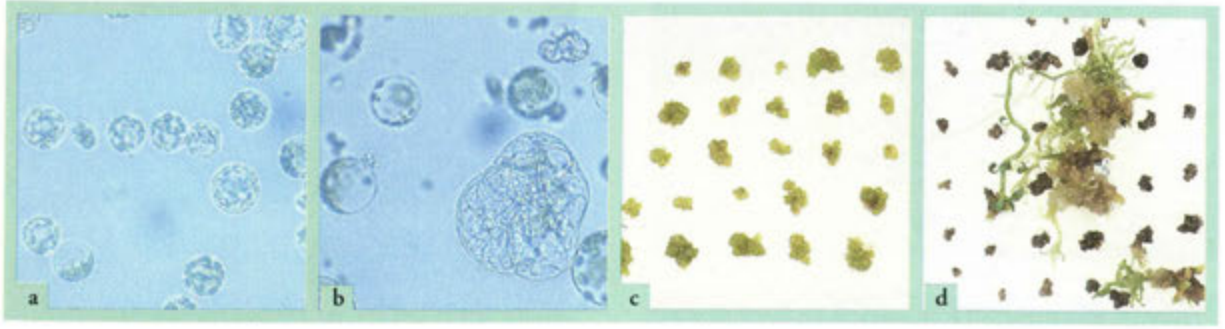

Figure 1 a) Freshly isolated mesophyll protoplasts, b) first protoplast division, c) macroscopic callus, d) shoot regeneration.

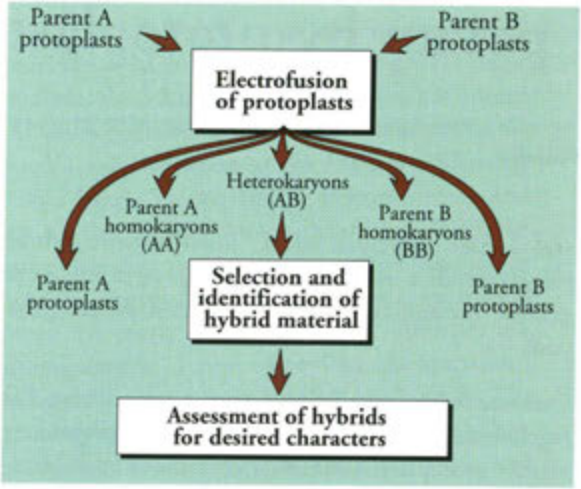

Figure 2 Basic strategy for potato somatic hybridisation.

the sequence of nutrient media containing plant growth substances, and physical conditions such as light and temperature. Of 12 dihaploid lines examined for protoplast isolation and culture, six were totipotent and Figure 1 shows the typical pattern of protoplast isolation, culture and regeneration for all lines. Two of the lines have good tuber shape and yield and are good fusion partners for somatic hybridisation with other lines with disease resistance characters. Up to 2 x 10^6 protoplasts per g fresh wt. of leaf mesophyll tissue could be isolated from these lines; 10-15% of the protoplasts divided, and 15-25% of calli regenerated shoots. In another line, mesophyll protoplasts were isolated readily, divided well and subsequently sustained good callus growth, but the calli did not regenerate shoots or roots. To obtain both intraspecific and interspecific fusions, leaf mesophyll protoplasts were placed in a Zimmermann Electrofusion apparatus. They were aligned by exposing to 30 sec of 100 V/cm a.c. and fused by two 20-40 µsec pulses of 1500 V/cm d.c. Fused protoplasts were plated on a sequence of nutrient media and shoot primordia were induced on a medium containing zeatin. Shoot elongation was encouraged with 6-benzylaminopurine and gibberellic acid and rooting promoted in the absence of plant growth substances or with α-napthylenacetic acid. Plants were obtained 4-5 months after fusion.

The strategy being pursued to create and exploit somatic hybridisation in potato is illustrated in Figure 2. An essential requirement of this approach is the ability to distinguish unequivocally somatic hybrids from unfused or self-fused protoplast regenerants. It was possible to identify interspecific somatic hybrid plants regenerated from the fusion products of a dihaploid *S. tuberosum* and *S. brevidens* using morphological features of the two parents. Three somatic hybrid plants out of five showed intermediate morphological characteristics for the leaf and stem shape, pigments on the stem and leaf (Fig. 3) and colour of flowers. The hybridity of these three somatic hybrids were further confirmed by esterase isozyme electrophoretic profile analyses which showed a summation of the parental profiles (Fig. 4). However, in the case of intraspecific somatic hybridisation none of the morphological characteristics of the two parents can be reliably used to identify somatic hybrid plants. Also, isozyme

Figure 3 Morphology of *Solanum brevidens* x PDH 417 as compared with parent types. *S. brevidens* (top left), PDH 417(top right), *S. brevidens* x PDH 417 somatic hybrid (bottom).

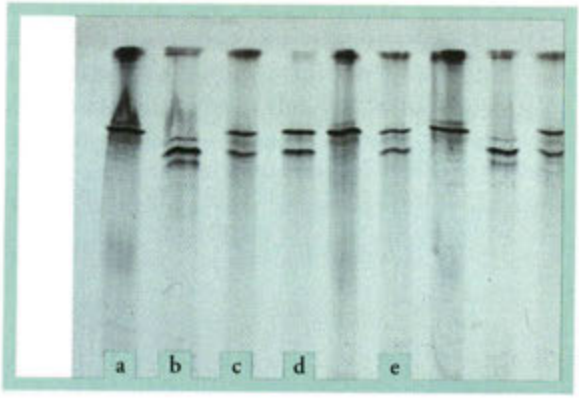

Figure 4 Esterase isozyme gel electrophoretic profiles: Lane a) PDH 417, b) *S. brevidens*, c) *S. brevidens* x PDH 417 somatic hybrid, d) *S. brevidens* x PDH 417 somatic hybrid, e) *S. brevidens* x PDH 417 somatic hybrid.

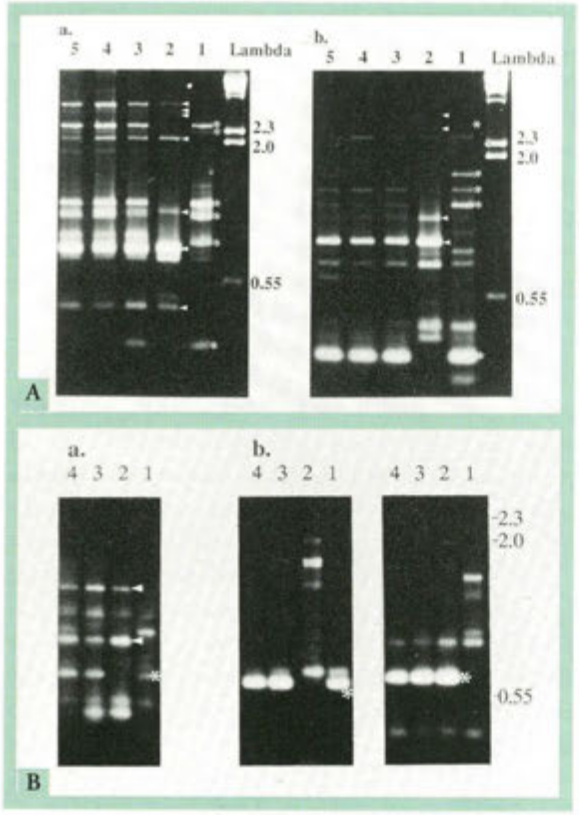

Figure 5 Molecular characterisation of somatic hybrids using Randomly Amplified Polymorphic DNA (RAPDs) markers:
A) Identification of inter-specific hybrids. DNA from *S. brevidens* (Lane 1) and PDH 417 (Lane 2) and 3 somatic hybrids (Lanes 3,4 and 5) were used as template for PCR amplification using (a) primer 1, and (b) primer 4. Products were fractionated on 1.5% agarose gels. Unique dominant amplified bands from *S. brevidens* (*) and PDH 417 (arrows) are highlighted.
B) PDH 40 (Lane 1), PDH 727 (Lane 2) and intra-specific hybrids 7 and 10 (Lanes 3 and 4) analysed with (a) primer 34 which generates dominant polymorphic bands (highlighted) in both parents and (b) with primers 30 and 32 which generate dominant amplification products in 727 and 40 respectively.

analyses and staining isozyme gels for total protein failed to distinguish the parental lines. A recently developed molecular technique termed 'random amplified polymorphic DNA' (RAPD) has proved to be a highly convenient technique capable of providing suitable molecular markers for somatic hybrid identification. This technique uses short nucleotide sequences (10 nt) to reproducibly amplify segments of genomic DNA. Polymorphisms amongst the amplification products can be detected after ethidium bromide staining of an agarose gel. The technique is more convenient to use than RFLP analysis because it uses smaller amounts of DNA and hence less plant material is required. Furthermore, it does not involve the use of radioactivity. The technique has been used successfully to confirm the hybridity of the three interspecific somatic hybrid plants obtained following protoplast fusion between *S. brevidens* and a dihaploid and to identify intraspecific somatic hybrids where no other identification markers have been available previously. The intraspecific and interspecific somatic hybrid plants obtained have molecular profiles which are combinations of the parental lines (Fig. 5).

In conclusion, we have demonstrated that potato dihaploids with complementary biological traits can be resynthesised to form tetraploids. This procedure can be achieved rarely by conventional sexual hybridisation and it promotes greater precision to the assembly of tetraploid genotypes from pre-selected donor dihaploid genotypes. Additionally, we have shown that interspecific somatic hybrid plants between a dihaploid line and a sexually incompatible wild *S. brevidens* can be produced to introduce useful disease resistance genes from wild species into cultivated potato breeding lines. Both the range and the nature of these intra- and interspecific hybrids are unique and provide a valuable genetic resource for both fundamental and applied research.

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Cellular and Environmental Physiology

H.V. Davies

The cellular and environmental physiology programme is geared towards an understanding of the processes and factors which regulate plant growth and development. The majority of the research is strategic in nature, the emphasis on more applied projects having declined in response to a reduction in near-market research. However, external funding has been secured from several bodies to continue specific applied projects, including those which have wider implications for environmental protection. The scientific expertise available in cellular and environmental physiology facilitates an in depth examination of control processes operating at molecular, cellular, tissue, organ and whole plant levels. It also allows the description, quantification and prediction of many biological and physical functions through mathematical biology.

Research in cellular and environmental physiology can be sub-divided into four categories: cell biology, soil-plant dynamics, environmental physiology and weed ecology. Significant and essential complementarity exists between the categories, an essential feature of multidisciplinary research programmes.

Within the Soil-Plant Dynamics group efforts are concentrated on understanding and quantifying the processes by which water and nutrients are supplied to roots from the soil, the extent to which uptake depends on the properties of the root system and the importance of physiological interactions between root and shoot. Investigations on a field-scale have established the magnitude of the variation in water and nutrient supply, the major consequences of such variation for yield and quality of arable crops, and the scale of impact nitrate pollutants have on the environment. A non-disruptive technique has been developed which is demonstrating, for the first time, the influence of root age on the capacity for nutrient uptake in the soil environment. The equivalent of only ten percent of the root system of a wheat plant may be actively involved in nitrate uptake, illustrating the dangers of treating such systems as networks of identical parts. An understanding of the interactions between plant

and microbial systems in nutrient acquisition will continue to be developed by focusing on systems less heterogeneous than the soil environment. The use of individual roots or small root systems in combination with the soil microbes with which they interact will be exploited as model experimental tools. In collaboration with chemists in the Cell Physiology group the effects of root exudates, both qualitative and quantitative, on microbial activity are also studied. Plant age and the extent of root microbial contamination have significant effects on amino acid exudation profiles.

Studies on root development are also underway within the Environmental Physiology group as part of a more extensive programme on drought tolerance in potato. The responsiveness of several plant parameters to water stress have been determined and are now being used to assess the combinations of characters conferring drought tolerance. The relative importance of shoot and root in determining the response to water-stress has been examined using grafts between cultivars contrasting in rooting characteristics. Drought increases the proportion of assimilate partitioned to the root system and the response is related to the genotype used to provide the shoot in the graft i.e. it is "top-driven". The levels of leaf hydration (relative water content) associated with leaf death differ between genotypes. The influences of shoot and root on the diurnal cycle of plant water status, stomatal conductance and photosynthesis are under scrutiny.

Water and nutrient supply tend to go hand in hand, particularly where elements such as nitrogen are concerned. The provision of adequate supplies of nitrogen to crop plants is essential if productivity is to be optimised under prevailing environmental conditions. Near-infra-red reflectance spectroscopy (NIR) techniques have been developed for the rapid assessment of the nitrogen content of potato plants. Other, more rapid methods, are being validated. This will assist decision making on the quantity of nitrogen to be added to optimise yield, thereby reducing potential pollution problems. The soil itself can provide substantial quantities of nitrogen from organic matter, emphasising the importance of analysing the population dynamics of, and interactions between, soil microflora and microfauna and the subsequent effects of these interactions on nutrient cycling. For example, the branching of saprophytic fungal colonies has been shown to be fractal in nature.

The Soil-Plant Dynamics and Environmental Physiology groups have an involvement in revealing the consequences of climate change for future Scottish agriculture, and in co-ordinating related research activities funded by SOAFD.

The Weed Ecology group has continued to increase its activities in weed population dynamics, amply supported by outside contracts to research the effects of rotational and permanent set aside management, reduced herbicide inputs into cereal rotations and organic farming on seed populations in the soil. Appropriate methods for statistical analysis of seed-bank data are being investigated and will be used to assess the long-term consequences of proposed alternative crop and field management strategies. Results to date indicate that, from the weed seedbank point of view, reducing herbicide usage by halving the dose of a conventional, comprehensive cereal weed control programme is more effective in avoiding an explosion in weed-seed populations than strategies for treating weeds only if, and when, they attain specific threshold densities. Research to quantify the effects on seed potato crops of accidental contamination with cereal herbicides has shown that a widely used sulphonylurea cereal herbicide has adverse effects on tuber number, shape and quality at as low as 0.5% of the normal field dose. A problem-solving herbicide database, Microherb, was launched in 1990 as a joint venture between SCRI, Queen's University Belfast and DANI. In the continuing search for an alternative to dinoseb for raspberry cane desiccation, two chemicals, sodium monochloracetate and fomesafen, have progressed to final stage trials.

Within the Cell Physiology group a programme has been initiated on the uptake and transport of foreign (xenobiotic) molecules, including herbicides, by higher plants. A novel xenobiotic anion transporter has been discovered, located on the vacuole membrane of certain higher plant cells, which may assist in sequestering potentially toxic molecules from the cytoplasm. An alternative transport system, fluid - phase endocytosis, has also been demonstrated. It is induced by a rapid plasmolysis/deplasmolysis cycle in both onion epidermal cells and pollen grains. The possibility of exploiting this phenomenon to introduce biologically active molecules into the embryo sac, using the pollen grain as a vector, is under examination. Another technical innovation has been the development of a pressure micro-injection system, allowing measurement of cell turgor whilst studying, concurrently, the transport of fluorescent probes. Cell turgor plays a central role in the uptake of sucrose and its conversion to starch in potato tubers. The enzyme sucrose synthase,

which catalyses the initial breakdown of sucrose in developing tubers, has been shown to be sucrose inducible in this tissue. The enzyme fructokinase, which catalyses the specific phosphorylation of fructose released from the action of sucrose synthase has been purified, its kinetic properties analysed and antibodies raised to the protein. Similarly, acid invertase and alkaline inorganic pyrophosphatase have been purified and N-terminal sequences and antibodies obtained. The purification of proteins involved in carbohydrate metabolism has proceeded with a view to cloning the relevant genes, producing transgenic plants and quantifying the effects of modifying gene expression on biochemical pathways. Nuclear magnetic resonance spectroscopy (NMR) has been used extensively to develop an understanding of the transport properties of the amyloplast membrane. Methods for non-destructive monitoring of metabolic pathways continue to be developed. They will be used to address the problem of seed dormancy in woody species as part of a new initiative. The latter includes an examination of molecular changes associated with dormancy break at low temperature. These techniques have been used with great success during the past year to isolate genes which are highly expressed only during the period of tuber formation in potato tubers. Isolation of such genes provides the opportunity to explore the molecular control of differentiation. Although new isoforms of alpha and beta tubulins appear on tuber formation, the genes isolated by differential hybridisation have little homology with sequences currently available in data banks.

The sink to source transition in potato tubers

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

Carbon compounds, manufactured in the leaves of green plants by photosynthesis, are moved continuously from their sites of production in the leaves (the 'sources') to sites of utilisation or storage (the 'sinks'). In nearly all crop species, sucrose is the major transported carbon compound, reaching the sinks by a specialised long-distance transport system, the phloem. In several crops the sinks (eg. roots, tubers or grains) perform predominantly a storage function within which the sucrose, and other transported carbon compounds such as amino acids, are converted into insoluble, polymeric forms such as starch and protein.

There has been considerable worldwide interest in determining the basic factors which regulate the fluxes of sucrose throughout a plant, a major aim being to manipulate both the rate and direction of carbon flow. One of the major disadvantages in attempting to study sugar transport within a single plant is that the

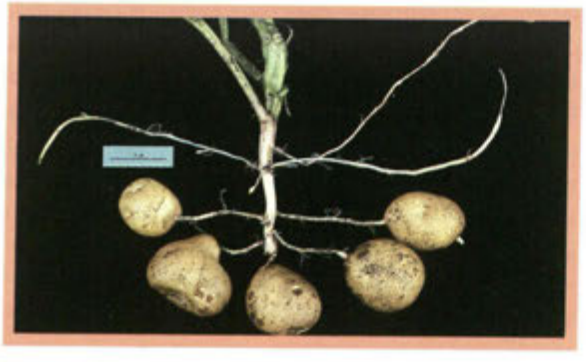

Figure 1a Growing (sink) potato tubers. The tuber receives sucrose via the phloem of the connecting stolon.

Figure 1b Sprouting (source) potato tubers. The sprouts receive sucrose via the phloem of the parent tuber.

sources and sinks are often both spatially separated and anatomically different, making the mechanisms involved in sugar transport extremely difficult to compare. Within a single potato tuber, however, a sink to source transition occurs with no apparent change in internal anatomy. During this transition the major direction of carbon flux within the tuber completely reverses. The potato tuber therefore provides a unique system within which to study the basic transport processes involved in sugar transport. This article summarises the results of several experiments aimed at elucidating the basic cellular changes accompanying the sink to source transition in the potato tuber.

The tuber as a sink In rapidly growing potato plants sucrose is continually transferred from the shoots to the growing tubers where it is converted rapidly into starch in the large storage parenchyma cells of the central (perimedulla) region of the tuber. Indeed, the growing potato tuber can be considered as a massive storage sink in which starch represents about 70% of the final tuber dry matter (Fig.1a).

The tuber as a source Following shoot senescence (sometimes induced prematurely in 'early' crops by burning down the shoots) the tubers cease growth and a period of dormancy ensues. Under appropriate conditions sprouting may follow. During sprouting the tuber functions as a source of carbon, transferring sucrose from the storage parenchyma cells, via the intricate phloem network of the tuber, to the developing shoots (Fig. 1b). The major direction of carbon flux within the tuber is consequently reversed during the sink to source transition. Anatomical evidence indicates that starch mobilisation occurs initially around existing vascular regions of the perimedulla, the same tissue in which 'unloading' of sucrose from the phloem predominated in the growing tuber.

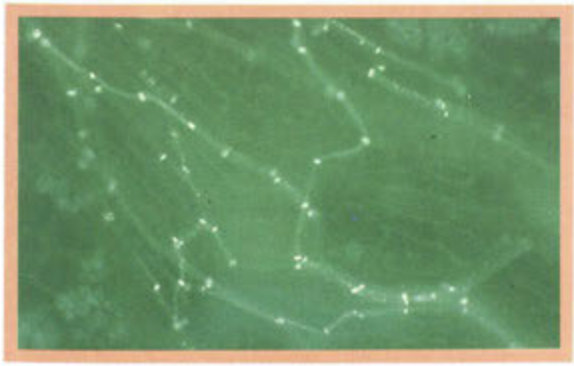

Figure 2 Sucrose is unloaded from the internal phloem network, shown here as anastomosing strands under the fluorescence microscope.

The transport characteristics of sink tubers The sucrose translocated into the growing tuber arrives via the stolon in the phloem. In the perimedulla the phloem branches out to form an anastomosing network with the storage parenchyma cells never more than a few cells away from an internal phloem strand (Fig.2). We have studied the ultrastructure of this short-distance pathway and also the factors which influence the transport of sucrose along it.

It was important to determine whether the sucrose delivered to the storage cells was moving in the apoplast (the continuum of cell walls throughout the tuber) or the symplast (the interconnected protoplasts of cells, functionally joined across the walls by specialised microchannels, the plasmodesmata) since the pathway of transport determines, to a large extent, the subsequent fate of the imported sucrose.

An ultrastructural study of the types and frequencies of plasmodesmata connnecting the phloem to storage cells revealed the presence of a continuous symplastic pathway. This is shown diagrammatically in the form of a 'plasmodesmagram' in Figure 3 in which the relative frequencies of plasmodesmata traversing different cell types are revealed.

Additional, more direct, evidence that assimilates may move from cell to cell via plasmodesmata was obtained by microinjecting membrane-impermeant

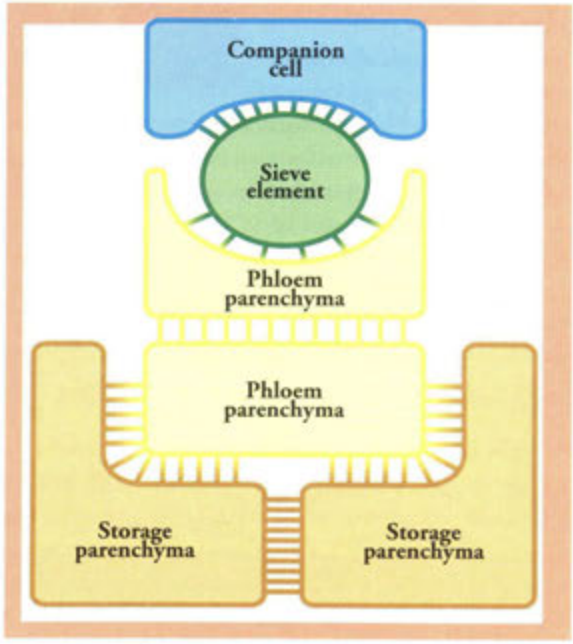

Figure 3 Schematic representation ("plasmodesmagram") of the plasmodesmatal frequencies between the sieve element and contiguous tissues (courtesy A.J.E. van Bel).

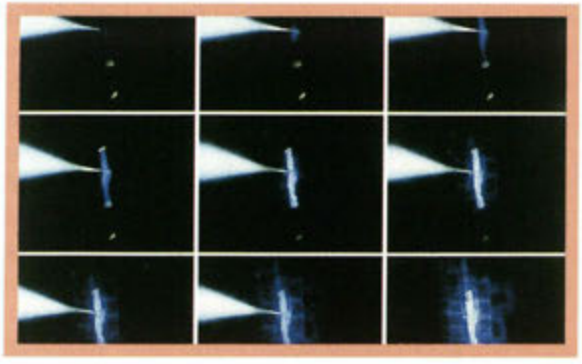

Figure 4 Microinjection of the fluorescent membrane-impermeant probe cascade blue into a single sink sieve element. The dye has spread via plasmodesmata to adjacent cells.

fluorescent probes directly into sink sieve elements and observing their subsequent intercellular spread into adjoining parenchyma elements using fluorescence microscopy. This approach involved the construction at SCRI of a microinjection facility, specifically with the aim of studying the permeability characteristics of plasmodesmata. A typical microinjection sequence into a sink sieve element (the individual conducting elements of the phloem) is shown in Figure 4. In this instance the impermeant probe Cascade Blue subsequently spread both longitudinally into other sieve elements and also laterally into adjoining parenchyma cells. The approach of microinjection is also being used at SCRI to study the influence of virus infection on plasmodesmatal conductance (see...) and a recent technical modification has incorporated an in-line micro pressure probe, allowing the turgor pressure of cells to be monitored simultaneously with the microinjection of probes.

In vivo physiological approaches also provided evidence for symplastic transport in the sink potato tuber. In one series of experiments two adjacent solute-collecting wells were made into the phloem-rich perimedulla of the tuber, one acting as the treatment containing a variety of solutions such as metabolic inhibitors and the other as the control containing buffered osmotica only. Following the formation of the wells, the shoot was pulse labelled with $^{14}CO_2$ and the arrival of ^{14}C sucrose into the collecting wells was monitored by sampling the well contents at regular intervals (Fig.5). The results of a typical experiment are shown in Figure 6 in which the treatment wells contained a plasmolysing solution (800mM sucrose or mannitol), and the control well aqueous buffer only. Plasmolysis of the storage cells (severing plasmodesmatal connections between cells) greatly restricted the entry of ^{14}C sucrose into the wells, providing further evidence that the sucrose delivered to the storage cells followed a symplastic pathway. Chromatographic analysis of the solutions entering the wells revealed that sucrose was not broken down by the enzyme invertase during its transit to the storage cells. As a result of several experiments, we concluded that the transport of sucrose could occur passively in the absence of metabolic energy and symplastically in the growing 'sink' tuber, the continuing conversion of sucrose to starch in the cytosol of the storage cells maintaining the necessary concentration gradient to allow 'downhill' sucrose unloading from the phloem.

Figure 5 Sampling of solute-collecting wells in a growing potato tuber.

Turgor-sensitive starch synthesis In order to separate transport events occurring in the pathway leading to the storage cells from those occurring within the storage cells themselves, we began a series of *in vitro* experiments examining the ability of isolated storage cells to take up sucrose and subsequently convert it to starch. Initially, we found that sucrose

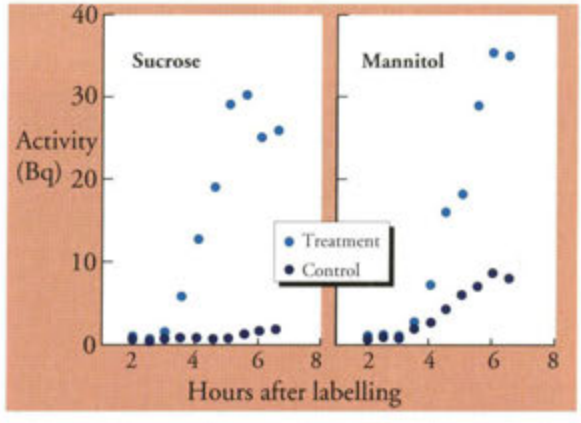

Figure 6 Efflux of ^{14}C solutes into solute collecting wells is severely inhibited by 800mM sucrose and 800mM mannitol. Efflux into control wells is unaffected.

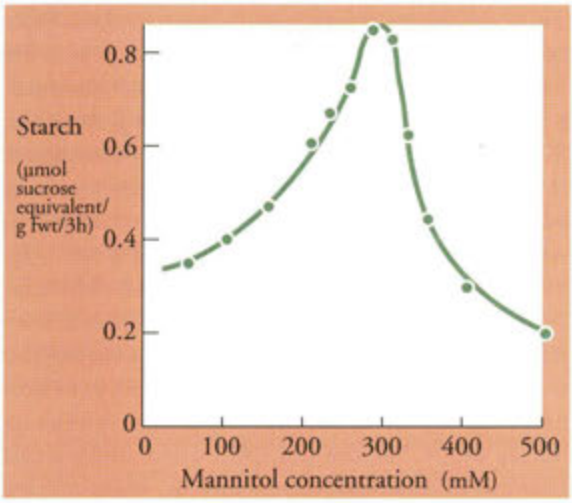

Figure 7 Turgor-sensitive starch synthesis in sink potato tubers.

was a poor substrate for starch synthesis unless the external osmotic environment was adjusted to 300mOsmol with mannitol. Under these conditions the storage cells showed considerably enhanced uptake of sucrose across the plasmalemma, with a concomitant increase in the rate of starch synthesis. In one experiment, the ability of storage cells to convert sucrose to starch was examined over a wide range of external mannitol concentrations. Starch synthesis showed a distinct osmotic optimum at 300mM mannitol (Fig. 7). We now believe that enhanced sucrose uptake and starch synthesis arise as a result of the stimulation, by reduced turgor pressure, of active, carrier-mediated sucrose transport at the plasmalemma. This affect appears to be mediated via the plasmalemma H^+-ATPase, which acts as an osmotically sensitive pump at low turgor pressure. The turgor sensitivity of starch synthesis declines gradually as tubers age and is essentially absent from mature, harvested tubers.

Sink isolation experiments In order to terminate suddenly the import of carbon into the sinks we detached half of the tubers in a population of plants at the stolon using a razor blade, thereby simulating the effects of early 'burn down'. The cessation of sucrose import into the tubers induced a rapid (<20h) loss in the turgor sensitive sucrose-uptake component when discs from the treated tubers were subsequently incubated in ^{14}C sucrose *in vitro* (Fig. 8). Sink isolation also greatly inhibited the capacity of the discs from excised tubers to synthesise starch *in vitro*. However, the ability of the isolated storage cells to take up sucrose across the plasmalemma was completely unaffected for up to 7d following sink removal. Thus, starch synthesising capacity, rather than the ability of the plasmalemma to transport sucrose, was lost rapidly following sink isolation. Measurements of the levels of several enzymes involved in carbohydrate metabolism showed that predominantly sucrose synthase, recently shown at SCRI to be a sucrose-inducible enzyme in potato tubers, was severely inhibited by sink removal, providing evidence for the hypothesis that this particular enzyme may be an important determinant of sink strength. In experiments in which the hexoses glucose and fructose (sugars which effectively bypass sucrose synthase) were supplied to tuber discs in parallel with sucrose, rates of conversion of glucose and fructose to starch were vastly greater than with sucrose, providing additional evidence that sucrose synthase activity may limit the rate of conversion of sucrose to starch.

The transport physiology of source tubers When dormant tubers were allowed to sprout in the dark, reserve mobilisation occurred from regions surrounding the internal phloem strands. Isolated storage discs from the perimedulla of sprouting (source) and growing (sink) tubers were supplied with exogenous sugars in parallel. In marked contrast with sink-tuber discs,

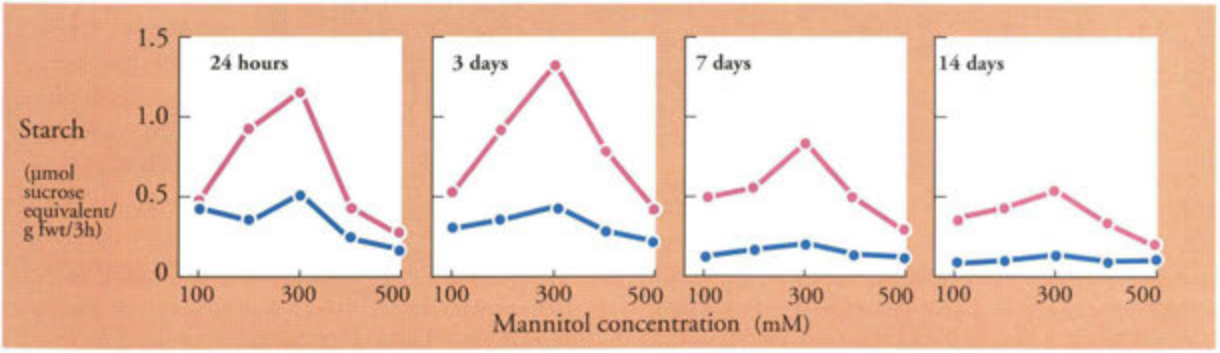

Figure 8 Decrease in turgor-sensitive starch synthesis following sink isolation. The tubers were detached from their stolons (sink isolation treatment - blue lines) and examined for their ability to convert sucrose to starch relative to control tubers (pink lines) at 24 h, 3 d, 7 d and 14 d following sink isolation.

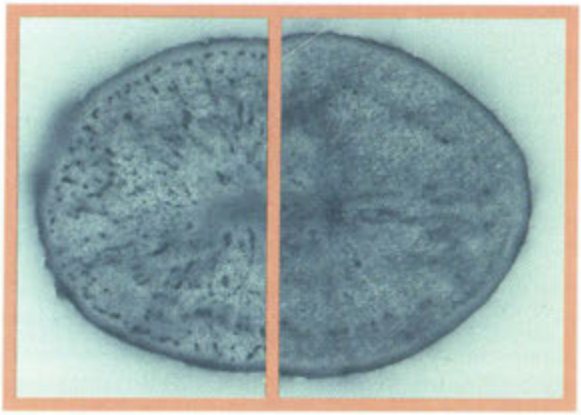

Figure 9 Inhibition of phloem loading by PCMBS, a blocker of the membrane-bound sucrose carrier protein. (a) Control (on the left) shows loading (dark areas) of the internal phloem strands. (b) On the right, PCMBS eliminates loading leaving only background, diffusional, sucrose uptake.

uptake of sucrose into the storage parenchyma of source-tuber discs was essentially diffusional and was insensitive to both turgor pressure and a range of metabolic inhibitors. It appears that as the tuber ages the sucrose-uptake capacity of the storage parenchyma cells decreases and their ability to synthesise starch is gradually lost. As shown above, both these changes can be accelerated rapidly by cutting off the sucrose supply by sink isolation.

Thus, the storage parenchyma of source tubers differ markedly in their membrane transport properties from those of sink tubers. Furthermore, a major transport change occurs at the phloem of source tubers. When slices of sprouting potato tubers were incubated in solutions containing ^{14}C sucrose the resulting autoradiographs showed evidence for considerable ^{14}C accumulation at the sites of the internal phloem (Fig. 9a). However, if the adjacent tuber slice was first incubated in the sulphydryl compound PCMBS, a known inhibitor of the plasmalemma sucrose-carrier protein, then loading of the internal phloem strands was eliminated and the tissue showed only general diffusional uptake of ^{14}C sucrose (Fig. 9b). These experiments provided evidence that sucrose loading, unlike sucrose unloading, occurs across the plasmalemma of the sieve element-companion cell complex. That is, source potato tubers load from the apoplast, not the symplast.

A model for the sink to source transition The model shown in Figure 10 is derived from several anatomical and physiological studies of the pathways of sucrose transport in sink and source tubers and incorporates a number of the features addressed in this article.

In the sink tuber sucrose is unloaded symplastically and continues to move via plasmodesmata until it enters the cytosol of the storage parenchyma cells. Here it is cleaved by sucrose synthase. The remainder

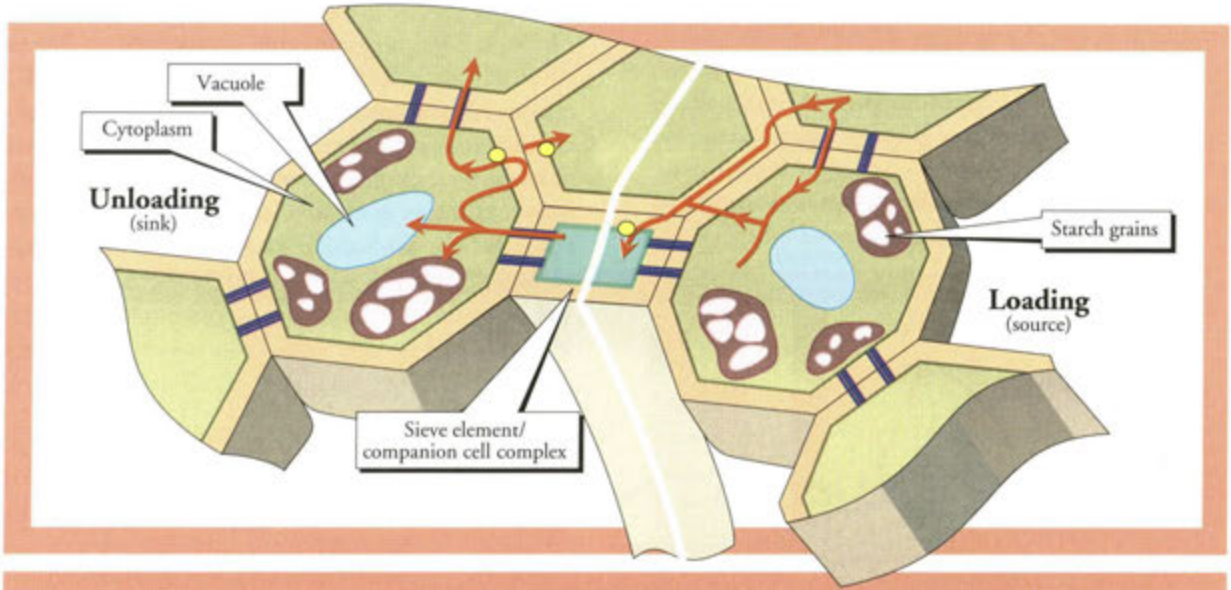

Figure 10 Schematic model for the sink to source transition in the potato tuber. Note that unloading the sink tissue occurs via plasmodesmata, followed by sucrose storage in the vacuole and starch synthesis in the amyloplast. The active sucrose transport system on the plasmalemma functions as a retrieval mechanism for sucrose escaping to the apoplast. Loading in the source tissue occurs via the apoplast and across the sieve element plasmalemma. Inactivation of the retrieval mechanism on the storage cells allows diffusion to the apoplast.

of the sucrose enters a vacuolar storage pool which is constantly turning over. The turgor-sensitive, active transport mechanism on the plasmalemma of the storage cells functions as a retrieval mechanism for sucrose which may potentially escape by diffusion to the apoplast. That is, the cell has to expend energy to maintain sucrose in the cytosol. We have suggested that this retrieval pump may also function to regulate cytosolic sucrose levels in response to the changing diurnal turgor pressure of the storage cells. The net result of the unloading pathway in sink tubers is that sucrose is predominanly maintained in the cytosol.

In source tubers degradation of starch occurs and cytosolic sucrose levels build up. The retrieval pump is no longer active on the storage-cell plasmalemma. Sucrose may move from cell to cell via plasmodesmata but is also now free to diffuse to the apoplast. From the apoplast the sucrose can be actively loaded into the phloem by sucrose-H^+ co-transport, ie. across the plasmalemma of the sieve element-companion cell complex, a process that is sensitive to the compound PCMBS (see Fig. 9b).

According to the above hypothesis the major change which occurs during the sink to source transition is in the predominant sucrose transport pathway (apoplast v symplast) which prevails at any one time. Implicit for the above hypothesis to operate, however, is a fundamental change in the plasmodesmata connecting the phloem with surrounding tissues, allowing them to function during phloem unloading but to be sealed (or inactivated) during phloem loading. Future attention will be paid to the permeability properties of plasmodesmata connecting the phloem with contiguous tissues.

Calcium and physiological disorders in potato tubers

H.V.Davies.

The potato tuber is susceptible to many diverse types of defects. In addition to infectious diseases, nematodes and insects, potato plants and tubers can also be affected by a large number of non-infectious physiological disorders. These are often referred to as non-pathogenic, non-parasitic or abiotic disorders. It is probably more appropriate to regard them as physiological disorders since causal factors include improper cultural practices, soil moisture and fertiliser regimes and other physical or chemical factors that modify the normal growth and development of potato plants and tubers. It follows that the severity of specific disorders will vary from year to year and from growing site to growing site. Disorders may be visible externally, which makes it relatively easy to reject affected tubers. Some disorders are only visible once the tuber is cut, sliced, peeled, cooked or processed into fried products. A high incidence of any physiological disorder in the crop will affect its value for both the fresh market and processing outlets. As it is imperative that the highest quality of potato is available to the end user, for whatever purpose, an understanding of the factors and processes which give rise to the problems is imperative if a solution is to be found and the overall quality of the potato improved for both home and overseas markets.

Internal Rust Spot - a calcium related physiological disorder Internal Rust Spot (IRS) is a physiological disorder of potato tubers which is characterised by rust coloured lesions, of various size, in the medullary tissues internal to the vascular ring. Lesions are generally more pronounced towards the apical end of the tuber. The disorder is induced by several environmental factors including restricted or irregular supplies of water, high temperatures, or by conditions which are generally unfavourable to uniform rates of tuber growth. For example, a high incidence of IRS has been reported in cv. Pentland Squire grown in Bologna, Italy and in cv. Arran Banner grown in the Lebanon. As to the basic underlying cause of IRS, there is a strong body of evidence implicating a role for calcium. Although many soils have a high calcium content, calcium ions are transported within the plant primarily in the xylem stream, a high phosphate con-

tent precluding substantial transport within the phloem network. Water potential gradients within the potato plant favour xylem transport into the foliage, which has a high calcium content. Tubers, however, are likely to receive most of their water along with sucrose in the phloem mass-flow stream. Thus tubers have a very low calcium content by comparison with leaves, a feature shared by apples and tomatoes which are also susceptible to calcium-related disorders (bitter pit and blossom end rot). Higher rates of transpiration in hot, Mediterranean-type climates would be expected to exacerbate the problem of water potential gradients, and hence the partitioning of xylem-mobile elements between leaves and tubers. The low calcium content of tubers may be considered marginal for maintaining cellular integrity and normal functioning. A decrease in supply at critical stages in development clearly results in a loss of subcellular compartmentation and ultimately cell death.

It is possible to induce IRS by growing plants in an inert medium supplied with a controlled combination of nutrients. If, when tubers are approximately 5 cm diam., the concentration of calcium in the nutrient medium is reduced from 20 mol/m^3 to 1 mol/m^3 and tubers supplied with the lower concentration for the rest of their growing period, symptoms of IRS are induced. If, however, the supply of calcium is reduced around tuber initiation, cell necrosis occurs in the centre of the tuber rather than in the adjacent medullary tissues. This produces a disorder known as Brown Centre. Brown Centre is common in the cultivar Russett Burbank, grown predominantly in the USA to provide French fries for the major fast-food outlets. It is not commonplace in cultivars commonly grown in the UK. It would appear that calcium nutrition can be implicated in both disorders but that the timing of the stress determines which symptoms develop. There are reports that application of calcium sulphate to field-grown crops reduces the incidence of calcium-related disorders, although when all data are taken together the results are not conclusive. It is known that the development of IRS occurs during the rapid phase of tuber growth, affected areas often developing a translucent appearance before cell death occurs. The translucence results from the breakdown of stored starch.

Figure 1 Effect of restricting the supplying of calcium to growing tubers of Désirée (on the left) and SCRI clone 10337de40 (on the right). Brown lesions are termed Internal Rust Spot .

Distinct genotypic variation exists in the susceptibility of tubers to IRS. Figure 1 shows the differential response of cv. Désirée and the SCRI clone 10337de40 to a reduction in calcium supply during tuber development. Désirée is highly resistant to IRS, the clone highly susceptible. When the effect of genotype is examined in more detail a range of susceptibilities is found (Fig. 2). For example, in addition to Désirée, cvs. Ailsa and Maris Bard appear resistant, whilst cvs. Pentland Squire, Arran Banner, Kirsty and the SCRI clone 15075 are susceptible. Both of the SCRI numbered clones also develop a high incidence of IRS when grown under standard field conditions. This range in susceptibility can be exploited to examine the physiological and biochemical bases of resistance. One obvious feature, examined at an early stage in the research programme, was the effect of tuber calcium content on the incidence of IRS. Within any single cultivar a reduction in tuber calcium content is

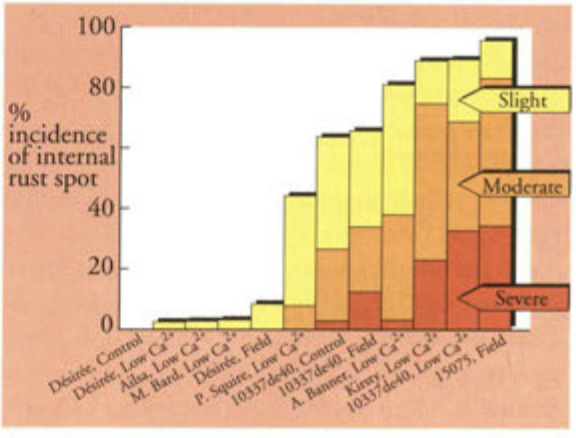

Figure 2 The incidence of IRS in tubers of potato genotypes grown in vermiculite with controlled calcium nutrition.
Control = calcium maintained at 20mM throughout tuber development. Low calcium = calcium supply reduced to 1mM after tuber formation.
The incidence of IRS in field grown tubers of certain genotypes is also shown.

