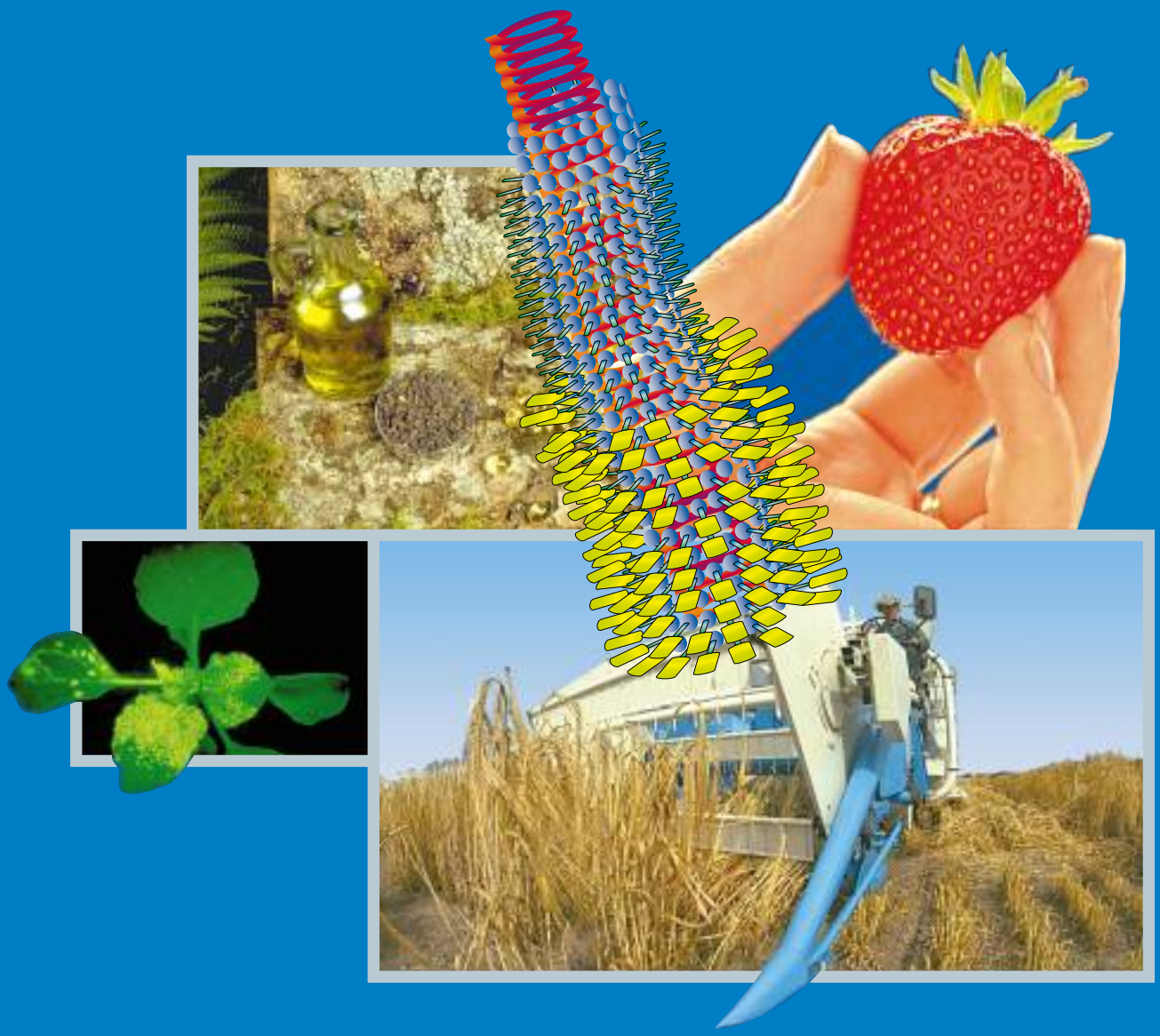
Scottish Crop Research Institute

Annual Report 1995



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

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

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

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

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Report of the Director

John R. Hillman

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

In a buoyant year of scientific discovery which marked the 50th anniversaries of the Yalta Conference, the end of World War II, and the formation of the United Nations (UN), the Allies celebrated the initiation of economically thriving democracies in Germany and Japan and international initiatives to promote global prosperity. Entertainment and the arts flourished. The nearly 360-year conjecture of Fermat's last theorem was finally concluded, but not Goldbach's Primes, The Kepler Problem, the Poincaré Conjecture and the Riemann Hypothesis!

The largest gathering of world leaders in history took place in New York in October to celebrate the 50th anniversary of the UN and to reaffirm the principles of the UN Charter that had taken effect on 24 October 1945. Reforms to the organisation were acknowledged but there was still overwhelming confidence in the ability of the UN to promote rather than enforce peace and social development around the world.

Economics

In 1995, the world economy was relatively robust, and the year was one of favourable economic performance, with a broad increase in the production of goods and services. According to International Monetary Fund (IMF) estimates, total output grew by

about 3.5%, largely similar to 1994. Focal points of conflict, however, impeded development and destabilised various economies. Problems continued in the former Yugoslavia, in impoverished areas of Africa - especially Rwanda, Burundi, Zaire, Somalia, Liberia and Sierra Leone - in Middle Eastern countries and in the republics of the former Soviet Union, especially Chechnya. Worrying disturbances occurred also in Mexico and Haiti. The causes of these conflicts related to ethnic, racial, religious, national and regional identities, stoked by poverty. Even stable democracies were not exempt from these influences, and fragmentation of countries remained a real issue.

The strong global economic performance disguised a pronounced slowdown in the more-developed countries (MDCs) as a whole. Here, economic expansion slowed to an estimated 2.5% in 1995 from 3.1% in 1994. Turbulence in the foreign exchange markets and pre-emptive rises in interest rates to suppress

inflation undoubtedly contributed to the downturn. The world's stock exchanges were characterised in 1995 by an accelerated rise following earlier stagnation. Derivatives contracts were subject to detailed reviews after a prominent UK merchant bank was made bankrupt by unauthorised transactions.

Once again, the economies of the less-developed countries (LDCs) grew faster than those of the MDCs, prompted by economic reforms, steady interest rates, export growth and an influx of capital funds. Total growth in LDCs reached 6%, a rate exceeding birthrate and sufficient to lead to a small gain in living standards. The fastest economic growth was experienced in China particularly, and Asia in general, but the short-lived financial crisis in Mexico, and to a lesser extent in Argentina, slowed growth in Latin America.

Exchange-rate instability early in 1995 had an initial negative impact, starting with the collapse of the Mexican peso and leading to the outflow of much-needed capital funds from many LDCs. Mexico accepted the terms imposed by the USA as a condition for receiving an aid package of $20 billion. The IMF and the Swiss-based Bank for International Settlements agreed to disburse additional substantial contributions to stabilise the Mexican peso and to prevent the country from defaulting on its debt. During the year, as economic growth in the MDCs slowed, central bankers in North America, Japan and Germany allowed short-term interest rates to fall.

In contrast to the relaxation in monetary policy, many governments of the MDCs continued with tough policies to reduce their budget deficits with the aim of attaining properly balanced budgets. Thus, 1995 was another year of tight public spending and tax reforms. In the European Union (EU), economic measures to reduce the public-sector deficit to 3% of gross domestic product (GDP) by 1999 were implemented rigorously, in order to meet the convergence criterion stipulated in the Maastricht Treaty.

Urban and rural employment growth was disappointing. Although there was a reduction in the average unemployment rate in countries belonging to the Organisation for Economic Co-operation and Development (OECD), to 7.9% in 1995 from 8.1% in 1994, this meant that at least 33.6 million people were seeking work in the OECD countries.

There were three factors which favoured the maintenance of high unemployment and instability in employment in North America and Europe. Firstly, competitive pressures arising from an opening up of international markets depressed wages and profitability. Secondly, in the modern economy and polity, inflation is deemed to be worse than unemployment. The unemployed can be viewed as a stabilising force against those wage demands which force up prices for goods and services. Thirdly, the modern welfare state has within its structure, features that are adverse to employment. The employer is faced with substantial direct and indirect non-productive costs in addition to wages. These costs can be lessened by not employing more staff, resorting to overtime, taking on temporary workers, deploying new labour-saving technologies, contracting out or demanning.