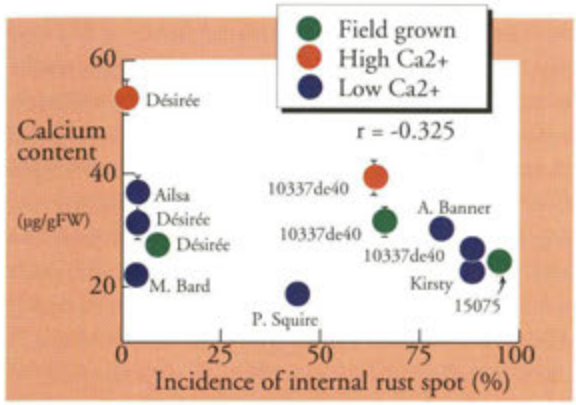

Figure 3 The relationship between tuber calcium content and incidence of IRS in a range of cultivars.

associated with an increase in IRS. However, by comparing the response of several cultivars it is evident that there is no clear threshold below which the disorder is induced. For example, a calcium concentration of 30 µg/g fresh weight may be associated with a 0% or 90% incidence of IRS, depending on cultivar (Fig. 3). Across the cultivars used the correlation (r) between calcium content and percentage incidence of IRS was -0.33 (Fig. 3). Poor correlations are also found when the concentration of other elements (sodium, magnesium, phosphorus, potassium, boron, aluminium, manganese, iron, cobalt, copper, zinc, strontium, molybdenum and barium) are related to the severity of IRS.

Subcellular localisation of calcium One of the problems faced when attempting to relate calcium content to the severity of calcium-related disorders is that calcium can be present in both physiologically active and inactive forms. Active forms include not only the free ion but also the calcium salts of organic acids, chlorides and nitrates and calcium reversibly bound to proteins and pectins. Inactive forms would include the insoluble phosphate, oxalate, carbonate and silicate. The majority of calcium in the tuber appears to be easily extracted indicating that a high proportion is in a soluble and, potentially active, form. By growing tubers in the presence of radioactive ^{45}Ca and localising its distribution using micro-autoradiography it has been possible to confirm that there are few cells which contain calcium oxalate, a potentially inactive form of calcium. Most of the calcium appears to be located within the vacuole, probably as soluble salts. At the subcellular level the distribution of calcium has been localised by electron microscopy by forming antimonate complexes with the ion. This has shown that calcium deposits are also associated with the vacuole membrane and with mitochondria and plastids (possibly as phosphate precipitates). It has also revealed that calcium is restricted mostly to companion cells and phloem parenchyma but that it occurs to a lesser extent in sieve elements. This agrees with information obtained from tubers grown in the presence of ^{45}Ca. Localised "hot-spots" of radioactivity are associated with regions occupied by both phloem bundles and xylem tissue. The intense labelling in the region of phloem bundles may be due to the formation of insoluble calcium phosphate. However, one cannot completely discard the possibility that a proportion of tuber calcium is derived from phloem transport. The question of what proportion is derived from phloem and xylem transport or from uptake directly from the soil solution through the tuber surface is an important one, but has not been resolved adequately. It has been suggested that stolon roots, located very near the tuber, play an important role in supplying tubers with calcium.

Neither the antimonate precipitation procedure nor the experiments with ^{45}Ca revealed significant quantities of calcium associated with the cell wall (calcium pectate). This is surprising as a considerable proportion of calcium in plants is believed to be wall-bound. The techniques for subcellular localisation of calcium have not yet been applied to cultivars susceptible or resistant to IRS. Variation in susceptibility cannot, therefore, be attributed to differences in subcellular compartmentation. However, the concentration of calcium in the apoplast of cells from a range of genotypes grown under low and high calcium supplies has been determined using a micro-electrode system. Whilst apoplastic concentrations decrease in all cultivars examined following a restriction in calcium supply, there is again no relation between absolute concentration and susceptibility to IRS.

Plant cells contain a calcium binding protein known as calmodulin, which, through its interaction with free ionic calcium can bring about substantial changes in cell functioning. In potato tubers grown under a low supply of calcium the calmodulin content is increased and the proportion of membrane - bound calmodulin disproportionately so. Also, of four genotypes examined, the two IRS-susceptible genotypes contained the lowest calmodulin levels. Thus, in a low calcium environment, resistant genotypes should be more sensitive to gating reactions as far as calcium metabolism is concerned. In a collaborative project with the University of Edinburgh, molecular techniques are employed to manipulate the expression of the calmod-

ulin gene in potato tubers. The importance of calcium-calmodulin interactions to a range of developmental and metabolic processes as well as to the development of physiological disorders can then be assessed.

Antioxidant status and susceptibilty to IRS Calcium has an important function in maintaining membrane integrity. Disruption of cellular organisation caused by a loss of integrity when calcium supply is limiting would allow uncontrolled chemical reactions to occur. These include deleterious oxidative reactions arising from metabolic processes within the cell. Since it is likely that such reactions would proceed via free radical processes, electron spin resonance (ESR) has been used to examine the lesions which typify IRS. This has shown that tissue necrosis is accompanied by the production of a free radical signal, an increased intensity of Fe^{3+} features, and a decrease in the intensity of Mn^{2+}. Changes in metal signals can be explained by oxidative reactions, with Fe^{2+} in healthy tissue being oxidised to Fe^{3+} and Mn^{2+} to either Mn^{3+} or Mn^{4+}. The formation of IRS appears to be primarily the result of metal ion oxidation (largely Fe^{2+} to Fe^{3+}) and is accompanied by some free radical production. The possibility of a more significant role for organic free radicals cannot be discounted because of mutual annihilation of free radicals during polymerisation reactions.

Calcium has a significant effect on membrane fluidity. It is conceivable, then, that the onset of IRS is associated, primarily, with the integrity of the plasma membrane and the endomembrane system. Loss of integrity would pre-dispose the cell to metabolic disturbance, upsetting the balance between oxygen free radical production and quenching by endogenous antioxidants. This could lead to the observed oxidation of metal and organic components within the cell resulting in cell death. This sequence of events would provide one explanation as to why individual cells may be affected by IRS whilst neighbouring cells remain unaffected.

The occurrence of uncontrolled oxidative reactions is exacerbated by the presence of iron, which exists in significant quantities in potato tubers. Low molecular weight iron complexes with the ability to catalyse the formation of $OH^{\cdot}$ from O_2 and H_2O_2 are thought to exist within the cell. Normally this reaction is limited by the presence of antioxidants such as ascorbic acid (vitamin C), α-tocopherol (vitamin E) and enzymes such as superoxide dismutase, catalase and peroxidases. These control the intracellular levels of O_2 and H_2O_2. Production of $OH^{\cdot}$ from O_2 and H_2O_2 results in redox cycling between Fe^{2+} and Fe^{3+} oxidation states, explaining the large increase in Fe^{3+} in necrotic tissue. A detailed examination of the membrane lipid free fatty acid and sterol composition of an IRS - susceptible and resistant genotype grown under both low and high calcium regimes has shown little difference in membrane composition between treatments and cultivars. This does not suggest that calcium stress is likely to affect membrane fluidity differentially in these genotypes. A comparison has also been made of the permeability properties of discs of tuber tissue isolated from a range of genotypes grown under high and low calcium supplies. Again there is no indication that membranes of IRS-susceptible types become more "leaky" (i.e. lose their integrity), more severely than resistant types.

In conjunction with the screening of IRS-affected tissues with ESR, the enzymatic and non-enzymatic antioxidant status of cv. Désirée (IRS-resistant) and the SCRI clone 10337de40 (IRS-susceptible) have been compared. Ascorbic acid is present in significant concentrations in both genotypes and there is no difference in the activities of ascorbate free radical reductase. The activity of the second ascorbate re-cycling enzyme, dehydroascorbate reductase is more than 100% higher in the resistant cultivar, however. Thus, Désirée has an apparently greater potential for regenerating ascorbate, although this is not reflected in the ascorbate : dehydroascorbate ratio. Levels of glutathione are also significantly higher in Désirée and a greater proportion of glutathione is recovered in the reduced, functional (for antioxidative purposes) form than in the clone. This correlates with a glutathione reductase activity almost 50% higher in Désirée. Glutathione is, like ascorbate, found in the cytosolic compartment, but can be compartmentalised within various subcellular organelles. It will recycle ascorbate as well as scavenging oxygen radicals. The compound α-tocopherol is a lipid-soluble antioxidant and occupies a key position in the defence against harmful oxidative reactions. It is the only known phenolic compound that will specifically guard against peroxidative damage within membranes. The level of α-tocopherol is low in both genotypes, re-enforcing the position that the potato tuber is, with regard to membrane lipids, likely to be susceptible to oxidative damage. Of the enzymic antioxidants, peroxidase, but more significantly superoxide dismutase (SOD) activity, is higher in Désirée than in the susceptible clone.

Quantitative changes in SOD have been determined for a range of cultivars grown under high and low calcium regimes. A negative correlation ($r > -0.73$) has been found between the incidence of IRS and the maximum catalytic activity of the enzyme. Isoelectric focusing of SOD isozymes extracted from ten potato cultivars has shown that three major isozymes are common to all genotypes but that qualitative and quantitative variation exists in other isoforms. Three of the four IRS-resistant cultivars, Shelagh, Moira and Désirée, possess the maximum number of isoforms, whilst susceptible cultivars Cara and Teena possess the lowest number. There is, therefore, some relation between SOD phenotype and susceptibilty to necrosis but, as with total enzyme activity values, the relation is not exact.

In conclusion, the physiological disorder IRS can be induced by manipulating the supply of calcium to developing tubers. A controlled nutrient environment has been developed for assessing the extent to which cultivars are resistant. Genotypes with an improved capacity for dealing with deleterious oxidative reactions appear to have an improved resistance towards IRS, but there is no evidence that membrane lipid and sterol composition affects resistance. Apart from the role of calmodulin in modulating the response to a restricted calcium supply, one other avenue remains to be tested. This is that cultivars differ in the capacity to synthesise the enzyme polygalacturonase, an enzyme regulated by calcium and which assists in the degradation of plant cell walls.

Micro- and minitubers in potato genetics and production

D.K.L. MacKerron, M. Coleman, & B. Marshall

A range of techniques, known collectively as tissue culture, have been developed recently to multiply and manipulate plants. In these techniques, plants are generally grown aseptically *in vitro* in artificial, controlled environments. At the most basic level small pieces of undifferentiated tissue, called callus, can be grown from single cells, but a callus then needs special treatment with mixtures of plant growth regulators (PGR) if it is to develop recognizable organs. A faster and more reliable method is to use small pieces of meristematic tissue from which small intact plants can be grown.

The potato is well-suited to micropropagation because the development of the vegetative shoot is extremely flexible. Under suitable conditions, e.g., low temperatures and less than 8h light, each axillary bud has the potential to differentiate to form a tuber. PGR and the supply of sucrose to the developing buds are important factors in determining the level of success in setting tubers. In recent years emphasis has been placed on developing a system that is reliable and widely applicable but, so far, none of the protocols used on intact micropropagated plants has allowed the production of one tuber per node.

In a variation of the technique small, micropropagated plants are transferred to a compost and are grown in a glasshouse under carefully controlled conditions where they produce a crop of small tubers *in vivo*. To distinguish the two differing origins of these tubers, the ones produced *in vitro* are called microtubers and those produced *in vivo* are called minitubers. The two kinds are sometimes referred to collectively as M-tubers.

One special use of minitubers at SCRI is to provide control plants in progeny trials because their size is comparable to that of tubers produced by plants grown from true seed.

Micropropagation has several advantages; it allows rapid multiplication of new genotypes, or of clones from established cultivars; the plants are disease-free and a large number can be grown in a small area in controlled environments, allowing year-round production. Furthermore micro- or minitubers are easily handled, stored, and transported.

These advantages give M-tubers a place in the commercial production of high-grade seed and could lead to a reduction in the number of field-grown genera-

Figure 1 Microtubers produced *in vitro* on nodal cuttings.

tions between pre-basic seed and commercial seed tubers if certain difficulties and uncertainties can be overcome. These include the probable price of M-tubers and the reliability of the yield of crops grown from them.

In addition to the advantages of M-tubers in commercial propagation systems, they are also being used at SCRI in physiological, pathological, and genetical studies.

Production of M-tubers Potato microplants are grown under sterile conditions on Murashige & Skoog (M&S) agar-medium with PGR to give a good plant habit with extension of the internodes. The plants are then divided into cuttings of one node each which are placed on fresh M&S medium with added PGR and a high concentration of sucrose. The cultures are incubated at 16°C in darkness after an initial period of short days to induce tuberization. Microtubers are visible after 14d and they continue to develop over an 8-week period (Fig. 1). This method produces one microtuber per node in a synchronous manner and provides physiologists, biochemists, and molecular biologists with a system to study the early molecular and biochemical events associated with tuber initiation and development. Since they are produced aseptically, microtubers may also be used in studies of disease development.

Molecular changes associated with tuberization Developmental processes, such as the formation of plant storage organs, involve the differential expression of genes. In tuber initiation and formation the plant redirects its development but very little is known about the molecular basis of the changes that take place. Studies on the complex regulation of the initiation and growth of stolons and tubers made *in vivo* are made difficult by the variable rates of development on the several stolons of a whole plant. The *in vitro* system provides several advantages over the conventional system, including the absence of an influence from a mother tuber, the free choice of genotype, the possibility of using genetically manipulated material, and, most importantly, the ability to produce large numbers of tubers synchronously under uniform conditions.

***In vitro* screening** Earliness of tuber set in 12 cultivars is being evaluated in experiments with microtubers by measuring stolon production and length, first signs of tuber swelling, tuber fill, and stolon death. In another test, resistance to *Phytophthora infestans* is being assessed using microtubers of three cultivars that exhibit different levels of resistance in the field. If the results of *in vitro* screening correlate well with field and glasshouse tests then the use of microtubers will provide a valuable screening method for assessing disease resistance and may also be used to screen new genotypes for earliness of tuber set.

Biochemical analysis The enzymes involved in starch and sucrose biosynthesis in developing tubers can be studied in intact microtubers instead of in tuber discs cut from conventional tubers.

Genetic transformation Genetic transformation of potatoes can be accomplished using the *Agrobacterium tumefaciens* transformation system and microtubers have been used recently in *Agrobacterium*-mediated transformation of tetraploid potato genotypes and wild species. This system has great potential in protoplast fusion studies for the transfer of traits from wild species into conventional cultivars, avoiding the problems of sexual incompatability. The protoplast fusion techniques require appropriate selection methods to detect hybrid genotypes. Thus, a kanamycin marker-gene carried in the transgenic lines, will allow transformed material to be selected post-fusion on an antibiotic-containing medium.

Agronomic methods Minitubers, produced in a glasshouse from plantlets propagated *in vitro* combine the advantages of *in vitro* plantlets, freedom from disease, and rapid, year-round production, with those of conventional tubers, ease of storage and of handling during transport. They lack some of the disadvantages of microtubers produced *in vitro*, e.g. low multiplication rate, and the large mass of conventional seed tubers. However, their low metabolic reserves is a potential disadvantage and they might not achieve their potential in the field unless their agronomy is fully understood. If plants produced from minitubers

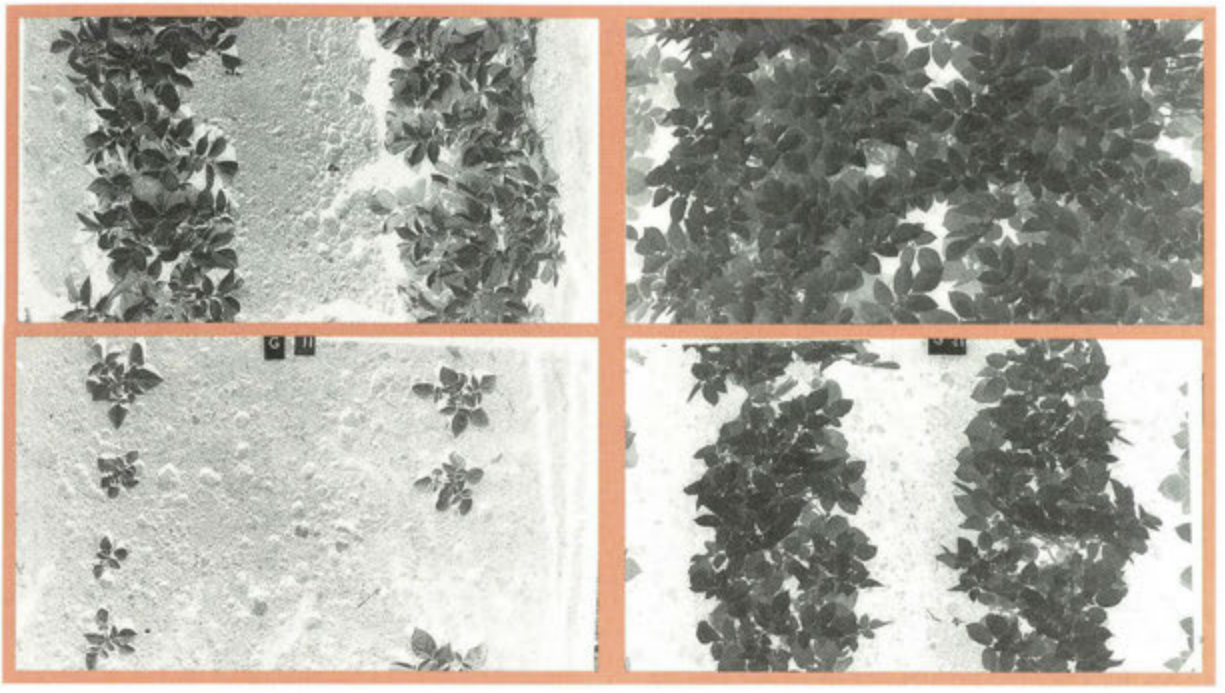

Figure 2 High contrast images of foliage cover obtained using infra-red sensitive film. The upper two photographs show foliage cover at 12 and 32 d after emergence from potato seed of conventional size and the lower two photographs from minitubers on the same days.

can perform economically as well as those from conventional high-grade seed then they will compete successfully in that part of the market. Furthermore, if their performance can be manipulated to match or nearly match that of conventional seed then they might even compete in wider seed markets. The success of minitubers in the seed chain depends on the balances between health, cost, and performance.

Minitubers are small with low metabolic reserves and they should not be planted too deeply nor too soon because their reserves may be inadequate to support the growth of the sprout to the surface or to recover after frost. In addition, they may be more prone to herbicide damage than plants from conventional seed.

Minitubers produce plants that are initially both small and single stemmed, although they branch later, and appropriate planting densities need to be devised to obtain suitable populations of stems and daughter tubers. The initial growth rate of young plantlets that grow from minitubers is slower than from conventional seed but chitting or presprouting may hasten the early growth from them.

Canopy development and growth Dry matter pro-

Figure 3 Plants grown from conventional seed (on the left) and 10-15mm presprouted minitubers (on the right) at 15/m^2, in early July.

duction can be described as the compound result of three processes: the interception of sunlight, the fixation of that energy into dry matter, and the partitioning of the dry matter into the tubers. Interception of sunlight can be examined with a photographic technique (Fig. 2) which provides a permanent record of the structure of the canopy. Infra-red sensitive film and an appropriate filter enhance the contrast between leaf and soil surfaces, enabling the use of an image analyser to process the photograph. The proportion of the ground covered by foliage, together with a measure of the daily receipt of solar energy, provides an estimate of the amount of energy that is intercepted.

In a field experiment, minitubers of cv. Désirée were graded into three sizes, 5-8mm, 8-10mm, and 10-15mm, subjected to two main chitting regimes and planted at three population densities. The plants grown from the minitubers were contrasted with plants grown from conventional seed. In plants grown from minitubers, 50% emergence was delayed by as much as 2 weeks, the spread of emergence was longer and ground cover reached 50% 27d after plants from the conventional seed (Fig. 3).

Pre-sprouting minitubers reduced the time taken to 50% emergence and to 50% ground cover compared with plants from non-sprouted tubers. The sprouts on minitubers were weaker than those on conventional seed tubers (Fig. 4) and plants from the smallest minitubers were the slowest to emerge. Consequently, the condition of the seed-bed is critical for minituber plants. Initially, small ridges were formed but once the plants had emerged the height of the ridge was increased. Ridging up may be repeated, thereby providing an opportunity to control weeds mechanically.

The effects of the treatments were largely independent of each other, for example, larger seed and presprouting each advanced emergence while the effect of using smaller seed could be partially offset by presprouting or by increasing planting density.

Figure 4 Plants 33 d after planting in a glasshouse to illustrate differences in vigour. Left, plant from conventional seed; Upper row of 5 plants - 10-15mm seed presprouted; Lower row of 5 plants - 10-15mm seed unsprouted.

The canopy of the minituber plants appeared to be less mature throughout the season and so compensated slightly for the effect of delayed canopy closure on the amount of light intercepted. The conventionally-grown plants senesced earliest even though irrigation was provided.

Total tuber yields from all the treatments were at acceptable commercial levels although yields and grade distributions differed.

The number of tubers produced was relatively insensitive to seed-tuber size or to chitting; the greatest response observed was to planting density. However, in adjusting plant population to optimize the total number of daughter tubers there may be little or no advantage in going beyond the intermediate density used in these experiments ($8/m^2$) even if small seed (25-35mm) are acceptable.

The efficiency of crop root-systems in nutrient uptake

D Robinson

Crops grow by acquiring resources from their environment: nutrients and water from below ground; oxygen, carbon dioxide and light from above (Fig. 1). Agriculture is concerned with making conditions favourable for the acquisition of these resources, so as to produce crops cost-effectively and with minimal environmental damage. It is concerned also with breeding varieties of crops better able to use the resources that are available, or to withstand the impact of potentially destructive pests, diseases and weather.

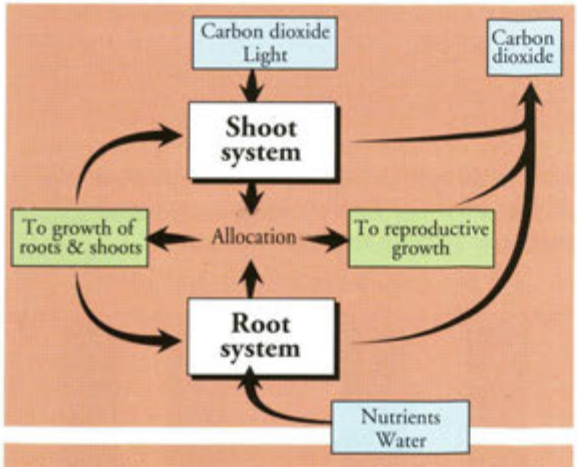

Figure 1 Schematic representation of the allocation of resources between roots, shoots and reproduction.

Many agricultural practices are aimed at modifying the growth and activity of crop root systems. Fertilizers are applied to be taken up by the roots. The soil is tilled so that root growth is unimpeded. The soil can be consolidated to ensure good contact between the roots of emerging seedlings and the surrounding soil particles. However, the inaccessibility of root systems means that they have rarely been the direct targets of conventional breeding programmes. Some features of root systems probably have been selected indirectly, as their operation is coupled physiologically to that of the more accessible parts that lend themselves more readily to phenotypic selection.

Root systems exhibit considerable phenotypic plasticity. They grow in an environment that is structurally, chemically and biologically variable, in time and in space. They have to perform several different functions simultaneously. They absorb water and nutrients, the mobilities of which in soil depend on the soil's texture and dryness, and on local chemical and biological processes. They anchor the plant into the ground. The roots of some species store assimilates for growth at a later time in the season. To perform these diverse functions, flexibility in form and physiology within a root system is essential.

In root crops (e.g. beet, carrot and turnip) a swollen taproot is the harvestable commodity. But in most food crops, the allocation of assimilates into roots represents an investment needed for the later development of reproductive structures (seeds or tubers) for which those crops are grown (Fig. 1). To some extent, assimilates allocated to roots can be scavenged and recycled into reproductive growth. However, a significant fraction of the assimilate allocated into roots cannot be recycled. It may be "fixed" in metabolically recalcitrant materials such as lignin and cellulose, or respired and the carbon lost as carbon dioxide. Provided that the fraction of assimilate allocated to the non-harvested parts of the plant is commensurate with the utility of those parts in, for example, resource capture, its loss from the crop is acceptable. Root systems represent a large fixed cost of assimilate in the physiological economics of plant growth, about half the total mass of a cereal crop. So the efficiency of root systems is important for obtaining the most cost-effective output (the crop) from the inputs (resources), and for making the best use of the inputs. Fertilizers are the most obvious of the inputs for which efficient use is desirable. But pesticides, herbicides and the expenditure of energy (fossil fuel) in spraying and tillage operations are also important.

Studying how root systems work The effectiveness of roots *as systems* has traditionally been studied in a crude way. Roots are sampled from the field, washed or picked out of the soil with considerable effort. The total amount of root per unit volume of soil or per unit ground area is then calculated. This is then used

as the basis for other calculations, e.g. how much nutrient was taken up by the crop per unit of root produced. Such an approach assumes that all parts of the root system contribute equally to the functioning of the system, as no means are available to distinguish between the contributions made to net uptake by roots of certain size- or age-classes. It also ignores a fundamental, and interesting, feature of a root system, that it is *a population.*

A root system is not produced in a once-and-for-all developmental event, like the limb of a mammal. It consists of a changing number of functional units, comprising juvenile root tips connected to progressively older parts in a complex branching structure. It is not easy to identify "individuals" in such a population, as its age structure is continuous, from root tips backwards, rather than discrete as between the generations of an animal population. Detailed studies of root demography are in their infancy compared with the many advances made in understanding the population biology of shoot systems. Because a root system is a population, innate variabilities exist within it even before localized non-uniformities of the environment influence its behaviour. Much of our current work aims to understand how the functioning of discrete parts of a root system contribute to the operation and efficiency of the whole.

The efficiency of wheat roots in nitrate uptake An opinion expressed frequently in the literature is that because roots are so physiologically adept at absorbing nitrate, and because these ions are so mobile in moist soil, any nitrate present should be taken up readily by plants with normal densities of roots. But this is not what happens, as the following experiment illustrates.

Spring wheat was grown in either unfertilized soil, or soil to which the equivalent of 200 kg N/ha had been applied. Plant growth and uptake of nitrogen, and the concentration of nitrate in the soil solution around the roots were measured at intervals over 100 days. The minimum concentration of nitrate measured in the soil was 0.4 mol/m^3, enough theoretically to drive a rate of uptake adequate to meet the demand of a high yielding crop if all of its roots had access to nitrate. However, the unfertilized plants did not make use of this freely available nitrate even though they were clearly nitrogen deficient: their final dry weights were a fifth and their total uptake of nitrogen only an eighth of those of plants to which fertilizer had been applied. Even these plants took up far less nitrogen than theory predicted. We calculated that

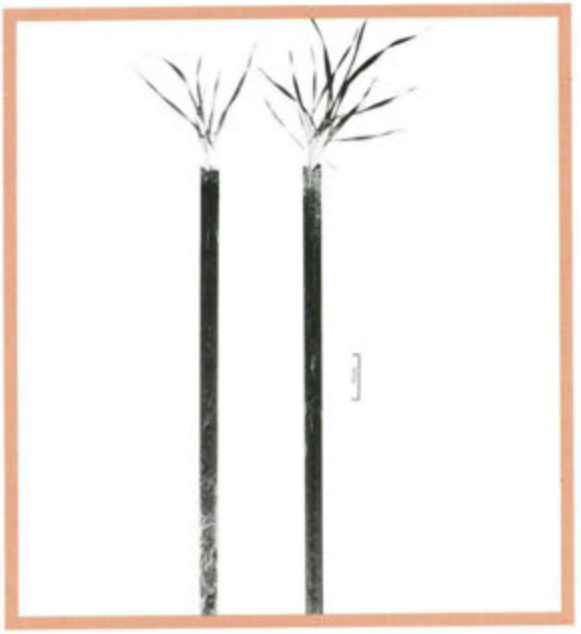

Figure 2 35 d-old-spring wheat plants growing in containers that allow repeated access to their root systems. The front cover of each container has been removed to reveal the soil and visible roots.

11% of the roots were apparently active in nitrate uptake in the unfertilized plants, and only 4% in those that received fertilizer.

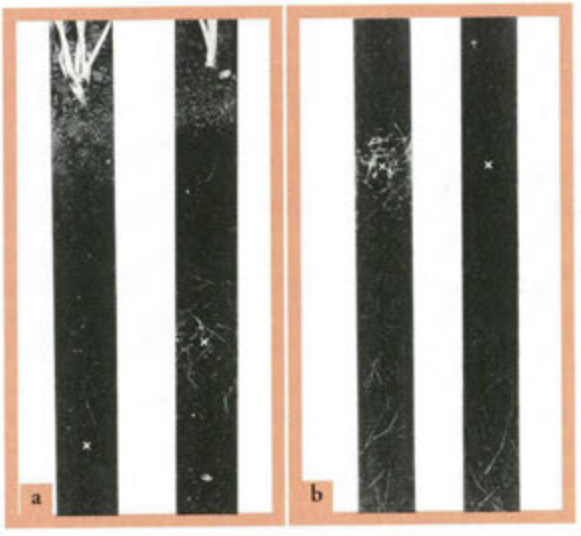

Figure 3 Close-up of opened containers showing the proliferation of new lateral roots in nitrogen deficient plants around the points (x) to which nitrate had been applied eight days previously. (a) Nitrate applied to old roots, fertilized plant on the left; (b) nitrate applied to young roots, fertilized plant on the right.

The cause of this apparent inefficiency is unknown. It is possible that there is a physiological constraint on nitrate uptake capacity in certain parts of the root system, but this is not consistent with the demonstrable demand of the nitrogen deficient plants for more nitrate than was available to them. Nor is it consistent with the many reports of accumulation of nitrogen in the plant in excess of the immediate need for it in growth. An alternative explanation is that some parts of root systems, especially of mature plants, have poor physical contact with the soil from which nutrients are supplied, although it is doubtful whether it could be as little as 10% or less. Poor root-soil contact might occur in older parts of the root system in which cortical senescence can be extensive. Senescent cortices could lose turgor and collapse, causing the root to shrink in diameter. This would disrupt contact between the surface of the root and the wall of the pore in which it had grown.

The contributions made to nutrient uptake by roots of different age are not known. If old roots are less effective than young ones, then a root system with a high proportion of old roots would be less efficient than one containing many young roots. The capacities of wheat roots of different ages to respond to, and absorb, localized supplies of nitrate was examined by growing plants in long, narrow containers that allow almost linear root systems to be produced (Fig. 2). Parts of root axes located towards the bottom of such a container would be relatively young, while those near the top, older. A removable front cover to the container allowed repeated access to the growing root system, so that the approximate age of the roots at a certain depth was obtained. This would not have been possible if plants had been grown in a conventional shallow plant pot.

After 33 days' growth, 200 µl of solution containing a high concentration (2,000 mol/m^3) of nitrate labelled with ^{15}N tracer was applied to soil overlying young roots at the bottom of the container, or over old roots at the top. Eight days later, nitrogen-deficient plants had produced a luxuriant growth of new lateral roots around the point at which the nitrate had been applied (Fig. 3). The roots of fertilized plants did not respond. The response of the nitrogen deficient plants occurred whether young or old parts of the root system had been supplied locally with nitrate. The responsiveness of young roots to nitrate is well-known. This experiment showed that older roots can be equally responsive.

The roots of the nitrogen deficient plants took up c. 88% of the nitrate that was applied. Fertilized plants acquired only 40%, demonstrating that discrete parts of root systems can be very efficient in capturing nitrate from the soil. This contrasts with the view obtained from the first experiment that whole root systems are highly inefficient. The amount of nitrate taken up was directly proportional to the density of roots in that part of the soil to which it had been applied. Nitrate uptake is usually assumed to be independent of the length of roots in the soil, because of the high mobility of nitrate ions in soil. Our results show that this assumption is not always valid.

These experiments provide no information about the *physiological* capacities of young or old roots to take up nitrate. The morphological changes induced in nitrogen deficient plants meant that nitrate was taken up largely by very young roots regardless of the depth at which nitrate was supplied. The above experiment was repeated, but the uptake of ^{15}N was measured after only 24 h, too short a time for any morphological changes to occur. No differences were found between the rates at which nitrate was taken up by old or young roots.

This work is continuing, to define more clearly the influence of responses to localized supplies of nitrate on the uptake of other nutrients, and to manipulate root-soil contact and the degree of clumping of roots in soil so as to quantify the impact of these physical factors on a root system's efficiency. In the longer term, the supply of assimilates to the root system will be controlled and subsequent effects on nutrient uptake measured, to obtain information about both the physical and physiological constraints on the attainment of efficient root systems.

Chemistry

M.J. Allison

This report presents an overview of the progress made during the year by the Chemistry Department, the Fibres Group and the Nuclear Magnetic Resonance Group. In addition there are reviews of one research topic from each group.

The main aim of the work of the Chemistry Department is the resolution and estimation of chemical compounds important in determining crop quality. A key element in achieving this objective is the development of applications of current instrumentation. During the year the Institute purchased a peptide synthesiser from Applied Biosystems (Model 431 A). This instrument which is shown in Figure 1, was housed in Chemistry. The synthesiser was commissioned and, to date, two short peptides have been successfully synthesised. In both cases a high degree of purity was achieved (a hexamer separated on a reverse phase-HPLC system is shown in Figure 2). Progress was made in determining the optimum conditions for the extraction and resolution of different plant proteins by RP-HPLC. An improved extraction procedure resulted in a single step, relatively clean preparation of barley and wheat glutelins. This advance enabled a ready assessment of the relationships between high molecular weight storage proteins of cereals and various quality attributes. In addition the use of different solvents to extract potato tuber proteins proved to be helpful in attempts made to identify cultivars from their RP-HPLC chromatographs.

Figure 1 Peptide synthesiser, model 431 A from Applied Biosystems.

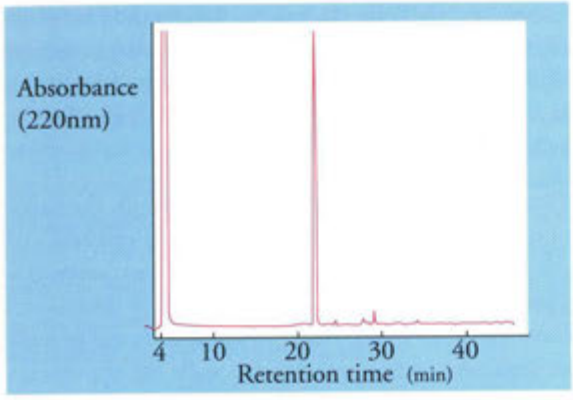

Figure 2 A hexamer peptide resolved on an RP-HPLC system.

The marketing potential of our NIR software package developed jointly by Chemistry and SASS was enhanced by the addition of a demonstration program to aid efficient use of the software. This demonstration program was completed by the end of the year, so that both programs could be exhibited together at the Pittsburgh conference in March 1991. Our thermal desorption system for the collection and analysis of volatile chemicals was also improved this year by the construction of an entrainment apparatus for sampling by gentle suction. Collection columns with specific stationary phases can now be used either for passive or active sampling of volatile chemicals from plants or soils.

Tracermass work during the year included anlayses of ^{15}N incorporation into viral coat protein for NMR investigations. The Dumas combustion module of the Tracermass was used for nitrogen analysis of samples having only small amounts of material (30 mg or less) available. Plasma emission spectrometric and x-ray fluorescence methods were developed for the estimation of sodium and chlorine respectively as a means of detecting sodium monochloracetate (SMA), which is a potential dinoseb replacement. Applications of the x-ray fluorescence technique revealed that there are considerable matrix effects for the estimation of chlorine from raspberry flour using this method. Later in the year, a rapid colorimetric test was developed for chlorogenic acid in potato tubers. This is a main factor causing after-cooking blackening (ACB) in potatoes. The method could be used to screen progeny from crosses for their ACB potential. An HPLC method for the estimation of glucosinolates was scaled down so that small amounts of plant material from rapidly cycling brassicas could be assayed qualitatively and quantitatively for glucosinolate content. The assay procedure was also applied to transformants to monitor changes in glucosinolate expression.

This is the first full year for use of the new NMR facilities at Dundee University. These are being continually developed and applied to research problems in the programmes of SCRI and other SARIs. Of particular note this year has been the acquisition of a microimaging accessory, the fundamental principles and applications of which are the subject of a separate research review. Conventional spectroscopic applications have been concentrated on three principal research areas:-

1. soluble plant cell wall fractions, using ^{1}H and ^{13}C spectroscopy and celluloses extracted from a range of plants using both solution and solid state ^{13}C NMR spectroscopy

2. *In vivo* studies of low molecular weight chemical species in the nematode *Longidorus elongatus* in order to identify the main fatty acid and sugar constituents and to investigate seasonal variations in their relative levels

3. mobile groups in the pepper ringspot tobravirus in which conventional ^{1}H spectra in H_2O and D_2O buffered solutions have revealed peaks consistent with proline, serine and asparagine. Peak overlap, however, means that 2-dimensional techniques will be needed for positive identification of the mobile section of the virus coat protein.

The Fibres Group was set up to investigate the potential for the production of plant fibres from annual and perennial plants. It has a number of objectives, the first being the isolation and identification of fibres from fibre-producing herbs, shrubs and trees and their characterisation by chemical and physical methods. Amongst the species being investigated are brassicas, nettles, flax, cereal straw and miscanthus. Closely allied to this is a programme on conventional and novel processes for the extraction and modification of plant fibres to suit the industrial user. Of particular concern are processes which will have minimal effects on the environment and, to that end, enzymatic and biomimetic processes are given a high priority. Work is also being carried out on the control of differentiation and development in plant fibre cells. For a number of justifiable reasons, flax has been chosen as the model plant and the initial focuses of the research are lignification and non-cellulosic polysaccharide deposition. This aspect is described later in greater detail.

The fourth objective is the potential of NMR spectroscopy for the evaluation of plant fibres. The insolubility of plant fibres made them unsuitable for evaluation by NMR until the introduction of cross-polarisation magic-angle spinning (CP-MAS) techniques.

The evaluation of several varieties of brassicas as potential sources of pulp for paper production confirmed that at least medium quality pulp was possible even though the process had not been optimised. The primary composition of the brassica fibre had an effect on the yield of pulp but had an insignificant effect on its quality. Investigations on the use of cereal straws as a source of fermentable carbohydrate showed that a pretreatment to remove the bulk of the lignin was essential for extensive hydrolysis of the fibre polysaccharides by commercial "cellulases". As revealed by CP-MAS NMR spectroscopy, the method used to remove lignin had an effect on the secondary structure of the residual cellulose. The most likely changes are in the crystallinity of the cellulose which will affect its potential as a source of fibre.

Considerable importance is being placed on the function of cyclodimers of phenolic acids in cell walls of the Gramineae. The hypothesis that dimerisation is a photochemical reaction within the wall is questioned from the observation that the monomers are present in both cereal straw and flax cell walls but the dimers have not been detected in flax. GC-MS has been used for the analysis of the dimers and has assisted a detailed investigation on the influence of steric and electronegative substituents on the pathway of photochemical dimerisation in cinnamic acids.

The use of high performance liquid chromatography for the separation of plant proteins

M.J. Allison

For many years gel electrophoresis in its various forms has proved to be a powerful tool for the resolution of a wide array of proteins from plants and animals. For some proteins, an electrophoretic separation based on molecular weight differences using polyacrylamide gels with sodium dodecylsulphate (SDS-PAGE) may resolve the different proteins clearly, or a better resolution may be achieved by electrofocusing the proteins (using electrical charge differences). Despite the success of electrophoretic techniques, there are other methods, such as HPLC which can be used to separate certain proteins which are difficult to resolve in a unidirectional electrophoresis system [1,2].

The success of HPLC in separating macromolecules is due at least in part to several advances made in the technology of column chromatography. One advance was the development of silica beads 5 to 10 µm in diam, which have been etched to give wide pores (300Å pore size) allowing access of high molecular weight (HMW) proteins. The development of stable chemical coatings for these beads, usually hydrophobic n-alkyl chains, meant that HMW proteins could enter the beads and interact with the non-polar stationary phase weakly or strongly, depending on the number and position of surface hydrophobic groups on the proteins. A second main advance of less recent origin arose from the use of solvent gradients in which solvents form the mobile phase, to elute solutes from the stationary phase. For cereal storage proteins, for example, a polar solvent, usually water, predominates at the sample injection stage over the non-polar solvent such as acetonitrile. As the chromatography progresses under high pressure the concentration of non-polar solvent increases until it competes with the stationary phase for bound solutes, and then successively elutes these solutes from the column in an increasing order of hydrophobicity. This system is referred to as reversed phase high performance liquid chromatography (RP-HPLC) and is a highly successful technique for the separation of small molecules and macromolecules.

RP-HPLC has been successfully used for the resolution of the storage proteins of cereals. As SDS PAGE separations are based on molecular weight and RP-HPLC separations are based on hydrophobicity, it is difficult to match peaks on RP-HPLC with protein bands on gels. It is possible to collect eluted RP-HPLC peaks and run these on gel electrophoresis systems. Alternatively, mutants deficient in certain protein groups can be used to cross-identify these groups separated by RP-HPLC and SDS PAGE. An example is shown in Figure 1 a and b.

Barley storage proteins When barley hordeins, which are proteins soluble in 55% isopropanol, are separated on SDS gels, they can be divided into four groups, A, B, C and D hordeins, on the basis of differences in migration rate. The B and C hordeins are present in greater amounts than the A or D and there is usually a high ratio of B to C hordeins. However in Risø 56, a mutant barley derived from cv. Carlsberg, the B hordeins are depressed such that there is a high C to B ratio. It can therefore be concluded from Figures 1 a and b that peaks with retention times between 30 - 50 min, are C hordeins whereas those between 55 - 80 min are B hordeins. These assignations have been confirmed by running eluted HPLC peaks on SDS gels.

For most proteins, there is usually a simple positive correlation between molecular weight and the number of hydrophobic groups present, but this is not the case for the hordeins in barley where the higher molecular weight C hordeins elute from the column earlier than the B hordeins. Studies on the HMW and LMW glutenins of wheat have shown that the former also elute earlier from the column than the latter. The HMW glutenins are rich in polar amino acids and have less leucine, isoleucine etc. compared to the LMW glutenins. Thus the early elution of HMW glutenins in RP-HPLC is due to fewer surface hydrophobic groups on HMW glutenins compared to LMW glutenins. The same is likely to be true for the C and B hordeins in barley. It is evident from these results that RP-HPLC provides a different separation method to electrophoretic techniques, and can complement information from different forms of electrophoresis.

An example of the use of RP-HPLC as an additional separation aid occurred in a survey of European barleys aimed at cultivar identification. In this survey a letter was assigned to the different forms of C hordeins, and the B hordein patterns were numbered. The SDS PAGE patterns obtained for both spring and winter barley cultivars were often indistinguishable for a number of cultivars, so that they were assigned to the same groupings. For example, 15 cultivars were typed as group 1A and nine as group 3B. Thus the hordein patterns differed between groups 1A and 3B but were indistinguishable within these groups when an SDS PAGE unidirectional separation method was applied. As a test of HPLC resolving power, a number of cultivars within each of the groups 1A and 3B were subjected to RP-HPLC chromatography on a synchro-pak column using an acetonitrile/water gradient to elute the column. Two kinds of separation criteria were used to distinguish protein patterns. One kind of polymorphism is observed as a significant difference in column retention time for individual proteins or groups of proteins. The standard deviation for retention time measured for many samples is usually less than one minute, and is frequently within 30 seconds.