Inflationary pressures remained subdued in nearly all countries. With the exception of Turkey and Mexico, average inflation in OECD countries was about 2.5% in 1995 compared with 2.7% the year before. The median inflation rate in the LDCs declined from 11.5% in 1994 to 8% in 1995.

The rationale of controlling inflation is straightforward: low and stable inflation encourages investment and R&D, and should stimulate growth. There was debate in 1995 as to whether reducing already low inflation impedes economic activity creating, in turn, lacklustre growth prospects.

World trade remained upbeat during 1995, expanding to 8%, close to the rate of 1994. This strength was mainly due to increasing trade between the MDCs and recovery of trade in several of the former communist countries in Europe. With the exception of Africa, the IMF expected the debt burdens of the LDCs to remain manageable. Even though the overall debt level of the LDCs rose gently in absolute terms, it declined as a proportion of exported goods and services.

While the economic decline of the former centrally planned economies persisted for the fifth consecutive year, the rate of decline fell sharply to 2%, and was uneven across the region. Those economies that were most advanced in their structural reforms to embrace global trade, conventional payment systems and privatisation, *viz*. the Czech Republic, Hungary, Poland, Slovakia, Slovenia and Albania, produced impressive trade performances and growth rates in the 4-6% range. Where reforms were less advanced, as in the Transcaucasus and Central Asia, inflation was high and economic decline evident. In an attempt to help

the Ukraine bring about economic reform, Russia rescheduled 50% of the $4.4 billion it was owed. In its three years of independence, Ukraine accumulated $7 billion of foreign debts and could no longer operate most of its factories following the termination of gas supplies from Turkmenistan for non-payment of bills.

By the end of 1995, 10 Central and Eastern European countries (CEECs) had signed association agreements with the EU. These were Bulgaria, Estonia, the Czech Republic, Hungary, Latvia, Lithuania, Poland, Romania, Slovakia and Slovenia. More than half of their trade was with the EU, but early accession to the union was not expected, given the potential impact on EU industries if the market place was opened completely. With regard to agriculture, the EU Common Agricultural Policy is unlikely to be dismantled rapidly and the massive dependency of the CEEC work force on agriculture would pose insuperable difficulties for both parties.

The European Parliament, the legislative branch of the EU, approved a customs pact with Turkey. By adopting many of the regulations governing trade within the EU, Turkey would be allowed to participate in the EU market as an outsider.

For the EU, 1995 was a year of introspection, growing cynicism and internal debate over future development. The year began with the formal accession of Austria, Finland and Sweden, bringing the number of EU states to 15. By the end of the year there were disagreements over the velocity and direction of EU development. The declared objective of full monetary union and a single currency by 1999, highlighted the difficulties facing a majority of members in fulfilling the economic conditions necessary for participating in the single currency, as stated in a protocol to the 1992 Maastricht Treaty on European Union. There was broad agreement on a technical strategy to introduce the single currency, the Euro, in stages after January 1999. Prior to a 1996 special conference to review the Maastricht Treaty which laid down the shape of the EU's decision-making institutions and processes, a "reflection group" was set up to seek consensus and prepare for the intergovernmental conference. The UK Government steadfastly resisted moves to closer political union, including qualified majority voting for almost all areas of policy including foreign and defence policies, immigration, justice, and police cooperation, and also resisted an enhanced law-making rôle for the EU Council of Ministers and the directly elected European Parliament. In addition to preparations for the forthcoming intergovernmental conference, the attention of the EU was concentrated on the war in Bosnia and Herzegovina, high unemployment and the declining competitiveness of many of the EU national economies.

At the end of the year, the four-year civil war in Bosnia and Herzegovina officially came to an end when the presidents of Bosnia and Herzegovina, Croatia and Serbia signed a peace agreement, triggering the deployment of 60,000 North Atlantic Treaty Organisation troops to maintain peace.

Acting on behalf of Spain, the EU settled a bitter six-week fishing dispute with Canada over fishing rights in international waters off Newfoundland. Both sides agreed to observe in future, the quotas assigned to each country by the Northwest Atlantic Fisheries Organization.

Chile and Bolivia approved plans to seek membership of the Southern Core Common Market (Mercosur), joining Argentina, Brazil, Paraguay and Uruguay in a trade organisation that largely eliminated tariffs from goods traded within the market.

Representatives of the 18 economic powers that comprised the Asia-Pacific Economic Co-operation (APEC) organisation, met in Japan to sign a declaration outlining general principles for achieving free trade among themselves by the year 2000. The APEC framework document, drafted by Japan, contained no sanctions because participation was voluntary and each state was allowed to work out its own policies for attaining APEC's ultimate goals. MDCs were to strive to reach these goals by the year 2010. An important development at the meeting was the agreement by China to cut its import tariffs so that it could join the World Trade Organization (WTO). To the disappointment of Australia and the USA, there was little progress otherwise on more liberalisation in agricultural products. The East Asian countries of China, Japan, South Korea and Taiwan voted to move cautiously and protect their domestic markets. Together, the members of APEC represented more than one third of the world's population, and accounted for more than 50% of the world's economic production and 40% of world trade. The two most affluent members of APEC were Japan and the USA.

In 1995, Commonwealth membership rose to 53 countries with the admittance of Cameroon, and most interestingly Mozambique, the former Portugese

colony with no formal historic connection with the old British Empire. Nigeria had its membership suspended because of its abuse of human rights.

The World Trade Organisation (WTO) became effective in January 1995 after the protracted six-year Uruguay Round of negotiations by the member states of the General Agreement on Tariffs and Trade (GATT). In essence, the WTO was established to oversee the fair implementation of agreed rules governing international trade. In view of the fact that most countries, and trading blocs such as the EU, had erected complex market-distorting barriers to external trade in agricultural and horticultural produce - mainly to protect farmers, rural economic infrastructure and to safeguard food security - the overall result has been to foster an inefficient, uncompetitive global agricultural system. In contrast, manufactured goods have been exposed to greater competitive pressures. Wars, conflicts, market collapses, unemployment and unfair trade practices in an unstable world, however, can rapidly alter perceptions of market protection mechanisms.

At this juncture, a set of five principles has been applied to international agricultural trade: (i) sanitary and phytosanitary regulations to be based on science rather than prejudice or unjustified discriminatory treatment; (ii) non-tariff barriers to trade to be converted to equivalent tariffs, with all tariffs to be reduced by at least 36% over six years; (iii) export subsidies to be cut by at least 36% and the volume of subsidised exports to be reduced by at least 21% over six years; (iv) all member nations must allow entry of duty-free agricultural imports of at least 3-5% of domestic consumption; and (v) all subsidies to domestic producers of traded products would be reduced by at least 20% over six years.

Populations

Estimates prepared by the Population Reference Bureau indicated that by mid-1995, the world's population was 5,702 million, representing an increase of 88 million over 1994. The annual rate of population increase declined to about 1.54% in 1995 from 1.6% in 1994, a result of birthrate declines in both LDCs and MDCs. If the 1995 growth rate were sustained, the world's population would double within 45 years. More than 85% of the population growth in MDCs occurred in the USA. In 1995, 32% of the world's population was below the age of 15, but the figure was 38% in LDCs outside China. Only 5% of the population in LDCs was over the age of 65 compared with 13% in MDCs. Urbanisation trends continued, with 43% of the total population living in urban areas; in LDCs 35% of their population was classified as urban compared with 74% in MDCs. The share of world population growth occurring in LDCs increased to 98% in 1995, and child-bearing females of LDCs were averaging about 3.5 children each during their lifetimes, a figure slightly more than double that of MDCs.

Africa's population stood at 720 million and the growth rate was the fastest in the world; Latin America grew to 481 million and Asia to 3.5 billion. Europe recorded its first negative rate of natural increase in modern history, -0.1%, largely the result of steeply declining birth rates in the European republics of the former Soviet Union. Italy had the lowest total fertility rate (average number of children produced by a woman in her lifetime assuming that the rate of childbearing in a given year remains constant) of 1.21 compared with world's highest of 6.2 in sub-Sahara Africa.

Against a background of governments throughout the world attempting to reduce the numbers of arrivals of illegal immigrants and asylum-seekers, the worldwide refugee population decreased to approximately 14.5 million by early 1995. Nonetheless, the total number of persons of concern to the office of the United Nations High Commissioner for Refugees (UNHCR) had risen to 27.4 million, a figure excluding 2.8 million Palestinian refugees living under the mandate of the UN Relief and Works Agency for Palestine Refugees in the Near East, and also omitting the estimated 26 million other displaced persons. In April, thousands of Hutu in the Kibeho refugee camp in southwestern Rwanda were slaughtered.

Officials in Guinea reported in January 1995 that more than 30,000 refugees had entered their country from neighbouring Sierra Leone. According to the UN World Food Programme, nearly one fifth of the population of Sierra Leone's 4.6 million population had been forced to flee their homes. Early in 1995, there was a feeling of optimism that the civil strife in Angola was coming to an end, as the UN Security Council voted to dispatch 7000 peace-keeping troops to the area. The conflict there has claimed about 500,000 lives since Angola won independence from Portugal in 1975.

Food-Aid

A study of the food-aid needs of more than 60 LDCs by the Economic Research Service of the USDA, indicated that economically poor countries would need

about 14 million tonnes of food-aid during the 1995-1996 marketing year, an increase of 12% from the previous year. FAO independently estimated that globally more than 36 million people faced severe food shortages, with 23 million of these people living in sub-Saharan Africa. Throughout the world, in both urban and rural areas, many more faced the insecurity of chronically scarce and uncertain food supplies. With the obvious exceptions of war-torn Bosnia and Herzegovina, and some countries of the former Soviet Union, the bulk of food-aid needs were in parts of Africa, southern Asia and parts of Latin America.

Food emergencies in Africa included the aftermath of severe drought in southern Africa; Mozambique, Zambia and Zimbabwe experienced the greatest needs. Civil strife hampered access to food-aid in Mozambique and Angola too. Grotesque conflicts in Rwanda and Burundi generated food-aid emergencies which extended to the refugee camps in neighbouring Zaire and Tanzania. As a result of civil war, large numbers of the populations of Sierra Leone and Liberia required aid, as did over one million people in the Sudan. Poverty, coupled to limited potential to produce adequate food supplies, again caused chronic food shortages in Ethiopia.

Elsewhere, Afghanistan and Bangladesh were the main recipients of the food-aid directed towards Asia, although floods in North Korea were thought to herald severe shortages of food in that country. Food shortages were reported in Central Asia and Transcaucasia, essentially as a result of poor harvests, unrest and disruption of the normal channels for distribution. Food-aid was also required in Armenia, Azerbaijan, Georgia, Kyrgyzstan, and the troubled Russian republic of Chechnya. Chronic food shortages persisted in Bolivia, Guatemala, Honduras and Peru. As always, Haiti suffered widespread poverty and poor crop production.

The UN Food and Agriculture Organization (FAO) Food Outlook report for 1995 estimates that during 1995-1996, the total shipment of food-aid in the form of cereals was 7.6 million metric tons (mmt, or million tonnes), declining from 8.4 mmt in 1994 - 1995, and from 12.6 mmt in 1993 - 1994. Much of the decline during this period was attributable to reduced releases from the USA. Further analysis shows that not all food-aid shipments were directed to the countries most in need: 10% less food-aid in 1994-1995 was received by the officially classified low-income (average annual income below $1345 in 1993), food-deficit countries. To solve poverty is to solve the problem of feeding people.

Agriculture and food supplies

In the Bulletin of Statistics of the FAO, total agricultural production and food production were static in 1995, whereas *per capita* food production declined slightly.

Grains In December 1995, the US Department of Agriculture (USDA) predicted that the world supply of grain at the end of the 1995-1996 marketing year (i.e. the year-end or carry-over grain stocks) would fall from 297 mmt to about 229 mmt in 1994-1995, a fall of 23% and down 37% from 1992-1993. The term 'grain' encompasses wheat, rice, and coarse grains such as maize, barley, sorghum and oats. Worryingly, the present year-end stocks represent just 13.1% of annual world consumption, down from 16.9% in 1994-1995, and as such is a record low and a percentage less than that available during the world grain crisis of the early 1970s, when international politics were more confrontational and the global economy was much weaker than now. Estimates by the FAO indicate that grain production worldwide would need to increase by at least 4% to ensure a minimal level of food security in 1996. Although grain prices increased sharply during the trading year, world grain consumption declined from 1761 mmt to 1747 mmt. Grain use in 1995 was calculated to be 305 kg per person per annum, representing a drop of 2.6% over the previous year and a decline of 8% from the peak in 1986. The decline in grain use reflected the drop in grain production concomitant with a rise in population. Moreover, meat production was more efficient and there were effects of economic restructuring in the former Soviet Union leading to a 10% decline there in demand for meat, milk products and human food based on cereals. Nearly 40% of world grain is fed to livestock (cattle, pigs, poultry, sheep, horses and goats), and over a period of nine years when the quantity of grain fed to livestock had not increased, meat production increased by 22%. This increased efficiency most probably reveals the impacts of improved breeds and management systems, as well as a pronounced transfer from cattle production to poultry and pigs.

China shifted from being a major grain exporter in recent years to being a major importer. Rapid economic growth of 8-9% in 1994 on the back of 50% growth over the previous four years led to increasing personal income despite an annual population growth of 14 million. China and drought-stricken Morocco

were expected to increase markedly their grain imports. In Japan, there was declining livestock production and consequently grain imports were down, but meat imports increased.

Wheat production increased from 522 mmt in 1994-1995 to 533 mmt, coarse grains declined from 863 mmt to 787 mmt, and rice also declined marginally from 361 mmt to 359 mmt.

Oilseeds In November 1995, the USDA Foreign Agricultural Service estimated that global oilseed production declined from the record crop of 259.4 mmt in the trading year 1994-1995 to 253.2 mmt in 1995-1996. Most of the decline was accounted for by the pronounced reduction in soybean production down from 136.7 mmt in 1994-1995 to 124.5 mmt over the same period in 1995-1996. Groundnut production also decreased from 26.4 mmt to 25.5 mmt, and copra 5 mmt to 4.8 mmt, contrasting with rapeseed which continued the long-term increase in production to 33.6 mmt over the year. Sunflower seed production also increased, from 23.7 mmt to 25.4 mmt, as did cottonseed, up from 32.9 mmt to 34.6 mmt. Palm kernal production increased marginally, 4.6 mmt to 4.8 mmt. As a consequence of lower production but higher consumption, year-end stocks of oilseeds were forecast to decline by about 20% from the previous year.

Livestock, Meat and Dairy World meat production expanded more rapidly than population growth in 1995, especially in the LDCs where FAO estimated that meat consumption per person would be 4% higher than in 1994. Nevertheless, contractions in meat consumption also occurred in much of Africa and in the Middle East as well as in the former republics of the Soviet Union. Australia began to rebuild sheep numbers following the devastating effects of prolonged drought and continued to switch from grass-fed to grain-fed cattle. Early in 1995, the Australian Cotton Foundation announced that the nation's cotton industry would suspend the use of chlorfluazuron (CFZ) until further notice, following the banned importation of Australian beef in 1994 by the USA and Japan after learning that some cattle had been fed cotton meal contaminated with CFZ. According to both the FAO and the USDA Foreign Agricultural Service, milk production (fresh weight) continued to decline slowly in the MDCs, down from 345 mmt in 1994 to 342 mmt in 1995 but slight rises were noted in North America and Oceania. An increase from 180 mmt in the previous year to 184 mmt in 1995 was recorded in the LDCs. As a result of enhanced demand by importing nations, world prices of dairy products such as cheese, butter and non-fat dry milk generally increased.

Sugar Forecasts by the USDA Foreign Agricultural Service point to a new record of 118 mmt world centrifugal sugar production in the crop year 1995-1996, and although total consumption rose to 116.6 mmt from 114.5 mmt in the previous crop year, production was expected to exceed consumption for the second successive year. Accordingly, rebuilding of world stocks took place from the record low levels at the beginning of the 1994-1995 crop year. Analysis of the data reveals that most of the growth in consumption took place in Asia, the Middle East and Latin America. Consumption was static in the MDCs as a result of the trend towards the use of alternative sweeteners such as high-fructose maize syrup and low-calorie sweeteners.