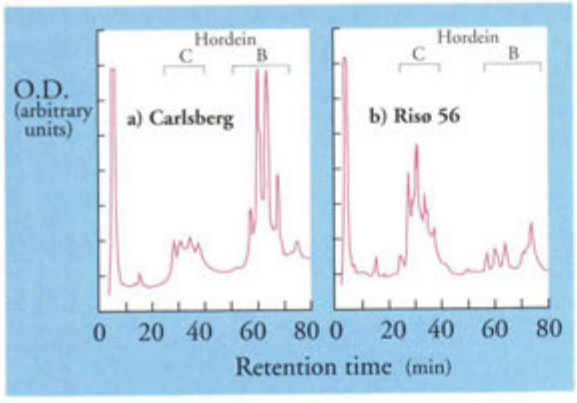

Figure 1 RP-HPLC chromatograms of hordein proteins from samples of the barley cultivars a) Carlsberg and b) Risø 56.

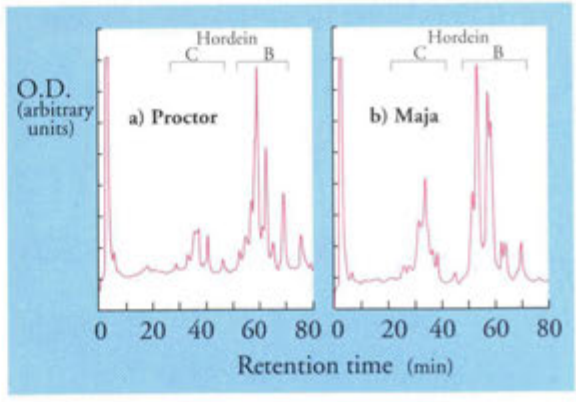

Figure 2 RP-HPLC chromatograms of hordein proteins from samples of the barley cultivars a) Proctor and b) Maja.

An example of variation in peak retention time of proteins from different cultivars is evident in the B hordein patterns of the cv. Proctor and Maja shown in Figures 2a and b. Both of these cultivars were electrophoretically typed as 1A. Separation on the basis of hydrophobicity, however, shows a marked polymorphism between the two cultivars. It is possible that such a marked polymorphism may be due to outcrossing during stock maintenance. In this case, however, hordeins of 15 cultivars from group 1A were subjected to RP-HPLC and five different patterns were observed.

A second type of polymorphism detected after RP-HPLC is not a qualitative pattern difference, but is apparent as a marked quantitative difference between proteins with the same retention time. Thus there is variation due to a difference in regulation of the amount of protein produced, and this may be attributed to a control gene mutation compared to the more usual polymorphism observed as a pattern difference for the structural genes. An example of the control gene type of difference was observed for the C hordeins of three cultivars collectively typed as 3B (Fig. 3). The main C hordein peak is quantitatively different in the cultivars Keg, Dram and Tern. This peak at 36 min retention time is a similar height to the B hordeins in Tern, but is significantly lower in Dram and Keg. Quantitative differences are readily observed in HPLC as the chromatograms are automatically integregated, whereas quantification of electrophoretic bands may be difficult if the bands are diffuse or distorted. Hordeins, of the cultivar, Terrax, also typed as 3B are qualitatively different to corresponding proteins in the 3B group particularly the late eluting B hordein peaks.

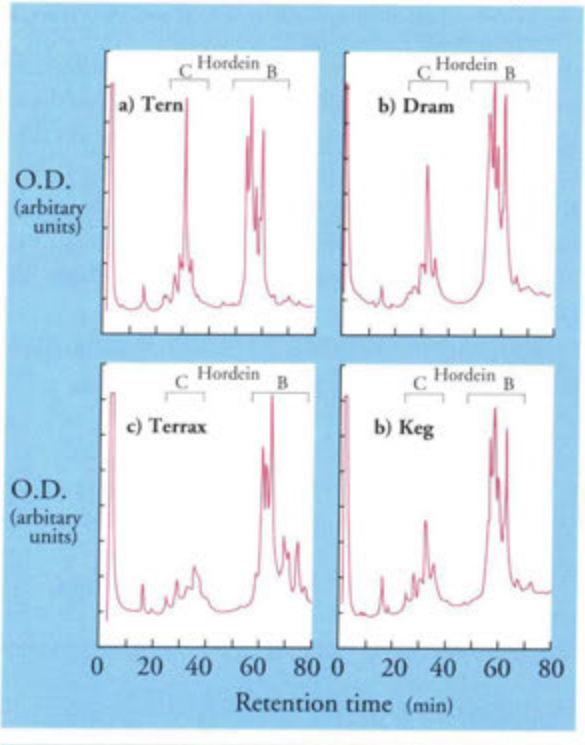

Figure 3 RP-HPLC chromatograms of hordein proteins from samples of the barley cultivars a) Tern, b) Dram, c) Terrax and d) Keg.

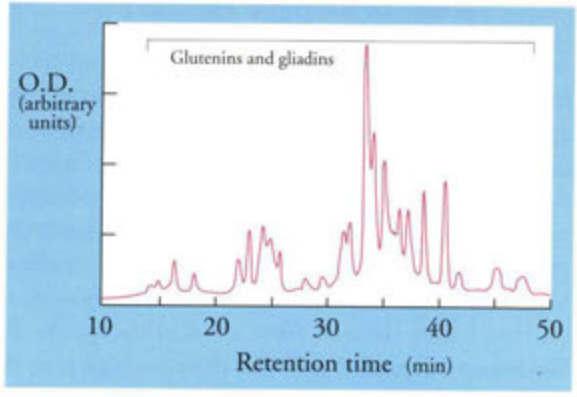

Figure 4 RP-HPLC chromatogram of the storage proteins of the winter wheat cultivar Camp Remy.

Wheat storage proteins RP-HPLC has also proved useful in separating the storage proteins of wheat cv. Camp Remy as shown in Figure 4. The difficulty of assigning protein peaks separated by RP-HPLC to corresponding electrophoretic bands is greater in wheat than in barley because of the further complexity of a hexaploid versus a diploid genome. Stepwise precipitation methods are routinely used to segregate the different storage protein groups in wheat grains. After precipitation of the gliadins (α,β, γ and ω types) and LMW glutenins, further precipitation and alkylation is used to isolate the HMW glutenins. It has been reported, however, that treatment of wheat-flour with dimethylsulphoxide (DMSO) solublises all of the

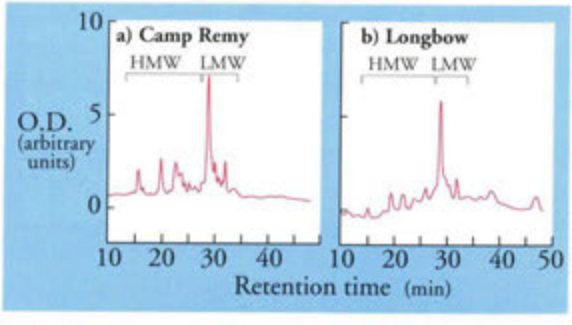

Figure 5 RP-HPLC chromatograms of LMW- and HMW-glutenins prepared by a DMSO method from the cultivars a) Camp Remy and b) Longbow.

albumins, globulins, gliadins and some of the starch, to leave a relatively clean preparation of glutenins with a similar resolution of HMW glutenins compared to the stepwise precipitation method. The DMSO method was used to prepare the glutenins of cv. Camp Remy, a winter wheat with good bread-making quality (BMQ) and from Longbow, a wheat with poor BMQ. The results are shown in Figure 5. It is clear that there are quantitative, as well as qualitative differences between these two wheat cultivars.

The DMSO method has also been applied to barley and glutelin proteins (equivalent to the wheat glutenins) from cv. Golden Promise are shown in Figure 6. This technique can therefore be used to resolve the high molecular weight glutelins of barley so that their relationship to barley quality can be investigated.

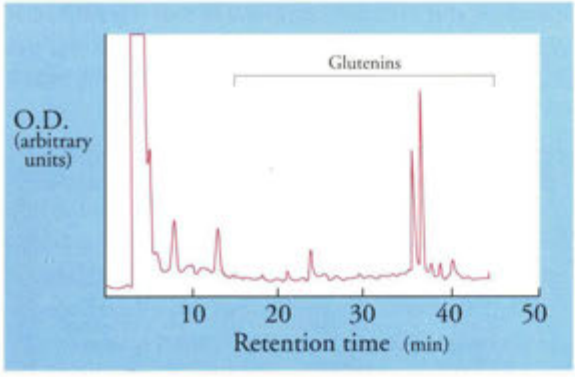

Figure 6 RP-HPLC chromatogram of the glutelin proteins from the barley cultivar, Golden Promise.

Potato proteins The extraction and separation of specific fractions of proteins is often an advantage when protein differences need to be highlighted. Proteins from potato tubers are readily soluble, and a large number can be resolved. It was observed, however, that if different solvents were used, some protein differences between cultivars were more clearly evident, especially where small differences in retention time were concerned. An example of this is shown in Figure 7 in which the alcohol-soluble proteins of the cultivars Cara and Pentland Dell show clear retention time differences. Thus the extraction of potato tuber proteins into different solvents may aid comparisons of protein peaks separated by RP-HPLC.

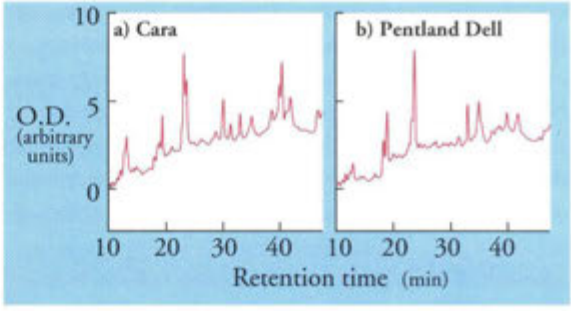

Figure 7 RP-HPLC chromatograms of the alcohol-soluble proteins of tuber samples from the potato cultivars a) Cara and b) Pentland Dell.

It is hoped that protein chromatograms obtained on RP-HPLC systems will continue to complement electropheretic separations. In the near future it is expected that it will be possible to apply voltage gradients to buffered solutions containing silica beads with their usual range of chemical phases, so that proteins can be resolved by electrophoresis and hydrophobicity using an electro-chromatography hybrid technique.

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Nuclear magnetic resonance microscopic imaging

B. A.. Goodman

NMR microscopic imaging is a technique that has evolved from a combination of the technologies of solution NMR spectroscopy and medical NMR imaging. The result is a technique, usually in the form of an accessory for conventional high-field NMR spectrometers, that is able to produce spatial information

(i.e. images) non-invasively with a resolution that is only slightly inferior to that achieved by optical microscopy.

Background to NMR Nuclear magnetic resonance involves transitions between spin states of nuclei and can, therefore, be observed only with nuclei that possess non-zero spin. In the presence of a magnetic field such nuclei behave like tiny bar magnets and orientate themselves (Fig. 1a). In the simplest case of spin 1/2 nuclei, which include the biologically important isotopes ^{1}H, ^{13}C, ^{15}N and ^{31}P, only two orientations can be adopted in a magnetic field; a low energy state, N_a, aligned with the magnetic field and a high energy state, N_b, opposed to the magnetic field. The population difference between the two states is proportional to the magnitudes of the magnetic field and the magnetogyric ratio, a proportionality constant that differs for each type of nucleus. In NMR spectroscopy nuclei of a selected isotope are excited from the N_a to the N_b state by a powerful pulse of electromagnetic energy (radiowaves) which lasts for a few microseconds. This pulse generates an oscillating magnetic field at right angles to the applied magnetic field, with the result that the direction of magnetization of the sample is changed by an angle, ϑ. Often the time of duration of the pulse is chosen so that $\vartheta=90°$, and is known as a 90° pulse(Fig. 1b). At the end of the pulse, the nuclei relax through interaction with local fluctuating magnetic fields back to their equilibrium states. During this process, a complex oscillating radiofrequency signal is emitted (Fig. 1c). This signal decays to zero as equilibrium is re-established and is known as the free induction decay (FID). The nature of the FID is determined by the chemical states of the nuclei in the specimen and Fourier transformation of the FID converts it to a spectrum. These various processes are shown diagramatically in Figure 1. Analysis of NMR

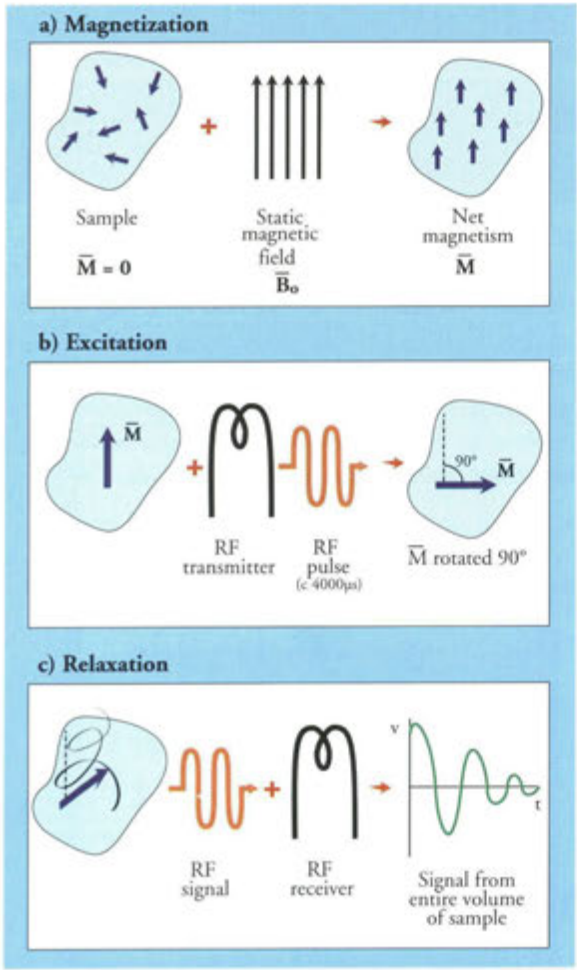

Figure 1 a) Magnetization of a sample containing magnetic nuclei by a static external magnetic field, b) change in direction of magnetization as a result of absorption of rf radiation, c) emission of rf signal as nuclei relax back to their equilibrium states after the external rf pulse.

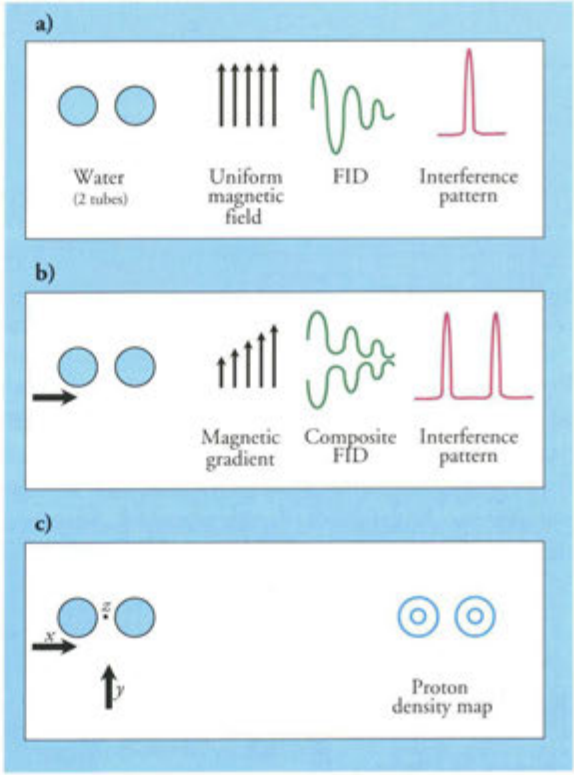

Figure 2 NMR traces (interference patterns derived from Fourier transforms of the FIDs) produced by 2 tubes of water in the spectrometer a) in a uniform magnetic field, b) with a 1-dimensional magnetic field gradient and c) with field gradients applied along 2 axes in the plane of the magnetic field.

spectra provides detailed information on the molecular composition of chemical species with the result that NMR spectroscopy is now one of the most widely used techniques in chemical characterization.

Principles of NMR imaging In a uniform magnetic field, all nuclei in a specimen that have the same chemical environments produce peaks in the same place in a spectrum (Fig. 2a). However, if magnetic field gradients are applied across a sample then spatial information can be obtained (Fig. 2b,c). In a typical measurement, the pulse sequence begins with a 90° pulse, that is shaped so that it produces a narrow radiofrequency bandwidth, being applied simultaneously with a field gradient along the z-axis. This has the result of limiting the spectrum to nuclei in a narrow slice in the xy plane. Application of a field gradient along the x- and y-axes generates a 2-dimensional data set, the Fourier transform of which yields the spatial distribution of spins in the imaging (xy) plane. Such images may be displayed on a TV screen, stored on recording tape, or produced as hard copy by photography or via a videoprinter. For most practical purposes NMR imaging is concerned with the distribution of protons in liquid-like environments and this is usually water. However, the NMR image is dependent upon a number of factors and is not simply determined by the concentrations of mobile protons. The chemical states of the protons and their relaxation properties all affect the image. Thus an NMR image potentially provides information about the chemical composition, morphology and dynamics of different regions of a specimen, and is not limited to providing structural information. Some recent applications that have been performed at SCRI in collaboration with the Department of Chemistry at Dundee University are reported below.

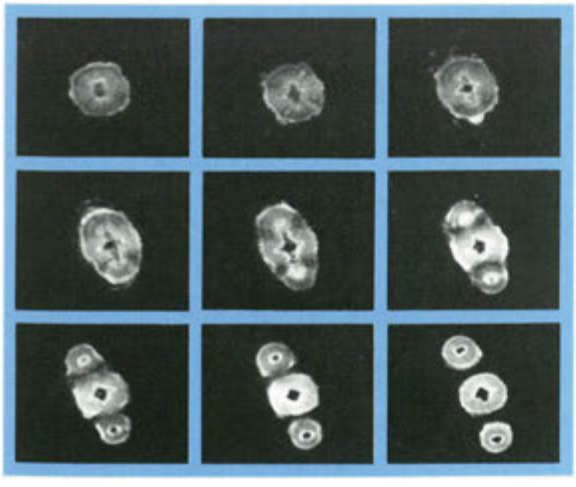

Figure 3 NMR images of 9 sequential slices (each 400μm thick) through the stem of a flax plant covering the region where the side-shoots (tillers) emerge.

Structural studies of plants The internal structure of plant specimens can be readily resolved using the NMR imaging technique. Figure 3 shows a series of images obtained from a flax plant from a point just below to just above the point of emergence of side shoots. The gross anatomical features are clearly revealed and these correlate well with optical micrographs obtained by conventional histological techniques on the same plant tissue. When short delay times are employed between the pulses used in the NMR experiment, the resulting image is dominated by components with short relaxation times. Thus in flax an image obtained with a short delay (Fig. 4b) shows enhanced intensity from the xylem tissue because of the presence of paramagnetic ions which shorten the spin-lattice relaxation time.

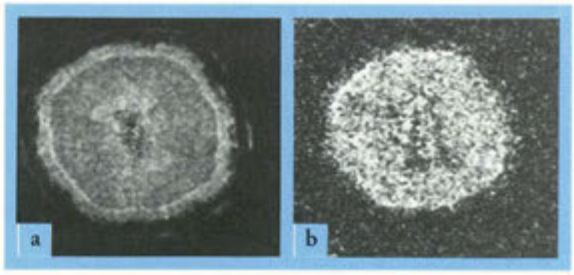

Figure 4 NMR images of the same slice through the stem of a flax plant just below the point of emergence of the side shoots taken with pulse delays of a) 2.0sec and b) 0.25sec.

Development of fruit and storage organs With soft fruits, such as raspberry and blackcurrant, and storage organs, such as potato, the gross histological features are clearly revealed (Fig. 5). However, the principal advantage of NMR imaging over other histological techniques is its non-invasive nature which permits the monitoring of internal changes in the development of a single specimen. Thus it is possible to investigate the changes that occur during fruit ripening or the development of *Botrytis* infection (Fig. 6).

Chemical shift selective imaging A further aspect of the NMR imaging procedure is the production of separate images from chemically distinct entities, such as water and lipid. An example of chemical shift selective imaging is shown in Figure 7 for an immature pupa of a Large White butterfly. With such specimens it is now possible to monitor directly changes in the levels and distribution of these components during develop-

ment. In Lepidoptera, major changes in gross morphology occur during the pupal stage and in mature specimens considerable detail is revealed in NMR images (Fig. 8). It should be noted that the specimen used to generate these images emerged two days after completion of the NMR experiment and laid eggs.

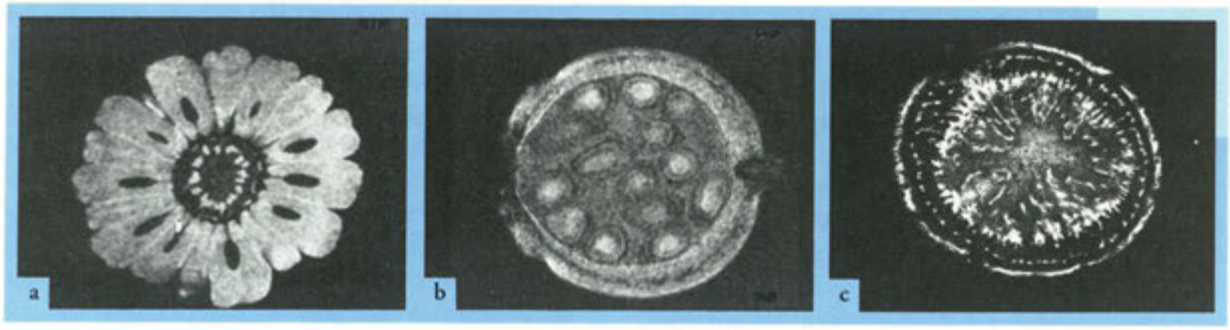

Figure 5 NMR images of a) a 500µm thick transverse slice through a raspberry fruit, b) a similar slice through a blackcurrant, and c) a 700µm thick slice through a sprouting potato (note the tip of a shoot in the top left hand corner).

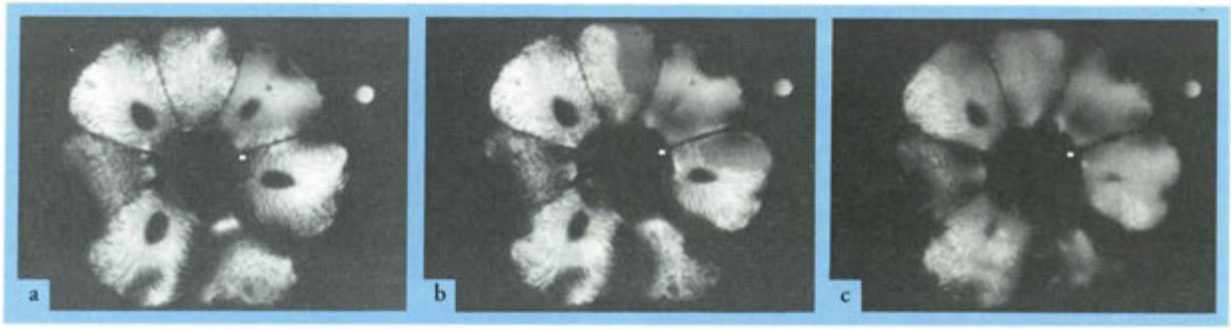

Figure 6 NMR images showing *Botrytis cinerea* in the fruit of a raspberry spreading from an inoculation wound in the drupelet identified by the phantom in the top right hand corner a) 1 day, b) 2 days and c) 4 days after inoculation.

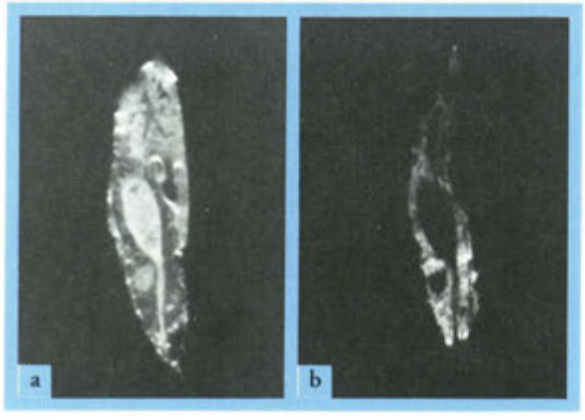

Figure 7 Chemical shift selective NMR images of an immature pupa of a Large White butterfly a) water and b) lipid distribution in a longitudinal slice approximately through the centre of the pupa.

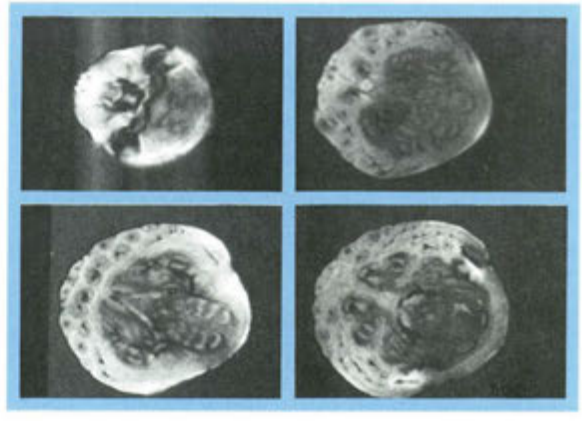

Figure 8 NMR images of 4 transverse slices (each 500µm thick and separated by 2mm) through a mature pupa of a noctyuid moth taken between the top of the head and the middle of the abdomen.

Control of differentiation in fibre cells

I. M. Morrison & G. J. McDougall

The Fibres Group at SCRI was set up to investigate the potential for new sources of plant fibre from annual and perennial species. More than 50% of the resources are being put into the identification of novel crops and the isolation of fibres with properties more closely aligned to industrial requirements than some of the existing raw materials. The other main thrust is to investigate the control of differentiation and development of fibre cells. The definition of what constitutes a plant fibre is vague and some opinions consider the term fibre to be synonymous with the cell wall. That view is especially prevalent in the field of both animal and human nutrition. We define fibre cells to be those cells which have a high length to width ratio and which occur in bundles such that the cells overlap each other to give additional strength. This definition is still rather vague but it does enable us to include, from one extreme, the textile fibres such as cotton and linen which have a length to width ratio in excess of 1000 down to the wood fibres used for paper pulp where the length:width ratio is between 50 and 100. Some of this diversity arises from the types of cell used for fibre and from which part of the plant it is isolated. Softwood fibres are mainly longitudinal tracheids which comprise up to 90% of the softwood volume while hardwood fibre cells are more diverse, reflecting the greater complexity of cell types in hardwoods. Non-woody fibres are isolated from different plant parts. Cotton is a fibre derived from seed hairs, flax (linen) is a bast fibre derived from the stem of a dicotyledon while sisal is an example of a leaf fibre which is isolated from the long leaves of a monocotyledon.

Our investigations into the control of differentiation and development of fibre cells are targeted to improve either the yield of fibre or the quality of fibre. These processes are not necessarily distinct. Improved yield could result from a higher proportion of fibre cells, with the same composition, per unit plant volume or from the same number of cells but with larger cell walls. The former would be a desirable outcome while the consequence of the latter would depend on which constituents of the fibre were increased in concentration. An increase in cellulose concentration would be a desirable result but an increase in lignification would be detrimental.

Virtually all plant cell walls contain cellulose which is the major constituent of all walls except in seeds. Celluloses isolated from all sources have the same primary structure of a β-(1→4)-linked polymer of D-glucopyranose but the fact that each successive glucose residue in these linear chains is twisted through an angle of 180° to its neighbour means that each successive glucose residue does not present the same face and the polymer repeat is really a cellobiose unit. However the most important feature of each cellulose chain is its ability to hydrogen bond with adjacent chains in the same plane and with chains in other planes to form the well-characterised microfibrils. Thus the major differences between celluloses from different sources are in the average individual chain lengths and the proportions of crystalline (microfibrillar) and non-crystalline (amorphous) regions.

The other components of fibre cell walls are far more diverse. The non-cellulosic polysaccharides are subdivided into the hemicelluloses which are basically neutral polysaccharides of considerably shorter degrees of polymerisation than cellulose and which contain a number of different sugar residues, and the pectic polysaccharides which are acidic polymers based on galacturonic acid. One of the major functions of the pectic polymers would appear to be cell to cell adhesion while the hemicelluloses are reported to be more closely related to lignification. Lignin is a phenylpropane polymer formed by a random polymerisation of phenoxy radicals and is not a carbohydrate. Considerable evidence is available to suggest that lignin and hemicelluloses are covalently bound in the cell wall. Indeed the lignified (secondary) cell wall has been compared with reinforced concrete where the cellulose microfibrils are the steel rods and the lignohemicellulose matrix is the cement. Lignin certainly exerts strength and brittleness to fibres. Other constituents, such as acetate and phenolic esters, are present in cell walls and although minor in proportion, they may have major functions in wall structure and utilisation.

Flax fibres In our work on development and differentiation in fibre cells at SCRI, we have chosen flax (*Linum usitatissimum*) as the model plant for the following reasons. It is a well established fibre-producing

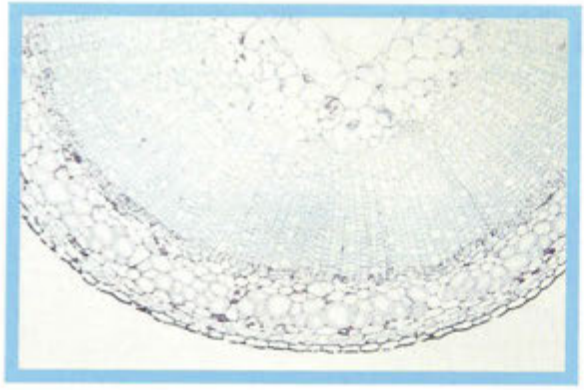

Figure 1 Half cross-section of fibre-bearing portion of flax at 9-10 weeks.

plant with the superior fibres being used for high quality linen and other fibres being used for canvas and paper. It is also readily grown in tissue culture and, having a small genome, should be amenable to genetic manipulation. The industrial requirement from most fibre sources is the production of as pure cellulose as possible but with minimum degradation. This usually results in a compromise with some non-cellulosic material remaining and some degradation of the cellulose.

As discussed earlier, improved fibre quality can be achieved from increased cellulose deposition or decreased lignin and/or non-cellulosic polysaccharide deposition. Increased cellulose and reduced lignin production are achieved by distinct biochemical routes although, since they are competing for the same metabolic pool, they are not mutually exclusive. It has been decided to concentrate on aspects of lignification and non-cellulosic polysaccharide production.

The biosynthesis of monomers involved as lignin building blocks is a multistep process but the polymerisation step is reported to involve peroxidase. The relationship between cell wall-associated peroxidases and lignification during the growth of flax fibres has, therefore, been investigated. The plants were grown under controlled conditions and two areas of the stem sampled at regular intervals, especially during the phase of rapid growth and expansion of the xylem radius. The cotyledonary node was taken as a reference point and one sample, hypocotyl, was taken below this point and the other, first internode, an equivalent distance above. The extent of lignification was first determined after 5 weeks growth when it was well advanced in the hypocotyl but barely detectable at the first internode. Thereafter, the percentage of lignified fibre increased at both sites, the rate of increase being greater in the hypocotyl at an early stage. However, the percentage in the first internode increased more rapidly nearer the end of the stem elongation phase and ended about 15% lower than in the hypocotyl. The presence of lignin was detected by fluorescence microscopy using ethidium bromide or by light microscopy using acid phloroglucinol.

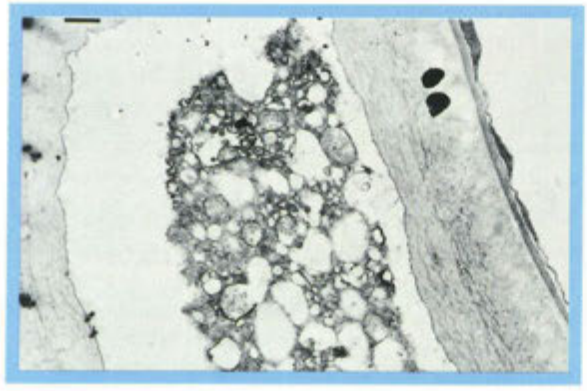

Figure 2 Electron micrographs of young developing fibre walls (c. 2 weeks old).

Peroxidase enzymes Cell wall-associated peroxidase isozymes were isolated in two fractions, one consisting of extracellular ionically-bound and free forms obtained by vacuum infiltration and the other was a covalently-bound fraction. They were isolated from the same regions of the stem which were analysed for extent of lignification and their activities were determined against several different substrates. The wall-associated activities reached a maximum which coincided with the period of greatest development of the xylem region but high levels were also obtained at the stage when lignification of fibre cells was increasing. A more detailed examination of the peroxidases was obtained from fibre-bearing tissue dissected from the central xylem core. Gel electrophoresis revealed the presence of several anionic and cationic isozymes but there were no major qualitative or quantitative differences in the pattern from plants of different ages. However, when peroxidases were isolated from flax callus and compared with those from xylem, it was observed that some of the cationic and anionic forms were missing (Fig. 3). Since, in general, peroxidases are ubiquitous enzymes, the absence of certain isozymes in callus tissue, which has a negligible lignin content, may indicate that the absent isozymes are involved in lignin biosynthesis.

Polysaccharides and phenolic acid The non-cellulosic polysaccharides of flax fibre have been isolated by differential extraction procedures and found to be

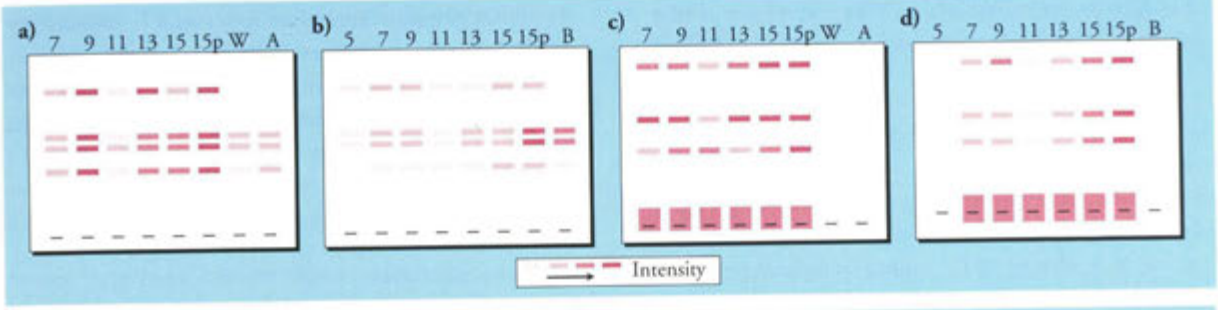

Figure 3 Peroxidase isozymes of flax stem and callus. a) and b) are acidic gels (cathode at the bottom) and c) and d) are basic gels (cathode at the top). a) and c) are hypocotyl samples and b) and d) are first internode samples. The figures are weeks of growth. (A) is Antares cultivar callus extract and (B) is Belinka cultivar callus extract.

similar to those found in softwood fibre. The xylose-rich polysaccharides, xyloglucan and (glucurono)-xylan, are present and have been identified by their characteristic limit digestion products using carbohydrase mixtures. The mannose-rich polysaccharides, glucomannan and galactoglucomannan, are also present being extracted with alkaline borate. The localisation of these polysaccharides in the separate layers of the secondary cell wall is currently being examined using cytochemical methods.

Other work has been concerned with the presence of cyclodimers of phenolic acids in cell walls. These dimers of ferulic and *p*-coumaric acids, mainly in the truxillic acid configuration, have been reported to be significant constituents of graminaceous cell walls. The hypothesis is that they are produced *in situ* by photodimerisation of the monomeric forms. Since the monomers are linked by their acidic function to hydroxyl groups in other wall polymers by ester bonds, the dimerisation process could be a potential source of structural integration. Experiments with flax have shown that its cell walls also contain the monomeric phenolic acids. Their concentration is of the same order as present in grasses but no dimers have been found. This suggests that dimerisation is less likely to be a light-induced process and the dimers may be formed enzymatically within the cell of grasses and transported as glycoconjugates to the wall for assembly.

Conclusion The involvement of specific peroxidase isozymes in lignification has not yet been confirmed but future work on excised fibre-bearing tissue and improved separation methods should provide the proof. Since lignification is likely to be a similar process in all plants, these results will be extended to more economically viable fibre-producing plants in our programme. Other work on establishing the architecture of cell walls will enable the use of more environmentally acceptable treatments to be used for the isolation of plant fibres.

Fungal and Bacterial Diseases

J.M. Duncan

A central theme of much of the work on plant genetics at SCRI has been, and will continue to be, plant disease resistance because it represents one way of controlling disease without recourse to expensive agrochemicals. The Mycology & Bacteriology Department also has an interest in the genetics underlying resistance and in the metabolic processes within the host and pathogen which result in a resistant plant. In future, most areas of work within the Department will be involved in some way with disease resistance, an area which includes some of the most important challenges in plant science.

Central to host resistance are the processes underlying host and pathogen recognition, but while it is generally accepted that early recognition of the potential pathogen by the plant host is crucial to effective resistance, little is yet known about the first steps in the process. In diseases where the pathogen exists as a series of well-defined physiologic races, it must be presumed that the initial recognition events between host and pathogen are due to the presence, or possibly absence, of constitutive elements, which are coded in some way by the genes in host and pathogen controlling vertical resistance and virulence respectively. Failure to recognise the presence of a pathogen and to respond to it by switching on the whole battery of cell processes, which together form host resistance, can result in extensive colonisation of a host as described in the article on downy mildew (*Peronospora* spp.) of *Rubus* by B. Williamson (p. 72). Highly specialised, indeed obligate, pathogens such as downy mildew do not provoke rapid host defence reactions and as a result can grow systemically through their host, thereby extending the parasitic phase of their life cycle. One practical outcome, other than the damage which eventually results, is the increased chance of unwittingly transmitting the disease in infected, vegetatively propagated material.

There are other important lessons to be taken from the work on downy mildew. Firstly, it demonstrates that resistance is the result of a dynamic interplay between host and pathogen, the outcome of which varies according to the circumstances. Altering environmental conditions or the physiology of the host can result in loss of resistance. In this case, physiological changes resulting from micropropagation, may have made plants more susceptible. This work also emphasises the specificity involved in plant disease and demonstrates the continuing requirement for intimate studies of the pathogens as well as the hosts.

Although the nature of the genes which code for the initial recognition events in pathogenesis may yet elude us, some of the subsequent events in the process

are known. The articles on the resistance of potato to *Erwinia* soft rots and blackleg, and on the induction of resistance in barley to mildew, demonstrate the considerable progress that has been made in unravelling the processes occurring in the early stages of host colonisation, enough to have reached the stage of being exploited at the practical level.

It has long been known that extracellular enzymes result in cell wall breakdown, an essential part of pathogenesis, but the realisation that they may also trigger the host's response is more recent. Highly specific elicitor molecules released by the action of extracellular enzymes stimulate various defence reactions in the host. The range of the responses is large and includes the synthesis of small molecules with antibiotic activity, phytoalexins, and pathogenesis related proteins, some of which may attack the pathogen directly. The various systems which are found in potatoes infected by soft rot bacteria are explained by G.D. Lyon (below).

The knowledge derived from fundamental studies on the biochemistry of host defence has been exploited by Lyon and Newton in their work on control of barley mildew by elicitor-like molecules from yeast. Extracts of this cheap and abundant starting material have been used to stimulate the natural defence response of the host. The results promise a cheap and environmentally acceptable future control strategy, which would be based on the induction of host resistance by the application of small quantities of non-toxic material.

A more recent discovery is that the pathogen may produce extracellular enzymes which disrupt the defence response of the host e.g. the proteolytic enzymes of *Erwinia* may destroy host enzymes essential to the defence reaction of potato. The numbers, types and actions of extracellular enzymes produced by various fungal and bacterial pathogens on their hosts, and how they relate to host defence mechanisms, are the subject of much research within the Department.

Concentration on disease resistance does not mean that other aspects of disease control have been neglected. As M.C.M. Pérombelon describes in his article, hot water treatment is a simple, cheap and effective means of controlling bacterial and fungal diseases of potato tubers after harvest which avoids agrochemicals. Other studies on potato tuber diseases have shown that their development can also be inhibited by the presence of antagonistic micro-organisms. Combining hot water treatment of tubers with applications of antagonists gave better control of blemish diseases than either treatment separately. Both strands of work have their origins in long term epidemiological studies on the survival and spread of a number of potato pathogens. Thus strategic research has pointed the way towards possible new control measures.

The biochemical basis of resistance of potatoes to *Erwinia carotovora*

G.D.Lyon & J.Heilbronn

Tuber soft rot and blackleg of stems caused by the bacterium *Erwinia carotovora* is an important disease of potatoes (Fig. 1) No satisfactory chemical control methods have been found and infection is presently limited by the use of 'clean' seed and good husbandry practices. Emphasis is also being placed on disease resistant cultivars to control losses and on the biochemical processes that underlye resistance mechanisms.

A characteristic feature of the potato-erwinia complex is the extent to which environmental conditions (in particular the oxygen status) can affect the expression of disease. Indeed some impairment of the host's resistance to *Erwinia* spp. is necessary for symptoms to develop and tuber rotting is most commonly observed when tubers are stored under conditions in which the level of oxygen within the tuber is significantly reduced. This impairment of resistance is important because it can be used to identify resistance genes whose products may not be expressed under anaerobic conditions.

The factors which are most likely to be responsible for the levels of resistance commonly observed in commercial cultivars of potatoes under aerobic conditions

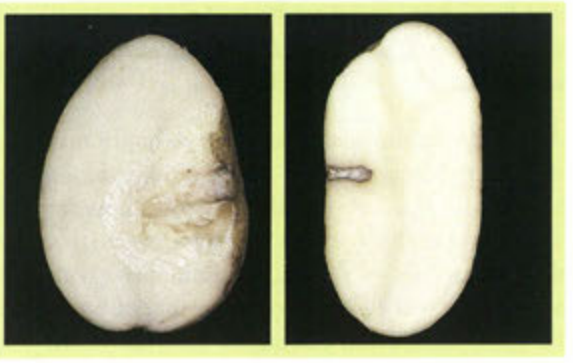

Figure 1 Rotting tuber under anaerobic conditions (left); resistance expressed under aerobic conditions (right).

are being studied at SCRI. Resistance is not due to a single factor, but to a number of mechanisms which limit infection by *Erwinia* spp. by directly affecting growth and spread of the bacteria and also by inhibiting enzymes involved in pathogenesis. This multicomponent system is similar to that operating against many plant pathogens but differs in that specific recognition mechanisms have not evolved within the potato/erwinia interactions. Thus erwinias can infect a broad host range and do not exist as specific races. The erwinia/potato interaction therefore offers an excellent opportunity to investigate horizontal resistance genes with the confidence that there are no race-specific genes present.

Infection route Soft rot bacteria are unable to penetrate the cuticle of plants directly and must therefore gain entry through wounds or natural openings. Wounds not only by-pass preformed physical barriers to penetration but may also provide readily available nutrients for bacterial growth. Further movement into the aerial parts via the stem occur through the vascular system from infected tubers, and a minimum threshold population of the pathogen may be a prerequisite for blackleg development.