Coffee A decline in total world production of green coffee was projected by the USDA Foreign Agricultural Service following poor weather conditions in Brazil. Total production was estimated to be 88.2 million 60-kg bags, down from the outturn of 96 million 60-kg bags in 1994.

Cocoa For the 1995-1996 crop year, world cocoa bean production was expected to increase from 2.49 mmt in the previous year to 2.6 mmt, a new record. Both Côte d'Ivoire and Ghana registered record harvests in 1994-1995 as trees reached peak performance in good growing conditions and under improved management. As the world's major source of cocoa in 1994-1995, Côte d'Ivoire was forecast to produce another record-harvest. Brazil and Malaysia forecast declines in production.

Cotton A rise in world cotton production from 85.5 million 480lb bales in 1994-1995 to 89.3 million 480-lb bales in 1995-1996 was projected in November 1995 by the USDA Foreign Agricultural Service. Flooding, heavy rain, pests and diseases (most notably whitefly-transmitted cotton leafcurl geminivirus) caused a disaster for cotton growers in Pakistan. Falls in production also occurred in southern Africa, particularly Tanzania and Zimbabwe. Cotton growing was revitalised in Peru and Uzbekistan, the latter receiving a World Bank credit to this end. As projected consumption of 86 million 480-lb bales (circa 19 mmt) was less than production, year-end stocks probably accumulated.

Rubber The International Rubber Agreement, a pact sponsored by the UN to stabilise the prices of natural rubber and to encourage continued cultivation, was renegotiated during 1995 but was not ratified. Neither producers nor suppliers could agree on the efficacy of the Agreement.

Tobacco Notwithstanding adverse publicity in most MDCs, world manufacture and consumption of tobacco products were not unduly influenced in 1995. World production of raw tobacco declined from the 1994 figure to a level of 6.4 mmt because of large year-end stocks. Milder tobacco brands expanded at the expense of the darker, pungent and more socially offensive tobaccos. Significantly, in 1995, the US Food and Drug Administration (FDA) began the process of classifying nicotine as an addictive drug, eventually permitting the FDA to assert justification over the sale of tobacco products and providing a legal fulcrum for afflicted smokers.

Wood, Paper and Pulp

Analysis of prices reveals that the global wood supply was under pressure in 1995. Producers harvested smaller and younger trees than hitherto, and products based on wood residues were to the fore. Lumber mills in the western USA were forced to close by environmental restrictions, reducing the total number by 9% to 383. US, European and especially the Asian markets focused on South America and Russia for forestry resources. Brazilian softwood log exports attained 780,000 cubic metres in 1995, and Chilean forestry exports were anticipated to grow by 50%. Data on exports from Russia were lacking.

Whilst the International Standards Organization was engaged in developing international certification criteria for timber and products from sustainable forests, numerous certification initiatives such as "eco-labelling" were launched by organisations and individual companies in the USA and EU. Without the spur of legislation, it was unclear as to whether consumers would pay more for certified wood products. Related to this theme were developments in the regulation of international trade in forest products. Bilateral disagreements between the USA and Canada over Canadian exports of softwood lumber were resolved during the year. Membership of the EU enabled Finland and Sweden, the largest exporters of wood products in Europe, to bring expertise and to have voting rights in deciding future policies on the trade in wood and wood products within the EU countries. These Nordic countries would also assist in formulating policies to meet northern-temperate zonal needs in general within Europe.

The wood-panel industry increased production and capacity, predicated to some extent on demands from the furniture industry in Asia. Natural disasters, such as the Kobe and Sakhalin Island earthquakes in 1995, were expected to raise demands for prefabricated dwellings using structural laminated timbers and products which are resilient to earthquake damage.

At this juncture, it would appear that all sectors of the world marketplace in paper and pulp, from newsprint to recycled fibre, witnessed price increases in 1995. In 1994, the last year for which complete data were available, world pulp, paper and board production increased by 6.7% over 1993 values to 268.5 mmt, much of the increase taking place in Asia. Pulp production rose by 5.4% to 171.5 mmt in 1994 but the share of pulp employed in papermaking declined somewhat. The trend towards the use of recycled fibre in de-inked pulp was constrained by a shortfall in supplies. By far the biggest impediment to expansion of the pulping and cellulose industries was the shortage of cheap high-quality wood fibre, forcing the industries to examine low-fibre and "tree-free" paper and alternative fibre crops.

Food processing and retailing

Investments in food processing and retailing were attracted to the emerging markets of China, India, South Korea, Central and South America, and South Africa. In the MDCs, there was a slight fall in the sales of "low-fat" and "diet" foods, compensated by increases in the sales of vegetarian products and more traditional products including fatty foods. Perhaps as a result of relatively lax advertising laws in Japan, sales of so-called "health-promoting" functional foods prospered. Incidences of food poisoning increased regardless of the extensive nutritional and preparation instructions included on labels. Several of the major European food businesses expanded into global operators, and EU proposals to permit the use of vegetable fats to replace cocoa butter in chocolate manufacture prompted complaints from cocoa bean producer nations in Africa, South America, Indonesia and Malaysia.

Own-label or private-label products increased their market share globally, but the large international food-processing companies differed in their approach to the supply of own-label products to the major retailer chains.

New food products launches were fewer than in 1994. Various lactic fermented products were marketed explicitly as beneficial to human health; low-fat vegetarian products emerged at a slower rate than in recent years.

Use of the sweeteners acesulfame K, aspartame, cyclamate, neohesperidin DC, saccharin and thaumatin were permitted in the EU Sweeteners Directive effective from the end of 1995, but acceptable daily intake limits were prescribed.

Craft brewing of beers was a worldwide feature for both large and small brewing companies in 1995, but in the US and Europe the total production volume of beer was barely changed. For the distilled spirits industry, only the Asian market expanded rapidly, but in an attempt to overcome a staid image, marketing was directed towards the 20-35 year age group. New blended spirit products, especially those with fruit flavours and spices, came to market. A somewhat mixed year was reported for wine producers. Australian, Californian, New Zealand, South African and South American wines grew in stature in terms of export volumes and reputation, and the wine industry in some of the republics of the former Soviet Union made progress in adapting to export markets. In Europe, Italy experienced poor weather conditions for vine cultivation, and a dry hot summer followed by heavy rain troubled producers in France and Germany.

Fruit-juice and soft-drinks processing units expanded rapidly throughout 1995. Numerous new products coupled with intensive marketing campaigns were pronounced features of the soft-drinks industry. The market grew by about 2% over 1994 when North America accounted for 46% of soft drinks consumption, compared with 31% in Western Europe and 18% in Asia.

Environment

Floods and drought had enormous economic, social and environmental impacts in 1995. Flood management policies were under review in North America, northwestern Europe, Morocco, Egypt and North Korea. Sections of Belgium, France, Germany and the Netherlands were placed in a state of emergency following January floods. Drought lingered in the Caribbean, northern Mexico and northeastern USA. Large-scale dam-building projects in China and India were severely criticised on environmental and social grounds.

One trend of relevance to the life sciences was the introduction of new technologies for the mining industry to address environmental concerns. Bio-oxidation processes, bio-recovery and bio-remediation collectively represent key areas of high-priority scientific and industrial endeavour.

In September, the Intergovernmental Panel on Climate Change (IPCC) posted a draft of its report on the Internet (World Wide Web), which concluded that the observed increase in global mean temperature of 0.3°-0.6° C was unlikely to be solely due to natural causes. In fact, the threat of global warming dominated environmental concerns, validating the prescience of the SCRI report "Global Warming: The Implications for Agriculture and Priorities for Research", released in 1989.

Delegates from the 166 countries that signed the 1992 UN Framework Convention on Climate Change in Rio de Janerio held the so-called "Conference of the Parties" in Berlin in March 1995. From this meeting came the Berlin Mandate which acknowledged that the target agreed at the Rio Summit of returning carbon dioxide emissions to their 1990 levels in the MDCs by the year 2000 was inadequate and that further reductions post-2000 would be necessary. A permanent secretariat was to be established in Bonn together with a negotiating group representing the major power blocs. The IPCC would remain the principal advisory body.