Low molecular weight inhibitors The most important of the resistance mechanisms operating against *E.carotovora* may be the production of antibacterial phytoalexins such as rishitin (Fig. 2). Phytoalexins are low molecular weight antimicrobial compounds that are both synthesized by, and accumulate in, plants after exposure to microorganisms and are regarded as factors limiting growth of pathogens in many plants. Rishitin is absent in healthy potatoes, is induced by erwinias under aerobic conditions, and is absent under anaerobic conditions, thus correlating well with the observation that tubers are very susceptible under anaerobic conditions. In addition, rishitin concentrations under aerobic conditions are much reduced at temperatures above 20°C, again correlating with greater amounts of rotting at these temperatures.

Cells of *E.carotovora* in rotting tubers which were known to contain rishitin showed damage characteristic of that caused by rishitin *in vitro* when examined by electron microscopy confirming that rishitin was effective *in planta.*

Phytoalexin accumulation is not the only component of resistance and other compounds may also contribute to the resistance observed in infected tubers. Phenolics contribute to the defence mechanisms of many plants and may function by directly inhibiting bacterial growth, by inhibiting wall degrading enzymes, or as precursors in the formation of physical barriers.

Several phenolics including caffeic, cinnamic, ferulic, salicylic, sinapic and vanillic acids, together with scopoletin and coniferyl alcohol occur in potatoes and inhibit growth of *Erwinia* spp. *in vitro.* Phenolics differ in the extent to which they inhibit growth, for example, chlorogenic acid was ineffective at 1mg/ml, whilst salicylic acid caused significant inhibition at 0.1mg/ml.

In addition to restricting growth of pathogens, phenolics may also inhibit enzymes involved in pathogenesis. However, the wall degrading enzymes produced by *E.carotovora* were tolerant of most phenolics, for

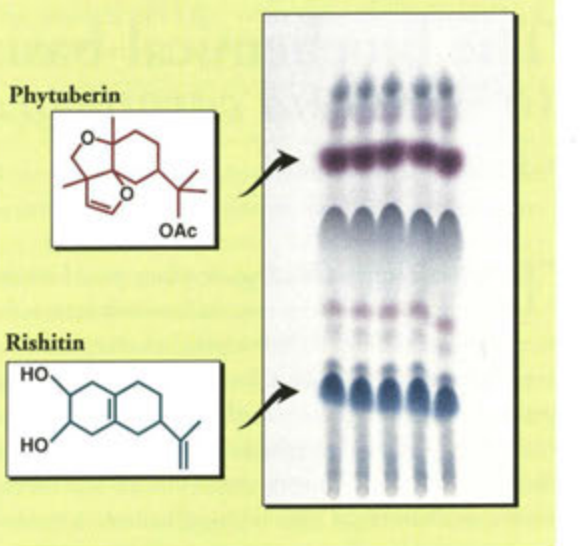

Figure 2 Thin layer chromatogram of sesquiterpene phytoalexins extracted from potato tubers infected with *E. carotovora* under aerobic conditions.

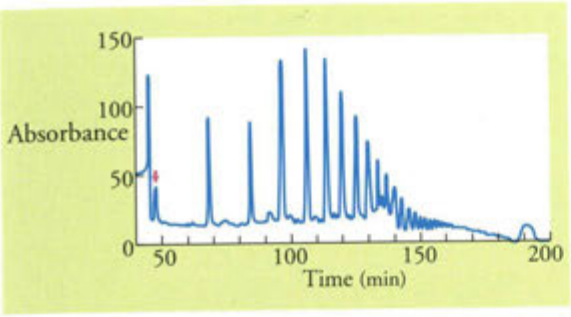

Figure 3 Preparative HPLC purification of oligogalacturonides with a degree of polymerization between 2-24 released from polygalacturonic acid by PGL. The arrow indicates dimers.

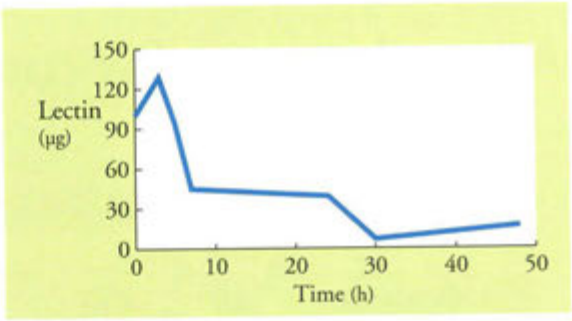

Figure 4 Degradation of potato lectin by an extracellular metalloprotease purified from *E.carotovora*. Data obtained using a laser densitometer to determine the amount of protein in each lectin band in different lanes (representing different incubation times) of an SDS gel.

example, polygalacturonic acid lyase (PGL) was not inhibited by benzoic, caffeic, chlorogenic, ferulic, *p*-coumaric, protocatechuic, salicylic, sinapic, syringic or vanillic acids at 200µg/ml and polygalacturonase (PG) was unaffected by most of them, although it was strongly inhibited by caffeic acid at 100µg/ml.

Although phenolics have been shown to affect the growth and enzyme activity of *E.carotovora*, there is no quantitative data on their concentration in cultivars differing in levels of resistance.

Resistance elicitors Although plant cell walls are barriers to infection by many microorganisms, *E.carotovora* is well adapted to colonise plant tissues and produces copious quantities of wall degrading enzymes which enable it to penetrate plant tissues. During wall degradation, enzymes such as PGL degrade pectin into smaller fragments. We have developed rapid techniques for quantifying and purifying wall fragments by HPLC (Fig. 3) and have shown that some of them, known as oligogalacturonides, are recognised by potato cells and elicit phytoalexin biosynthesis. The amount of PGL produced by the pathogen affects the amounts of elicitor-active pectin fragments released. Thus, whilst some PGL is necessary to release the fragments, very high levels of PGL quickly break down the elicitors to form inactive products unable to induce phytoalexins. These results in part explain why potatoes produce less rishitin above 20°C when PGL is more active and degrades the pectin fragments quicker than at lower temperatures. For effective elicitation of resistance the optimum sized pectin fragments must remain intact for a maximum time.

Other types of biological activity associated with the degradation products of pectin have been detected, eg extracts which are cytotoxic and inhibit lettuce root growth, and others which stimulate growth of potato tuber cells, but they have not yet been investigated in sufficient detail to determine their role in the interaction between *E.carotovora* and potato.

Isolates of *Erwinia* spp. recovered from the same host tend to be serologically related suggesting that some recognition reaction with the host plant may take place. Compounds involved in this recognition reaction have not yet been identified.

Proteins It is probable that proteins also play a role in resistance to *E.carotovora* and we have found an interaction between the phytoalexin rishitin and potato lectin (a hydroxyproline-rich glycoprotein). The lectin does not bind to healthy cells of *E.carotovora* but does bind to cells treated with rishitin which removes an outer layer from the bacteria exposing new receptor sites for the lectin.

We have recently purified an extracellular metalloprotease from *E.carotovora* and shown that it is not inhibited by potato chymotrypsin inhibitors that are reported to be involved in aphid resistance. The protease degrades potato lectin *in vitro* (Fig. 4) thus providing the first experimental evidence that a protease from a plant pathogen is capable of degrading plant proteins associated with resistance. Additionally, we have obtained polyclonal antibodies to the protease and are developing ELISA techniques to quantify the protease *in planta*. Other antibodies will be developed to quantify proteins involved in pathogenicity and those associated with resistance.

A novel system for controlling plant disease

G.D.Lyon, A.C.Newton & T.Reglinski

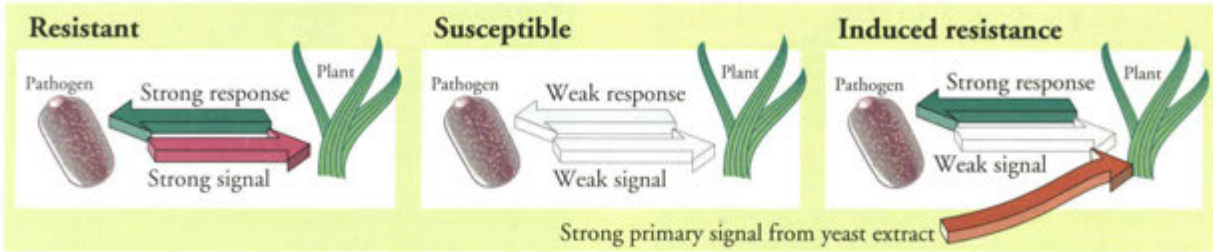

Figure 1 Schematic presentation of molecular events occurring during induction of resistance. Susceptibility occurs because of the failure of the plant to receive a strong stimulus from the pathogen. Application of the yeast extract supplements the 'message' from the pathogen thereby priming the plant to produce a strong response when a potential pathogen is present.

Plant diseases have to be controlled to maintain the high quality and yield of food and ornamental crops. A number of techniques which include the use of quarantine procedures, disease-free seed and root stocks, resistant cultivars, and application of pesticides have been used to achieve disease control. However, breeding for disease resistance and application of pesticides have not always provided lasting solutions to the problems because the pathogen frequently adapts to overcome the control mechanism.

At SCRI fundamental research into the nature of disease resistance in a number of host/pathogen interactions is providing information to develop new methods of disease control in the field. These studies have shown that the mechanisms involved in conferring resistance to disease differ in detail between plant species and between pathogens, but that there are also broad similarities between them. Thus resistance is due to a combination of physical and chemical barriers which are either preformed or induced only after infection. Phytoalexins are compounds produced by a plant in response to infection by a pathogen and which limit the growth of that pathogen. They are a very important component of resistance and are produced when the plant detects the presence of specific compounds, phytoalexin elicitors, which are released by the pathogen.

Plants are susceptible to infection when they fail to respond quickly to the presence of the pathogen. Thus resistance can be increased if the plant's response is accelerated. We are utilizing this concept by applying resistance elicitors derived from bakers yeast to "prime" the resistance mechanisms so that the plant responds more quickly to the pathogen (Fig. 1). This quickening of the plant's response does not in itself involve the production of high levels of phytoalexins within the plant and results in no detrimental effects.

Control of barley mildew We have demonstrated by laboratory and glasshouse experiments that mildew (*Erysiphe graminis* f.sp. *hordei*) on detached leaves or whole plants of barley (Fig. 2) and oats can be reduced by application of the elicitors in yeast

Figure 2 Right: Glasshouse grown barley plants cv Golden Promise inoculated with mildew. Left: comparable plants sprayed 3 times with yeast extract.

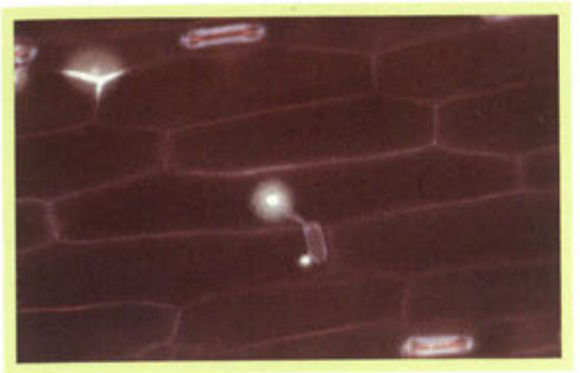

Figure 3 Germination of mildew spore on barley leaf showing penetration and host response (central fluorescent papilla surrounded by fluorescent zone). Stained with aniline blue in lactophenol.

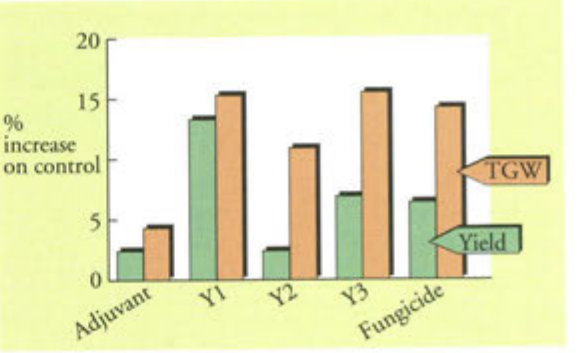

Figure 4 Effect of various yeast extracts on yield and thousand grain weight of spring barley cv. Triumph in field experiments.

extracts. At a cellular level resistance to mildew is characterised by production of structures in the cell wall called papillae around the point of attempted infection (Fig. 3). Application of yeast extract prior to inoculation with mildew increases the speed of papillae formation which may be crucial in resistance expression. In addition, application of yeast extract to barley leaves causes a rapid rise in activity of phenylalanine ammonia lyase, the first enzyme in the biosynthesis of phenylpropanoids which are important components of resistance, again providing direct evidence that the yeast extracts are working through the plants own resistance mechanisms.

Field experiments in which mildew infection was assessed both visually and by an ELISA technique, confirmed that the extracts reduced the level of mildew in both spring and winter barley. Whilst a cultivar such as Golden Promise, which is very susceptible to mildew, can be partially protected from infection using yeast extracts, the level of control and yield achieved is not as good as that obtained with a systemic fungicide. However, with cultivars which already possess some resistance to infection, e.g. Triumph, the levels of disease reduction and yield have been comparable to those after fungicide application (Fig. 4).

Further development of this disease control method is required. However the final formulation of a product will have a significant effect on the efficacy of the treatment and will determine the frequency of spray application and whether it must be used in conjunction with fungicides. Results already suggest that these yeast extracts have commercial potential for the control of mildew on cereals. In addition, because of the similarities in recognition and response reactions to a range of plant pathogens, we believe that these yeast extracts will also be effective against diseases in other crops and will represent an additional, environmentally attractive weapon in the control of plant diseases.

Acknowledgements We would like to that the Home-Grown Cereals Authority for financial support of the yeast work and Nickerson Seeds Ltd for the kind gift of soybean seed.

Hot water treatment to control seed borne blackleg and fungal blemish diseases of potatoes

M.C.M. Pérombelon

Potato seed tubers are frequently infected by several bacterial and fungal pathogens, namely *Erwinia carotovora* subsp. *carotovora* (tuber soft rot), *E. carotovora* subsp. *atroseptica* (Eca, tuber soft rot and blackleg), *Polyscytalum pustulans* (skin spot), *Helminthosporium solani* (silver scurf), *Phoma foveata* (gangrene), *Fusarium* sp. (dry rot), *Rhizoctonia solani* (black scurf) and *Colletotrichum coccodes* (black dot). The diseases are primarily seed borne and infection is initially superficial or in lenticels and wounds.

Disease develops when environmental conditions reduce host resistance and favour growth of the pathogens and, in the case of blackleg, it is related to the number of pathogenic bacteria on the tubers. Blackleg symptoms are rare when there are less than c. 10^3 bacterial cells per tuber[1]. Disease control has relied on seed certification schemes to ensure the production of healthy stocks but as they have relied on roguing and visual inspection of the growing crop and harvested tubers, latent infections escape detection. It is now known that blackleg incidence in a mother crop has little bearing on the level of latent infection by Eca of the progeny tubers. Tubers can be contaminated in soil by bacteria rotting from mother tubers in the absence of blackleg, and during mechanical handling with contaminated machinery. Similarly, there are no visual symptoms of infection by some fungal pathogens immediately after harvest.

Chemotherapy has been used with varying degrees of success to control the fungal pathogens but has failed to control erwinias because the bacteria are protected in lenticels and in suberised wounds. The success of fungicide treatments depends on timing and the amount of soil present on the tuber surface and some of the fungi have developed resistance to some fungicides.

Hot water treatment Attempts to treat potatoes with dry or moist heat to control diseases have been unsuccessful, mostly because of adverse effects on appearance and sprouting. In contrast, hot water treatment of tubers was shown to reduce fungal infection in laboratory experiments[2] and more recently tuber contamination by erwinias[3] . Heat is transferred to a solid more efficiently from water than air and provided the thermal death point of the pathogens is reached without affecting the viability of the eyes, disease-free tubers can be produced.

Two different hot water treatment methods were tried in the early eighties to control blackleg commercially. In one, one tonne boxes of seed tubers were dipped in water at 45°C for 30 min[4]. Although initial results were promising, several problems became evident; (1) it was difficult to restore the water to the required temperature uniformly and rapidly within the tuber mass after the boxes were placed in the water, and (2) treated tubers could not be dried uniformly and rapidly in a bulk system even when forced ventilation was used. The other method is a patented continuous flow hot water treatment system developed by Aberdeen Biotechnology with the support of SCRI. It

Figure 1 Continuous hot water treatment equipment: prewashed tubers being fed to hot water tank.

does not suffer from the problems associated with a bulk dipping system and produces dry tubers free from soil with a uniformly low level of contamination and good sprouting ability [5,6].

Treatment of tubers occurs continuously in four stages: (1) tubers are unloaded into a hopper and pass through a cold water pre-washer in which soil from the tuber surface is removed, (2) the washed tubers then pass over an inspection table where any rotting tubers present can be removed, (3) tubers then move to the hot water plant which consists of a revolving wheel with perforated baskets on the periphery each holding c. 50 kg tubers, (4) tubers are dried with high pressure air jets before boxing or bagging. Heat absorbed by the tubers ensures that any residual moisture on the surface evaporates quickly after leaving the machine. Adequate ventilation after treatment is essential to remove residual heat and prevent condensation on the tubers. The present machine is mobile and can treat 5-7 t/h (Fig. 1).

Susceptibility of pathogens to heat Laboratory studies showed that the thermal death points of erwinias *in vitro* were 50-52°C and that in naturally contaminated tubers of different cultivars subjected to different temperature-time combinations, contamination was lowered to a barely detectable level at 53°C for 7 min, 55°C for 5 and 59°C for 3 min. The choice of suitable temperature-time combinations can be determined experimentally by comparing the effect of a selected range of treatments on tuber contamination and crop performance in the field. Heat tolerance of 3-wk-old cultures of several fungal pathogens on Cellophane at different water temperatures for 5 min showed that all were progressively affected by rising temperatures within the range 53-57°C.

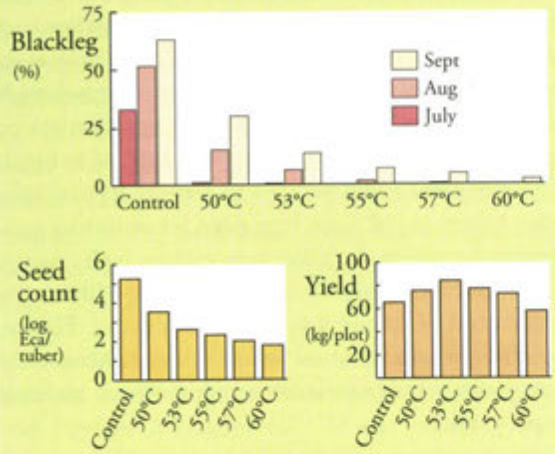

Figure 2 Effect of hot water treatment of cv. Désirée seed tubers at different temperatures for 5 min on disease incidence, Eca on seed tubers, and on crop yield.

Figure 3 Effect of hot water treatment (55°C, 5 min) of cv Désirée seed tubers in April on plant growth. a) treated plot b) untreated control plot.

Field trials

Blackleg The effects of treating naturally contaminated seed tubers of cv. Désirée in April at different temperatures for 5 min on the incidence of blackleg and crop growth and yield in the field are shown in Figure 2. Seed contamination level by Eca and blackleg incidence were progressively reduced with increasing temperature; disease incidence increased as the season progressed but was greater at the lower treatment temperatures; emergence was delayed with increasing temperatures but significant blanking occurred only at 60°C. The effect of the treatments on yield was complex; delayed emergence retarded crop maturity while a low incidence of blackleg increased yield and compensation by neighbouring plants probably negated any effect of blanking on yield.

These results and others indicated that treatment at 55°C for 5 min is optimum for blackleg control, crop growth and yield (Fig. 3). The high levels of blackleg obtained in the untreated plants was attributed to high seed contamination and exceptionally wet growing conditions favourable for blackleg development.

Fungal diseases The same temperature-time combination treatment used for blackleg control (55°C, 5 min) was applied to tubers of cv. Maris Piper naturally infected with different fungal pathogens. Tubers were stored at different temperatures following treatment to favour growth of the various fungi. Inocula of skin spot, silver scurf and black scurf were virtually eliminated by the treatment regardless of its timing and storage periods (Fig. 4). Results for black dot were inconclusive partly because of a low initial level of infection and partly because of difficulties distinguishing live from dead tissue. Excellent control of gangrene was obtained while infection remained superficial but was less good if treatment was delayed until deep lesions had formed in store.

Preliminary tests have indicated that hot water treatment can partially control powdery scab and although the pathogen was not eliminated, numbers of viable spores were reduced after treatment. Here also the depth of the lesion in the seed tuber appeared to be important.

Cultivar reaction Hot water treatment may cause reduced, late or irregular emergence in some cultivars,

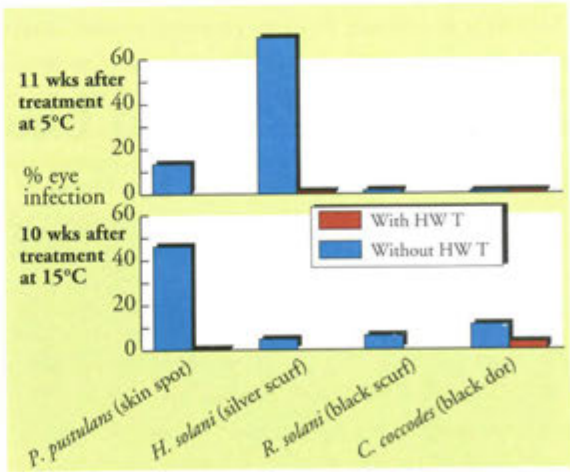

Figure 4 Effect of hot water treatment (HWT) on the viability of different fungal pathogens in the eyes of naturally infected cv. Maris Piper tubers 11 wks after treatment.

especially when treated late in the storage season. Susceptibility to damage appears to be related to some unknown feature of the cultivar and the physiological age of the tubers but not to their maturity characteristic. When seed-sized tubers of eight cultivars were treated at monthly intervals from December to April, plant growth and yield were reduced more by later than earlier treatment and some cultivars were affected more than others. By the end of June, growth was similar to that of the untreated controls regardless of time of treatment in cultivars Désirée, Marfona, Kondor and Record, while growth was poorer than untreated controls in plants of cultivars Maris Bard and Wilja from seed treated after February and in Estima and Maris Piper from those treated after December. Tuber yields of all cultivars were reduced by treating seed in March and April, except in Record which was never affected by treatment.

Conclusions Blackleg is an intractable problem and although it can be eliminated in axenically produced nuclear propagation material and reduced by shortening multiplication periods with mini tubers, contamination of seed stocks can occur rapidly by several different routes[6]. Until resistant cultivars are produced, the continuous flow hot water treatment is the only practical control measure which ensures the production of dry tubers with a low level of erwinia contamination. In addition, the treatment is a practical alternative to fungicide treatment for controlling superficial fungal pathogens with the advantage that it is not affected by soil on the tubers. The technique would allow the introduction of biological control agents after treatment to prevent recontamination and to protect progeny tubers in the field. The eyes of treated tubers often become heavily infected with saprophytes which may behave as antagonists to pathogens and improve the persistence of control. The treatment could be used to prevent sprouting in ware tubers stored for a long time for industrial processing by raising the water temperature high enough to kill the eyes or greatly retard sprouting without the application of sprout suppressant chemicals. Finally, tubers after treatment are free of soil and therefore of nematodes which would be an advantage to the seed export trade.

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Downy mildew in blackberries and raspberries

B. Williamson, Wendy A. Breese[1] & R.C. Shattock[1]

Downy mildew (*Peronospora rubi*) is extremely rare on wild blackberries and raspberries in the UK and there have never been serious outbreaks of the disease until the mid 1980s. Consequently, the Rubus cane fruit industry was unprepared for the destructive outbreaks which occurred at the propagation stage on blackberries and blackberry x red raspberry hybrid cultivars (eg Boysenberry and Tummelberry) in south east England.

In new Zealand a disease of Boysenberries call 'dryberry' is said to be caused by *Peronospora sparsa*, the fungus which also causes the downy mildew of roses. This fungus is morphologically identical to that found on wild and cultivated cane fruits, but it has never been shown whether the pathogen from roses can infect blackberries and Rubus cane fruits and, conversely, if the fungus on cane fruits can infect roses.

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The MAFF Plant Health Division imposed a nil tolerance for downy mildew during inspections of plants presented for certification as soon as the disease was discovered in newly propagated stocks. This action was based primarily on the character of the disease under New Zealand conditions where it systemically infects plants and spores are airborne. There was a complete lack of information about how it may spread to affect raspberry and blackberry plantations in the UK.

Micropropagation has been introduced in the UK to multiply stocks of new cultivars and to avoid viruses and *Phytophthora* root rot. It was expected that downy mildew would also be controlled by this method. However, numerous stocks of newly-weaned plants of Tummelberry, and blackberry cultivars Loch Ness, Merton Thornless and Bedford Giant became severely affected by downy mildew and had to be destroyed. Similar outbreaks of downy mildew on micropropagated blackberries, hybrid berries and red raspberries were also reported in the mid 1980s in California and Washington.

Inoculation experiments In 1988 a MAFF-funded research project was established jointly between the University College of North Wales, Bangor (UCNW) and SCRI to study the biology of *P. rubi* on rubus cane fruits. Experiments were performed in the laboratory, in polytunnels and in the field at Bangor where relatively little risk to the industry was perceived. The aim of the work was to define the conditions for spore production and germination, to establish the relative susceptibility of current UK rubus cultivars and test whether roses and blackberries could be cross-infected by the same fungal isolates.

Work at Bangor showed that the rose cv. Can Can became infected at the same time as a range of cane fruits exposed to heavily-infected Tummelberry plants in a polytunnel. In collaboration with ADAS at Efford EHF, it was shown also that Tayberry plants became infected when they were exposed amongst roses in a fungicide trial to control rose downy mildew. Both these experiments presented strong circumstantial evidence that downy mildews on both roses and cane fruits can cross-infect.

It has now been shown repeatedly in the laboratory that isolates of mildew from roses can infect young detached leaflets of blackberries, red raspberries and their hybrids. Sporangiospores (asexually produced) germinate freely on leaves over a wide temperature

Figure 1 Mycelium of *P. rubi.*

range, penetrate the leaf surface and establish a network of mycelium between living cells, sending fine tubular feeding haustoria into cells. The fungal mycelium was traced from leaf laminae, through the stalks and into stems by fluorescence microscopy.

The fungus also infected the flowers and fruits of red raspberries, blackberries and hybrids where it was found growing systemically in petals, stamens, and sepals and it penetrated the ovary walls to form a mycelium within developing embryos and drupelets. This type of infection gave rise to misshapened fruits as described in New Zealand. The malformed berries were composed of red and green drupelets of variable sizes. Some apparently healthy fruits were also infected. The hybrid berries Boysenberry, Tummelberry and Tayberry were more susceptible than the blackberries, and the raspberries were the most resistant of the cultivars tested.

Field trials It is not known whether new stocks of plants carrying downy mildew constitute a serious threat to established plantations in the UK. In field trials, dry weather persisted during the spring and early summer in two successive seasons and mature Tummelberries exposed to infector plants failed to

Figure 2 Malling Delight fruits with downy mildew.

Figure 3 Symptoms of downy mildew on leaves of Boysenberry.

develop significant disease, even after use of overhead irrigation. Nevertheless, some leaves and fruits became infected and further studies of these plants may show whether the disease can persist and initiate an epidemic.

The recent outbreaks of downy mildew on micropropagated blackberries and hybrid berries were most probably caused by spores produced on infected wild or commercial roses where the disease has caused significant damage for many years. At weaning, the plants are succulent and highly susceptible to infection which will be difficult to control without expensive spore-proof facilities. Exhaustive microscopical studies of *in vitro* mother plants used to produce micropropagated plants commercially have shown that these stocks were entirely free of all fungal pathogens.

Disease control Up to twelve sprays of fungicide have been necessary to control downy mildew on Boysenberry in New Zealand and it is imperative that new plantations in the UK are established from healthy certified stocks to avoid the possibility of long-lived systemic infections becoming established in roots and crowns of plants. Metalaxyl is effective against the fungus, but there is no label-approved use on rubus cane fruits under glass or at the propagation stage. Resistance to phenylamide fungicides has developed in other closely related fungi and it is therefore important to avoid this type of fungicide during propagation, otherwise new fungicide-resistant strains will arise and be disseminated worldwide on new germplasm.

Closer links between the rose and cane fruit industries in Europe are now required to tackle this disease that seems to affect both crops. Fungal isolates from a wider spectrum of cane fruits and rose hosts should be examined to further define the taxonomic status of the pathogen and to devise control measures that can operate within the constraints of both industries.

This airborne pathogen also produces persistent resting spores (oospores) in leaves and flowers and it will probably be difficult to eradicate if it becomes established in UK..

Plant Viruses

B.D. Harrison

In the Annual Report of the Scottish Horticultural Research Institute for 1966, I wrote that we aim not only 'to identify the causes of diseases and to find ways of avoiding them' but also 'to study in more depth some of the basic properties of the viruses', including those involved in virus replication, host range and vector transmissibility. Although much has been achieved during the intervening 24 years and the relative emphases on different facets of the programme have changed, our broad approach remains the same and consists of four main themes: molecular biology, conventional and non-conventional resistance, mechanisms of transmission by vectors, and virus detection, characterisation and variation.

In the Molecular Biology programme, several virus genomic molecules have been sequenced. The nucleotide sequences of DNA-1 and DNA-2 of Indian cassava mosaic geminivirus (ICMV) proved to be typical for whitefly-transmitted geminiviruses. DNA-1 has substantial sequence similarities with other whitefly-transmitted geminiviruses whereas DNA-2 has only limited similarities, even with African cassava mosaic geminivirus (ACMV). Also the 'common region' in ICMV DNA is different from that of ACMV except for a region near the stem-loop structure. These differences therefore support the view that ICMV and ACMV should be considered separate viruses.

The nucleotide sequence of raspberry ringspot nepovirus RNA-2 was completed and is described on p. 84. Determination of the sequence of raspberry bushy dwarf virus RNA-2 confirmed that RNA-3, the subgenomic messenger RNA for viral particle protein, is derived from the 3' end of RNA-2. The 5' half of RNA-2 encodes a single protein that has slight sequence similarity to comparable proteins that are implicated in cell-to-cell movement of alfalfa mosaic and tobacco streak viruses.

Sequencing of the 10 kb genomic RNA of parsnip yellow fleck virus (PYFV) has progressed well. The 5' end was found to encode virus coat protein whereas the 3' half encodes regions of a polyprotein that have similarities to the polymerase and protease of both cowpea mosaic comovirus and poliovirus.

Hybridisation tests with cDNA clones specific for either RNA-1, RNA-2 or RNA-3 of potato mop-top furovirus (PMTV) indicated that their sequence similarity is confined to ends of the molecules. No similarity could be detected in other regions, suggesting that the virus has a tripartite genome. RNA-2 contains the coat protein gene together with three other open reading frames which encode proteins that have similarities to those derived from the 'triple gene block' of barley stripe mosaic and other viruses.

Comparisons of the genomic RNA sequences of strains of potato leafroll luteovirus (PLRV) from Scotland, The Netherlands, Canada and Australia showed that the Scottish strain has 119 nucleotides at its 5' end in place of a sequence of 14 other nucleotides in the other strains. However, it was found that less than 1% of PLRV-RNA molecules in particles of the Scottish strain have these extra nucleotides, which are virtually the same as those in a chloroplast RNA species. This is considered to be evidence that PLRV-RNA and chloroplast RNA recombine in plants, or have done so in the past. Other work on PLRV RNA has identified the 5' end of the subgenomic mRNA for coat protein at a point 212 nucleotides upstream from the coat protein gene.

In the Virus Resistance programme most of the work done was on resistance of potatoes to PLRV. The evidence for a single dominant gene which restricts PLRV concentration, and the effect of transformation with the PLRV coat protein gene are described on p. 78. Virus resistance mechanisms continue to be explored in a project which is linked to the International Potato Center, Peru and funded by ODA. Commercialisation of the PLRV coat protein gene is being undertaken by the Nickerson International Seed Company.

The processes involved in cell-to-cell movement of viruses constitute potential targets for genetically engineered resistance in plants but are still poorly understood. Examples of the kind of information that can be got with the aid of a new method of injecting individual trichome cells with virus inocula or fluorescent materials at controlled pressure are detailed on p. 86.

Application of alkaline phosphatase-based ELISA to detect viruses in viruliferous insects as part of the programme on Mechanisms of Vector Transmission is limited by the low virus content of the insects and by interference in the assay caused by endogenous phosphatases. The use of penicillinase-based ELISA to detect okra leaf curl geminivirus (OLCV) in its whitefly vector, *Bemisia tabaci*, has proved advantageous. With the aid of a monoclonal antibody that reacts with OLCV in triple antibody sandwich ELISA, the virus could be detected in about half the individual *B. tabaci* cultured on infected okra plants. The virus content of the insects did not increase greatly with an increase of acquisition access period from 1 to 7 days. It decreased slowly when insects were transferred from okra to cotton, a non-host of OLCV, but could still be detected for at least 18 days. There was no evidence for OLCV replication in the whiteflies.

In further work on groundnut viruses from Malawi, evidence was obtained that the sap-transmissible causal agent of groundnut streak necrosis is dependent on another agent which infects the weed *Tridax procumbens*, but not groundnut, for its transmission by aphids. The interaction between the two may be similar to that needed for transmission of carrot mottle umbravirus by aphids.

In the programme on Virus Detection, Characterisation and Variation, raspberry veinbanding disease was shown to be caused by rubus yellow net virus (RYNV) in association with raspberry leaf mottle virus (RLMV) and not, as previously thought, RYNV plus black raspberry necrosis virus (BRNV). However, infection with BRNV or raspberry leaf spot virus intensified the veinbanding symptoms induced by RYNV + RLMV. Gooseberry plants affected by veinbanding disease were found to contain a virus which has bacilliform particles of 130 x 30 nm that superficially resemble those of RYNV and the newly described 'badnaviruses', and which is mechanically transmissible to *Nicotiana occidentalis*.

Raspberry bushy dwarf virus has become prevalent in England and France in the raspberry cultivar Autumn Bliss, which develops a severe yellows disease, and resistance-breaking isolates of the virus were identified in commercial plantings of Tayberry and raspberry cv. Delight in Kent.

Detailed studies of the viruses known as narcissus yellow stripe and narcissus latent have culminated in the conclusion that they are closely related and may be strains, and that they constitute the first members of a new virus group typified by having filamentous particles about 650 nm long containing a particle protein of more than 40 kDa, pinwheel cytoplasmic inclusion bodies and a pattern of RNA translation products

unlike that of other viruses with filamentous particles. A fuller account is given on p. 80.

Panels of monoclonal antibodies (MAbs) were prepared to okra leaf curl geminivirus (OLCV) and solanum nodiflorum mottle sobemovirus (SNMV). The OLCV MAbs detected virus isolates from the Ivory Coast and Nigeria, and should be useful for virus detection and identification. Of the SNMV MAbs, some reacted with intact virus particles but others only with denatured coat protein, which could be detected in immunoblots. Reactions with MAbs to potato mop-top furovirus provided evidence of only slight antigenic variation between isolates. The epitopes were found by immunogold staining to be located either on the surface of the sides of the rod-shaped particles or at one of their ends. To identify epitopes detected by MAbs produced to PLRV particles, a set of overlapping hexapeptides was synthesised, representing the complete amino-acid sequence of the PLRV coat protein. Three of the MAbs reacted with peptides representing an amino acid sequence close to the N-terminus of the coat protein, suggesting that this is an immunodominant region. Further work on variation among South American isolates of PMTV and PLRV is planned in an EEC-funded collaborative project with the International Potato Center, Peru.

A valuable side-product of the work on MAbs was the development of a hybridoma cloning supplement which eliminates the need for mice to act as sources of peritoneal macrophages to be used as feeder cells. The medium is to be commercialised by Life Technologies Inc.

Several geminiviruses, including African (ACMV) and Indian cassava mosaic (ICMV), euphorbia mosaic and Indian tomato leaf curl (ITomLCV) were detected in plant extracts by the polymerase chain reaction (PCR) using degenerate primers specific for relatively conserved sequences in the stem-loop structure within the common region of the viral DNA, and in the particle protein gene in DNA-1. ITomLCV was purified in small amounts and found to have a distinctive epitope profile in ELISA with MAbs to ACMV or ICMV. This profile was conserved in virus isolates from different fields and the similar profiles of virus isolates from infected weeds indicated that they are reservoirs of the virus. In collaboration with V. Muniyappa (University of Agricultural Sciences, Bangalore) and A. Varma (Indian Agricultural Research Institute, New Delhi), geminivirus isolates from eight crop legumes in different parts of India were detected in ELISA with MAbs to ACMV or ICMV. They showed two main types of epitope profile, with small variations within each type. Isolates from *Lablab purpureus* were of one type whereas those from horsegram, black gram, cowpea, pigeon pea, French bean, lima bean and soybean were of a second type. This pattern of reactions supports the view that these isolates represent two distinct geminiviruses. Further tests with the MAbs, in collaboration with G. Thottappilly, International Institute of Tropical Agriculture, Nigeria and others, detected geminivirus isolates in several weed species and in castor bean, but not in yellows-affected cowpea. The virus in castor bean was antigenically indistinguishable from West African isolates of ACMV but the weed isolates were very different. The patterns of antigenic variation in geminivirus isolates from cassava, okra and tomato are described on p. 88.

The extent of variation in epitope profiles of viral particle protein is a measure of variation in only one viral gene, whereas hybridisation with cDNA probes can be used to assess variation in all parts of the genome. This approach was used to compare several luteoviruses. Probes for sequences in open reading frame (ORF) 1 or 2a at the 5' end of PLRV RNA did not react with other luteoviruses whereas probes for sequences in ORF 2b or 5 reacted with barley yellow dwarf virus-RPV (RPV) RNA and a probe for ORF 3, encoding the virus particle protein, reacted with RNA of RPV, groundnut rosette assistor, carrot red leaf and beet western yellows luteoviruses. Thus some parts of the luteovirus genome are more strongly conserved than others.

Resistance to potato leafroll luteovirus

H. Barker, B. Reavy, A. Kumar, M.A. Mayo, R.M. Solomon-Blackburn & J.A.T. Woodford

Potato leafroll luteovirus (PLRV) occurs in all countries where potatoes are grown and is the most economically important potato virus, causing large crop losses in many regions (Fig. 1). Its main vector is the aphid *Myzus persicae*, and its spread can be partly controlled by the use of insecticides. However, partly because of the growing desire to minimise the use of agrochemicals, there is increasing interest in producing resistant cultivars. Although single genes for resistance to other common potato viruses are effective and in use in breeding programmes, previous research on PLRV has failed to identify comparable resistance genes in potato. The moderate degree of resistance expressed by some potato genotypes appears to be controlled by multiple genes. We describe below two additional types of resistance, one under simple genetic control and the other produced by genetic engineering.

Figure 1 Symptoms of potato leaf roll virus.

Resistance under simple genetic control Use of ELISA to determine PLRV concentration has led to the identification of a type of resistance which is expressed as a substantial restriction on the amount of virus that accumulates in infected plants. For example the virus titre in Pentland Crown (a resistant cultivar) is typically only 5 to 20% of that in Maris Piper (a susceptible cultivar). This restriction of virus multiplication is expressed both in plants with primary infection (current-season infection) and in those with secondary infection (tuber-borne infection). There has been no deliberate selection for this trait in breeding programmes and the occurrence of this type of resistance is therefore fortuitous. Nevertheless, it is valuable because plants that possess it, although infected, remain poor sources of virus for vector aphids (Fig. 2).

	Sampling date	Virus content: Leaves (ng/g)	Virus content: Aphids (pg/insect)	Transmission to test plants (%)
Maris Piper	4 June	660	980	58
	20 June	1500	452	13
	13 July	513	0	2
	3 August	1000	0	0
Pentland Crown	4 June	20	0	0
	20 June	60	0	3
	13 July	45	0	0
	3 August	30	0	0

Figure 2 Acquisition and transmission of potato leafroll virus by *Myzus persicae* from field-grown potato plants.

The consequences of using this resistance were tested in 1989 in a field experiment. The experiment sought

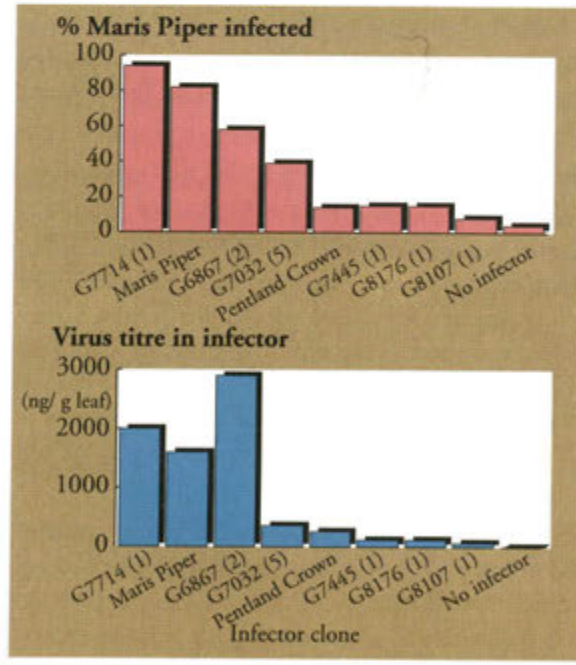

Figure 3 Percentage of plants infected with PLRV in plots of Maris Piper containing infector plants of clones with differing virus concentrations.

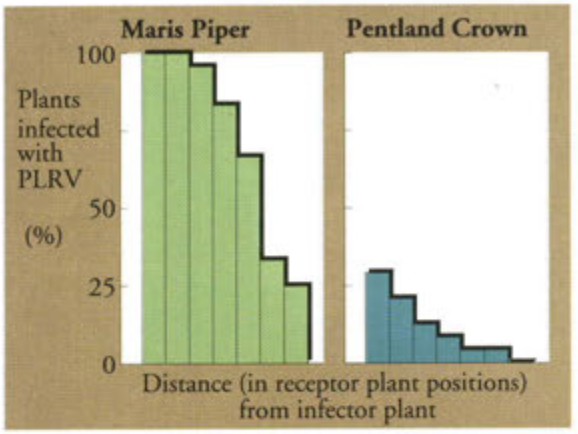

Figure 4 Infection with PLRV* in receptor plants up to seven positions from infector plants of Maris Piper and Pentland Crown.
*24 plants tested by ELISA at each position

to estimate the spread of PLRV from infected plants of a range of clones, that differ in the concentration of PLRV reached in secondary infection, to plants of a virus-free receptor cultivar (Maris Piper). In this experiment, virus was spread much less from plants of clones in which little PLRV accumulated than from plants of clones which contained large amounts of virus (Fig. 3). For example, there was 6-fold less spread of PLRV from plants of cv. Pentland Crown, a resistant genotype, than from plants of the susceptible cv. Maris Piper which contained 6-fold more virus than Pentland Crown plants. Furthermore, virus spread over a greater distance from infector plants of Maris Piper than from those of Pentland Crown (Fig. 4). Resistance to PLRV accumulation is therefore a valuable phenotypic trait which should be selected in breeding programmes.

To determine the inheritance of this trait, a resistant and a susceptible parent have been crossed and their progeny tested for segregation into phenotypic classes based on PLRV concentration in plants with secondary infection. Progenies obtained both from this cross, and from selfing the resistant parent, segregated into two phenotypic classes with either high or low virus concentration. A progeny obtained by selfing the susceptible parent did not segregate, all genotypes having a high virus concentration. In these tests, the relative numbers of plants with high and low PLRV concentrations fit the hypothesis that the resistant parent has a dominant major gene for resistance in a simplex state and the susceptible parent is homozygous recessive (Fig. 5). Knowledge of the inheritance of this trait will facilitate its systematic use in breeding programmes.