Further discussions were held on the substances covered by the Basel Convention on international trade in hazardous wastes, deflecting attempts to prevent an extension of the numbers of banned substances. By September, the 89 signatory countries to the Convention agreed to an extension which forbids the 25 OECD members from transporting wastes to non-OECD members for recycling after 1997. During the year, the major lead-producing countries of Australia and Canada blocked an agreement in the OECD to reduce the amount of lead in the environment, encouraging instead a "voluntary action plan".

A downside to the reduction of industrial emissions of sulfur dioxide was reported in 1995. Sulfur depositions fell by 80% from the late 1970s to 1995. Vegetation across a swathe of Europe may be showing signs of sulfur deficiency, with crop diseases increasing and crop yields falling; oilseed rape and other brassicas were the worst affected.

The first comprehensive UN report on global biodiversity, released in November, estimated that there were about 15 million plant and animal species, of which only 1.75 million had been formally identified.

Environmentally-friendly, alternative energy systems suffered limited market demand and research funding in 1995. Crude conventional market analyses of production costs restricted any large-scale developments to remote locations where alternative energy sources had a competitive advantage. Current predictions point to some of these sources reaching competitive parity in 20 years with oil priced at $20 a barrel.

Soil erosion, encroachment on natural ecosystems, pollution, overfishing, loss of biodiversity, excessive demands on forests, modified atmospheric composition leading to climate change, fresh-water use exceeding the sustainable yield of aquifers and shared river systems, and the irresponsible wholesale ignorance of sustainability remained bleak symptoms of mankind's activities on the planet. Crop and food production were universally assumed to benefit from ongoing improved efficiency and yield, emphasising the urgency to maintain investments in research and development.

United Kingdom perspectives

In the United Kingdom, the pace of economic growth slowed in 1995 to an annual rate of 2.5%, down from 4% in 1994, under the impact of higher taxes and interest rates introduced in 1994, and combined with a slackening demand for exports by its major trading partners. This rapid slowdown in economic activity, coupled with subdued wage and price inflationary pressures, led to lower interest rates before the end of the year. The public-sector deficit for 1995-1996 was some £6 billion higher than the revised target of £23.5 billion, largely attributable to lower taxation revenues. A cautious, tax-cutting budget was announced in November, the reduction in taxation counterbalanced by reductions in public spending. During the year, the unemployment rate declined to 8.1% compared with an EU average of 11.4%.

The rate of return achieved by UK industrial and commercial companies (ICCs, both quoted and unquoted but excluding pharmaceutical companies) declined in 1995 to 9%, contrasting with 9.5% in 1994, according to the Office for National Statistics. To what extent future trends might be influenced by the incorporation of innovation in companies is not wholly clear. The 1996 UK R&D Scoreboard published by the Department of Trade and Industry, noted that R&D investment by companies in the UK rose by only 4.2% in 1995, compared with increases of 6% and 7.5% in the two previous years. Since sales increased by 9% and profits by 18% (i.e. there was a decline in R&D intensity) the results were disappointing, especially in a year when public-sector R&D funding declined. The pharmaceutical industry accounted for nearly one third of the R&D undertaken in the UK.

The R&D intensity of companies based in the UK was less than half that of companies in competing countries, with the exception of Italy and Norway. Of the sectors relevant to SCRI (alcoholic beverages; breweries; chemicals; diversified industrials; extractive industries; food manufacturers; health care; paper, packaging and printing; pharmaceuticals; food retailers; textiles and apparel; tobacco, and water) there were certain companies that performed at or close to the top of the world R&D league.

In May, a government-appointed committee chaired by Lord Nolan published its first reports on the standards of conduct in public life. The Nolan Committee is expected to have profound effects on individuals and organisations that utilise public funds.

Unlike other members of the EU, farming prosperity in the UK improved markedly in 1995, for the fourth successive year, and overall profits attained levels akin to those in the early 1980s. Support payments, however, were around £3000 million as a result of CAP reform and the devaluation of sterling. The arable sector thrived and there was a sharp upturn in the fortune of horticulture, an area of activity in the UK barely affected by CAP inputs. Investment levels increased as a safeguard against short-term fluctuations in interest rates and land prices rose by around 25% in the year.

In production terms, the agricultural and horticultural output of the UK reflects the constraints of land area and climate. Comparisons of production with Europe and the rest of the world (Ministry of Agriculture, Fisheries and Food Internet site http://www.open.gov.uk/maff/stats/) reveal that the UK arable and horticultural sectors were a small pro-

portion of European production. A valuation of £6,798 million was given to the major UK arable and horticultural crops at 1995 prices. On the other hand, the industry was highly efficient. For wheat, barley, potatoes, cabbage, onions, carrots and tomatoes, for example, the crop yield on a kg per ha basis was considerably greater than the average for the rest of Europe or the world.

According to the Forestry Commission, only 10% of the UK land area (2.45 million ha) is forested, but is expanding at a rate of 20,000 ha per year. In 1994, production of wood and wood products was 7.6 million m^3 of which 3.5 million m^3 was softwood sawlogs, 0.3 million m^3 hardwood and 3.1 million m^3 particle board, fibreboard and paper board, levels that met just 15% of UK demand.

As a share of the UK economy in 1995, agriculture and horticulture constituted *circa* 1.5% of the Gross Domestic Product (GDP), approximately £9 billion, and forestry only 0.05%. These figures belied the importance of successful value-adding industries with larger GDP valuations, downstream and upstream, and were based on current market valuations of primary production. Perturbations to the supply and low costs of primary produce would have dramatic effects on these secondary industries and commerce in general.

Evaluation of the non-monetary costs and benefits of agriculture, horticulture and forestry is fraught with difficulty. Perceived and real beneficial effects include such aspects as sustainability (e.g. carbon sinks, atmospheric cleansing, waste disposal, recycling of nutrients); hydrological benefits; creation and preservation of landscapes, habitats, ecological refugia and dispersal corridors; and provision of recreational facilities. Perceived and real detrimental effects include the reduction of biodiversity, excessive use of water for irrigation, alteration of rural lifestyles with the onset of modern farming methodology, pollution, adverse impacts on animal dignity and welfare in highly intensive systems, amended landscapes and restrictions on access to the countryside.

Agriculture, horticulture and forestry represent the major UK land use industries and are a bastion against unfettered urbanisation. There are also huge regional variations in their relative roles in the local economy. Increasing efficiency of production inexorably leads to declining employment and hence political influence as alternative forms of rural employment take hold. To this scenario must be added the pressures of cheaper imported supplies, competition with other forms of land use, and calls for reduction in the costs of the CAP. Realisation has dawned that R&D, access to intellectual property and innovation are the routes by which these primary industries can thrive in open world markets, meeting consumer preferences and avoiding degeneration to peasant industries competing solely on the grounds of cheap labour costs.

In future Reports I shall analyse the biotechnology industry and SCRI's involvement in this rapidly expanding area of wealth creation and intellectual challenge.

UK food plants

The current range of home-grown and imported plants consumed in the UK is large (Table 1), although most are of minor importance and diets can be depressingly narrow. Advancements in modern retailing arrangements and marketing efforts, aided by changes in lifestyle and a massive increase in professional catering, all raise demands and expectations in the quality and type of food rather than quantity. Even though there are residual historical, ethnic, social and economic factors that favour specific diets, growing health awareness and hedonism have become incompatible with restricted, narrow supplies and regional seasonality of produce. Blemish-free and safe produce, meat and milk products are universal expectations of processors, retailers and consumers alike, and dominated by the perceptions of urban dwellers.

Economists recognise that neither import substitution nor a trade deficit justify unfettered agricultural and horticultural production in the present- day international trading environment, even if the land resource and labour were available. Economic justification is a prerequisite.

Plant breeders and geneticists, physiologists, biotechnologists, pathologists, agronomists and engineers, using advanced mathematics, chemistry and physics are providing new, improved, low-input cultivars with extended growing seasons, as well as better cultivation, protection, harvesting and storage technologies. This R&D combines fundamental, strategic and applied research approaches, usually within a single project. Natural tolerance or resistance to biotic and abiotic stress whilst sustaining quality, yield performance and efficiency of production are foremost in the minds of major institutions such as SCRI, but to the intellectual challenges are added products for the rapidly expanding biotechnology, food processing and environment industries. We aim to deliver (for the UK) plants, products, processes and concepts that meet

dietary, health, environmental and wealth-creating needs and to play a key role in rural prosperity.

Scotland's climate and latitude have both advantages and disadvantages for agriculture, horticulture and forestry. There are relatively few pests and diseases and "green-bridging", the overwintering of pests and diseases is rarer than in most competing countries. Scottish plant material is typically of high-health status. The long summer daylengths favour a productive, albeit short, growing season. Growing habitats are diverse, with wide temperature ranges and varying degrees of exposure to wind and salt. Excellent grass production on the wetter areas enables livestock production to function with inputs often lower than elsewhere. By and large, supplies of fresh water are plentiful. Transport costs to the major markets are a problem, however. Production units in Scotland differ enormously in their size, sophistication of opera-

1. *Poaceae (Gramineae)*

The cereals - barley, oat, rice, rye, wheat
Coarse grains - maize, sorghum
Forage grasses
Sugar cane

Grass fruits (caryopses or grain) are the largest single source of carbohydrate on earth.

2. *Leguminosae*

Forage legumes
The pulses - adzuki, blackgram, broadbean, chickpea, cowpea, haricot, horsegram, jack, kidney, lablab, lentils, lima, mat, mung, pea, pigeonpea, soya, string- and snapbean, sword, vetches, yambean

Proteinaceous, sometimes contain toxins, allergens or haemagglutinins. Also produce oils.

3. *Solanaceae*

Aubergine	**Potato**
Capsicum	**Tomato**

4. *Cruciferae*

Broccoli	**Kohl-rabi**
Brussels sprout	**Mustard and Cress**
Cabbage	**Oilseed rape**
Cauliflower	**Radish**
Chard	**Swede**
Chinese leaves	**Turnip**
Kale	

Proteins, oils and carbohydrates. The most important group of green vegetables.

5. *Chenopodiaceae*

Beet - fodder, garden, spinach, sugar
Mangold
Spinach
Swiss chard

6. *Liliaceae*

Chives	**Onion**
Garlic	**Onion** - Egyptian, spring, Welsh
Leek	**Rakkyo**

7. *Rosaceae*

Soft fruit - blackberry, currant (black/red/white), gooseberry, hybrid berries, raspberry, strawberry
Stone fruit - almond, apricot, cherry, damson, greengage, nectarine, peach, plum
Top fruit - apple, medlar, pear, quince

8. *Cucurbitaceae*

Courgette/Zucchini	**Melon**
Cucumber	**Melon (water)**
Gourd	**Pumpkin**
Marrow	**Squash**

9. *Umbelliferae*

Carrot	**Parsley**
Celeriac	**Parsnip**
Celery	

10. Major imported families

Anacardiaceae - mango, cashew
Araceae - cocoyams, dasheen, yautia, taro
Bromeliaceae - pineapple
Convolvulaceae - sweetpotato
Euphorbiaceae - cassava
Musaceae - banana, plantain
Palmae - date palm, sago palm, oil palm, betel nut
Rubiaceae - coffee
Rutaceae - citron, grapefruit, lemon, lime, orange, pummelo, tangerine
Sterculiaceae - cocoa, cola
Theaceae - tea

11. Miscellaneous horticultural plants

Artichoke	**Macadamia**
Avocado	**Mulberry**
Blueberry & cranberry	**Okra**
Breadfruit	**Papaya**
Chestnut	**Passionfruit**
Endive	**Pecan**
Fig	**Pistachio**
Grape	**Pomegranate**
Guava	**Rhubarb**
Hazelnut	**Tamarind**
Lettuce	**Walnut**

Also includes flavourings, fumatories, herbs and spices and masticatories.

Table 1 Range of food plants consumed in the UK.

tion, levels of investment, profitability and relationships with customers. Our R&D, regulatory, advisory and educational base is impressive - there are five Scottish Agricultural and Biological Research Institutes (SABRIs; Scottish Crop Research Institute, Hannah Research Institute, Macaulay Land Use Research Institute, Moredun Research Institute, Rowett Research Institute), the Scottish Agricultural College, and the Scottish Agricultural Science Agency. All these bodies are members of the Committee of Heads of Agricultural and Biological Organisations in Scotland, which also includes the Fisheries Research Service, Forestry Commission and the Royal Botanic Garden, Edinburgh, and have long histories of achievement in the international arena (see also the SCRI 1994 Annual Report).

Man is the only animal to cook food, a process which widens the range of species consumed. Compared with fresh plant food, cooking modifies the appearance, taste and texture, frequently removing anti-nutritional, toxic and other components, and affecting cell integrity. Vitamins and micronutrients may be lost, too, but more cellular material is made accessible to gut secretions. Only in recent times has attention been given to antioxidants and free-radical scavengers in the diet, to dietary fibre, to personal bioremediation possibilities by dietary intake, and to the psychology of food choice. A future challenge is the problem of processing sewage resulting from the modern diet and medication.

My 1994 report detailed two activites which were heralded in the landmark science White Paper *Realising our Potential: A Strategy for Science, Engineering and Technology* (Cm 2250, May 1993), namely the UK Technology Foresight Programme (TFP) and the Multi-Departmental Scrutiny of Public Sector Research Establishments (PSREs). Both activities took place in 1994 and both involved SCRI.

Technology Foresight Programme

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

In April 1995, the Agriculture, Natural Resources and Environment (ANRE) Panel of the TFP (which I chaired), published a report along with 14 other panels that together covered all the major UK industrial and service sectors. Summaries of the Panel Report and the overarching report from the TFP Steering Report are presented in my 1994 Report of the Director. The ANRE Panel recommendations called for investment in research in key areas, better coordination of research and its integration into specific technologies, and for more investment into public perception and understanding of new technologies.

Agriculture, Horticulture & Forestry	John Hillman	SCRI & MRS Ltd
Chemicals	John Beacham	ICI
Construction	Herb Nahapiet	UK Detention Services Ltd
Defence & Aerospace	Tony Edwards	Messier-Dowty Int.
Energy	Gordon MacKerron	University of Sussex
Financial Services	Michael Hughes	Barclays de Zoete Wedd
Food & Drink	Peter Lillford	Unilever Research
Health & Life Sciences	Mark Ferguson	University of Manchester
IT, Electronics & Communications	John Taylor	Hewlett Packard Laboratories
Leisure & Learning	Peter Wallis	SRU Ltd
Manufacturing, Production & Business Processes	David Grant	GEC plc
Marine	David Goodrich	British Maritime Technology Ltd
Materials	John Campbell	Cookson Group plc
Natural Resources & Environment	Kerry Turner	University of East Anglia
Retail & Distribution	Graham Winfield	Booker-Tate Ltd
Transport	Stephen Gibbs	Docklands Light Railway

Table 2 List of Technology Foresight Panels, Phase 2, and their Chairmen.

Members

J R Hillman	Scottish Crop Research Institute
J Braunholtz	Horticultural Development Council
P Chisholm	The Wellcome Trust
J Evans	Forestry Commission
A B N Gill	National Farmers Union
G Harrington	Meat & Livestock Commission
R B Heap	Babraham Institute
T Hegarty	SOAEFD
B J Legg	Silsoe Research Institute
B J Miflin	Institute of Arable Crops Research
J MacArthur Clark	Consultant
P Maplestone	BBSRC
C H McMurray	Department of Agriculture for Northern Ireland
R Morrod	Zeneca Agrochemicals
J S Marsh	University of Reading
J F Oldfield	Farmer and HGCA
J Sherlock	MAFF
M Tricker	NERC
R Turner	British Society of Plant Breeders
J Vowles	Agricultural Engineers' Association
David Rawlins (Secretary)	Office of Science and Technology

Methods of working

- Sub-Groups-
  - Livestock Systems(J MacArthur Clark)
  - Forestry & Wood Products (J Evans)
  - Plant Systems (R Turner)
  - Foresight Challenge Awards Group (R B Heap)
- Short & medium-term projections
- Cross-Sector/Panel activities
- Dissemination of new report
- Visionary concepts
- Promoting & monitoring coordination of priority setting between research sponsors
- Providing inputs to EU programmes
- Directory of databases

Remit

- Dissemination of Panel findings and recommendations
- Development of networks, both regional and national
- Promote implementation of findings to Private Sector, Research Councils, Government Departments, Universities, Research Institutes
- Monitor progress
- Review recommendations

Lifespan

- Five years, but dynamic

Table 3 Agriculture, Horticulture & Forestry Sector Panel.