	Number of genotypes - Low: high PLRV concentration		
	Results obtained	Results expected * Ratio	Numbers
G7445(1) x Maris Piper	58:40	1:1	49:49
G7445(1) x G7445(1)	26:9	3:1	26:9
Maris Piper x Maris Piper	0:21	0:1	0:21

* Assuming 'low concentration' character is controlled by a single dominant gene occuring in a simplex state in the resistant parent (G7445(1)), and the susceptible parent (Maris Piper) is homozygous recessive.

Figure 5 Numbers of genotypes in progenies from crosses between Maris Piper (susceptible) and G7445(1) (resistant) with high and low PLRV concentrations.

Genetically engineered resistance Genetic engineering methods offer a new approach to correcting deficiencies in crop plants, such as lack of virus resistance, without compromising good commercial attributes. In recent years, virus-specific resistance has been induced in several species by genetic transformation with sequences encoding the coat proteins of viruses in several taxonomic groups. However, little information is available for luteoviruses which, unlike the other viruses tested, are confined to phloem tissue and are not sap-transmissible. In all examples so far examined, the protection is manifested as prevention of infection, or as a delay in the onset of virus accumulation and/or symptom development in systemically infected leaves. However, once the transgenic plants become systemically infected, the virus may reach concentrations similar to those in control plants.

Plants of two potato cultivars (Désirée and Pentland Squire) were transformed with *Agrobacterium tumefaciens*

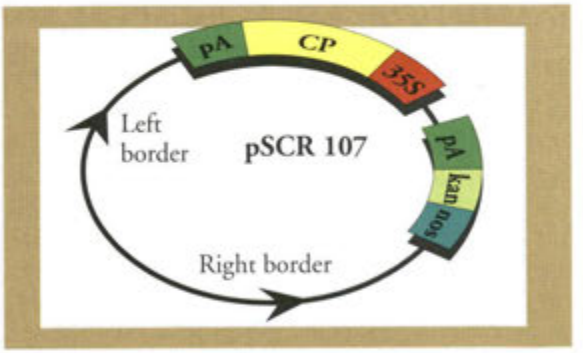

Figure 6 Diagram of plasmid pSCR 107. The sequence between the left and right borders is inserted in the plant genome.
pA = nopaline synthase polyadenylation or terminator sequences.
CP = cDNA encoding potato leafroll virus coat protein.
35S = cauliflower mosaic virus 35S promoter.
kan = Tn5 neomycin phosphotransferase (NPT II gene).
nos = nopaline synthase promoter.

containing a binary expression vector. The vector (plasmid pSCR 107) contained sequences that encode the coat protein of PLRV under the control of the 35S promotor from cauliflower mosaic virus (Fig. 6). The genomic DNA of six transgenic clones of Désirée and ten clones of Pentland Squire was analysed by Southern blotting and all were found to contain a single copy of the PLRV coat protein gene. Northern blotting showed that RNA transcripts containing PLRV coat protein mRNA were readily detected. PLRV coat protein could be detected in the plants of some transgenic clones by using sensitive immunoblotting techniques. Tubers were collected from transgenic plants that became infected following inoculation either by large numbers of viruliferous aphids or by grafting with scions from infected plants. These tubers were used to grow plants with secondary infection. In contrast with the results of studies with coat protein-mediated resistance to viruses in other taxonomic groups, less virus accumulated in the transgenic plants of some clones than in control plants. For example, in plants of two particular transgenic clones, one of Désirée (clone B1) and one of Pentland Squire (clone C4), the PLRV concentration at different sampling times was about 15% and 30% respectively of that in control plants. Some typical data from these and some other transgenic clones are shown in Figure 7.

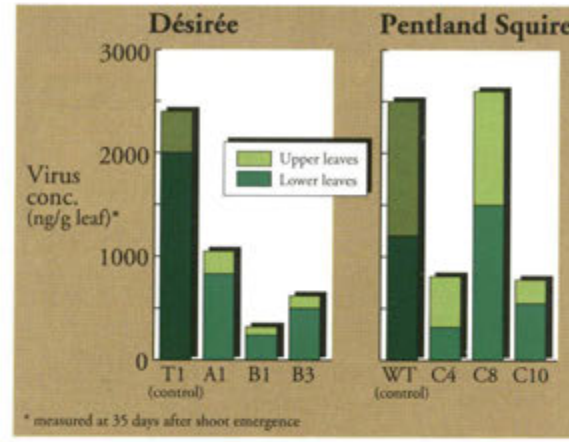

Figure 7 Comparison of PLRV accumulation in transgenic and non-transgenic potato clones.

Thus, transformation with sequences encoding the PLRV coat protein introduces into plants a measure of resistance to the virus. Interestingly, the expression of this resistance resembles that controlled by a conventional gene (see previous section). Experiments are in progress to determine whether the underlying mechanism of resistance is the same in both instances and whether these two types of resistance will complement one another.

Progress in research on narcissus viruses

W.P. Mowat, S. Dawson, G.H. Duncan & D.J. Robinson

The U.K., which has long been the world's largest producer of narcissus, now leads the world in the production of virus-tested (VT) stocks of this crop. Pre-eminent in this development are Scottish farmers who have incorporated narcissus into a mixed arable and livestock farming system. The provision of this new environment for the crop, in contrast to the intensive culture on traditional specialist bulb farms, has been a key factor in the successful establishment of the Scottish VT stocks programme (Fig. 1). Previous SCRI reports have documented much of the progress of this joint programme with the industry in the provision of VT mother plants, the development of a

Figure 1 Virus-tested narcissus stocks on a traditional Scottish arable-livestock farm.

rapid propagation method, epidemiological studies, and the design and performance of control measures to prevent re-infection. In addition, and central to the successful development and operation of the programme, was the need to identify, and provide reliable detection methods for, the viruses infecting the narcissus crop. Fourteen viruses have been reported from commercial narcissus stocks grown in the UK, two of which seem to occur so rarely as not to be considered of practical importance. Of the twelve commonly occurring viruses, four are transmitted by aphids, five by nematodes and three by unknown means. The nematode-borne viruses are well known and important pathogens of other crops, have a wide natural and experimental host range and have been comprehensively studied and characterised. By contrast, the other seven viruses have restricted host ranges and all but two are confined to their natural bulbous host species, a restriction which has hampered the study of their properties and the production of specific antisera to their particles for use in identification and detection. Of the 12 common viruses, six have isometric particles, five have filamentous particles and one has rod-shaped particles.

Viruses with isometric particles. Arising from the VT stock programme, two previously unrecognised viruses with isometric particles were found and antiserum produced to detect them. The first, narcissus tip necrosis virus (NTNV), has been provisionally placed in the carmovirus group on the basis of the physical and chemical properties of its particles (*c.* 28 nm diam., Fig. 2). An unresolved inconsistency was the detection of two major proteins (42 kDa and 39 kDa) in the particles whereas only one was expected. Although it is likely that the smaller protein is a degradation product of the other, the subsequent discovery of another virus with particles of a similar size, narcissus Q (NQV), which can occur in mixed infection with NTNV, offered a possible alternative explanation. Preliminary results from our current studies indicated that the particles of NTNV and NQV each contain a single protein of molecular weight *c.* 40 kD and 36kDa, respectively. Thus it seems that the smaller of the two proteins previously reported for NTNV particles may well have been that of NQV. In contrast to NTNV which has a nucleic acid of *c.* 1.6 x 10^6Da mol. wt, that extracted from NQV particles seems to be unusually small, about 1 x 10^6Da. Further work will be required to show whether NQV has affinities with other known viruses, or whether it is a novel kind of virus. Both NTNV and NQV occur commonly in stocks of several cultivars but their mode of transmission is unknown. Field studies, however, have shown a slow and erratic pattern of spread for NTNV, which parallels that of a third virus, narcissus mosaic potexvirus, whose natural mode of spread also remains to be discovered.

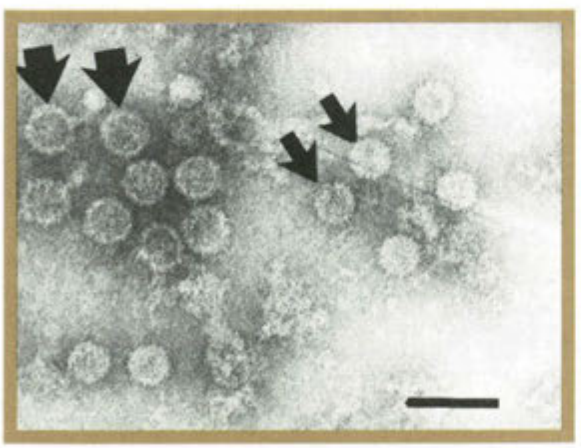

Figure 2 Isometric particles of narcissus tip necrosis (smaller arrows) and narcissus Q (larger arrows) viruses in sap extract from a naturally infected narcissus plant (Bar = 50 nm).

Figure 3 Symptoms of narcissus yellow stripe disease in leaves and flowers of narcissus cv. Rembrandt.

Virus associated with yellow stripe disease One of the long standing and important virological problems in narcissus is the identification and detection of viruses with filamentous particles *c.* 750 nm long, which occur commonly and often in low concentration, in plants of many important cultivars. Particles of this size are typical of potyviruses which are aphid-transmitted, have a particle coat protein of *c.* 35 kDa and characteristically produce another protein which assembles in the cytoplasm of infected cells into inclusion bodies called pinwheels (Fig. 4). The first of these viruses for which the disease aetiology was established, and a specific antiserum produced, was narcissus late season yellows (NLSYV). We have found that NLSYV is widespread in commercial stocks and often

Figure 4 Cytoplasmic inclusion bodies in thin sections of cells of narcissus with yellow stripe symptoms (a) and *Nicotiana clevelandii* infected with narcissus latent virus (b) and maclura mosaic virus (c). Black dots are particles of gold colloid bound to antibodies prepared to CI protein from narcissus with yellow stripe disease. (Bars = 500 nm).

causes no overt disease. Thus, although three other viruses, narcissus degeneration, narcissus white streak and narcissus yellow stripe (NYSV) (Fig. 3) have previously been described as having 750 nm long filamentous particles associated with specific disease symptoms, the particles observed in these plants could have been those of NLSYV. Although we established that narcissus degeneration virus was serologically distinct from NLSYV, the antiserum prepared to its particles also contained antibodies to NLSYV particles, thus confirming the potential fallibility of naming viruses by association of virus particles and disease. In white streak-affected plants all the particles detected were serologically related to NLSYV. The cause of white streak disease therefore remains uncertain. The association of potyvirus-like particles with yellow stripe disease was less straightforward. An analysis of experimentally-infected VT narcissus plants which developed yellow stripe symptoms failed to reveal particles of potyvirus length (*c.* 750 nm) but showed that all contained particles (*c.* 650 nm long) of narcissus latent virus (NLV) a member of another group of aphid-borne viruses, the carlaviruses. However, pinwheel cytoplasmic inclusion (CI) bodies (Fig. 4) typical of potyviruses were consistently found, suggesting that a potyvirus was also present. Having failed to detect the 750 nm particles of NYSV described by others, the CI protein was therefore selected as an alternative virus-specific antigen for the serological detection and identification of the virus. Gold-labelled antibodies prepared to a protein of *c.* 76 kDa, isolated from fractionated extracts of NYSV-infected narcissus, were used to show that this protein is a constituent of the CI bodies seen in electron micrographs of tissue sections (Fig. 4). The antiserum to this CI protein also reacted specifically in ELISA with leaf extracts from yellow stripe-diseased narcissus plants so providing a means of detecting and identifying the putative potyvirus.

A further link between NLV and yellow stripe disease was obtained from surveys of naturally infected narcissus plants in which the two were invariably associated. On re-investigating the properties of NLV, our findings on particle morphology (*c.* 660 nm x 13 nm), host range and aphid transmissibility were in agreement with previous reports but the apparent mol. wt of the particle protein was *c.* 45 kDa, which was substantially larger than that of other viruses with filamentous particles (Fig. 5). Moreover, NLV-infected plant cells contained potyvirus-like CI which were serologically related to those of NYSV and also to those of definitive potyviruses. The similarity of the distinctive properties of NLV and those of the virus

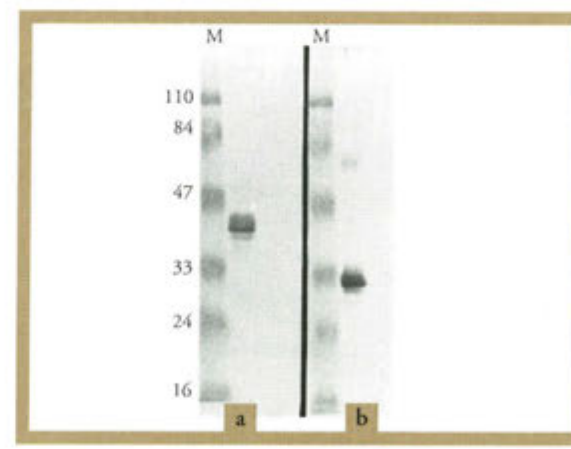

Figure 5 Electro-immunoblot analysis of sap containing particles of narcissus latent virus (a) and potato Y potyvirus (b) electrophoresed in a SDS-polyacrylamide gel and treated with antiserum to the homologous particles (figures are mol. wt of marker proteins (M) in kDa).

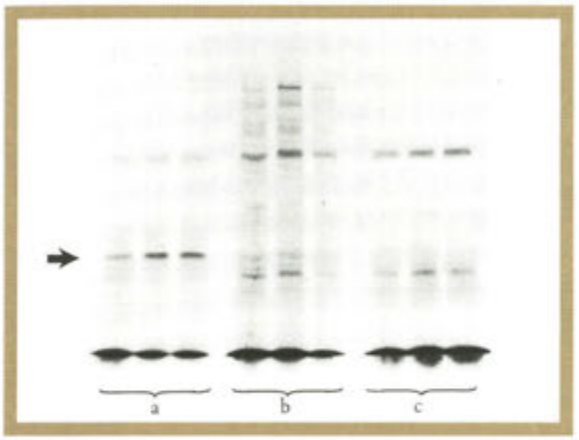

Figure 6 Autoradiogram showing *in vitro* translation products from a) narcissus latent virus RNA b) tobacco etch potyvirus RNA and c) no added RNA. Arrow indicates major NLV specific product of 25 kDa.

cultures in narcissus with yellow stripe symptoms led us to conclude that these were not two distinct viruses as had previously been thought. However, confirmation of this conclusion awaits the reproduction of the disease on return of NLV to narcissus.

Evidence for a new virus group It remained unclear whether NLV/NYSV is a carlavirus, as previously thought, or a potyvirus, as suggested by the pinwheel type of CI. A basic and definitive property of potyviruses is the organisation of the genome as a single gene. The gene product is cleared into a number of high molecular weight proteins, including precursors of the coat and CI proteins, when virus RNA is translated in a cell-free system (*in vitro*). The *in vitro* translation of NLV/NYSV RNA, however, produced only one major product of low mol. wt (*c.* 25 kDa) (Fig. 6) which did not react with antisera to virus particle protein or CI protein. Indeed this product has no obvious affinities with those yielded by *in vitro* translation of RNA from viruses belonging to any of the described groups with filamentous particles. Our serological tests with some potyviruses and carlaviruses, virus groups in which serological inter-relationships commonly occur, also failed to establish affinity with either of these virus groups. It appears, therefore, that NLV/NYSV has properties that distinguish it from all recognised virus groups and that it should not be classified either as a carlavirus or as a potyvirus. Furthermore, the distinctive combination of particle of carlavirus length (*c.* 650 nm), a potyvirus-like CI and particle protein of high mol. wt shown by NLV/NYSV is shared with only one other virus, maclura mosaic virus (MMV). The taxonomic affinity of NLV/NYSV and MMV is further supported by serological relationship between their particles and CI proteins (Fig. 4c). These viruses seem to represent a new virus group.

Figure 7 The detection of narcissus latent virus in sap extracts of naturally infected narcissus plants by dot-blot ELISA. Red colouration indicates the presence of the virus.

Serodiagnostic techniques Many of the 12 viruses commonly infecting narcissus have no experimental indicator host species and the most appropriate means of detection is therefore by serodiagnosis. Reliable virus indexing is crucial for the provision of VT mother plants, epidemiological studies and the certification of the health of stock during propagation in the VT stocks programme. To be cost effective, it is imperative that virus-indexing for the Certification scheme uses one type of test for all viruses. The $F(ab')_2$ form of ELISA which is designed to provide sensitivity and broad serological reactivity was initially adopted as an appropriate test for this purpose. However, although this method proved to be more sensitive than an infectivity assay (usually the most sensitive means of detecting viruses) for tomato black ring virus in narcissus, it was unreliable for the detection in sap extracts of particle antigen of narcissus late season yellows and narcissus latent viruses, and the CI protein of NYSV. Attempts to improve sensitivity by exploiting the amplifying potential of biotin-avidin systems showed that these increased absorbance readings but not sensitivity. Dot blotting of sap extracts on untreated nitrocellulose membrane, using a protein A-enzyme ELISA system, did provide the required enhancement of sensitivity for all three antigens (Fig. 7). We found it necessary, however, to incorporate the surfactant Tween 20 in the extraction buffer to obtain specific reactions, a radical modification of the

conventional method. A reliable detection method by ELISA is now available for the routine assessment of health of VT stocks for eight of the nine viruses likely to spread in the Scottish bulb growing region but the reliable detection of the wide range of serological variants of tobacco rattle virus is yet to be solved. Antisera produced to degraded forms of the coat protein combined with the dot-blot form of ELISA broadened the spectrum of reactivity but did not detect all isolates. An answer to this final problem of detection may come from other approaches that are being tested at SCRI, such as the detection of a non-structural protein of the virus or obtaining a monoclonal antibody to a conserved epitope of the virus coat protein.

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The genome of raspberry ringspot virus: gene structure and expression

M.A. Mayo, O. Acosta, V.C. Blok, J. Wardell, A. Manoukian, C.A. Jolly & D.J. Robinson

Raspberry ringspot virus (RRV) can cause a serious disease in some cultivars of raspberry. Figure 1 illustrates one such outbreak. The occurrence and extent of these outbreaks in the late 1940s were a major factor in the genesis of the Scottish Horticultural Research Institute, one of the two progenitors of SCRI. Much work was therefore done to control the disease, and outbreaks are now less common. The virus itself has been studied in some detail and the results obtained have contributed to knowledge about plant viruses more generally. RRV is a founding member of the nepovirus group, was the first nepovirus shown to have a bi-partite genome, the first in which pseudo-recombinants were produced so allowing determinants for biological properties to be assigned to one genome part or the other, the first in which different nematode species were shown to be the specific vectors of serologically distinguishable virus strains, and the first shown to survive in the field in dormant weed seeds. In particular, the smaller genome segment, RNA-2 (4 kb), was found to encode the coat protein, to determine nematode vector specificity and to control some kinds of disease symptom.

Figure 1 An outbreak of disease in cv. Malling Jewel raspberry in eastern Scotland caused by raspberry ringspot virus infection.

This research has been taken further in recent years by taking advantage of new molecular biological methods of analysis. One aim has been to determine the number and location of genes in the RRV genome. The first two phases of this work, to identify the RRV-specific proteins produced *in vivo*, and to determine the nucleotide sequence of RNA-2, have been completed.

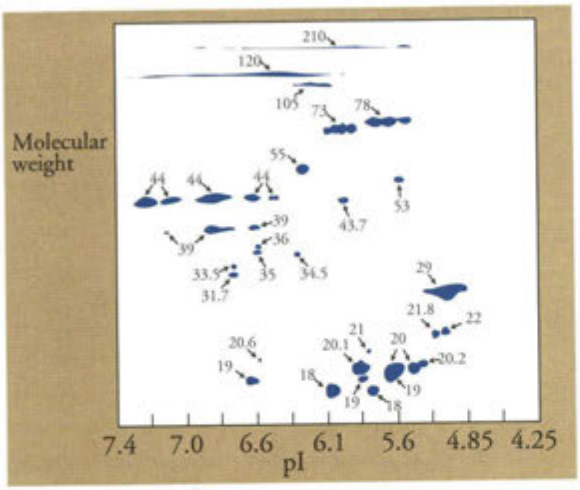

Figure 2 Autoradiographic analysis by two-dimensional polyacrylamide gel electrophoresis of proteins synthesized in tobacco protoplasts. Diagram shows the positions of proteins present in RRV-infected protoplasts but not in mock-inoculated protoplasts.

RRV-specific proteins in protoplasts When RRV RNA was translated *in vitro*, the result suggested that each RNA was translated into one large polyprotein. However the coat protein is smaller than the RNA-2 translation product and this must therefore be cleaved to form mature virus proteins. RRV-infected protoplasts have been used in attempts to detect these proteins *in vivo*. After inoculating tobacco mesophyll protoplasts with virus or buffer (control), ^{35}S-methionine was incorporated into proteins. Proteins synthesized in virus-infected and mock-inoculated protoplasts were then compared by 2-dimensional gel electrophoresis. Figure 2 shows the positions of the virus-induced polypeptides detected. Most of them were not synthesized in protoplasts infected with a different nepovirus and are therefore encoded by the RRV genome. Many of the proteins therefore are probably intermediates in the conversion of two large polyprotein translation products into functional proteins. Where several polypeptides differ in charge but not size, they may well be charge isomers of one polypeptide.

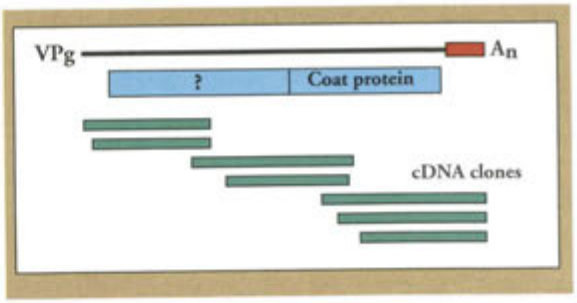

Figure 3 Diagram of RRV RNA-2. The RNA (black bar) terminates in either a genome-linked protein (VPg) or a poly(A) sequence (An). The sequences shared with RNA-1 are shown as a red box and the open reading frame as a blue box. The positions of the principal cDNA clones are shown as green boxes.

Nucleotide sequence of RRV RNA-2 To analyse further the proteins encoded by RRV RNA, the nucleotide sequence of the RNA was determined and the amino acid sequence of the putative translation product was deduced. RNA from RRV particles was reverse transcribed and cDNA was cloned in plasmid vectors. Cloned cDNA representing all of RNA-2 (Fig. 3) and some of RNA-1 was sequenced. The sequence of RNA-2 confirms that the RNA encodes a large polyprotein with a molecular weight of c.120 kDa. Like with other nepoviruses, almost all of the 3' non-coding region of RNA-2 (3928nt) is the same as that of RNA-1 (Fig. 3). The N-terminal sequence of RRV coat protein (kindly determined by Dr M. Edwards, IVEM, Oxford), made it possible to deduce that, as with other nepoviruses, this protein is the C-terminal portion of the RNA-2 polyprotein (Fig. 3). Figure 4 shows the amino acid sequence at the cleavage site. The cleavage site sequence (GC^A) resembles that at which some alphavirus polyproteins are cleaved to form structural proteins but is unlike the

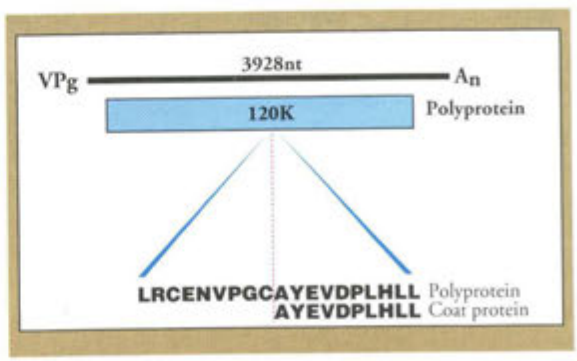

Figure 4 Diagram showing the deduced location of the site of cleavage of RRV coat protein from the polyprotein translation product of RNA-2.

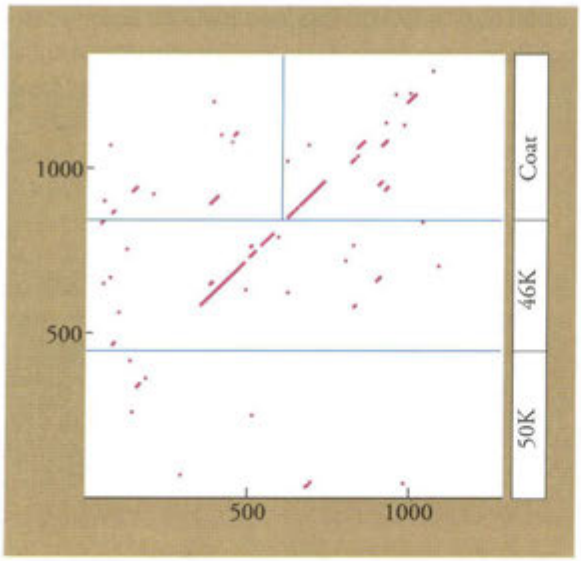

Figure 5 Comparison of the sequences of the RNA-2 translation products of RRV RNA-2 (horizontal) and tomato black ring virus RNA-2 (vertical). Numbers are amino acid residues from the N- terminal end. The known positions of cleavage are indicated by blue lines.

cleavage sites in any plant virus polyproteins or picornavirus polyproteins.

Comparison with nucleotide sequences found for RNA of other viruses shows that RRV RNA-2 is most like RNA-2 of tomato black ring virus (TBRV). Figure 5 compares the amino acid sequences of RRV and TBRV polyproteins. The coat proteins are about 22% identical and much more similar when chemically similar amino acids are scored. TBRV RNA-2 polyprotein is thought to yield three proteins by proteolysis (Fig. 5) and it is suggestive that RRV RNA-2 polyprotein, which is smaller, resembles that of TBRV RNA-2 for much of the length of the coat protein and the adjoining putative 46 kDa protein but not at all in the remainder of the sequences. If, as seems likely, RRV and TBRV have evolved from a common origin, the N-terminal region of the polyprotein, which may form a third protein (as for TBRV) or be part of a larger protein with two domains, has been less constrained from variation than the C-terminal part.

Cell to cell movement of viruses and plasmodesmatal permeability

P.M. Derrick, H. Barker & K.J. Oparka

The genome of many plant viruses is thought to encode at least one protein which is essential for cell-to-cell spread of infection. Furthermore, the 'transport proteins' of a few of these viruses bind within or near plasmodesmata, the most likely route of intercellular virus movement in plants. Although several lines of evidence suggest that transport proteins of viruses in different taxonomic groups may not all function in the same manner, at least part of the role of all of them is to cause a change in the properties and structure of plasmodesmata, thereby enabling viral nucleic acid or virus particles to pass from cell to cell.

Information on virus-induced modification of plasmodesmatal function is of interest not only to virologists, but also to transport physiologists and cell biologists, because of the insight it may give to the processes involved in plasmodesmatal function and regulation in non-infected plants. From a virological standpoint, the information may reveal processes that must occur if a plant is to be a host to a virus and conversely, indicate ways in which plants are, or could be made, virus resistant.

During recent years, the use of microinjection techniques in plants has revealed that, although apparently rather variable in structure between both tissues and species, plasmodesmata studied to date have remarkably similar properties in relation to their permeability to solutes. In taxonomically diverse species, only molecules of a molecular weight of less than about 1

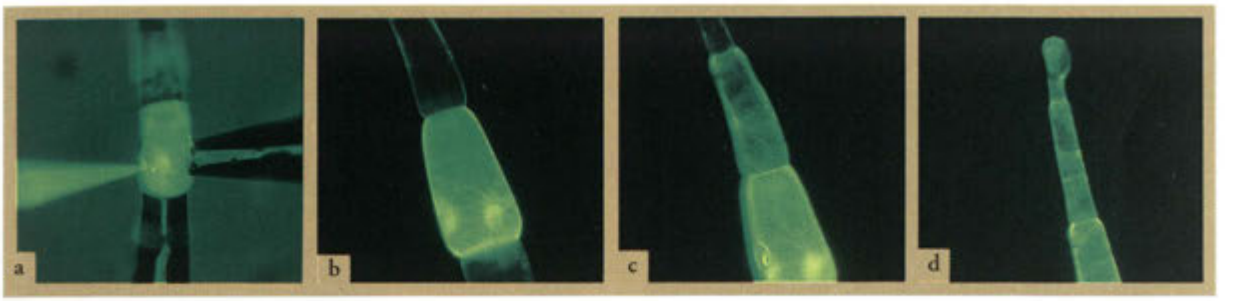

Figure 1 Microinjection of Lucifer Yellow CH into a *N.clevelandii* leaf trichome, showing symplastic movement of a membrane impermeant dye. Mol.wt of Lucifer Yellow CH = 457. (a) Injection of a trichome cell. Note the large diameter holding pipette supporting the trichome during injection. (b) c.1 min, (c) c.2 min and (d) 4 to 5 min after injection.

kDa can pass from cell to cell via plasmodesmata, a feature shared with gap junctions, the parallel structures linking animal cells. For example, by microinjection of membrane-impermeant fluorescein-labelled oligopeptides into the cytoplasm of *Nicotiana clevelandii* epidermal cells, we showed that the largest molecule able to move to neighbouring cells, i.e. via plasmodesmata, had a molecular weight of 749 Da[1].

When fluorescent probes were microinjected into cells of leaves systemically infected with tobacco rattle virus (TRV), tomato black ring virus or potato virus Y, no effects of virus infection on plasmodesmatal permeability were detected. However, plasmodesmata of plants infected with carrot mottle virus had decreased permeability[1], possibly because this virus induces the formation of filamentous cell wall outgrowths originating at plasmodesmata. The experiments with the other three viruses therefore failed to detect any long term effects of infection on plasmodesmatal permeability. Moreover, evidence from other sources suggests that transport proteins may be synthesised or active for only a relatively short period early in the virus replication cycle. An experimental system with a more closely controlled spread of infection was therefore needed to examine the virus-plasmodesma interaction at a time soon after inoculation. To this end, we have used leaf trichome cells of *Nicotiana clevelandii* in which movement of fluorescent materials from one cell to another can be observed readily and movement of virus from the trichome cells to the leaf lamina can be arrested by cutting off the trichome. A method was devised for injecting the cytosol of individual trichome cells at controlled pressure to minimise disturbance of the cell contents[2]. Using this system, the largest fluorescein-labelled peptide able to move from an injected cell to a neighbouring cell in non-infected trichomes had a molecular weight of 749 Da the same as for epidermal cells. When purified TRV particles were injected into trichome cells, necrotic lesions developed in the leaf lamina at the base of 67% of the injected trichomes.

Before examining the molecular size exclusion limit of plasmodesmata of infected trichome cells, the time needed for virus to spread through the trichome was established to enable the period during which the transport protein was active to be estimated. Trichomes were cut off at the base at various times following injection of a single distal cell with purified TRV particles and lesion formation used to indicate whether the virus had spread into the leaf lamina (Fig. 2). In this way, the times required for the virus to cross one, two or three cell walls, depending on the position of the cell injected, could be deduced. Following injection, at least 4 - 5h were needed for TRV to move across one cell wall. When TRV-inoculated cells were individually reinjected 4 - 5h later, fluorescein-labelled oxidised insulin A chain (mol.wt = 2921) and fluorescein-labelled dextran (mol.wt =

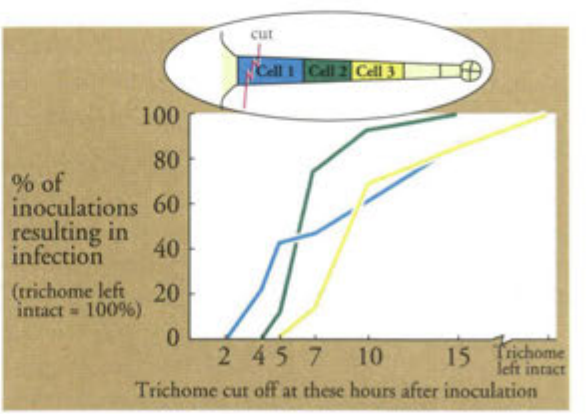

Figure 2 Movement of TRV through *N. clevelandii* leaf trichomes. Inset shows position of cut through trichome base.

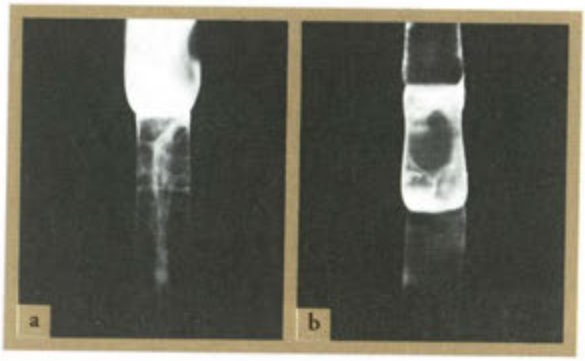

Figure 3 FITC-labelled dextran (Mol.wt = 4400) (a) moving out of a trichome cell that was injected 5 h previously with TRV and (b) restricted to the injected cell that was inoculated only 2 h previously with TRV. Photographs taken at 5 min (a) and 30 min (b) after injection of the fluorochrome.

4400) moved rapidly out of the injected cell (Fig. 3). In contrast, these probes did not move out of cells which had not been pre-injected with TRV nor out of cells injected with TRV only 2h previously, thus demonstrating that TRV infection increased plasmodesmatal permeability to solutes. This is the first direct evidence that virus infection has such an effect.

Future work will enable the size of the increase of the plasmodesmatal exclusion limit to be estimated. However, in other work[3], plants genetically transformed to express the transport protein gene of tobacco mosaic virus showed a considerable increase in the plasmodesmatal exclusion limit to between 9.4 kDa and 17.2 kDa, a size much smaller than viral genomic RNA molecules. Considering the functions of the TRV and tobacco mosaic virus transport proteins, it is clear that these and perhaps other factors, must have effects in addition to causing a non-specific increase in plasmodesmatal permeability.

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Patterns of antigenic variation in whitefly-transmitted geminiviruses

B.D. Harrison, M.M. Swanson, P.F. McGrath & D. Fargette

Geminiviruses cause many of the world's most economically important virus diseases of crops in tropical and sub-tropical countries. Some of the viruses are transmitted by whiteflies (*Bemisia tabaci*) but others have leafhopper vectors. The most important diseases caused by the whitefly-transmitted viruses are cassava mosaic, tomato leaf curl and yellow leaf curl, tobacco leaf curl, yellow mosaics of many legume species, and leaf curl or mosaic of cucurbits, pepper, okra and cotton. In cassava alone, the value of crop losses in Africa caused by mosaic is thought to exceed £200 million annually. We have studied many of these whitefly-transmitted viruses, and have prepared a series of diagnostic reagents of wide applicability.

Of these reagents, polyclonal antisera and monoclonal antibodies (MAbs) have proved to be invaluable and have been used extensively in ELISA. Early work[1] in which polyclonal antisera were used in immunosorbent electron microscopy instead of ELISA showed that serological relationships between different whitefly-transmitted geminiviruses are common whereas leafhopper-transmitted geminiviruses are not serologically related to the whitefly-transmitted viruses, and most are not related to one another. The production

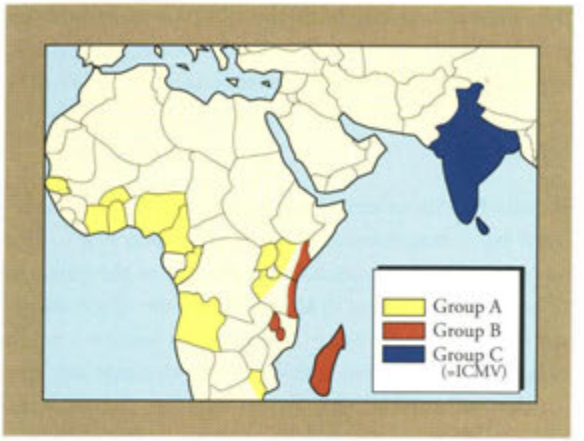

Figure 1 Geographical distribution of geminivirus antigenic types from mosaic-affected cassava.

of panels of MAbs to particles of the whitefly-transmitted African cassava mosaic (ACMV) and Indian cassava mosaic (ICMV) geminiviruses has enabled us to investigate these relationships in more detail, and to assess the extent of variation among isolates of the same virus.

Initial tests, in which crude extracts from mosaic-affected cassava leaves from plants sent from a range of countries were tested for reactivity with 17 ACMV MAbs, showed unexpectedly that some of the virus isolates were not closely related to others. They fell into three categories: Group A, reacting with at least 14 of the MAbs; Group B, reacting with 4 to 9; and Group C (=ICMV), reacting with only 2 or 3[2]. Tests with MAbs to ICMV have confirmed this grouping. Group C isolates reacted with almost all these MAbs, Group A with about half of them and Group B with only one. Moreover, the groups can also be distinguished in other ways, such as ability to infect and to multiply at different temperatures in *Nicotiana* spp., and the extent of the nucleotide sequence similarity of the smaller of two genomic DNA species. Thus groupings based on different criteria coincide.

When the geographical origin of isolates of the three groups is mapped (Fig. 1), it can be seen that each group of isolates occurs in a different region. Group A isolates occur in many countries of West Africa, and in South Africa, Uganda, western Kenya and western Tanzania. In contrast, Group B isolates occur in coastal Kenya, coastal Tanzania, Malawi and Malagasy, and Group C isolates occur in India and Sri Lanka. This pattern of distribution, and the apparent absence of similar virus isolates in South America, led us to suggest that all cassava was geminivirus-free when originally brought from the Americas across the Atlantic Ocean by Portuguese colonists and traders in the 16th to 18th centuries, that after introduction to locations in West Africa, East Africa and India it became infected by geminivirus isolates which already occurred in these regions in other plant species, and that the virus isolates then endemic in West Africa differed from those in East Africa and the Indian subcontinent[3]. Group A isolates would have spread with cassava cultivation across much of Africa from west to east. If this hypothesis is correct, one might expect to find cassava-infecting geminiviruses in wild plants in the three regions. Indeed, an isolate from a wild euphorbiaceous species from Malagasy has proved to be antigenically indistinguishable from Group B isolates of ACMV, although there is now no way of knowing whether the plant was infected by inoculum from other such wild plants or from cassava.

Leaf curl and yellow leaf curl of tomato occur in many tropical regions ranging from Central America to Australia and cause serious crop losses in many countries. Some of the ACMV and ICMV MAbs can be used to detect these viruses but, as with cassava mosaic, isolates from different geographical regions share different combinations of epitopes with ACMV and ICMV. From their epitope profiles, the tomato viruses examined to date can be assigned to four main groups with different geographical distributions (Fig. 2).

Figure 2 Geographical distribution of geminivirus antigenic types from leaf curl- or yellow leaf curl-affected tomato.

Group 1 occurs in Mediterranean countries, Senegal, Egypt and Yemen, Group 2 occurs in India, Group 3 in Thailand and Group 4 in Mexico, USA (Florida) and, probably, the West Indies.

Geminiviruses also occur widely in okra (*Abelmoschus esculentus*). However, isolates of okra leaf curl virus (OLCV) from West Africa have very different epitope profiles from those of bhendi (okra) yellow vein mosaic virus (BYVMV) from India. Indeed, OLCV shares many epitopes with ACMV (Group A isolates) whereas BYVMV has an epitope profile very like that of ICMV. Tests of several other geminiviruses from different host species in India[4] for reactivity with the full range of ACMV and ICMV MAbs show that although they differ in epitope profile, many of them have a general resemblance to ICMV in their patterns of reaction. Similarly, geminivirus isolates from the southern United States and Central America have a general similarity in epitope profile notwithstanding their different host ranges.

Summarising these results, we reach the remarkable conclusion that geminiviruses causing the same disease in cassava or tomato in different continents have different epitope profiles, whereas distinct geminiviruses which have non-overlapping host ranges but occur in the same geographical area show a general similarity in epitope profile. Within the geminivirus group, evolution seems to have proceeded differently in different continents, either by parallel or convergent changes in different progenitor viruses occurring in the same region, or by adaptation of a different progenitor geminivirus occurring in each region to a variety of host species.

All whitefly-transmitted geminiviruses for which vectors are known are transmitted by the same species, *Bemisia tabaci*. Moreover, the vector specificity of different members of the geminivirus group seems to be determined by the specificity of their coat protein[1,5]. This raises the question of whether *Bemisia tabaci* may occur as different biotypes in different geographical regions, with each biotype acting to select virus isolates with a coat protein that has structural features particularly suited for transmission by that biotype. If this situation exists, both the differences in epitope profile of viruses causing the same disease in different regions, and the epitope similarity of different viruses occurring in the same region, could readily be explained.

B. tabaci contains esterase enzymes which can be separated by electrophoresis in polyacrylamide gels[6]. We have adapted this approach for analysis of the patterns of esterases occurring in single whiteflies. Such analyses show that although the esterase patterns of *B. tabaci* from one plant species in one country are very similar, substantial differences exist in the patterns given by *B. tabaci* from the same host in four different regions: India, Malawi, Ivory Coast and United States. Further work is needed to establish whether geminiviruses are preferentially transmitted by the *B. tabaci* biotype from their source region and to exploit further the unique opportunity to study the nature and causes of evolutionary change in this important group of plant viruses.

We are indebted for financial support to the Overseas Development Administration (Project R4356), the Natural Resources Institute (Project X0060), the European Economic Community (Project TS2A-0137-C(CD)) and the Institut Francais de Recherche Scientifique pour le Dévelloppement en Coopération (ORSTOM) and also, for their co-operation and help in providing infected plant material, to colleagues in many countries who are too numerous to list.

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Nematode and Insect Pests

D.L. Trudgill

Research on nematodes and insects at SCRI is broadly concentrated on genetics and molecular ecology; host parasite recognition and interactions; pest ecology, biology and control; and vectors of plant viruses.

We are internationally recognised as a centre for research on nematodes which transmit viruses to a range of economically important crops and collaborate closely with our virological colleagues and with nematologists world-wide. The objectives are to identify and elucidate the mechanisms of virus retention in the vector (Fig. 1) and of vector specificity. Recent progress includes the demonstration of a high degree of specificity between serotypes of tobraviruses and species of *Trichodorus* and *Paratrichodorus*, and the identification of carbohydrates associated with virus particles retained in the oesophagus of *Xiphinema diversicaudatum*. Also, in contrast to the high degree of specificity observed between most European viruses and their nematode vectors, our current studies on viruses from N. America are revealing a comparative lack of specificity, with each virus being transmitted by several different species of longidorid nematode.

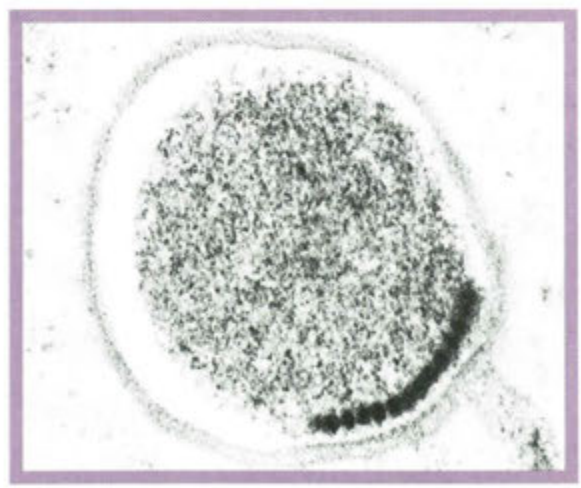

Figure 1 Cross section showing particles of arabis mosaic virus lining the stylet lumen of *Xiphinema diversicaudatum*.

Figure 2 Double dart moth (*Graphiphora augur*), a new pest of raspberry.

Other work in collaboration with a Dutch group is examining the resistance of plants transformed with the tobacco rattle virus (TRV) protein coat gene to nematode-transmitted TRV.