Also recommended was that in the subsequent phases of the TFP, consideration should be given to splitting the ANRE Panel into more focused panels, and this was done in the Summer of 1995, as phase two of the TFP was launched by the Office of Science and Technology (OST).

My responsibility is now for the Agriculture, Horticulture and Forestry (AHF) Sector Panel whose membership and activities are detailed in Table 3. Reports will flow from the new AHF Panel in 1996.

Prior Options

In its response to the Scrutiny Reports, the Government announced in 1995 a series of Prior Option reviews, each addressing the actual and potential relationship of the PSRE in question to others in similar or related areas of activity, with an eye to potential privatisation or rationalisation. Reviews would also take explicit account of the outcome of the UK TFP, and the requirements of customer Government Department. The findings of the reviews, which were to be conducted in three tranches in late 1995 and through 1996, would be considered by Ministers collectively to ensure that all cross-departmental aspects had been fully covered.

The Scottish Office established a single review team to cover SCRI, the Scottish Agricultural Science Agency (SASA, East Craigs), and the Macaulay Land Use Research Institute (MLURI), as three of 11 establishments in the Agriculture and Plant Science grouping - the first tranche. An Interdepartmental Steering Committee chaired by Professor Tom Blundell of the BBSRC was also established to co-ordinate all the reviews in this grouping, with a remit to report to Ministers by the end of March 1996.

Announcements of the reviews of SCRI, SASA and MLURI were made by the Secretary of State for Scotland on 23 November 1995. Comments were invited by 8 January 1996, and comments were also invited in a general notice which appeared in the December edition of 'Government Opportunities' (published on 24 November 1995). Several other bodies were consulted directly.

The review focused on five key questions and on the privatisation guidance issued by OST, Office of Public Service and the Treasury. The Key questions were: Is the function needed?; Must the public sector by responsible for funding the function?; Must the public sector provide the function itself?; What is the scope for rationalisation?; How will the function be managed?

The final outcome of the Prior Options exercise was expected to be announced towards the end of 1996.

The Scottish Crop Research Institute

SCRI is a non-profit-making limited company established under the Companies Act, has charitable status and is a Non-Departmental Public Body because over 50% of the total funding is received from Scottish Office, Agriculture, Environment and Fisheries Department (SOAEFD), and all members of the Governing Body are appointed by the Secretary of State for Scotland. Staff are not formal civil servants, but are members of the SOAEFD Superannuation Scheme, and SOAEFD funds any redundancies, the site, and much of its fabric and capital equipment. There is also a Management Statement and Financial Memorandum embodying the formal relationship with SOAEFD.

SCRI is a special centre of international excellence, bridging the public and private sectors, and noted for the high-quality, innovative research encompassing its entire programme. Whilst many PSREs in recent years have been closed, merged or rationalised, SCRI has evolved dynamically, expanding in a highly competitive market. In the last 15 years the site at Mylnefield has received huge investments in buildings, state-of-the-art scientific facilities, and new scientific staff. Independent Visiting Group reports; performance indicators (e.g. refereed papers per scientist, cost per refereed paper, grant income, EU income etc.); market impact measures (e.g. university courses presented, research student training etc.); attractiveness for senior visiting scientists, governmental teams and companies; value-for-money; and rate of expansion, are unequalled within the research service. Within the last six years the establishment of the dramatically successful, award-winning technology transfer company, Mylnefield Research Service Ltd, and the appointment of its outstanding Managing Director, Dr N.W. Kerby, has complemented the efforts in redirecting the science and linking with universities and other institutions at home and abroad.

As detailed in the SCRI Corporate Plan, the mission of the Institute is to undertake an integrated programme of fundamental and strategic multidisciplinary research of the highest quality on agricultural, horticultural and industrial crops, their pests and diseases, and on processes common to all plants; to create and protect wealth, and to improve the quality of life and the environment. It aims to increase knowledge of the basic biological sciences, to improve crop quality and utilisation, to improve biodiversity, agricultural sustainability and diversification, and to develop environmentally benign methods of protecting crops from depredation by pests, pathogens and weeds. A broad multidisciplinary approach to address the mission is a special strength of the Institute, employing and fostering the disciplines of genetics and breeding,

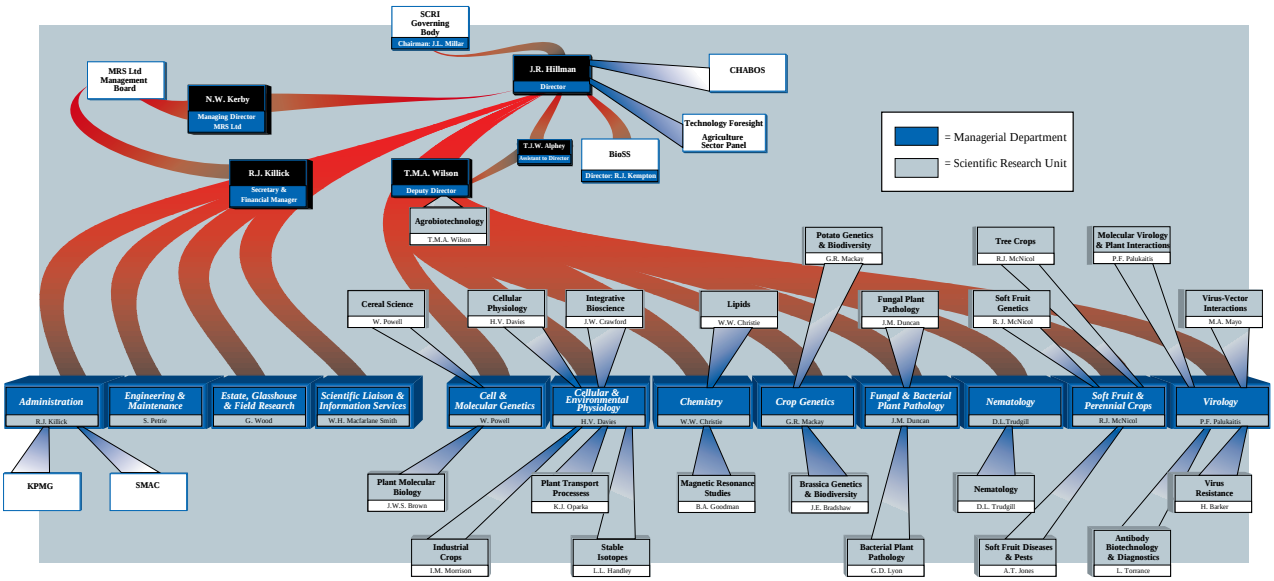

Figure 1 SCRI Departmental and Research Unit Structure.

molecular and cellular biology; pathology (virology, bacteriology, mycology, nematology and entomology); physiology (metabolic, environmental and developmental); chemistry and biochemistry; agronomy; ecology (molecular ecology, vegetation dynamics, bioremediation); serology; physics; mathematics and statistics. In 1995 the day-to-day scientific management and focus of the Institute was restructured with the formation of 22 research units .

The range of skills from fundamental studies on genetics and physiology, through agronomy and pathology to glasshouse and field trials with exploitation of SCRI-based international genetic resources in a region of high phytosanitary conditions, is unique within the UK. Synergistic and complementary liaison with other research organisations, universities and colleges in the UK and abroad is an integral part of the scientific development of the Institute. Such links are continually being developed and strengthened. SCRI and MRS Ltd actively seek research contracts from Government Departments and agencies, levy boards, grower organisations, international agencies, commercial companies, local government and trust funds.

SCRI acts as a parent body for Biomathematics and Statistics Scotland (BioSS), formerly the Scottish Agricultural Statistics Service (SASS), which acts as a unit of SCRI under the able leadership of R A Kempton. BioSS was set up in 1987 to cover the biomathematical and statistical needs of the five SABRIs and SAC. High-level consultancy, training and research inputs from BioSS give a major advantage to the SABRI and SAC research programmes. The next Visiting Group to BioSS was scheduled for early 1996.