The objective of research on pest biology and ecology is the acquisition of knowledge relevant to improving pest control whilst reducing pesticide inputs. Link group studies with St Andrews University are exploring the influence of microclimate on pest and predator populations of insects in unsprayed raspberry plantations (Fig. 2). The factors controlling populations of nematodes have been extensively studied and currently include research on host plant effects, the role of bacterial parasites, and the distribution of predatory nematodes. Ectoparasites, root-knot and cyst nematodes are all studied, but research on potato cyst nematodes (PCN) is central to many of our interests. Research aimed at modelling PCN population dynamics and effects on yield in the field are nearing completion. Previously, all such models have been derived from glasshouse studies, but we have used mixtures of potato clones with different levels of resistance to produce field plots with a wide range of population densities of PCN. Precise experiments are then made using cultivars with different levels of resistance to nematode multiplication and tolerance of damage. SCRI-developed models are used to fit curves to the data and are being developed to predict the relation of nematode population increases to crop yield losses under different cropping patterns and pesticide application regimes.

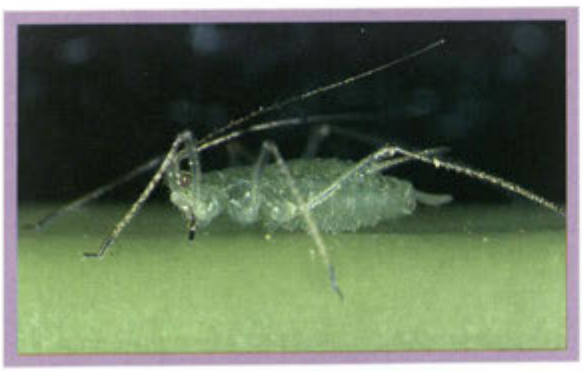

Figure 3 Large raspberry aphid (*Amphorophora idaei*) feeding on leaf petiole.

Another objective, focused on PCN, is to identify the distinct introductions into Europe from S. America, to track their subsequent spread and determine their spectrum of virulence on resistant potatoes. This is a form of "nematode archaeology" in which the tools are virulence studies, isozyme electrophoresis and analysis of nematode genomic and mitochondrial DNA. Our results so far show that *Globodera pallida* is genetically much more heterogenous than *G. rostochiensis*, they confirm that pathotypes Pa1 and Pa2/3 are distinct introductions and that UK populations classified as pathotypes Pa2 and Pa3 derive from the same introduction. They also indicate that a unique populations of *G. pallida*, virulent on cv. Morag which is normally *c.* 80% resistant, is derived from a previously unrecognised introduction.

A similar approach is being used to determine relationships between species, races and biotypes of parthenogenetic root-knot nematodes and aphids. Selection for, and the genetics of, virulence are key areas of investigation which link with both the molecular studies and those on recognition systems. The recognition studies cover a wide area. They include research on the nature and role in resistance to raspberry aphids (Fig. 3) of the leaf-surface factors which has implicated several volatiles; the recognition of nematodes by fungal and bacterial parasites where lectins have been applied to probe the nature and role of glycoproteins on the nematode cuticle; and the role of nematode amphids (sense organs on the head). The effects of root exudate chemicals and electrical fields in the detection of roots by nematodes and the production of polyclonal antisera and monoclonal antibodies as probes to identify nematode surface components and their role in eliciting a hypersensitive response in resistant plants are also being studied. The susceptible condition in cyst and root-knot nematode host plants also involves a recognition process in which the cells fed upon become enlarged and multinucleate. This change is thought to be induced by saliva injected by the nematode and studies on

nematode salivary gland cells have shown that they contain numerous granules which stain positively for nucleic acids and which react with an antibody to RNA.

These basic investigations will be continued in the future with the emphasis on understanding mechanisms. There is no doubt that molecular biology offers exciting possibilities for identifying genes and mechanisms, and the recent development of techniques for amplifying small amounts of DNA are ideally suited to relatively small animals such as nematodes and insects.

Modelling pest damage and reproduction

M.S. Phillips, D.L. Trudgill & C.A. Hackett

An ability to model nematode populations and their effects on crop yield is central to the development of efficient and reliable strategies for the control of pests whilst reducing the use of pesticides. Building models allows us to understand the principles governing the life cycle of the pest, the damage it causes and the way both pest and host interact with each other and the environment.

Potato cyst nematodes (PCN) are persistent, damaging pests of potato whose relationships we have been modelling. There are two species, *Globodera rostochiensis* and *G. pallida* (Fig. 1) and between potato crops, they survive as eggs in the soil. Populations decrease by *c.* 30% per annum when potatoes are not grown, but can increase fifty-fold in the presence of a susceptible crop. Potatoes are the only field host in the UK and exudates from the growing roots of both resistant and susceptible cultivars stimulate PCN juveniles to hatch from their eggs in large numbers. The juveniles invade potato roots where they settle and develop into adults and produce the next generation. In the UK there is usually only one generation in a season. Control is by crop rotation, resistant cultivars and by relatively toxic nematicides which are applied to 22,000 ha each year. *G. rostochiensis* is well controlled by resistant cultivars such as Pentland Javelin and Maris Piper but, as yet only a few cultivars with partial resistance are available against *G. pallida*, and modelling at SCRI has been concentrated on this latter species.

Figure 1 Females and cysts of *Globodera pallida* on potato roots.

Population density Initial work in pots on susceptible cultivars showed that as the initial population density of PCN in the soil at planting increased, so its multiplication rate decreased (Fig. 2). This is because increasing number of invading juveniles caused increasing damage and loss of root tissue thereby increasing the competition between them. This crowding caused a progressive shift in the sex ratio of the developing juveniles from mainly females at low population densities to mostly males at high population densities.

In partially resistant cultivars, laboratory experiments have shown that the shift towards a larger proportion of the juveniles becoming males occurs at lower population densities than in susceptible cultivars. Even so,

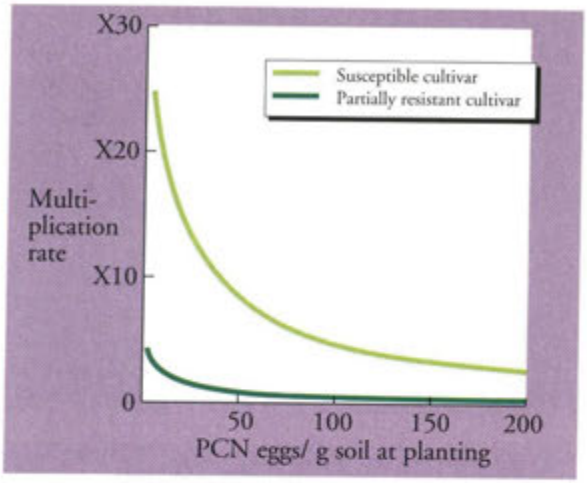

Figure 2 The relationship between multiplication of PCN and initial nematode population density.

sufficient females may be produced at low population densities for the population to increase, albeit to a lesser extent than on susceptible cultivars (Fig. 2).

Effect on yield Damage is proportional to the population density of PCN at planting but our field trials have shown that cultivars vary in the amount of damage sustained. Some are able to withstand attack with relatively little loss of yield whilst others suffer proportionally much more at the same population levels of nematodes. Such genotypes are termed tolerant and intolerant respectively.

To explain the effect of the interaction between partial resistance and tolerance on potato yields and PCN population increase, field trial sites were prepared with replicated plots spanning a range of population densities. These trials showed (Fig. 3) that each nematode causes proportionally more damage at low rather than at high population densities. This effect of PCN on yield can be adequately described by an inverse linear relationship which requires two parameters, the maximum yield when no nematodes are present and another describing the rate of yield loss as the PCN population density at planting increases. The rates of yield loss in these trials were similar for many cultivars

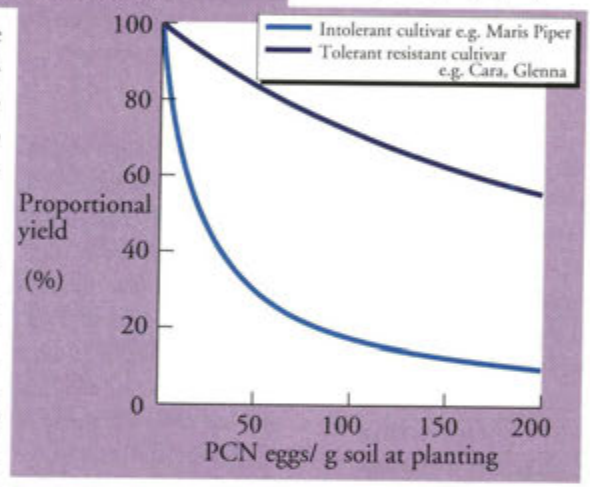

Figure 3 The relationship between yield and initial nematode population density.

or genotypes but yields of several, e.g. Cara, Glenna and clone 12243 were much less affected i.e. they were more tolerant (Fig. 3).

Although in the potato plant tolerance and resistance are independently inherited they interact to affect the rate of nematode multiplication. A tolerant susceptible cultivar e.g. Cara whose roots system will be less damaged by PCN than that of an intolerant cultivar will support higher rates of nematode reproduction, especially at the higher population densities. Consequently the effects of nematode competition within the roots will be less in a tolerant cultivar and multiplication rates will be higher especially at higher levels of initial population density. Hence, repeated by growing such a cultivar in the same field will build up very high populations of PCN.

These principles apply also to partially resistant genotypes (Fig. 4). The breeders clones 12243 and Heather are both *c.* 80% resistant to *G. pallida* and similarly reduced the rate of reproduction at low initial population densities where damage to the plants was small. As the initial population level increased, the intolerant clone 11233 was progressively more damaged and the already reduced multiplication rate was further restricted. In contrast, the tolerant clone 11243 con-

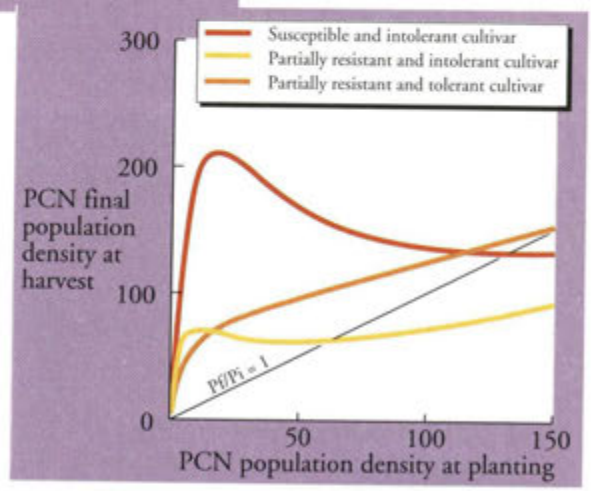

Figure 4 The relationship between final and initial nematode population density following potato crops with different levels of resistance and tolerance.

tinued to grow relatively well even at higher nematode densities thereby allowing the population to increase. It can be seen from Figure 4 that, in spite of its partial resistance, in heavily infested fields, the tolerant clone supported greater multiplication rates than the susceptible but intolerant cv. Maris Piper. Indeed, frequently growing the partially resistant clone 12243 would eventually produce higher and more damaging population levels than would be achieved by growing susceptible cultivars with a similar frequency. Consequently, for use in PCN infested land without nematicides, potato breeders need to combine partial resistance with a high level of tolerance. Even so, such cultivars would have to be grown on reasonable rotations if PCN population densities were to be progressively decreased.

Pest prediction as an aid to control

J.A.T. Woodford & S.C. Gordon

Half a million species of insects are potential plant pests but few thrive in Britain, particularly in the north of the country where many are near the limit of their geographical distributions. In these circumstances routine insurance control is costly, unnecessary and unwise, especially where it relies on the use of pesticides which will damage natural enemies and may hasten the development of resistant pests. Nevertheless, changes in the weather and other factors lead to sporadic outbreaks of pests which seriously reduce crop yields and quality. The aim of pest prediction is to reduce uncertainty for the grower so that control measures are applied only in those years when the damage is likely to exceed the cost of treatment, (sometimes termed the economic threshold). The ideal prediction system involves farmers monitoring pest incidence and using computer-based expert systems to make timely control decisions. However, monitoring requires expertise and is time-consuming. Also, given favourable conditions, pest infestations can increase so rapidly that damage occurs before a problem is detected and control measures applied. In practice, therefore, strategic forecasting systems operating at a regional or national scale have often been developed. These give earlier, less detailed warnings of the risk of pest attack but have the disadvantage that growers are not given specific advice on the levels of pests in their own crops.

Damage to plants is usually a function of degree of pest infestation and its duration, i.e. pest-days. However, for pests that affect crop quality, or are responsible, directly or indirectly, for infecting crops with other pathogens, the date of infestation in relation to crop growth can be more important than the size of the infestation. Prediction methods for two such pests, the raspberry cane midge and potato aphids transmitting virus have been developed at SCRI.

Raspberry cane midge Outbreaks of raspberry cane midge (*Resseliella theobaldi*) are indirectly responsible for serious yield losses in many raspberry-growing

Figure 1 Raspberry cane midge larvae feeding beneath the rind of first year raspberry cane.

Figure 2 Midge blight in raspberry, cv. Glen Clova (left), healthy (right).

areas of Europe. The larval feeding sites at the base of first year canes (Fig. 1) become infected by pathogenic fungi which cause wilting and death of fruiting canes in the following year (Fig. 2). This damage known as midge blight can be prevented by timely control of the midge. Insecticidal control is most effective if applied against the overwintering adult midges shortly after they emerge from the soil in the spring. Sprays applied at this time minimise the size of subsequent, potentially more damaging generations that attack the canes during the harvest period when insecticides can no longer be applied. It is difficult to time these control measures because midge emergence is dependent on soil temperature and shows wide annual and geographical variations. From an understanding of the biology of *R. theobaldi* and details of the dates of oviposition in the spring, we were able to develop a model to predict oviposition dates.

To monitor oviposition dates, oviposition sites for female midges were provided by making short slits in young canes. Counts of egg numbers were used initially by advisers to warn growers of the onset of midge activity in the spring and although the procedure gave an accurate assessment of risk in the plantations sampled, it was very labour intensive and required trained staff. In practice, therefore, only a few sites were monitored. To develop a strategic forecast, temperatures from the agro-meteorological site at

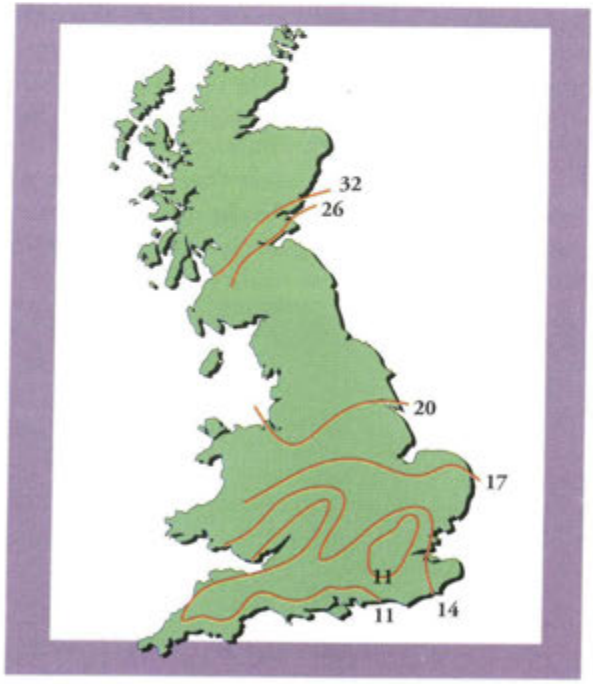

Figure 3 Predicted date (days from May 1) of emergence of raspberry cane midge in Britain in 1989.

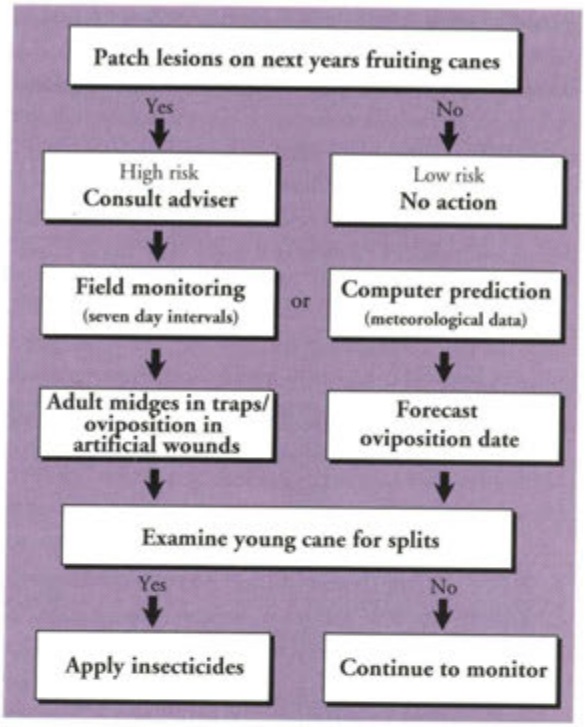

Figure 4 Raspberry cane midge decision making guide.

SCRI were accumulated from 1 March until the date when the first *R. theobaldi* eggs were found.

Over an 8-year period, *R. theobaldi* started to lay eggs at Invergowrie at dates between 18 May to 10 June and there was a poor relationship between the starting date and accumulated value of the daily mean air temperatures or 9 a.m. soil temperatures. However, in collaboration with meteorologists at the MAFF, we combined air and soil temperature measurements to derive estimates of the daily maximum and minimum temperatures at 10 cm below soil level. Accumulations of these daily mean values provided a much closer fit to the observed dates of midge oviposition. Fit was further improved by only accumulating temperatures above a base temperature of 4°C which was assumed to be the lowest temperature at which the midge is able to develop. Egg laying was predicted to start when the accumulated temperature reached 339 day degrees above the base temperature. The date when this value is reached varies with the aspect and location of plantations, for example, south-facing slopes are warmer than level or north-facing sites. Similarly, observed oviposition dates in plantations in East Anglia were 2-3 weeks earlier than those in Tayside. However, the strategic forecast compensates for these variable effects. The forecast has been developed and operated since 1989 by MAFF meteorolo-

Figure 5 Peach-potato aphid, *Myzus persicae* (left) and potato aphid, *Macrosiphum euphorbiae* (right).

gists, using temperature records from climatological stations within about 40 km of raspberry plantations. The aim is to make a first prediction at least 2 weeks before the expected date of oviposition, and to update the prediction at weekly intervals to allow for recent temperature fluctuations. Fig. 3 shows the dates for the onset of oviposition by *R. theobaldi* predicted by the forecast model in 1989.

The final stage in rationalising control decisions for *R. theobaldi* (Fig. 4) does require growers to examine their plantations. In plantations where oviposition is predicted before young canes begin to split there is less risk of damage and control can be delayed or omitted altogether.

Potato aphids Scottish seed production owes its origins to the lower rates of spread of potato leafroll virus (PLRV) and potato virus Y transmitted by aphids, especially *Myzus persicae* and *Macrosiphum euphorbiae* (Fig. 5). In most years, aphids do not colonise potato crops in Scotland until late June or July. By this time, plants growing from infected tubers, which are the main sources of infection, should have been removed by roguing. Although roguing usually prevents increase of aphid-transmitted viruses in crops with low initial infection levels, problems may develop in warm years because aphids colonise the crop and spread virus before any infected plants can be rogued. This situation occurred in the mid-1970s leading to second outbreaks of PLRV. Granular insecticides applied at planting were shown to prevent aphids multiplying on young infected plants and controlled the spread of PLRV. However, the routine use of systemic granular insecticides has not been advocated because of the risk of selecting resistant aphids. Therefore, methods have been developed for predicting early migrations of potato aphids and for monitoring the early stages of crop colonisation in time to warn growers either to apply granules before planting, or to spray untreated crops. Analysis of records from suction traps, which catch flying aphids, showed that temperatures in January and February had most effect on aphid survival and time of migration to potato. Consequently, knowledge of these temperatures, together with estimates of the levels of virus in the seed crop, are used to determine the need for granules. Spring temperature also influences virus spread by affecting aphid activity and multiplication rate. Aphid numbers on potato crops are monitored by entomologists from SAC and spray warnings are issued when more than two *Myzus persicae* or 10 *Macrosiphum euphorbiae* per 100 plants are found. Good control of virus spread is achieved if sprays are applied when the warning is received but not if they are delayed. Together these two predictive schemes have prevented the unnecessary over-use of insecticides, but currently there are weaknesses in the amount of information available on the post-harvest levels of virus in seed crops which emphasises the importance of data on winter weather conditions for deciding whether to use granular insecticides.

Acknowledgement: Figure 3 kindly provided by National Agrometeorological Unit, ADAS, Wolverhampton.

Biochemical approaches to assessing nematode heterogeneity

M.S. Phillips, M. Fargette & J. Gonzalez

Within populations of plant pathogens such as nematodes, virulence against a wide range of plant resistance genes is likely to be associated with a high degree of genetic heterogeneity. Cyst nematodes and root-knot nematodes are sedentary endoparasites of plant roots in which the juvenile nematodes tunnel into the root, settling to feed at a specific site where they induce the host to form enlarged, multinucleate cells which provide a continuous, rich supply of food.

Potato cyst nematodes have a narrow host range confined to solanaceous plants and two species, *Globodera pallida* and *G. rostochiensis*, have been introduced into Europe from S. America. They reproduce sexually and have a diploid number of chromosomes. Research at SCRI is directed towards determining how many introductions occurred, how did they differ in their complement of virulence genes which enable them to overcome plant resistance, where the introductions were spread, and has the degree of heterogeneity been decreased during the process of spread from field to field. Three species of root-knot nematode, *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, have wide host ranges and world-wide distributions. Some populations from Africa are morphologically similar to *M. incognita* but differ from this and the other species in their ability to overcome several types of resistance. Root-knot nematodes differ from cyst nematodes by reproducing by an asexual cloning process. Hence, within populations, and certainly within single female lines, there is likely to be no heterogeneity. The objectives of our research were to determine the extent of heterogeneity within and between populations, and to determine the relationships between the three species and the virulent populations from Africa.

Isozyme studies Various techniques are available for assessing the degree of heterogeneity of organisms and isozyme analysis has been used extensively at SCRI for this purpose. Isozymes are variants of enzymes with the same function, but which, due to a small mutation differ slightly in the electrical charge they carry and they can be separated and visualised on gels by electrophoresis.

Several different isozymes have been included in the studies and overall the results showed that there was much greater heterogeneity in potato cyst than in root-knot nematodes. Up to 15 esterase bands were identified in gels made with proteins from different populations of *G. pallida*, whereas each root-knot nematode species had at most only five bands. The species of root-knot nematode were distinct but within each there was little variation; two esterase bands were identified for all the populations of *M. incognita* studied, two for *M. arenaria* and only one for *M. javanica*. Analysis of acid phosphatases led to the same conclusion that there was very little variation between populations within a species of root-knot nematode and that variation within a population was extremely rare. The consistency of isozyme patterns means that they can be used to identify reliably species of individual root-knot nematodes, a procedure which is difficult using conventional morphometrical techniques.

The isozyme phenotypes of the populations of root-knot nematodes from Africa were all identical, but differed from those of the other species. Consequently, in spite of their morphological similarity to *M. incognita*, the populations must be considered a distinct species.

Isozyme patterns within potato cyst nematodes were very variable and *G. pallida* was more variable than *G. rostochiensis*. The evidence supports the view that there is one pathotype (Ro1) of *G. rostochiensis* in Europe and UK which is avirulent on cultivars with the H1 resistance gene such as Maris Piper and which was derived from a single introduction, probably of only a few cysts. Populations of *G. rostochiensis* from continental Europe belonging to pathotypes virulent on Maris Piper (Ro3 and Ro5) revealed greater heterogeneity indicating possibly two further introductions of this species. Interestingly, pathotype Ro4 of *G. rostochiensis* from Germany, which like Ro1 is avirulent on Maris Piper, has isozyme phenotypes similar to Ro5, suggesting that it is derived from the Ro5 introduction but has lost the gene which confers virulence.

UK populations of *G. pallida* showed a high degree of isozyme heterogeneity with large variations between populations and between individuals within a population. Populations classified as pathotype Pa1 have distinct banding patterns, supporting the view that they were derived from a distinct introduction, probably into Northern Ireland. There were no consistent differences between UK populations classified as pathotypes Pa2 or Pa3. This suggests that these pathotypes, which differ only slightly in their ability to multiply on partially resistant cultivars such as Morag, were derived from the same introduction. Some populations showed less isozyme heterogeneity than others, indicating that there has been a progressive loss of heterogeneity from the original introduction of *G. pallida* pathotype Pa2/3. One population of *G. pallida*, virulent on cv. Morag which is normally *c.* 80% resistant, displayed some isozyme bands that were similar to those derived from pathotype Pa1, whereas others resembled Pa2/3. It is unclear whether this population was derived from a distinct introduction or whether it is a hybrid between Pa1 and Pa2/3 but the results show that isozyme phenotypes are useful in assessing the patterns of introduction and spread of both species of potato cyst nematodes.

Natural plant products as probes and pesticides

W.M. Robertson, A.N.E. Birch, B. Boag & J.M.S. Forrest

The presence of pesticide residues in ground water has led to increasing public concern, especially where the chemicals have contaminated drinking water supplies. Adverse effects on wildlife have also highlighted the need for less toxic pesticides and these concerns have given added impetus to the aim of finding environmentally benign methods of controlling nematodes. Plants have developed several forms of defence mechanisms against pathogens during the course of evolution and detailed studies of them may provide more acceptable control methods than the synthetic chemicals currently in use. Lectins from plants are proteins with special properties which suggest that they may be involved in defence against potential pathogens. Their affinity for particular carbohydrates is a property of great interest and has led to their use as probes for cell surfaces in both plant and animal systems.

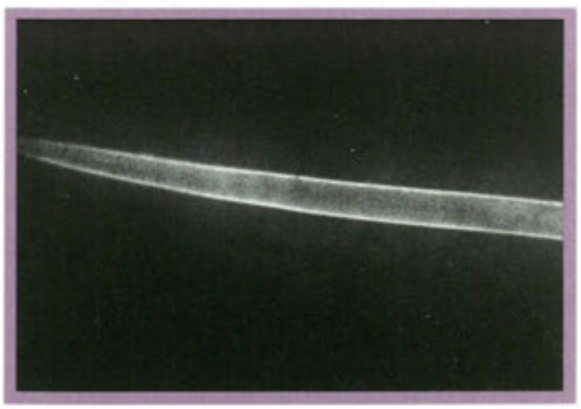

Figure 1 *Anguina tritici* labelled with WGA-TRITC.

The interface between nematodes and the external environment is provided by a thin outer epicuticle which forms the first line of defence for nematodes against chemicals or pathogens. The epicuticle is separated from the underlying nucleated cells by a thicker collagenous cuticle and evidence indicates that it is a dynamic structure capable of rapidly shedding surface components. Although inspection of the cuticle by light or electron microscopy suggests a uniform structure with annular grooves and ridges interspersed with openings for vital body functions, tests with a panel of different lectins conjugated with a fluorescent dye present a different picture. For example, when the invasive stage of *Anguina tritici*, a nematode pest of cereals, is taken from the soil, it is covered for most of its length by the carbohydrate N-acetyl glucosamine (Fig. 1). However, nematodes taken from fresh cereal galls are covered with the wheatgerm lectin which has bound specifically to the carbohydrate on the epicuticle surface. It is possible that the covering of wheatgerm lectin may prevent the plant recognising the presence of the nematode and this avoids triggering the plant defence system. In the virus vector nematode *Longidorus elongatus*, wheatgerm lectin labels the somatic receptor openings (Fig. 2) but not the cuticle surface, and in potato cyst nematode (PCN),

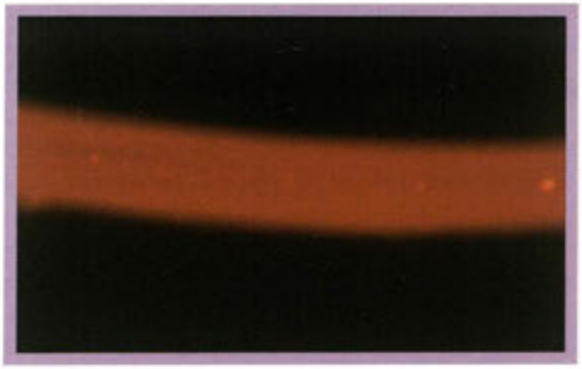

Figure 2 *L. elongatus* labelled with WGA-TRITC.

Figure 3 Healthy and distorted *G. rostochienensis* cysts.

Globodera spp., it labels only the secretions from the two sensory (amphidial) glands on the nematode head. A series of various lectins has revealed differences in labelling between pathotype Pa_1 and three other pathotypes of *Globodera.*

Differences in the surfaces of nematode genera and species has also been demonstrated by fungi parasitic on nematodes. Twenty-three species of nematodes from 13 genera screened with the nematophagous fungi *Arthrobotrys dasguptae* showed that the adhesive knobs which trap the nematode prior to infection attached to some species only and within genera there was up to a 160-fold difference in the numbers which attached. Pre-treating the nematode with a range of lectins and carbohydrates alone or in combination did not affect the success of attachment suggesting that carbohydrates were not involved.

In contrast, it has been demonstrated recently that the mannose-specific lectin concanavalin A binds to the amphids and inhibits the development of PCN. This effect was dependent on pH and concentrations of 5-10µg/ml were more effective than 100µg/ml. Other natural plant products with nematicidal activity, such as rishitin, have a normal dose-related response and are repellent at low concentrations but kill *in vitro* at and above 100µg/ml.

Current work funded by the British Technology Group has identified further natural plant products with nematicidal activity. The choice of bioassay affects the assessment and effectiveness of a plant product, e.g. a novel nematicide which gave only 30% control of PCN female development also induced smaller distorted cysts which hatched poorly when compared with cysts from untreated plants (Fig. 3). The combined effects produced more than 70% control. Further studies into the plant products resulting from resistant reactions and compounds present in resistant plants will continue the search for more natural means of pest control than is currently employed in agricultural practice.

Oilseed rape and wildlife

B. Boag, W.H. Macfarlane Smith & D.W. Griffiths

The first suggestion that oilseed rape could have a harmful effect on wildlife was made during the winter of 1986/1987 when Onderscheka and Tataruch published results showing that large numbers of roe deer in Austria died or suffered ill-health as a result of feeding on this crop. Brassicas are known to contain antimetabolites including glucosinolates and S-methyl cysteine sulphoxide (SMCO) and many, but by no means all, of the symptoms observed in the deer were typical of kale anaemia poisoning. As all the oilseed rape grown in Austria was of the double-low type, i.e. are low in erucic acid and glucosinolates, the harmful effects on animals were particularly associated with this variant of the crop. The glucosinolates are thought to be the chemicals which impart the bitter peppery taste to brassicas and since the new double low varieties probably have less of these chemicals in the leaves they are probably more palatable than the

old single low varieties. This was confirmed when, during the winter of 1987/1988, a study made by the Game Conservancy found rabbits, hares and to a certain extent roe deer preferred double to single low varieties. During 1988/1989 staff at SCRI investigated rabbit grazing of single- and double-low oilseed rape and of forage rapes with conventional glucosinolate content and of low progoitrin levels. Initially the rabbits appeared to be attracted to graze double-low oilseed rape in preference to the other three rapes. Subsequently all the rapes were grazed equally and almost 100% plant loss caused. The double-low oilseed rape showed significantly higher levels (30%) of SMCO for much of the experiment than was recorded in the single low variety. In all cases substantial modification of the amounts of individual glucosinolates occurred as a result of rabbit damage.

Widespread public concern persisted throughout the EEC at the possibility of wildlife being harmed by oilseed rape and in 1989 MAFF commissioned a three year research project with SCRI, the Game Conservancy and MLURI to investigate the problem.

The study was divided into two distinct areas: 1) the Game Conservancy and SCRI investigated the relationship between roe deer grazing oilseed rape in the wild and chemical analysis of the rape and 2) SCRI and MLURI monitored the effect of oilseed rape in the diet on roe deer health and the influence of chemical changes in crop quality on intake.

The field studies are being conducted in Scotland by the Game Conservancy in areas where the prevalence of oilseed rape and the overwinter conditions are most similar to the situation in Austria where roe deer mortality was observed. Over the last two winters, roe deer have been radio-tracked using an all-terrain vehicle fitted with direction-finding equipment which allows the amount of time spent on the different crops and in the woods to be monitored. Preliminary results show that the roe deer feed regularly on oilseed rape but also feed on other crops along field margins and in woodland. Other research using night vision goggles has indicated that while roe deer preferred oilseed rape in December and January they avoided the crop in February and March spending most of their time eating winter barley. These changes may have been related to the significant increase in glucosinolates observed over this period.

The work to be done at MLURI will investigate if the amount of double-low oilseed rape eaten in the wild, as determined by the data from the radio-tracked animals, has a detrimental effect on the health of roe deer.

It is envisaged that information gathered from this collaborative exercise will allow us to make recommendations to farmers about their agricultural practices and land use which will ensure that roe deer are not poisoned by oilseed rape in Great Britain.

Reference

Onderscheka, K. & Tataruch, F. (1987). Publication of the Research Institute for Wildlife Sciences at the University of Vienna College of Veterinary Medicine.

Scottish Agricultural Statistics Service

R.A. Kempton

The Scottish Agricultural Statistics Service (SASS) provides statistical and mathematical support to the five SARIs, SAC, SOAFD Agricultural Scientific Services and, on a contract basis, to other organisations in the agricultural, environmental and food sectors.

During the year there was progress in all aspects of SASS work: the development of research initiatives; strengthening of on-site consultancy services; expansion of the training programme; installation of a new computing system; and increase in international activities and external funding. The cross-institute structure allowed SASS to respond positively to the new scientific directions, organisational changes and funding constraints currently affecting government-sponsored research in the UK.

As research programmes change, SASS is increasingly involved in introducing statistical and mathematical methods into new scientific areas. Application areas identified for particular attention include chemometrics, environmental modelling, image analysis, molecular biology, food science, plant variety and seeds testing, and expert systems. Research progress has required a multidisciplinary approach involving statistics, mathematics, the latest computer technology and close contact with scientists and their problems. Achievements in two of these areas, chemometrics and image analysis, are illustrated in following research reports.

Our training programme continued with some 250 scientists attending courses in statistics and mathematical modelling during the year. Courses were also commissioned by the Forestry Commission Northern Research Station and ADAS.

A major enhancement of computing facilities was made at SASS Headquarters and several SARIs. This represented the first stage in a policy to standardise on UNIX as the operating system for SOAFD-funded organisations in the 1990s. The new systems have

improved communications between sites and opened up new opportunities for image processing and other computer-intensive methods. At SASS Headquarters, a more distributed computing system has resulted in substantial savings in operating staff time and computer maintenance charges.

Among a number of international ventures, SASS contributed to a major SARI initiative to strengthen scientific collaboration with the nations of Eastern Europe. Following a number of exchange visits, good links now exist with biometricians in Czechoslovakia, Hungary and Poland, particularly in the area of crop science and environmental modelling. SASS has contributed to two EC Concerted Action Programmes and currently holds two EC-funded research projects. Links with international environmental agencies have given rise to a number of contracts for monitoring wildlife abundance which now provide a major source of funding. SASS statistical expertise in the traditional areas of field and animal experimentation is still widely sought by scientists in the Third World, where maximising food production remains a paramount importance. Contacts with statisticians from Brazil, China, Kenya, Nepal, Nigeria and Zimbabwe have led to exchange visits and training assignments.

Computer visions of the future

C.A. Glasbey & G.W. Horgan Scottish Agricultural Statistics Service, University of Edinburgh

Sight is the most important sense we have. A large part of our brains is used for seeing and we are superb at it. Vision has always been the most important way in which scientists acquire information; whether it be Galileo gazing at the night sky, Charles Darwin watching finches on Galapagos, or Benoit Mandelbrot looking at fractal coastlines. In recent years many fresh views on the world have been obtained from new instruments and machines such as electron microscopes, medical scanning systems and satellites. Simply looking at these new images may not be sufficient though, because they can be far from what we are used to, or we may want to measure areas or shapes. Computers play an essential role in the collection of many new types of images and can also help us to interpret them. Four examples of ongoing work undertaken by SASS ranging from microscopic level up to the U.S. Space Shuttle are described in this paper.

Soil structure Pores in soil are home for many small organisms which are of interest to scientists at the Macaulay Land Use Research Institute. A cross-section through a soil aggregate area in which the white corresponds to soil material and the black to pores obtained by scanning electron microscope is shown in Figure 1a. Unfortunately, study of the structure of pores using cross-sections is very limited. For example, it is impossible to determine the proportion of the pores in the aggregate (about 1mm across) which are connected to the outside. However, by defining a mathematical model of the soil structure in three dimensions, it is possible to study pores by using a computer to generate simulations of the model.

A cross-section of a computer simulation of our model (termed a Boolean process) is illustrated in Figure 1b. Soil material was simulated by randomly positioned, overlapping spheres with radii drawn from an exponential distribution. Pore connectivity can be measured from these three-dimensional simulations. For example, although only half the pores appear to be connected to the exterior in the above figures, simulation in three dimensions of a cube of the same size (scaled to represent $1mm^3$) shows that over 99% of pores are connected. Figure 1c shows a simulated pore network in three dimensions. Using the model we can also predict that a protozoan of 20 μm diam outside the soil cube could reach only 11% of the pore space.

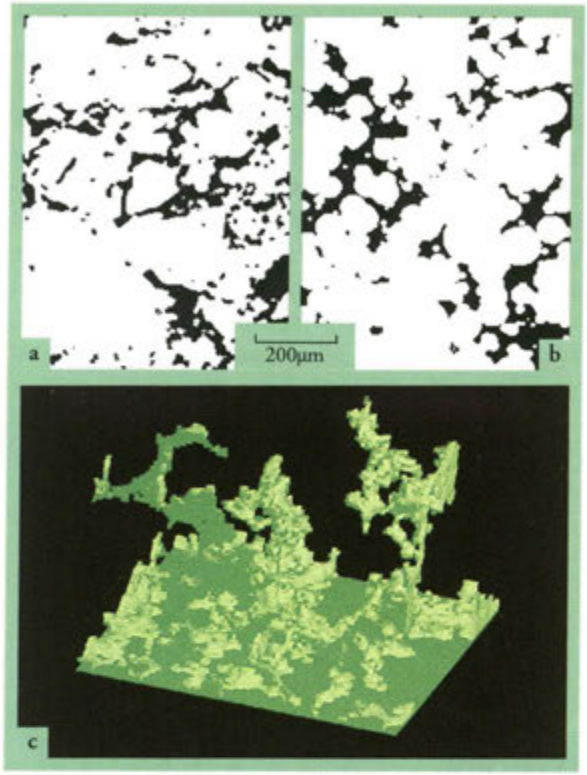

Figure 1 Soil pores and simulation: (a) magnified cross-section through a soil aggregate, with soil pores shown in black, (b) simulation of pore distribution modelled by a Boolean process, and (c) three-dimensional view of simulated soil pores.

Comparison of electrophoretic patterns Malaria is a serious endemic disease in the tropics with the pathogen occurring in many forms. Work at the School of Genetics in Edinburgh University is concerned with studying this variation.

Gel electrophoresis is a method of separating proteins on the basis of molecular weight and electrical charge. Figure 2 shows proteins from two strains of malaria separated by this method. It is necessary to determine which proteins are in different positions in the two samples, after allowing for unavoidable distortions of the gels. Visually this comparison is a difficult and tedious process, but we have developed a computer algorithm which corrects the distortion and then superimposes colours as illustrated in Figure 2c. Coincident spots appear as black, whereas those that differ in location are red or green.

In the work so far we have used only a simple algorithm to correct for distortions. The affine transformation can be visualised if this page is tilted and

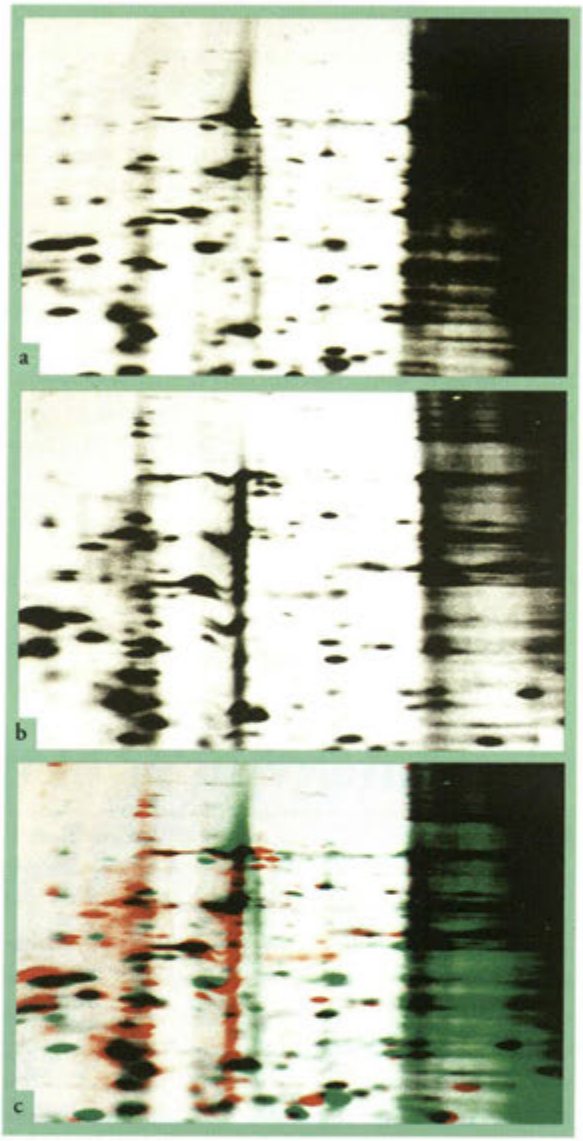

Figure 2 Electrophoretograms of two strains of malaria from West Africa and Brazil, and superposition: (a) and (b) black and white pictures of protein spots, (c) combination of (a) and (b) after transformation.

rotated: the normal rectangle is seen as a diamond shape. We are now working on more complex transformations to correct for more subtle distortions which arise due to imperfections in gels. These treat the image as though it were printed on a sheet of rubber which can be differentially stretched.

Magnetic resonance imaging Magnetic resonance imaging (MRI) provides views of tissues within a living body. The Rowett Research Institute has been using this technique in its study of human nutrition. Images are formed by exposing subjects to large oscil-

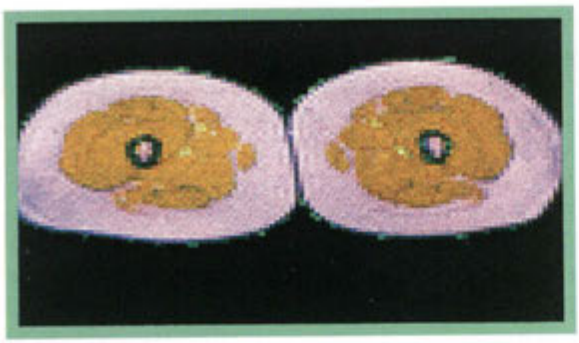

Figure 3 Magnetic resonance image of a pair of human thighs.

lating magnetic fields of short duration, and then measuring the decay in these oscillations using an array of sensors. The image in Figure 3 is a pair of human thighs in cross-section, with colours arbitrarily assigned. The flat bases indicate where the patient was lying on the bed. Within each thigh four, roughly concentric, regions are visible. Moving from the innermost to the outermost, these are bone marrow, bone, muscle and fat.

Our work (in co-operation with the Department of Mathematics and Statistics, Edinburgh University) aims to detect tissue boundaries as a first step towards the automatic classification of regions in MRI. The boundary can then be used to estimate the area of the region it encloses. Alternatively, it could be compared with an 'ideal' boundary representation of a given tissue to detect abnormalities. The algorithm we have been using smooths the image to remove small irregularities in the intensity values while leaving the large differences, which occur at the boundaries between different tissue types, more or less intact. After smoothing, a calculation of the differences in neighbouring intensity values within the image shows that within a tissue region the gradients are small, while at boundaries the gradients are large. Graphical output from a computer shows the boundaries for various degrees of smoothing as detected by the algorithm. If there is too little smoothing, many spurious edges are shown (Fig. 4a); further smoothing provides results which compares favourably with the colour image (Fig. 4b), but if smoothing is carried too far the two thighs tend to merge (Fig. 4c). We have been looking for ways to obtain the optimum amount of smoothing by computer.

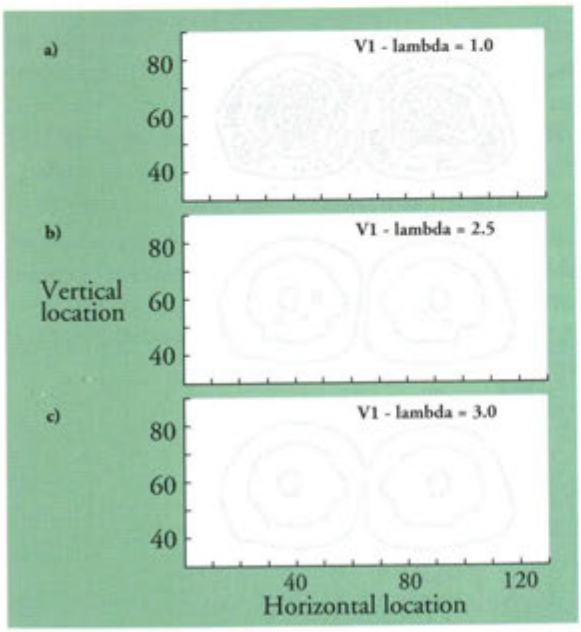

Figure 4 Output from a computer algorithm for detecting boundaries between different tissues in Figure 3 involving a) too little smoothing, b) about the right amount, and c) too much.

Satellite images Satellites have observed the earth's surface passively by measuring the reflected rays from the sun in the visible and infra-red parts of the spectrum. However, new satellites about to be launched will beam microwave radiation and receive reflected

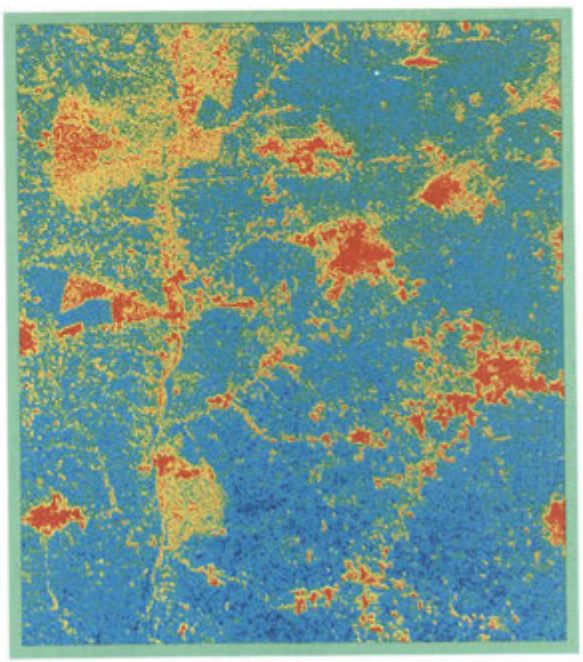

Figure 5 False-colour synthetic aperture radar image of central Spain. This picture was taken by the U.S. Space Shuttle in November 1981, in black and white. In the false-colour representation different shades of grey have been shown instead as different colours.

signals from the earth's surface irrespective of darkness or clouds, in the same way that the Magellan spacecraft is presently mapping Venus. Different images of the earth's surface will be obtained showing features such as surface texture and moisture level. A false-colour synthetic aperture radar (SAR) image of central Spain, recorded by the U.S. Space Shuttle in 1981 is shown in Figure 5.

Interpretation of SAR data is in its infancy, and is made difficult by the large amounts of speckle (like TV interference) present. Traditionally this has been overcome by averaging radar returns over several adjacent observations on the ground. For example, by averaging the original 40m resolution data over 200m square areas, the success in identifying land cover in Figure 5 rose from 39% to 51%. It rose further to 66% when measures of texture in an area were also used. When these data were combined with a conventional satellite (Landsat) image of the same region, a 78% success rate was achieved.

This approach is empirical, and makes no use of statistical knowledge concerning the distribution and correlation of reflected radar signals. We are currently developing appropriate statistical models which should assist the estimation of the true radar backscatter distribution for each part of the image, and thereby the identification of vegetation cover and measurement of its characteristics.

Statistical aspects of near infrared spectroscopy

J. W. McNicol and I. A. Cowe

Determining the chemical composition of plant tissues has traditionally been a slow and complex matter involving sample preparation and wet chemical analysis. Recently, however, new spectroscopic methods have been developed which largely eliminate sample preparation and wet chemistry. In effect an instrument can be trained to "see" the composition of a sample without the need to destroy the sample in the process. A comparable analogy might be a gardener recognising the difference between a ripe and an immature raspberry and from that information guessing the amount of sugar present in the fruit. By using light beyond the visible range of wavelengths and into the near infrared (NIR) we can determine composition much more accurately than is possible by visual means.

When light at a specific wavelength is directed towards a sample, some of that light will be reflected, some will be absorbed by specific molecules and some will be transmitted through the sample. The proportion of light falling into each of these categories will depend largely on the wavelength chosen and on the nature of the sample. A Near Infrared Spectrophotometer is an instrument designed specifically to measure the light reflected or transmitted by samples at specific points in the NIR spectrum. Some instruments measure only a small number of predetermined wavelengths, others use the full range of NIR wavelengths recording at every second or perhaps every fourth nanometer. In this article we shall assume that the instrument measures reflected rather than transmitted energy, and that a spectrum consists of approximately 700 data points measured at 2nm

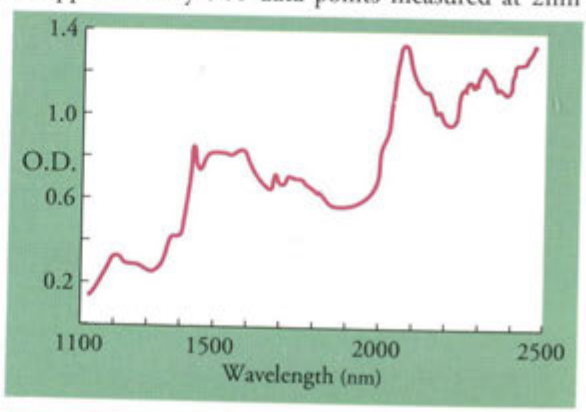

Figure 1 Near infrared spectrum of sucrose.

intervals from 1100 to 2500nm. A typical NIR spectrum, a sample of crystalline sucrose, is shown in Figure 1.

Applications of near infrared spectroscopy NIR spectrophotometers have several advantages over traditional wet chemical methods for analysing samples. Firstly sample preparation is simple. In some cases the sample must be ground to a powder before being placed in a sample cup and presented to the instrument; for other samples, such as small seeds, grinding may be unnecessary and they can be inserted directly into the instrument. Secondly, analysis is rapid in that each sample spectrum is derived within a few seconds and several different constituents can be estimated from the same spectral measurements. In the example that follows, both protein and moisture were estimated from the spectra of a set of wheat flour samples.

Given these advantages, NIR has become a popular screening technique with a wide range of applications. It is used routinely to estimate digestibility and nitrogen in forage crops such as grass, straw and silage. Several of the characters used in assessing the quality of wheat and flour in wheat-breeding programmes, such as protein, moisture and grain hardness, are assessed by NIR. Sugar levels, used to assess the quality of curing practice in tobacco, and fat and protein levels in milk powder are other examples of situations where NIR has taken over from traditional, slower laboratory methods.

Statistical analysis Two features of NIR analyses create the need for a substantial statistical input. Firstly there is the calibration problem. In NIR spectra, absorbance bands for different constituents overlap and measurements at a single point in the spectrum correlate poorly with the concentration of any constituent. By taking measurements at several points we overcome this problem. The additional wavelengths in effect "unscramble" the relationship between spectral response and concentration.

Thus for each application the instrument must be programmed to recognise spectral features of the constituent of interest in a set of typical NIR spectra. Calibrations are specific to the material used in their derivation. For example, a protein calibration for ground wheat samples will be similar but not identical to one for ground barley samples. The basis of a calibration relationship is that increases in the concentration of the constituent of interest in the sample are

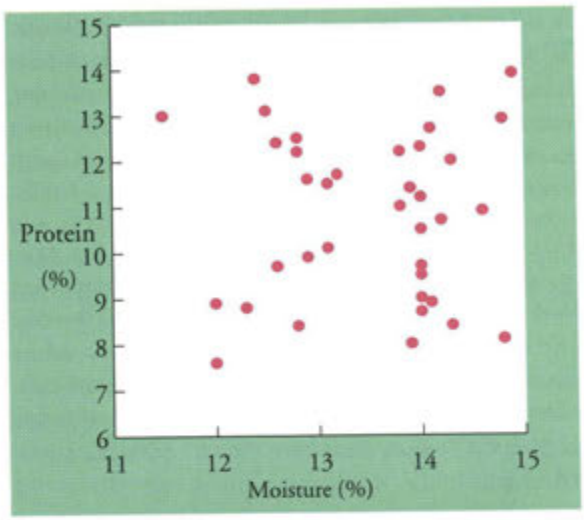

Figure 2 Protein and moisture values of 39 wheat samples.

matched by increases in the optical density (OD) at wavelengths associated with that constituent.

Secondly the volume of data from a typical NIR exercise is so great that some degree of data reduction is necessary if calibration relationships are to be derived which are not so complex as to be worthless in practice. This has traditionally involved selecting from the 700 wavelengths a subset of only four or five which together will successfully predict the constituent of interest.

Example data set The statistical techniques used to analyse NIR spectra will be demonstrated using a set of 39 samples of ground wheat flour obtained from the Flour Milling and Bread Research Association, Chorleywood, England. The samples were chosen to provide a reasonable range of protein and moisture levels and to have a low protein/moisture correlation (Fig. 2). NIR spectra were recorded at 2 nm intervals

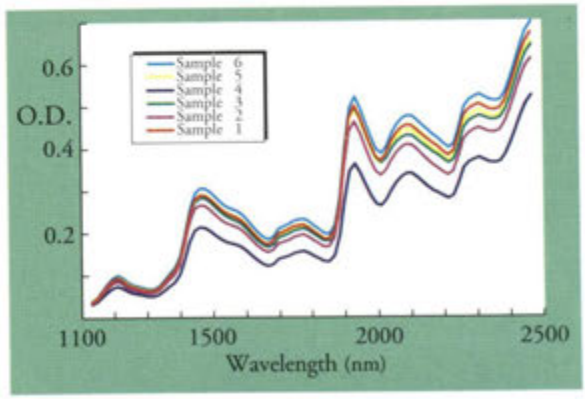

Figure 3 NIR spectra of six ground wheat samples.

from 1120 to 2480nm. Thus each spectrum consists of 681 values, with 26,559 values in total for all the samples. Figure 3 shows the spectra of six samples, selected to show the range of spectral shapes. A figure showing 39 samples would simply be a more crowded version of the same.

It is interesting to note from this figure that the spectra do not have local peaks at wavelengths associated with protein (1500, 1980 and 2204nm). However the sample with the consistently lowest OD values does have the lowest protein value among the six samples in the figure and the sample with the consistently highest OD values does have the second highest protein value.

Conventional statistical analysis The first attempts at calibration were based on simple equations involving only a few wavelengths. For example one possible moisture calibration equation for these samples is

%Moisture = 14.4 + 85.4 x OD1930 - 88.4 x OD2300

where OD1930 denotes the energy of a sample measured at 1930nm. This equation provides a simple recipe for estimating the moisture content of a wheat sample when the OD values of the sample at 1930 and 2300nm are known.

The method used to derive this equation is straightforward. A search is made across the spectrum to identify that wavelength whose OD values have the highest correlation with moisture, in this case 1930nm. A calibration equation is then created involving this one wavelength only. A second search is then made among all possible equations involving two wavelengths, one of which is fixed at 1930nm. The equation which demonstrates the closest agreement between the calculated values and the true moisture values becomes the calibration equation.

Although this procedure is conceptually simple it involves a considerable amount of computer processing time. In the moisture example, the number of equations which have to be derived is 681 x 680. Further, if it is felt worthwhile to consider the possibility of more than two wavelengths in the equation the amount of computing time can increase exponentially.

This procedure can also be criticised on the grounds that it provides very little insight into the information held in the spectral data. Two wavelengths are shown to be related to moisture but nothing else is revealed.

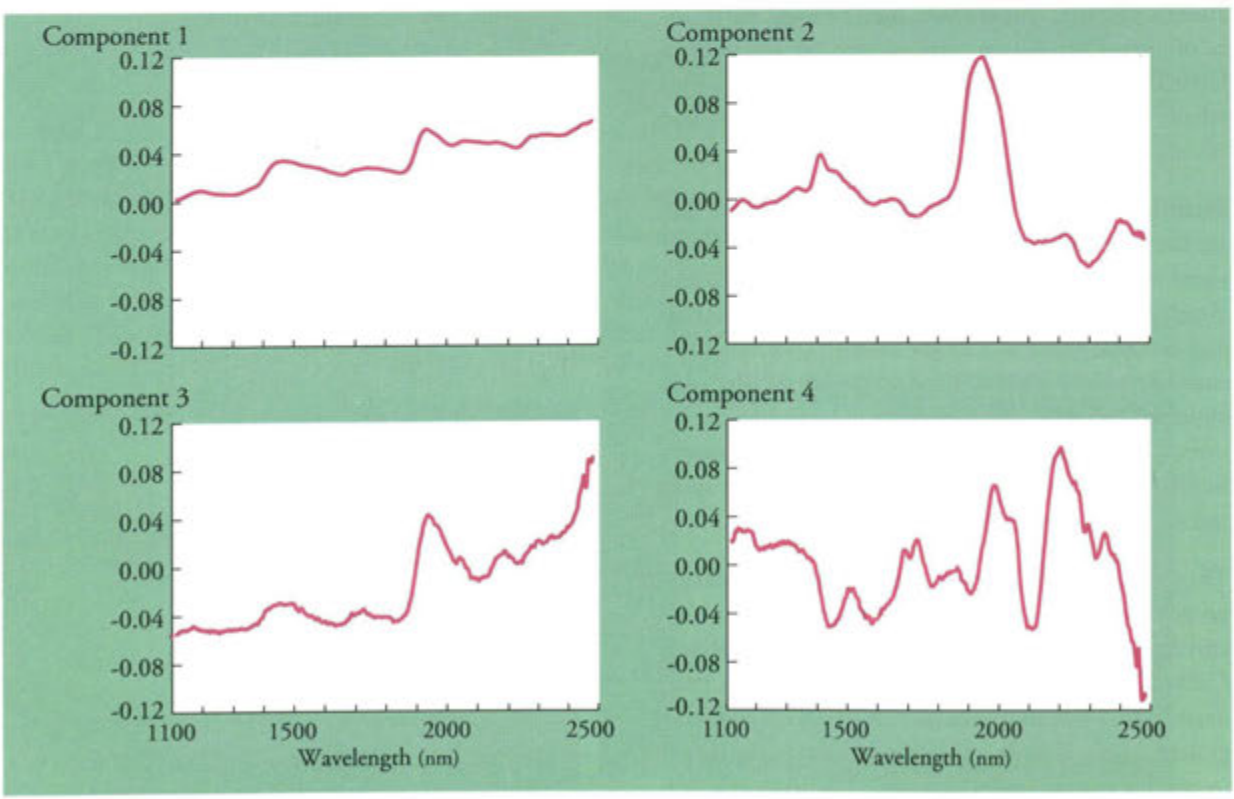

Figure 4 First four principal components of the ground wheat samples.

Principal components analysis One approach to revealing information from a large body of data is to use a 'multivariate' statistical technique, that is a technique designed to cope with many observations on each sample. NIR spectra can be thought of as 700 observations each on single samples and therefore suitable for such an approach.

The most appropriate multivariate technique is called principal components analysis (PCA). This approach attempts to divide all the variation in the spectral data into convenient 'components'. Each of the 700 wavelengths contributes to every component but to a different degree. These contributions are called the component 'weights' and the greater the weight the more the wavelength influences the component. Further, each component can be thought of as an axis along which each sample can be measured. These sample measurements are called component 'scores'. A sample will have a score on every component axis. Principal components are derived in such a way that the first component always has more variation than the second component which in turn has more variation than the third component and so on.

Principal components analysis, therefore, provides the potential for extracting the information held in the spectral data. Partitioning of the variation is a convenient way of discovering which constituents in the samples are contributing most to the variation in the OD values. By correlating the scores of a principal component with constituent values derived by analytical methods, we can also determine how closely a component is related to those constituents.

Principal component analysis of Chorleywood data Figure 4 shows the weights of the first four principal components of the Chorleywood data. The second component is the most straightforward to interpret. The wavelengths around 1934nm have by far the highest weights. Most of the other wavelengths have weights close to zero. The area of the spectrum around 1934nm is known to be related to moisture. Further, Figure 5 shows the close relationship between the sample scores for these components and the laboratory derived moisture values with a correlation coefficient of 0.97. Thus we can confidently associate this component with moisture. It is also worth noting that this moisture component accounted for only 1% of all the variation in the spectral data, a result which seems puzzling at first.

The fourth component has relatively large weights around 1980 and 2204nm, areas of the spectrum associated with protein. There are also large negative weights at 2100nm, an area of the NIR spectrum associated with starch. In wheat grains, starch and protein are negatively correlated. Therefore, these three 'peaks' point to the fourth component being related to protein and starch. Its scores have a correlation of 0.7 with the laboratory assessments of protein, confirming the protein association. This component accounted for only 0.1% of all the spectral variation, much less than the moisture contribution.

The first component, which accounts for 98% of all the spectral variation, has a correlation of 0.7 with the laboratory protein assessments, yet does not highlight any specific region of the spectrum. This component can be shown to be related to particle size, i.e. the size of the individual flour particles after the whole grains have been ground to a powder. It is very difficult to control particle size when grinding whole grains and large differences between samples are known to occur. The correlation with protein is consistent with the fact that particle size is related to grain hardness which in turn is related to protein.

The third component also has a peak at the water band centred on 1934nm but the scores are uncorrelated with moisture. Unlike the second component which correlated highly with oven dried moisture, this component is related to bound water which cannot be driven off by oven drying. Subsequent components account for so little variation that they can safely be ignored.

The PCA has shown that particle size is by far the most dominant source of spectral variation in these

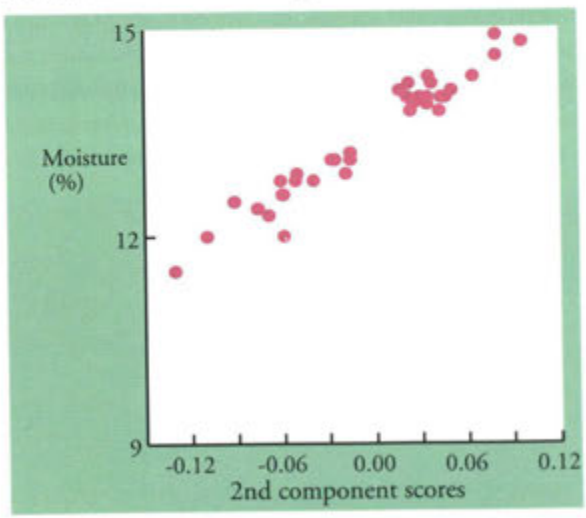

Figure 5 Scores of the second principal component and the corresponding moisture values.

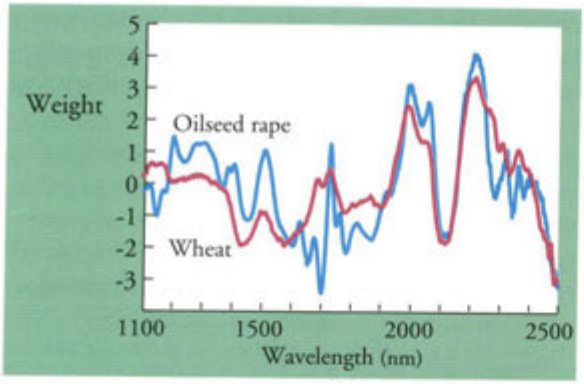

Figure 6 Estimated protein spectra for wheat and oilseed rape.

data while protein and moisture effects, the features in which we are most interested, account for only a very small amount of NIR variation. Nevertheless, using the technique of principal components we are able to detect and measure their influence.

Principal components have further advantages. They can be used to derive calibration equations of the type described above but instead of using wavelengths, the calibration equation uses components and because there are far fewer components, seldom more than 10, selection of appropriate components for an equation is much simpler. For example, the protein equation for the Chorleywood data includes components 1,3 and 4 only. These components can be combined to form an estimate of the complete NIR spectrum of protein. Figure 6 shows this estimate.

Estimations such as this are very useful in highlighting areas of the spectrum which are important in a constituent. Figure 6 also displays the estimate of the protein spectrum derived from the protein calibration for a set of oilseed rape samples. The figure confirms that the two proteins are similar but suggests areas of the spectrum where there are differences, thus providing pointers to the nature of the differences.

Conclusions The patterns shown in the Chorleywood data are typical of those revealed by principal components analysis of NIR spectral data. Where grinding of the samples has taken place, a particle size component is always present. It accounts for the majority of the variation and has the same shape as the first component in Figure 4. If protein or moisture is present in the samples component shapes very similar to the second and fourth components in Figure 4 are found. Recognisable patterns for oil and sucrose have also been established.

Not only has PCA successfully identified the presence of constituents in samples and quantifying them, it has also allowed comparisons to be made between different sets of samples. The different protein spectrum estimates from wheat and oilseed rape have already been described. In another study using PCA it has been possible to monitor the protein, cell wall and starch contributions to the malting process in barley by estimating the hot-water extract spectrum on successive days of a malting run.

Such detailed interpretations of NIR spectra are not possible without the use of advanced statistical methods and these methods in turn depend on the availability of considerable computing power. The current desk-top microcomputer now has that level of power and hence the use of multivariate statistical methods to analyse data from modern analytical instruments such as NIR spectrophotometers is increasing rapidly. This particular combination of statistics and chemistry has become a discipline in its own right, with its own new name - Chemometrics.

Research Services

Data Processing

R.J. Clark

The new SCRINet local area network with Sun workstations at its centre has enhanced computing services available to scientists. They 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. Nearly 100 PCs are currently installed and this number is increasing.

The continuing need for high quality output of graphs and poster material justified the purchase of a new graph plotter. It is attached to SCRINet as a shared output device, that is, any user can produce coloured graphs from any PC on the network. Laser printers and lineprinters are similarly available via the network.

The transition from a mainframe based service, provided for many years by Edinburgh University, to one using Sun workstations entails considerable changes for all users. User training on the Unix operating system and network software occupied DP Unit staff over the latter part 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. The seminar room adjacent to the DP Unit affords facilities for an efficient teaching mix of lecture and practical experience. DP staff also prepare user-notes as training materials.

Services on the network are primarily for access to statistical packages like Genstat and Minitab, popular with users for many years on the mainframe comput-

Data Processing user area.

er. Other services, such as the relational database package Oracle and the Uniras graphics package are also mounted or planned. Support is being provided to move user-written mainframe software to the Sun, such as the CHIP genetic information package written in the Crop Genetics Department.

Direct connection to the Joint Academic Network (JANET) wide area network will take place soon. Access to mainframe services for large applications, like DNA sequencing, will continue to be required. Electronic mail facilitates collaboration between scientists at SCRI and co-workers throughout the U.K. and world-wide.

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.

The personal computer is regarded as another tool by many scientists. Microsoft Works is a standard package for applications ranging from papers to graphs and databases. More complex applications for displays and posters, using Supercalc, Harvard Graphics and Corel Draw, are produced in co-operation with Information services.

The Unit develops applications and provides training for users of a Quantimet 900 Image Analyser. A television camera is used to make rapid and reproducible measurements from a range of images, for example, leaf areas, ground cover from infra-red photographs, and potato tuber grading from 35mm slides. Plant roots and cells, and density of staining in nematodes, can be measured using a microscope.

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 1990 some 3,000 separate photographic

Preparing graphics on the Apple Macintosh Desk Top Publishing system.

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 publishing 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.

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

Field trials on Mylnefield farm, Invergowrie.

Part of the glasshouse range at Invergowrie.

carried out at the IAPGR farm at Blythbank and other off-station sites. The general crop husbandry is based on a long-term (20+ years) plan of land use and is consistent with good farming practice and sound business management. Unless otherwise specified by experimental requirements, the land is maintained at pH 6.5, high P and K status, not deficient in trace elements, no evidence of previous trial cropping, free from perennial weeds and volunteer crops, and, as far as possible, free from soil-borne pests and diseases.

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

The Department maintains the UK virus-free nuclear stocks of *Ribes, Rubus* and *Narcissus* used for first-stage commercial production of planting stocks. It is responsible for a total glasshouse area of 9000 m^2 with controlled steam heating and supplementary lighting. Individual glasshouse cubicles range in size from 12 m^2 to 350 m^2 and some are designed for specific research objectives, including quarantine and isolation. There is also a wide range of cold Dutch light, polytunnels and net structures.

Controlled environment cabinets range in size from 0.25 m^2 to 2.0 m^2 and in complexity from simple incubators to growth rooms where the levels of temperature, lighting and humidity can be programmed. Plants can be exposed to winter regimes to simulate frost damage or to the high light and temperature requirements for sub-tropical research.

Engineering and Maintenance Department

T. Hopton

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.

Growth cabinet maintenance.

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

The wide range of equipment and technologies present in the Institute offers a constant challenge to

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

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 a laboratory block, installation of an 11000 volt, 500 KVA mains transformer to feed all of the glasshouses and some of the service buildings, and the upgrading of fire alarms. Capital works completed under the direction of our architects were the laboratories and glasshouses for the Crop Genetics Department.

Mylnefield Research Services Ltd.

R.J. Killick

Mylnefield Research Services Limited is a wholly-owned subsidiary company of the Scottish Crop Research Institute. It was incorporated in November 1989 with the intention of facilitating the handling of external, especially commercial, income.

SCRI has charitable status which confers a number of tax privileges. For example, charities are entitled to an 80% remission on local rates which saves the Institute about £280,000 per annum. Loss of charitable status would therefore have serious consequences.

The level of funding from Central Government to AFRS institutes has been falling in real terms since the mid-1980s, although the SARIs, which are supported by the Scottish Office Agriculture and Fisheries Department, have fared relatively better than their English counterparts. At the same time, there has been an insistence that the beneficiaries of near-market research, not the tax payer, must pay for that work. Thus institutes have been obliged to seek non-Exchequer sources of money, such as private trusts, the EC, commerce and industry.

Only in very specific circumstances may charities trade for profit and the solution for many charities is to set up a separate trading company. The activities of the trading company earn profits on which corporation tax is payable. The net profit is then transferred each year to the charity via a deed of covenant, which allows the charity to reclaim the tax from the Inland Revenue.

In the case of SCRI/MRS a commercial sponsor seeking to utilise the specific expertise at Mylnefield will negotiate an appropriate contract with MRS Ltd which will charge a fee to cover all expenses plus a profit element. MRS will then sub-contract with SCRI at a price which just covers the full cost, so that neither profit nor subsidy occurs; the profit remains with MRS Ltd until almost the year end when it can be covenanted to SCRI. A more sophisticated arrangement would provide a halfway house charity. In this model the trading company covenants its profits to a trust which can act like a bank. This avoids the possible embarrassment to the charity of having too large a cash surplus at the end of its financial year.

Because MRS is independent of Government, it is free to act as its Directors think fit. For example, it could hold an ecu account or it could employ staff on conditions of service markedly different from those of the Civil Service. It is possible that MRS may employ a Commercial Manager to develop business and such an appointee would have a remuneration package more in line with commercial practice, such as including a

company car, than is possible under SCRI terms and conditions of service.

Mylnefield Research Services Ltd exists primarily to protect the Institute's charitable status and to expand the funding base. It will have some additional minor benefits; for example, it may be possible to recover more VAT than has hitherto been possible. The company did not trade in 1990, not least because the Governing Body, aware of the complexity of financial transactions between charity and trading company necessary to satisfy Inland Revenue rules, took the view that an accountant should first be appointed to SCRI's staff. This appointment was made in November, and we look forward to MRS beginning to trade in 1991 to the benefit of science at SCRI.

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 (4 April and 7 November) and the Crop Sub-Committees were active throughout the year particularly Soft Fruit and Forage Crops.

Travel grants authorised from the General Fund by the Committee were:-
£100 to Dr W. Macfarlane Smith, Crop Genetics Department, British Grassland Society Conference.
£300 to Dr K M. Wright, Cellular and Environmental Physiology Department, International Conference on Phloem Transport, Cognac, France.
£500 to Dr L. Torrance, Virology Department, Congress of Virology, Berlin, Germany.
£275 to Dr B. Boag, Zoology Department, International Nematology Congress, Veldhoven, The Netherlands.
Other donations made during the year were £1,000 to the European Association for Potato Research meeting in Edinburgh in July. International scientists visited the Institute during this meeting and several Institute staff were closely involved in its organisation. Approval was also given for a contribution to a video illustrating the role and work of the Institute which would also be used for training and demonstration purposes.

The Society has instituted two prizes. One is an annual award of merit for work carried out by field and glasshouse staff in the Estate Division. The second is a scholarship to enable a research worker to visit the Institute for between 6 and 12 months.

Membership of the Society was 314 on 31 December 1990.

Officers of the Society

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Vice-Chairman: A.M. Jacobsen, Mains of Catterline, Catterline, Stonehaven, Kincardineshire, AB3 2UL

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Extracts from the Accounts

Income and expenditure account for the year ended 31 March 1990

	1990	1989
Income		
Grants from DAFS	6,022,848	5,891,564
Research and contracts	3,586	-
Trading activities	96,137	146,861
SASS income	143,889	105,685
Bank interest receivable	8,401	-
Depreciation contribution from reserve	362,212	298,858
Other income	94,528	50,865
	6,731,601	6,493,833
Expenditure		
Staff costs	4,436,272	4,236,515
Supplies and services	1,094,384	1,307,761
Depreciation	468,101	405,710
Other expenditure	667,087	554,696
	6,665,844	6,504,682
Surplus/(deficit) for year	65,757	(10,849)
Balance from previous year	833,921	844,770
Balance carried forward	899,678	833,921

Balance sheet as at 31 March 1990

		1990		1989
Fixed assets				
Tangible fixed assets		11,013,063		9,799,398
Current assets				
Stocks	95,341		107,433	
Debtors	211,120		154,978	
Cash at bank and in hand	217,809		159,051	
	524,270		421,462	
Current liabilities				
Creditors				
Amounts falling due within one year	460,423		399,438	
Net current assets		63,847		22,024
Total assets less current liabilities		11,076,910		9,821,422
Represented by:				
Creditors				
Amounts falling due after more than one year		10,155,582		8,965,851
Capital and reserves				
Capital reserves		21,650		21,650
Income and Expenditure		899,678		833,921
		11,076,910		9,821,422

Staff list as at 31 December 1990

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

Crop Genetics Department (CG)

Head : G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol.[5]	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
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
W.T.B. Thomas, B.Sc., Ph.D.	SSO
Ruth M. Solomon-Blackburn, B.A., M.Sc.	HSO
S. Clulow, B.Sc., Ph.D.	HSO (Appt. Jan)
Mary Coleman, 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	ASO
P. Davie, O.N.C.	ASO
Norma Dow	ASO
Michelle Fleming, H.N.D.	ASO
Frances Gourlay, H.N.C.	ASO
R. Keith	ASO
A. Lorimer	ASO
Karen McIlravey, O.N.C., H.N.C.	ASO
Jane McNicole, H.N.C.	ASO
Ann Todd	ASO
D. Todd	ASO
A. Wilson	ASO
M.P.L. Campbell	P&GS (E)
Alice Bertie	EWII
Joyce I. Cairns	EWII
J.D. Fuller	EWII
Patricia Lawrence	EWII
A. Margaret McInroy	EWII
Moira Myles	EWII

Soft Fruit Genetics Department (SFG)

Head : R.J. McNicol, B.Sc.	UG7 (Prom. Apr)
M.R. Cormack, N.D.H.	SSO
R. Brennan, B.Sc., Ph.D.	HSO
Julie Graham, B.Sc.	HSO (Appt. Oct)
Sandra L. Gordon, H.N.C.	ASO
Amanda Thomson, H.N.D.	ASO

Cell & Molecular Genetics Department (CMG)

Head : W. Powell, B.Sc., M.Sc., Ph.D.[5,6]	UG7
J.W.S. Brown, B.Sc., Ph.D.[6]	UG7 (Appt. May)
B.P. Forster, B.Sc., Ph.D.	SSO
A. Kumar, B.Sc., Ph.D.	SSO (Prom. Apr)
R. Waugh, B.Sc., Ph.D.[6]	SSO (Prom. Apr)
S. Millam, B.Sc., Ph.D.	HSO
E. Baird, H.N.C.	SO
B. Harrower, H.N.D.	SO (Tr. from Zoo Jan)
Diane Davidson	ASO
Nicky Duncan, H.N.C.	ASO
M. Macaulay	ASO

Cellular & Environmental Physiology Department (CEP)

Head : H.V. Davies, B.Sc., Ph.D.[6]	UG6 (Prom. Sep)
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
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
D.C. Gordon, H.N.C.	HSO
R.A. Jefferies, B.Sc., Ph.D.	HSO
Heather A. Ross, H.N.C., C.Biol., M.I. Biol.	HSO
T. Shepherd, B.Sc., Ph.D.	HSO
M. Taylor, B.Sc., Ph.D.	HSO
J.S. Wiseman, S.D.H.	HSO
Katherine Wright, B.A., Ph.D.	HSO
I. Young, B.Sc., Ph.D.	HSO
Sandra Caul, H.N.C.	SO
A. Gardner, B.Sc.	SO
D.A.M. Prior, H.N.C.	SO
Susan Verrall, H.N.C.	SO
Gladys Wright, H.N.C.	SO
D. Crabb	ASO
G. Dunlop, O.N.C.	ASO
Margaret Garland	ASO
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

Chemistry Department (Chem)

Head : M.J. Allison, B.Sc., Ph.D.	UG7
I.A. Cowe, H.N.C.	SSO
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
Winifred M. Stein, H.N.C.	HSO
D.C. Cuthbertson, H.N.C.	SO
K. Taylor, H.N.C.	SO
Judith 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)

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
Susan E. Burrows, B.Sc.	SO

Mycology and Bacteriology Department (M & B)

Head : J.M. Duncan, B.Sc., Ph.D.	UG6 (Prom. Sep)
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
J.G. Harrison, B.Sc., Ph.D.	SSO
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
E. Marion Burnett, H.N.C.	SO
Jacqueline Heilbronn, H.N.C.	SO
D. Johnston, B.Sc.	SO
Naomi Williams, H.N.C.	SO
Sandra Millar, O.N.C., H.N.C.	ASO
Evelyn Warden	EWII

Virology Department (Vir)

Head : B.D. Harrison, C.B.E., B.Sc., Ph.D., Hon.D.Agr.For. C.Biol., F.I.Biol., F.R.S.E., F.R.S.[2,4]	UG5
A.F. Murant, B.Sc., Ph.D., A.R.C.S., C.Biol., F.I.Biol., F.R.S.E.[6]	UG6
H. Barker, B.Sc., Ph.D	UG7
A.T. Jones, B.Sc., Ph.D.	UG7
M.A. Mayo, B.Sc., Ph.D., C.Biol., M.I.Biol.	UG7
W.P. Mowat, B.Sc., Dip.Agr.Sci.	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
J.H. Raschké, H.N.C.	HSO
G.H. Cowan, H.N.D.	SO
Sheila M.S. Dawson, H.C.	SO
Anne C. Jolly, H.N.C.	SO
E.W. Milne, O.N.C.	SO
Wendy McGavin, B.Sc.	SO
Kara D. Webster, H.N.C.	SO (Tr. from CEP Aug)
Gillian L. Fraser	ASO
Anne Grant	ASO
Rena Reid	EWIII

Zoology Department (Zoo)

Head : D.L. Trudgill, B.Sc., Ph.D., C.Biol., F.I.Biol.[5,6]	UG6
B. Boag, B.Sc., Ph.D.	UG7
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
D.J.F. Brown, B.A., Ph.D., C.Biol., M.I. Biol.	SSO
S.C. Gordon, H.N.C.	SSO
R. Neilson, H.N.C.	SO
Sheena Lamond	ASO
Gaynor Malloch, B.Sc.	ASO
Anne Marshall	ASO

Data Processing Unit (DP)

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

Scientific Liaison & Information Services Department (SLIS)

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T. G. Geoghegan, A.B.I.P.P., A.M.P.A.	Chief Photographer
S.F. Malecki	Senior Photographer
G. Menzies	Photographer
I.R. Pitkethly, H.N.D.	Higher Graphics Officer (Appt. Oct.)
Ursula McKean, M.A., Dip. Lib.	Assistant Librarian
Lorna 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
Accountant : S.L. Howie, C.A.	SEO (Appt. Nov)
Assistant Secretary : D.L. Hood, B.Admin., L.T.I., A.I.I.M.	HEO
Personnel Officer : I. Paxton, H.N.C.	EO
Freida Soutar	HEO (Prom. Jul)
Catherine Skelly	EO (Prom. Sep)
Margaret Barnes	AO
Dianne Beharrie, Dip. Ed.	AO (P/T)
Rhona G. Davidson	AO (Appt. Dec)
Catherine McDougall	AO
Wendy A. Patterson, H.N.D.	AO (Appt. Dec)
Sarah-Jane Simms, H.N.D.	AO (Appt. Dec)
Lesley Wilkinson	AO
Kristy L. Grant, B.A.	AA (P/T, Appt. Jul)
Margaret M. Mills	AA (P/T)
Loraine Galloway	SPS (Prom. Jul)
Linda Butler	Typist
Joyce Davidson	Typist
Jean Findlay	Typist (P/T)
Sheena Forsyth	Typist (P/T)
Elizabeth Fyffe	Typist
Maureen Murray	Typist
Elizabeth L. Nicoll	Typist
Myra Purves	Typist

Engineering & Maintenance Department (EM)

Institute Engineer : T. Hopton, C.Eng., M.I. Mech.E.	SP&TO
R. Macdonald	HP&TO
S. Petrie, B.Sc.	HP&TO
D. Gray	P&TO
J. Anderson	Craftsman
D. Byrne	Craftsman
J.R. Caithness	Craftsman
D. Diduca	Craftsman
D. Hutcheson	Craftsman
E. Lawrence	Craftsman (Appt. Jul)
A. Low	Craftsman
K. Low	Craftsman
R. White	Craftsman
T. Purves	Caretaker
J. Rowe	Caretaker
R. Pugh	Boilerman
Janice McDonald	AO (P/T, Appt. Aug)
G.W. Pollock	PTO
G.C. Roberts	Craftsman (Appt. Jul)
I.M. Scrimgeour	Craftsman (Appt. Jul)
J. Heeney	Storeman II (Tr. from Admin. Apr)
J. Flight	Storeman II (Tr. from Admin. Apr)

Estate, Glasshouse & Field Experiments Department (EGF)

Head : W.I.A. Jack	SSO
G. Wood, B.Sc., Ph.D., F.E.T.C.	HSO
P.A. Gill, H.N.D.	HSO
D.S. Petrie	P&GS(D)
R.W. Reid	P&GS(D)
C.C. Carrie	P&GS(E)
A.D. Lindsay	P&GS(E)
A.W. Mills	P&GS(E)
R. Ogg	P&GS(E)
D.G. Pugh	P&GS(E)
B.D. Robertson	P&GS(E)
C.R. Dalrymple	EWI
E.A.M. Gardiner	EWI
J.P.T. Grant	EWI
W.D.J. Jack, B.Sc.	EWI
W.W. Killoh	EWI
N. McInroy	EWI
J. Mason	EWI
D.R. Simpson	EWI
J.R.K. Bennett	EWII
L.A. McNicoll	EWII
Gillian Pugh	EWII (Prom. May)
Angela Thain	EWII (Prom. May)
J.T. Bennett	EWIII
G. Dow	EWIII
D.K.L. Robertson	EWIII
P.W. Yeaman	EWIII
C. Conacher	EWIV (Appt. Nov)
B. Fleming	EWIV
I. Fleming	EWIV
A. Fuller	EWIV
Carol Taylor	EWIV (Appt. Nov)
J.K. Wilde	EWIV (Appt. Jun)
Lorna Doig	AO (P/T, Tr. from Admin Sep)

Scottish Agricultural Statistics Service (SASS)

King's Buildings, University of Edinburgh

Director :R.A. Kempton, M.A., B.Phil.	UG6
C.A. Glasbey, M.A., Dip. Math. Stats., Ph.D.	UG7
E.A. Hunter, B.Sc., M.Phil.	UG7
G.W. Horgan, B.A., M.Sc.	SSO
M. Talbot, F.I.S., M.Phil.	SSO
Helen K. Brown, B.A., M.Sc.	HSO
Janet M. Dickson, B.Sc.	HSO
A.D. Mann, B.Sc.	HSO
Karen Cattanach	HSO (Appt. Aug)
A. Sword	HSO (Appt. Oct)
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)
Henrietta Wallace	AA (Appt. Jul)
Karyn Linton	PS (P/T, Appt. Nov)
Amy G. Stewart	Typist (P/T, Appt. Jul)

Aberdeen Unit

Head :M.F. Franklin, B.Sc., M.Sc., Ph.D.	UG7
S.T. Buckland, B.Sc., M.Sc., Ph.D.	UG7
Jean M. Cooper, B.Sc., Dip. Math. Stats.	SSO
D.A. Elston, B.Sc., M.Sc.	HSO (Tr. from Edinburgh Jun)
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
Jackie Lyon	ASO

Cellular and Environmental Physiology

J.A.C. Smart, B.Sc.	HSO
Elizabeth A. Murant, B.Sc.	HSO (Appt. Jan)

Crop Genetics

Margaret Ramsay, 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
Hazel Thomson	ASO

Virology

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

Zoology

Sybill MacIntosh, B.Sc.	SO (Tr. from EGF Apt)

SOAFD Flexible Funding

Cellular and Environmental Physiology

Susan Smith, B.Sc., Ph.D.	HSO (Appt. Oct)
Ramanee Mann, B.Sc.	SO (Appt. Oct)
Sheena Rodger	ASO

AFRC / Dundee University

Cell and Molecular Genetics

Gillian Clark, H.N.C.	Res. Tech. (Appt. Jun)
G. Simpson, B.Sc., Ph.D.	Res. Asst. IA (Appt. Jun, Proms. Dec)
David Leader, B.Sc.	Res. Asst. (Appt. Jun.)
C. Simpson, H.N.D., B.Sc.	Res. Asst. (Appt. Jun.)
Petra Vaux, B.Sc., Ph.D.	Res. Asst. (Appt. Jun.)

BTG

Zoology

Irene E.Geoghegan	SO (Appt. Jan)
Sharon J. Dubbels	ASO (Appt. Jun)

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, Dott. Agr. Sci.	HSO
Edna Cuthbert, S.N.C., H.N.D.	ASO

HDC

Mycology and Bacteriology

Pamela H Scott, B.Sc.	SO

H-GCA

Crop Genetics

J-P. Camm, B.Sc., Ph.D.	HSO

Mycology and Bacteriology

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

Cellular and Environmental Physiology

Shona McIntosh	ASO

MAFF

Cellular and Environmental Physiology

Elizabeth Robertson	ASO (Appt. Jul)

ODA

Cell and Molecular Genetics

Stephanie Cooper-Bland, B.Sc., Ph.D.	HSO (Appt. Nov)
P. Lanham, B.Sc., Ph.D.	HSO (Tr. from M&B Jul)
Sarah Fennel, B.Sc.	SO (Appt. Aug)
Jennifer Watters, H.N.D.	ASO (Appt. Nov)

Virology

Vivian Blok, B.Sc., M.Sc., Ph.D.	HSO (Appt. Feb)
P.M. Derrick, B.Sc., Ph.D.	HSO (Appt. Nov)
Mary-Jo Farmer, B.Sc., Ph.D.	HSO (Appt. Apr)
P. McGrath, B.Sc., Ph.D.	HSO
Maud Swanson, B.Sc.	HSO

PMB

Cellular and Environmental Physiology

M. Young, H.N.D.	SO
Ailsa Smith, B.Sc.	EWV

Mycology and Bacteriology Department

Anne McLeod, H.N.D.	SO
G. McMillan	ASO

United Biscuits

Cell and Molecular Genetics

A.L. March	SO (Appt. Apr)
I Morrison	ASO

Miscellaneous Funding

Cellular and Environmental Physiology

Isobel Christie	Res. Asst.
Gina McPhail	Res. Asst. (Appt. Jul)

Resignations

Name	Dept.	Grade	Month
D. Adamson	SFG	ASO	September
N. Anderson	EM	Craftsman	May
S. Bowick	EM	Craftsman	July
E. D. Bowman	Zoo	ASO	September
I. Bradbury	SASS	SSO	April
A. G. Bruce	SASS	AA	May
M. Catley	CMG	HSO	April
D. Cawston	CMG	SO	March
I.R. Craigie	SASS	SSO	July
J. Davidson	Admin	Typist	May
C. Evans	SASS	PS	April
J. Fairbairn	SASS	Typist	April
D. Hedley	M&B	HSO	October
G. Martin	SLIS	HGO	August
L. McGurk	M&B	ASO	June
L. Monks	Admin	AA	June
A. Nicoll	EGF	EWI	November
P. Phillips	SASS	SSO	March
G.G. Pollock	EGF	EWIV	July
J. Robb	Zoo	SO	October
P.H. Scott	M&B	SO	June
A. Smith	CEP	ASO	August
M. Smith	EGF	EWIV	March
A.J. Soutar	SASS	SO	August
F.E.C. Stewart	CEP	ASO	September

Staff Retirements

Name	Dept.	Grade	Month
A. L. Bertie	Admin	AO	January
A. Davidson	EM	Craftsman	October
D. Mason	CEP	SSO	January
H. Taylor	CEP	SSO	February
E. Watt	EGF	EWIV	October

Redundancies, Voluntary and Flexible Retirements

Name	Dept.	Grade	Month
Alison M. Campbell	M & B	SO	March
P. Cromwell	Chem	ASO	January
J.N. Dick	CG	SO	March
G.M. Dobbie	CG	ASO	January
Hazel J. Duff	SASS	AO	November
R.J.A. Exley	SLIS	SSO	January
J. Muscott	SASS	UG7	August
Susan Rawlings	Zoo	ASO	March

Short term workers and visitors

Name	Country of Origin	Dept.	Month of Arrival	Length of stay
A. Abruzzese	Italy	CEP	July	3 weeks
D. Aeschlimann	Bolivia	Zoo	April	3 weeks
M. Betka	Germany	Zoo	January	1 month
B. Borkhardt	Denmark	Vir	November	1 month
S. Bouzoubaa	France	Vir	September	1 month
A.J. Bowie	England	SASS	July	1 year
A. Cassidy	Scotland	CMG	July	1 year
M. Coiro	Italy	Zoo	January	3 weeks
D. Cormack	Scotland	CMG	July	10 months
G. Demangeat	France	Vir	November	1 month
D. Deng	China	Vir	October	1 year
D. Fargette	France	Vir	January	3 years
Mireille Fargette	France	Zoo	January	3 years
R.S. Forrest	Scotland	Zoo	March	1 year
Shirley Fryer	England	CMG	October	1 week
L. Hackney	Scotland	CMG	September	1 year
Y. Hong	China	Vir	February	1 year
Emily Hoover	USA	SFG	March	9 months
Jacqueline Kelsey	Scotland	Vir	April	6 months
K. Knoll	Germany	CG	May	4 months
D. Liu	China	Vir	April	1 year
J. Luby	USA	SFG	March	9 months
D. Lynch	Canada	CG	November	6 months
Anne Manoukian	France	Vir	April	5 months
P. Moss	India	CMG	April	2 weeks
Anatolia Mpunami	Tanzania	Vir	May	2 months
V. Muniyappa	India	Vir	April	8 months
T. Natsuaki	Japan	Vir	September	9 months
R.W. Quene	Netherlands	SFG	May	3 months
Maria Sandgren	Sweden	Vir	September	1 month
Joyce Schoondergang	Netherlands	SFG	May	3 months
P. Spalding	Scotland	CEP	June	3 months
Miranda Spool	Netherlands	CMG	April	6 months
M. van der Gaag	Netherlands	M&B	March	4 months
A. Watson	Scotland	Zoo	November	8 months
Jennifer Watters	Scotland	CMG	July	10 months
J. Welch	Scotland	M&B	August	2 months
L. Wen	China	CMG	August	1 year
F. Wightman	Canada	DG	January	6 months
Ly Mee Yu	Scotland	SASS	July	2 months

Postgraduate Students

Name	Dept.	Subject
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.
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.
Sarah Fennel	CMG	Biochemical and molecular markers of *Arachis.*
Susan Finney	CMG	Anther culture technology in barley breeding.
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.
Julie Graham	SFG	*Agrobacterium spp.* as DNA vectors in soft fruit plants.
R.S. Hartley	CMG	Isolation and characterisation of retrotransposons from plants.
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.
Laura A. MacCulloch	Zoo	Localisation of plant roots by parasitic nematodes.
R.J. McNicol*	SFG	Investigations into running off in blackcurrants.
Jane Miller	Vir	Potato leafroll virus in protoplasts.
R. Moon	M&B	Transformation systems in *Phytophthora infestans.*
Elizabeth Murant	CEP	Endocytosis in plant cells.
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.
L.D. Ramsay	CG	Applications of biometrical genetics to swede breeding.
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.
Pamela Scott	M&B	*Phytophthora spp.* causing root rot disease of raspberries.
C. Simpson	CMG	Transposable elements from maize.
G. Simpson	CMG	Splicing of pre-mRNAs in plants.
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.
Joanne Wilde	CMG	Genetic fingerprinting of cocoa.
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.N.E. Birch	Germany	March	3 days
	USA	August	8 days
	Switzerland	December	4 days
V.C. Blok	India	February	2 days
	Germany	August	1 week
	France	September	4 days
B. Boag	Belgium	February	2 days
	The Netherlands	August	7 days
R.M. Brennan	Switzerland	June	14 days
K.M. Brierley	The Netherlands	October	1 week
D.J.F. Brown	The Netherlands	February	1 week
	Bulgaria	March	1 week
	Portugal	June	3 weeks
	Italy/Germany	July	3 weeks
	The Netherlands	August	1 week
J.W.S. Brown	West Germany	May	10 days
	West Germany/ Switzerland	September	8 days
S.T. Buckland	USA	April	1 week
	The Netherlands	June	2 weeks
	Hungary	July	1 week
	USA	July	1 week
	Eire	November	2 days
	USA	November	1 week
I.A. Cowe	Belgium	June	5 days
	USA	August	5 days
J.W. Crawford	The Netherlands	May	1 week
D.C. Cuthbertson	Belgium	June	5 days
M.F.B. Dale	Spain	May	4 days
H.V. Davies	Italy	May	3 days
	Canada	July	4 days
	USA	July	9 days
J.M. Duncan	Yugoslavia	November	14 days
D.A. Elston	The Netherlands/ Germany	April	2 weeks
J.M.S. Forrest	Germany	April	1 day
	Germany	November	1 day
B.P. Forster	India	February	14 days
	USA	August	5 days
	France	September	5 days
M.F. Franklin	Canada	July	3 weeks
C.A. Glasbey	Spain	April	4 days
	Hungary	July	1 week
S.C. Gordon	Germany	June	5 days
	France	July	1 day
B.S. Griffiths	Denmark	May	4 weeks
B.D. Harrison	India	January	2 weeks
	Finland	April	3 days
	USSR	May	1 week
	Germany	May	1 week
	Germany	August	10 days
	China	October	3 weeks
P. Hedley	Holland	June	3 days
J.R. Hillman	Japan	November	5 days
G.W. Horgan	Italy	July	2 days
E.A. Hunter	Holland	May	2 days
	Norway	December	4 days
N.L. Innes	Italy	March	2 days
	The Netherlands	May	4 days
	India	May	3 days
	USA	October	3 days
	India	November	5 days
	Peru	December	4 days

Name	Country Visited	Month of visit	Duration of visit
A.T. Jones	USA	August	1 week
	Germany	September	1 week
R.A. Kempton	Kenya	March	2 weeks
	Hungary	July	1 week
A. Kumar	Holland	June	8 days
	Czechoslovakia	November	10 days
G.D. Lyon	Soviet Union	December	15 days
G. Machray	Holland	June	3 days
	West Germany	November	3 days
G.R. Mackay	France	June	2 days
G.J. McDougall	France	September	1 week
W.H. Macfarlane Smith	Belgium	February	4 days
B. Marshall	The Netherlands	May	1 week
M.A. Mayo	France	February	3 days
	Germany	August	10 days
	Australia	November	2 weeks
	India	December	2 weeks
J.W. McNicol	Belgium	June	2 days
S. Millam	Holland	June	14 days
A.F. Murant	Germany	August	2 weeks
	France	September	4 days
A.C. Newton	Germany	August	2 days
	Denmark	January	4 days
K.J. Oparka	France	August	1 week
	France	October	1 week
M.C.M. Perombélon	Peru	February	16 days
	Mauritius	July	7 days
	France	September	5 days
	France	October	7 days
M.S. Phillips	Romania	September	12 days
W. Powell	The Netherlands	February	4 days
	USA	March	8 days
	Costa Rica	April	10 days
	The Netherlands	October	1 day
	USA	November	6 days
	India	December	10 days
K. Ritz	Germany	November	1 week
I.M. Roberts	India	October	4 weeks
	Italy	October	3 days
W.M. Robertson	Germany	November	6 days
D. Robinson	France	September	1 week
	Germany	October	1 week
D.J. Robinson	Germany	August	1 week
	India	September	2 weeks
	Italy	October	3 days
K.P. Scott	Germany	August	1 week
M.M. Swanson	Colombia	May	1 week
J.S. Swanston	The Netherlands	June	3 days
M. Talbot	Eire	June	2 days
	Luxembourg	July	2 days
	Germany	September	2 days
D.L. Trudgill	Tanzania	May	2 weeks
	The Netherlands	August	1 week
	Czechoslovakia	December	2 weeks
R. Waugh	Denmark	January	3 days
	Germany	May	10 days
	Holland	June	3 days
	West Germany	November	3 days
M.J. Wilkinson	Germany	April	7 days
J.A.T. Woodford	USA	August	1 week
F.G. Wright	Eire	October	2 days
K.M. Wright	France	August	1 week

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	Entomology Group Committee, AAB
	Member	International Organisation for Biological Control
		- Working group on Host Plant Resistance to Insects and Mites
		- Working group on Integrated Plant Protection in Vegetables
R.M. Brennan	Member	NFT Blackcurrant and Bush Fruit Panel
D.J.F. Brown	Secretary and Treasurer	European Society of Nematologists
J.W.S. Brown	Co-organiser	Scottish Plant Biotechnology Forum
S.T. Buckland	Member	Scientific Committee, International Whaling Commission
	Member	Working Group on Integrated Population Monitoring, British Trust for Ornithology
M.R. Cormack	Member	NFT Scottish Soft Fruit Panel
	Member	SDA Raspberry Industry Group
	Member	TRIO Adviser, Fruit Crops
	Member	SCRI/ASS/COSAC Liaison Group
	Member	Committee Soft Fruit Working Group
I.A. Cowe	Co-chairman	NIR '91 International Conference on NIR
D.C. Cuthbertson	Member	NIR '91 International Conference on NIR
H.V. Davies	Chairman	PMB research review; physiology and agronomy
	Member	British Plant Growth Regulator Group
J.M. Duncan	Membership Secretary	Council of the British Society for Plant Pathology
R.P. Ellis	Tech.Secretary	SSCR Cereals Group
	Member	BSPB Cereal Crop Group Consultative Committee
	BSPB Representative	Institute of Brewing Scottish Working Party
M. Franklin	Member	British Region Committee, Biometric Society
B.A. Goodman	Member	Royal Society of Chemistry, Mössbouer Discussion Group
	Member	CEC BCR exercise on an ESR
	Member	MAFF group on detection of irradiated foodstuffs
S.C. Gordon	Session Organiser	Crop Protection in Northern Britain
B.D. Harrison	Member	Advisory Committee, Advances in Virus Research
	Member	Advisory Committee for NERC Institute of Virology and Environmental Microbiology
	Member	Section 7 Committee, Royal Society
	Member	Research Grant Board F, Royal Society
J.R. Hillman	Member	AFRC Plants and Environment Research Committee
	Member	SOAFD Joint Management Board
	Member	Organising Committee for EAPR Triennial Conference 1990
	Member	ECRE Board of Management
	Member	GIUS, WSC, Technical Committee
	Member	NFT (Brogdale) Advisory Committee
	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
N.L. Innes	Member	Governing Board, CIP, Peru
	Chairman	Nominations Committee, CIP, Peru
	Secretary	Executive Committee, CIP, Peru
	Member	Examinations Board, M.Sc.Applied Genetics, University of Birmingham
	Member	Horticultural Quartet (ADAS/AFRC/SAC/SARI)
	Member	Royal Society of Edinburgh, Section Committee Biology (1)
	Member	BSPB Technical Advisory Committee
	Member	SCRI/ASS/COSAC Liaison Group
	Member	University of Dundee Botanic Garden Committee
	Vice-President	Association of AppliedBiologists
A.T. Jones	Chairman	ISHS, Working Group on Virus Diseases of Small Fruits
R.A. Kempton	Council Member	International Biometric Society
	Council Member	Royal Statistical Society
H.M. Lawson	Member	ADAS/IACR Weed Liaison Group
	Member	BCPC R&D sub-committee - Weeds
	Member	SCRI/SAC Weeds Group

Name	Position	Committee or Organisation
W.H. MacFarlane Smith	Member	BSPB Oilseed & Industrial Crop Group NPTC Plant Variety Development Panel
	Chairman	SCRI/ASS/SAC Forage Brassica Working Group
	Member	SCRI/ASS/COSAC Liaison Group
	Secretary	SCRI/SSCR Forage Group Sub-committee
	Member	Technical Survey Sectoral Quartet on Crop Production
G.R. Mackay	Member	Interdepartmental Committee of (SOAFD/E. Craigs) Breeders Quarantine Unit
	Chairman	Potato Section of EUCARPIA
	Member	Scientific Committee of 11th EAPR Triennial Conference
	Member	BSPB Potato Group
	Member	SOAFD (SARI/SAC) Potato Working Group
D.K.L. MacKerron	Chairman	Working group on Water Relations in Potato Production, EAPR Physiology and Agronomy Sections
	Secretary	SARI/SAC/ASS Potato Working Group
	Secretary	SSCR Potato Crop Sub-committee
	Member	SARI/SAC/ASS Liaison Group
B. Marshall	Member	AFRC Soil Science Steering Group
M.A. Mayo	Member	Executive Committee, International Committee on Taxonomy of Viruses
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	British Society for Plant Pathology Council
	Member	UK Cereal Pathogen Virulence Survey Committee
M.C.M. Pérombelon	Chairman	ISPP International Erwinia (soft rot) Group
	Member	COST 88 Working Group on Detection of Bacteria
D.A. Perry	Treasurer	Association for Crop Protection in Northern Britain
W. Powell	Co-organiser	Scottish Plant Biotechnology Forum
	Technical	Royal Society of Edinburgh,
	Secretary	Organising Committee on Opportunities and Problems in Plant Biotechnology
G. Ramsay	Member	SOAFD SARI/SAC Oilseeds & Protein Working Group
K. Ritz	Member	SAC/SARI Working Party on Organic Production Systems
I.M. Roberts	Chairman	AFRC Electron Microscope Advisory Group
	SafetyRepresentative	Royal Microscopical Society
D. Robinson	Member	AAB Plant Physiology Committee
D.J. Robinson	Member	Society for General Microbiology Virus Group Committee
	Member	Advisory Committee on Releases to the Environment
H.E. Stewart	Member	SARI/SAC Working Group on Organic Production Systems
M. Talbot	Member	Statistics Group of UK National List and Seeds Committee
	Member	Technical Working Party on Automation and Computer Programs of the International Union for the Protection of Plant Varieties
	Member	Statistics Committee of the International Seed Testing Association
W.T.B. Thomas	Member	AAB Plant Breeding Committee
B. Williamson	Member	Committee of British Association for Advancement of Science (Tayside & Fife Branch)
R. Wilson	Member	BSPB Oilseed Rape Group
J.A.T. Woodford	Session Organiser	British Crop Protection Council Conference - Pests and Diseases
	Regional Hon. Sec.	Royal Entomological Society
I M Young	Member	SAC Compaction Study Group

Editorial Duties

Name	Position	Journal Title
H. Barker	Editorial Board	*Annals of Applied Biology*
D.J.F. Brown	Editorial Board	*Nematologica Mediterranea*
J.M. Duncan	Associate Editor	*Journal of Horticultural Science*
	German Translator	*Potato Research*
M.F. Franklin	Editorial Board	*Crop Science*
B.D. Harrison	Editor	*AAB Descriptions of Plant Viruses*
	Editorial Board	*Proceedings B, Royal Society of Edinburgh*
J.R. Hillman	Managing Editor	*Crop Research*
	Publication C'tee	*Journal of HorticulturalScience*
	Editorial Board	*Agricultural Systems*
N.L. Innes	Editorial Board	*Agbiotech News and Information*
	Editorial Board	*Crop Research*
H.M. Lawson	Associate Editor	*Journal of Horticultural Science*
D.K.L. MacKerron	Associate Editor	*Journal of Horticultural Science*
M.A. Mayo	Editor (plant viruses)	*Journal of General Virology*
J.W. McNicol	Editorial Board	*Annals of Applied Biology*
I.M. Morrison	Editorial Board	*Journal of the Science of Food and Agriculture*
	Series Editor	*Advances in Plant Cell Biochemistry and Biotechnology*
A.F. Murant	Editorial Board	*Intervirology*
	Editorial Board	*Virus Research*
	Editor	*AAB Descriptions of Plant Viruses*
I.M. Nevison	Editorial Board	*Journal of Nutrition*
D.A. Perry	Editorial Board	*Crop Research*
W. Powell	Editorial Board	*Heredity*
	Editorial Board	*Potato Research*
	Editorial Board	*Crop Research*
D. Robinson	Associate Editor	*Journal of Horticultural Science*
D.J. Robinson	Editorial Board	*Journal of Virological Methods*
	Editorial Board	*Journal of General Virology*
D.L. Trudgill	Editorial Board	*Revue de Nematologie*
	Consulting Editor	*Plant and Soil*
R.L. Wastie	Editorial Board	*Potato Research*
	Editorial Board	*Annals of Applied Biology*
B. Williamson	Associate Editor	*Annals of Applied Biology*
J.A.T. Woodford	Editorial Board	*Annals of Applied Biology*

Awards and Distinctions

Name	Dept.	Degree/Award/Distinction/Appointment
J.W.S. Brown	CMG	Honorary Lecturer, University of Dundee
D. Diduca	EM	Certificate in Gas Safety
G. Dunlop	CEP	'High Standard Award' from Meteorological Office
I. Fleming	EGF	NPTC Certificate of Competence:Safe use of pesticides and ground crop sprayer
B.A. Goodman	DG	Honorary Lecturer, University of Dundee
J. Gorrod	DP	HNC Computer Studies
J.P.T. Grant	EGF	NPTC Certificate of Competence:Safe use of pesticides and ground crop sprayer
B.D. Harrison	Vir	Honorary Member, Society for General Microbiology; Honorary Doctorate of Agriculture and Forestry, University of Helsinki
D. Hutcheson	EM	Certificate in Gas Safety
N.L. Innes	Admin	Vice-President, Association of Applied Biologists
R.J. Killick	Admin	MBA (with distinction), University of Dundee
G.D. Lyon	M&B	Honorary Lecturer, University of Dundee
R. MacDonald	EM	Certificate in Gas Safety
G.R. Mackay	CG	Fellowship of Institute of Biology
J. Mason	EGF	NPTC Certificate of Competence: Safe use of pesticides and ground crop sprayer
K.J. Oparka	CEP	Peter Massalski Prize for meritorious research.
W. Powell	CMG	Broekhuizen Prize 1990; Honorary Lecturer, University of Dundee; Honorary Senior Lecturer, University of St Andrews
R. Pugh	EM	Certificate in Gas Safety
T. Reglinski	M&B	Ph.D. University of Dundee
A.D. Turnbull-Ross	Vir	PhD. University of Leicester
R. Waugh	CMG	Honorary Lecturer, University of Dundee
B. Williamson	M&B	Honorary Lecturer University of Dundee

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]

PU 01 Develop enhanced germplasm in potato and more effective methods of genetic manipulation and breeding

(a) Genetic architecture of potatoes and production of enhanced germplasm. [*Mackay G. R.*]
(b) Genetic architecture of traits of strategic importance to the UK seed potato industry. [*Mackay G. R.*]
(c) Genetics, exploration and exploitation of emergent techniques and conventional breeding/selection of potatoes. [*Mackay G. R.*]
(d) Develop new breeding material from primitive and novel germplasm. [*Mackay G. R.*]
(e) Develop and use methods for testing segregating potato populations for resistance to disease. [*Wastie R. L.*]
(f) Develop and use screening tests for biochemical compounds in potatoes. [*Allison M. J.*]
(g) The biochemical characteristics of good crisping quality in *Solanum tuberosum.* [IFS]. [*Duffus C.*]
(h) Maintain and evaluate the Commonwealth Potato Collection. [*Wilkinson M.J.*]
(i) The biochemical characteristics of good crisping quality in *Solanum tuberosum.* [*Mackay G. R.*]
(j) Incorporation of Birmingham Potato collection into the Commonwealth Potato Collection. [*Wilkinson M. J.*]
(k) Correlating glasshouse and field performance of true (botanic) potato seed populations. [*Clulow S. A.*]
(l) Development and evaluation of methods for specific applications of high-technology instrumentation for the SCRI research programme. [*Allison M. J.*]

PU 03 Develop enhanced germplasm in soft fruit and more effective means of genetic and vegetative manipulation

(a) Produce improved germplasm of raspberry and study relevant characters. [*McNicol R. J.*]
(b) Provide improved germplasm of blackcurrant and study relevant characters. [*Brennan R. M.*]
(c) Provide improved germplasm of blackberries and other *Rubus* fruits. [*McNicol R. J.*]
(d) Identify and select strawberry genotypes adapted to the Scottish environment and study genetics of factors involved. [*McNicol R. J.*]
(e) To evaluate genotypes and extend the genetic base available in novel fruit genera. [*Cormack M. R.*]
(f) Develop methods of using *Agrobacterium* spp. as vectors for introducing DNA into soft fruit germplasm. [IFS] [*McNicol R. J.*]
(g) Morphological and genotypic factors in relation to mechanical harvesting of soft fruits. [*Cormack M. R.*]
(h) Evaluate genotypes of and design production methods for raspberry and other *Rubus.* [*Cormack M. R.*]
(i) To evaluate genotypes and extend the genetic base available in novel fruit genera. [HDC] [*Cormack M. R.*]
(j) Evaluate genotypes of and design production methods for raspberry and other *Rubus.* [HDC] [*Cormack M. R.*]
(k) Introduction of exogenous DNA into *Rubus, Ribes, Fragaria* and other soft fruit genera using *Agrobacterium tumefaciens.* [*McNicol R. J.*]

PU 04 Develop more effective means of germplasm manipulation and produce enhanced germplasm of brassica crops

(f) Multiply and stabilise breeders' selections; trial selections in collaboration with other organisations. [*Mackay G. R.*]
(m) Improve brassica root crop germplasms. [*Vacancy*]
(n) Improve leafy forage brassica germplasms. [*MacFarlane Smith W. H.*]
(o) Genetics and nature of resistance and susceptibility to root flies in brassicas. [*Birch A. N. E.*]
(p) Inheritance and gene expression in brassicas. [*Middlefell-Williams J.*]
(q) Study quantitative genetics in brassicas. [*MacFarlane Smith W. H.*]
(r) Reproductive biology of *Brassica* spp. [*MacFarlane Smith W. H.*]
(s) Content, variability and role of sulphur containing metabolites, oil and protein in plants and seeds of *Brassica* spp. [*MacFarlane Smith W. H.*]
(t) *In vitro* selection for herbicide resistance in *Brassica* spp. [IFS] [*Ramsay M.*]
(w) Multiply and stabilise breeders' selections; trial selections in collaboration with other organisations. [Nickerson/Dalgety] [*Mackay G. R.*]

PU 09 The biology and control of diseases and pests of soft fruit crops in Northern Britain

(a) Epidemiology and pathogenesis of fungal pathogens of soft fruit. [*Williamson B.*]
(b) Prediction and assessment of damage caused by pests of cane and bush fruits. [*Woodford J. A. T.*]
(c) Role of nematodes in planting disorders of raspberry. [*Trudgill D. L.*]
(d) Properties, relationships and resistance mechanisms to *Rubus* viruses. [*Jones A. T.*]

(e) Produce virus-free stocks, assess virus resistance and index British and imported *Rubus* genotypes. [*Jones A. T.*]
(f) Devise diagnostic tests for reversion and other *Ribes* viruses and produce virus-free stocks. [*Jones A. T.*]
(g) Determine the cause of, and devise diagnostic methods for, strawberry June yellows. [*Jones A. T.*]
(h) Study the nature and properties of reversion and other *Ribes* viruses. [*Jones A. T.*]
(j) Epidemiology and pathogenesis of fungal pathogens of soft fruit. [HDC] [*Williamson B.*]

PU 11 Characterisation, effects and control of viruses of ornamentals

(a) Determine properties, relationships and detection of previously undescribed viruses from narcissus. [*Mowat W. P.*]
(b) Maintain virus-tested clones of narcissus and determine their health. [*Mowat W. P.*]
(c) Determine basis of effects of viruses on flower pigmentation. [*Mowat W. P.*]

PU 12 The biology and properties of non-indigenous plant viruses

(a) Characterise whitefly-transmitted viruses from cassava and other tropical crops.[ODA] [*Swanson M. M.*]
(b) Epidemiology and assay methods for groundnut rosette and groundnut rosette assistor viruses.[ODA] [*Murant A. F.*]
(c) Detection and properties of West African whitefly-transmitted geminiviruses.[EEC] [*Harrison B. D.*]

PU 19 The cellular and molecular basis of crop improvement

(a) Development of stable, single cell isolation and regeneration systems. [*Millam S.*]
(b) Exploitation of protoplasts and microspore systems in crop improvements. [*Millam S.*]
(d) Introduction of foreign genes into plants (genetic transformation). [*Kumar A.*]
(g) Genome organisation and structure at the nucleic acid level. [*Waugh R.*]
(h) Construction of detailed genetic linkage maps using molecular (RFLPs) and isoenzyme markers. [*Waugh R.*]
(j) Develop and utilise suitable aneuploid stocks for use in genetic linkage studies. [*Forster B. P.*]
(l) Gene isolation by insertional mutagenesis. [*Kumar A.*]
(n) Isolation, proliferation, and regeneration of plants from potato protoplasts. [IFS] [*Millam S.*]
(o) Transposon mutagenesis - a strategy for the isolation and cloning of important genes in potato. [IFS] [*Kumar A.*]
(p) UsnRNA-based transformation vectors for the delivery of antisense RNAs to plant cell nuclei. [IFS] [*Waugh R.*]

PU 20 Statistical and mathematical support for agricultural, environmental and food R&D

(a) Training scientists in statistics and use of statistical software. [*Glasbey C. A.*]
(b) Development and application of new statistical methods. [*Glasbey C. A.*]
(c) Statistical computing. [*Talbot M.*]
(d) Statistical research and consultancy for HRI. [*Bradbury I.*]
(e) Statistical research and consultancy for MLURI. [*Buckland S. T.*]
(f) Statistical research and consultancy for MRI. [*Hunter E. A.*]
(g) Statistical research and consultancy for SCRI. [*McNicol J. W.*]
(h) Statistical research and consultancy for RRI. [*Franklin M. F.*]
(i) Statistical research and consultancy for SAC. [*Hunter E. A.*]
(j) Statistical research and consultancy for DAFS Agricultural Scientific Services. [*Talbot M.*]
(k) Statistical support for PVRO. [*Talbot M.*]
(l) Statistical computing. [EEC] [*Talbot M.*]
(m) Statistical support for PVRO. [MAFF] [*Talbot M.*]

PU 21 Plant fibres

(a) Physical and chemical characteristics of fibre-producing herbs, shrubs and trees. [*Morrison I. M.*]
(b) Control of differentiation and development of fibre cells. [*Morrison I. M.*]
(c) Factors influencing the ease of isolation of fibres. [*Morrison I. M.*]
(d) Determination of the composition and structure of plant fibre and fibre products. [*Goodman B.A.*]

PU 22 Control of root development, growth and function

(e) Physiological and environmental factors influencing root growth. [*Young I. M.*]
(f) Determine physiological and environmental factors influencing the induction and proliferation of roots. [*Robinson D.*]
(g) Mechanisms of drought tolerance and interactions between water and nutrient supply in potato. [*Bengough G.*]
(h) Nutritional effects on grain quality of barley. [*Marshall B.*]
(i) Nutrient availability and inflow. [*Linehan D. J.*]
(j) Identification and quantification of root exudates. [*Shepherd T.*]
(k) Nutritional effects on grain quality of barley. [ext.] [*Marshall B.*]
(l) Soil microbial processes influencing the supply of nitrogen to organic crops. [FF] [*Ritz K.*]

PU 23 Agriculture and the environment

(b) The role of the soil microbial biomass in plant nutrition, pesticide degradation and its interaction with root exudates. [*Ritz K.*]
(c) Prediction and monitoring of weed populations and weed management strategies in crops, uncropped areas and in rotations. [*Lawson H. M.*]
(d) Biology and population dynamics of plant-parasitic nematodes. [*Boag B.*]
(e) Determine the factors influencing the activity of fungi and bacteria which attack nematodes. [*Boag B.*]
(g) The biology and ecology of entomophilic nematodes. [*Boag B.*]
(h) Biology and ecology of pest and beneficial arthropods associated with cane and bush fruit plantations. [*Woodford J. A. T.*]
(i) Methods of risk assessment and control of risks of the release into the environment of plants with alien genes. [*Mackay G. R.*]
(j) Risk assessment: transgenic plants containing virus satellite nucleic acid. [*Mowat W. P.*]
(k) Research into the performance of disease resistant genotypes in pesticide-free farming systems. [*Mackay G. R.*]
(l) Prediction and monitoring of weed populations and weed management strategies in crops, uncropped areas and in rotations. [H-GCA] [*Lawson H. M.*]
(m) Mechanisms of uptake, transport and modes of action of xenobiotics. [*Oparka K. J.*]
(o) Endocytosis in higher plants: The potential for uptake and targeted transport of foreign molecules. [IFS] [*Oparka K. J.*]

PU 24 Strategic studies on pests and pathogens

(a) Mechanisms of host recognition, resistance and susceptibility to insects, mites and nematodes. [*Trudgill D. L.*]
(b) Effects of vector and host on feeding behaviour in relation to virus acquisition and transmission by aphids. [*Woodford J. A. T.*]
(c) Role of neuroactive and other compounds in the development of nematodes. [*Robertson W. M.*]
(g) Nature and function of nematode and plant pathogenesis related proteins. [IFS] [*Forrest J. M. S.*]
(h) Genetic control of pathogenesis and changes in physiological races of fungal and bacterial pathogens of plants. [*Duncan J. M.*]
(i) Identify and elucidate the effects of pre- and post-formed host and pathogen compounds on disease resistance. [*Lyon G. D.*]
(j) Host and pathogen interactions: factors determining latency and host resistance. [*Williamson B.*]
(k) Electrophoretic and molecular techniques to determine groupings within invertebrate pests. [*Trudgill D. L.*]
(l) Biochemical processes in parasite development. [*Goodman B. A.*]
(n) Molecular genetical analysis of pectic enzyme production of *Erwinia carotovora* as affected by temperature. [IFS] [*Pérombelon M. C. M.*]
(p) Nature and function of nematode salivary secretions. [IFS] [*Forrest J. M. S.*]
(q) Investigate the control of pests by naturally occurring compounds. [BTG] [*Robertson W.M.*]
(r) Identify and elucidate the effects of pre- and post-formed host and pathogen compounds on disease resistance. [PMB] [*Pérombelon M. C. M.*]
(s) Role of fimbriae in the pathogenicity of soft rot erwinias on potato plants (blackleg). [IFS] [*Pérombelon M. C. M.*]
(t) Development of a new crop protection system using yeast extracts. [H-GCA] [*Lyon G. D.*]
(u) Determine the interactions between saprophytic and pathogenic microbial populations in the soil and on plants. [*Perry D. A.*]
(v) Expression and durability of partial resistance to mildew. [*Newton A. C.*]

PU 25 Basic and strategic studies on plant viruses

(a) Mechanisms of virus transmission by aphids. [*Murant A. F.*]
(b) Genome organization of viruses and molecular aspects of their biological behaviour. [*Harrison B. D.*]
(c) Enhance virus resistance by transforming plants with virus- related nucleic acid. [*Harrison B. D.*]
(d) Structure and function of the genome RNA of potato leafroll luteovirus. [*Mayo M. A.*]
(e) Genome organization and properties of gene products of plant picornaviruses. [*Reavy B.*]
(f) Mechanism of virus transport and intercellular movement in plant tissue. [*Barker H.*]
(g) Monoclonal antibodies to identify and analyse important epitopes on virus proteins. [*Torrance L.*]
(h) Methods for electron microscopy of viruses and virus vectors. [*Roberts I. M.*]
(i) Methods for detection and study of virus-related proteins and nucleic acids. [*Mowat W. P.*]
(j) Structure and function of the genome of raspberry ringspot nepovirus. [*Mayo M. A.*]
(k) Molecular biology of potato mop-top virus. [*Torrance L.*]
(l) Mechanisms determining specificity and efficiency of nepovirus transmission by nematodes. [*Trudgill D. L.*]
(m) Transmissibility of tobraviruses by nematodes: specificity and inhibition. [*Robinson D. J.*]
(n) Structure and function of genome RNA of raspberry ringspot nepovirus. [IFS] [*Mayo M. A.*]
(o) Genome organization of plant picornaviruses. [IFS] [*Turnbull-Ross A.*]
(p) Mechanism of virus transport and intercellular movement in plant tissue. [IFS] [*Derrick P.M.*]
(r) Structural analysis of viral proteins. [*Goodman B. A.*]
(s) Transmissibility of tobraviruses by nematodes: specificity and inhibition. [ext.] [*Robinson D. J.*]
(t) Characterization of the particle protein of pepper ringspot virus by nuclear magnetic resonance spectroscopy. [IFS] [*Brierley K. M.*]
(u) Characterization of the particle protein of pepper ringspot virus by nuclear magnetic resonance spectroscopy. [*Goodman B. A.*]

PU 26 Plant and Crop Physiology

(a) Mathematical analysis of plant and crop processes. [*Marshall B.*]
(b) The use of Artificial Intelligence to model crop production systems. [IFS] [*Marshall B.*]
(c) Physiological and biochemical regulation of carbohydrate transport and metabolism. [*Davies H. V.*]
(e) Mechanisms regulating the initiation and differentiation of plant storage tissues. [*Davies H. V.*]
(j) Quantify the effects of environment on growth and development in crop plants. [*MacKerron D. K. L.*]
(k) Physiological and biochemical processes limiting growth, development and quality of grain legumes. [*Oparka K. J.*]
(m) *In vivo* characterisation of metabolic processes in plants by NMR spectroscopy. [*Goodman B. A.*]
(n) Mathematical analysis of plant and crop processes. [H-GCA, EEC, CIP] [*Marshall B.*]
(o) Physiological and biochemical regulation of carbohydrate transport and metabolism. [EEC] [*Davies H. V.*]
(p) Quantify the effects of environment on growth and development in crop plants. [ext.] [*MacKerron D. K. L.*]
(q) To determine the factors modifying the transcription of genes controlling carbohydrate metabolism. [FF] [*Davies H. V.*]
(r) Sensitivity analysis of crop performance with development to aid crop management in an altered climate. [FF] [*Crawford J. W.*]

PU 27 Diseases and pests of arable crops

(a) Biology and pathogenesis of bacterial pathogens of potatoes. [*Pérombelon M. C. M.*]
(b) Survival and distribution of *Phytophthora infestans*. [*Duncan J. M.*]
(c) Immunodiagnostics for fungal plant pathogens. [*Harrison J. G.*]
(d) Interactions between tolerance, resistance and potato cyst nematodes. [*Trudgill D. L.*]
(e) Genetic basis of virulence in potato cyst nematode and effects of selection. [*Phillips M. S.*]
(f) Epidemiology of potato leafroll virus. [*Woodford J. A. T.*]
(g) Mechanisms, effectiveness and inheritance of virus resistance in potato. [*Barker H.*]
(h) Determine properties, transmission by vectors and identification of potato viruses. [*Torrance L.*]
(k) Biology and pathogenesis of bacterial pathogens of potatoes. [ext.] [*Pérombelon M. C. M.*]

(l) Interactions between tolerance, resistance and potato cyst nematodes. [PMB] [*Trudgill D. L.*]
(m) Mechanisms, effectiveness and inheritance of virus resistance in potato. [Nickerson] [*Barker H.*]

PU 28 The control, expression and manipulation of genes and gene complexes in cereals and legumes

(a) Biometrical genetics of barley and faba beans. [*Thomas W. T. B.*]
(b) Physiological genetics of barley. [*Ellis R. P.*]
(c) Genetics of biochemical components of cereals and beans. [*Allison M. J.*]
(d) Utilize biochemical markers in genetic studies. [*Ellis R. P.*]
(e) Analysis of resistance conferred by novel genetic components of cereals to fungal diseases. [*Newton A. C.*]
(f) Develop and utilize rapid screening tests for quality assessment in cereals. [*Allison M. J.*]
(h) Tissue culture and transformation techniques. [*Ramsay G.*]
(i) Trial extension crops. [Dalgety] [*Ellis R. P.*]

PU 29 The control, expression and manipulation of genes and gene complexes in cereals and legumes

(a) Characterisation of barley composition in relation to malting quality. [H-GCA] [*Ellis R. P.*]

PU 30 Effect of wounding and storage conditions on potato tuber contamination by soft rot erwinias

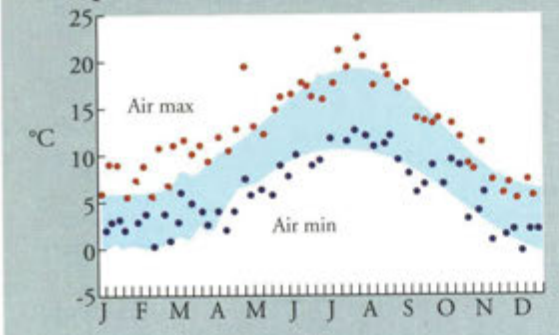
(a) Effect of wounding and storage conditions on potato tuber contamination by soft rot erwinias. [PMB] [*Pérombelon M. C. M.*]

Meteorological Records

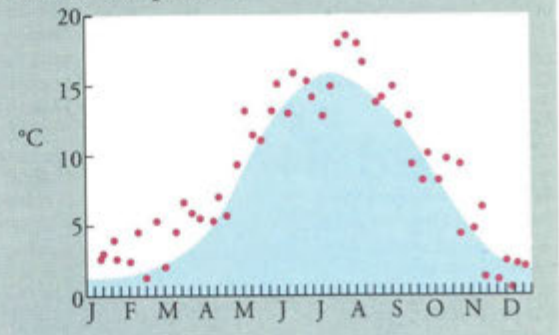
D.K.L. MacKerron

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

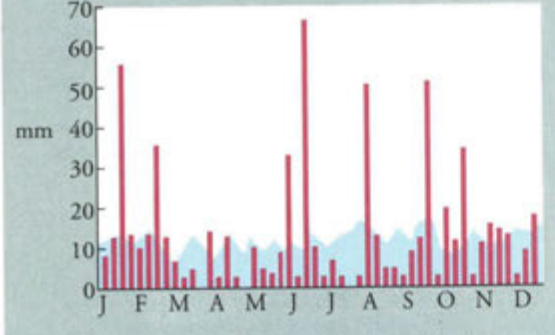
Air temperature



10cm soil temperature

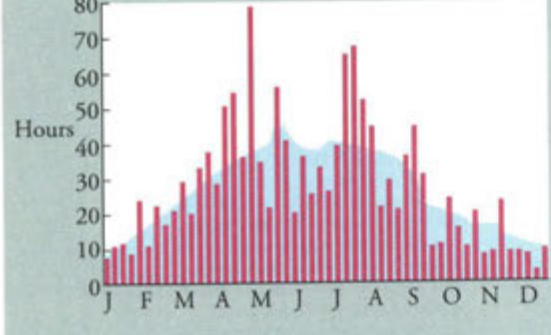


Rainfall



Soil moisture deficit

Sunshine



Run of wind

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
Hurley Research Station	Hurley, Maidenhead, Berkshire SL6 5LR	062-882-3631
North Wyke Research Station	Okehampton, Devon EX20 2SB	083-782-558
Welsh Plant Breeding Station	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	0970-828255
AFRC Institute of Engineering Research	Wrest Park, Silsoe, Bedford MK45 4HS	0525-60000
AFRC Institute of Food Research	Shinfield, Reading RG2 9AT	0734-883103
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	0273-678133
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

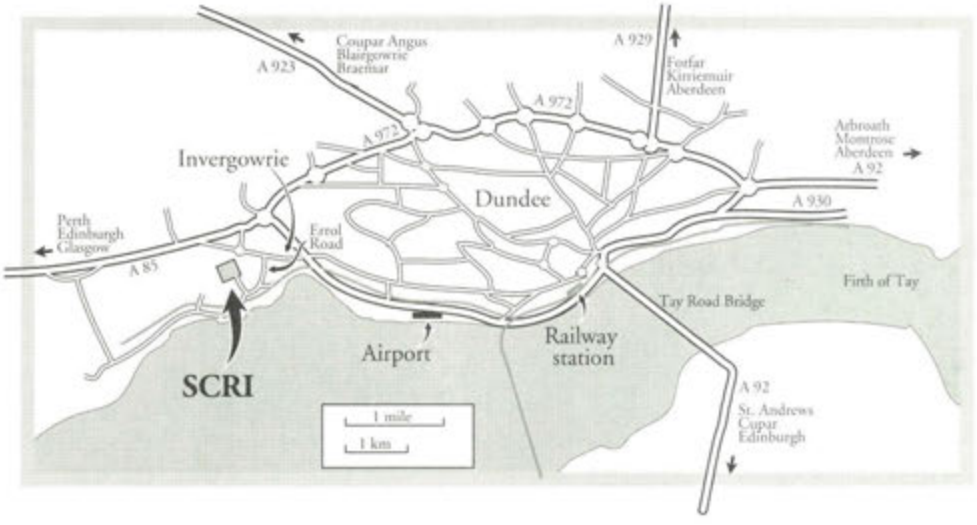
Scottish Agricultural Research Institutes

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 Crisp and Snack 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