Following the pattern of previous SCRI Annual Reports, this report details only a small selection of the research achievements of SCRI and MRS Ltd, briefly describes the commercial successes of MRS Ltd, and summarises the important linking role of the associated Friendly Society, the Scottish Society for Crop Research (SSCR; D.L. Hood, Secretary & Treasurer; T.P.M. Thomson, Chairman). Advances have been made in both fundamental and strategic science, with contributions to the protection and understanding of the environment, and discoveries are reported of direct and indirect benefit to agriculture, horticulture, forestry, land management and biotechnology. Dedicated and talented scientific and support staff in every department and section of the Institute, BioSS and MRS Ltd account for our stature, successes and delivery of achievements.

On behalf of the staff and Governing Body, it is a pleasure once again for me to acknowledge with gratitude the staff of SOAEFD for their continuing support of and commitment to our research programme and to our development. Regardless of the enormous pressures upon them, they function rigorously and fairly, as always, to the highest professional standards of British public service. Grants, contracts, donations, advice and joint participation in our activities from the SSCR, other governmental departments and their agencies, non-governmental agencies, grower levy boards, local and regional authorities, commercial companies, farmers and other individuals, and learned societies, are also warmly appreciated.

SCRI is buoyant and justifies its existence in every respect. We have every confidence in meeting future challenges; scientifically and commercially, our prospects are outstanding.

People & events

Tim Heilbronn

Retirements Two long-standing, senior members of staff left during 1995.

Dr Derek A. Perry retired on 7 April, after 33 years service. He was editor of the Annual Reports for the years 1990 to 1994, overseeing the change from the old-style report, to the current, highly acclaimed, full-colour report. As the Institute's principal spokesman, with responsibility for Scientific Liaison for all plant science research undertaken at the Institute, he held a key position, but he was also widely respected for his expertise as a plant pathologist.

He graduated from the University of Birmingham, and gained his PhD from the University of London in 1958 for work carried out at Rothamsted

Dr D.A. Perry.

Experimental Station. He spent four years in Uganda with the Empire Cotton Growing Corporation, and then joined the Mycology and Bacteriology Department of the then Scottish Horticultural Research Institute (SHRI) in 1962, with periods of secondment in Nigeria and Brazil in 1965 and 1982 respectively. During his career he acquired a very broad knowledge of plant fungal and bacterial diseases on a range of crops in both temperate and tropical regions. Within the international scientific community, he made his mark as one of the leading experts on problems associated with seed establishment, methods for seed vigour testing, and production methods to retain vigour. He was Chairman of the International Seed Testing Association Vigour Test Committee from 1974 to 1983, and Treasurer of the Association for Crop Protection in Northern Britain from 1986 to 1995. Since 1993 he has been Treasurer of the British Society for Plant Pathology.

Dr Roger L. Wastie, formerly Deputy Head of the Crop Genetics Department, retired in June 1995, after 21 years as a plant pathologist at first the Scottish Plant Breeding Station at Edinburgh, and latterly SCRI, but was still actively involved as joint supervisor of a number of research projects. He was also on the Editorial Boards of the Annals of Applied Biology and Potato Research. Sadly, his-well deserved retirement was to be cut tragically short when he died on 16 January 1996, from complications following surgery. After gaining a first class honours degree in Botany at the University of Oxford, in 1958, and his PhD at the University of Cambridge, in 1961, he worked at the Rubber Research Institute of Malaya, at Kuala Lumpar, for 12 years and became an acknowledged authority on pathology of *Hevea*. He then spent two years as Head of Biology at the Bentham Grammar School, Lancaster, before joining the Potato Division of SPBS in 1974. Following the merger of SPBS with the then SHRI, he was one of the last tranche to make the move north, transferring to Mylnefield in March 1989. Everyone who knew Roger will remember his loud, floral shirts, his odd socks, and his love of curries and afternoon teas. But above all he will be greatly missed by friends and colleagues for his gentle humour, and his wealth of expertise as a pathologist, for which he was well respected throughout the international scientific community.

Dr Pérombelon was presented with the MBE by her Majesty the Queen.

Honours Dr Michel C.M. Pérombelon received an MBE in the New Year's Honours List, for services to science, in recognition of his internationally renowned contribution to the understanding of erwinias and to the potato industry.

The American Oil Chemists' Society honoured Dr Bill Christie, Head of the Chemistry Department, with its annual H J Dutton Award in Lipid Chemistry. The Award is presented to an individual who has made a significant contribution to the development of methods for the analysis of fats and oils. Dr Christie has worked in the field of lipid analysis

for more than 30 years, and is considered to be at the forefront of research in natural oils. He joined the Department of Lipid Biochemistry at the Hannah Research Institute, Ayr in 1967, and moved to his current position in January 1993. His considerable accomplishments include the development of many novel methods of analysis and separation of complex mixtures of fats. He is the author of more than 250 publications, and his books on lipids have guided a generation of researchers.

Alison Roberts won the Katherine Esau Prize, which is awarded only every five years, for her studies using a novel new technique to look at movement of viruses within plants. The award of this prize at the International Conference on the Transport of Photoassimilates, held at the University of Kent, Canterbury, recognised a remarkable success story both for Alison, and for her colleagues at SCRI.

Alison Roberts

Senior Appointments Professor T. Michael A. Wilson, formerly Head of Virology, was appointed Deputy Director. He is internationally respected as one of the leading authorities on the molecular biology of plant viruses, and has continued to supervise a number of scientists engaged in biotechnology and crop resistance research, whilst taking on a leading role for the Institute. He graduated with first class honours in Biochemistry from Edinburgh University and was awarded a PhD from Cambridge University for structural studies on tobacco mosaic virus. He is a Visiting Professor at the University of Dundee and was, until his appointment to SCRI in March 1992, on secondment from the John Innes Institute for almost 3 years as Professor in the Center for Agricultural Molecular Biology at Rutgers University in New Jersey, USA. In 1993 he was awarded an Honorary Professorship by the Zhejiang Academy of Agricultural Sciences in China.

Dr Bill Macfarlane Smith was appointed Head of Scientific Liaison and Information Services with effect from 1 August. He studied botany at the University of Aberdeen, then completed his PhD on the genetics of strawberry whilst a member of staff at the University of Reading. His successful attempts to produce viable hybrids between wild species of strawberry and the shrub potentilla started a long career in breeding, firstly as head of the highly successful Rothwell Plant Breeders barley breeding team for Shell Nickerson, with specific interest in malting barleys. Later, as Technical Manager, he was responsible for liaison with all associated European seed and plant breeding companies and had special responsibility for plant breeding activities in Scotland and Eire. He joined the Scottish Plant Breeding Station, at Pentlandfield, Edinburgh, in 1978, and shortly afterwards became Head of the Brassica Department, with responsibility for breeding new varieties of swede and forage rape, and associated research. Following the merger of SPBS with the then SHRI he transferred to SCRI in 1983.

The Mylnefield Fellowship Dr Frederick Gildow, Associate Professor of Plant Pathology at the Pennsylvania State University, spent a year at SCRI as the first Mylnefield Fellow. This Fellowship was created to support research by visiting scientists in innovative areas of agricultural research, and was used by Dr Gildow to support a collaborative research project with Dr M.A. Mayo. Dr Gildow is one of the leading authorities on transmission of plant viruses by insects.

Visitors As in previous years there were several groups from Africa, The Americas, China and the Commonwealth of Independent States, including a high-ranking delegation from the Ministry of Agriculture of Turkmenistan, and a delegation from Ukraine, accompanied by Lord Sempill. There was an increasing number of groups from Scandinavia, with visitors from Denmark, Finland, Norway and Sweden, as well as a group of potato scientists from Iceland. Distinguished visitors during the year included members of the Scotia Agricultural Club, who held their AGM at the Institute and toured the site in May, and members of the Royal Commission on Environmental Pollution, led by Sir John Houghton CBE, FRS, who visited in April.

The Earl of Lindsay, Minister of State for Agriculture, Foresty and the Environment in Scotland, made a special point of visiting the joint SCRI/MRS/SAC/SOAEFD exhibit at the Scotgrow Exhibition at Ingliston on 11 October. The exhibit, promoting the Scottish raspberry industry, showed a unique collaboration between the scientific specialists, NSA Plants Ltd and Scottish Soft Fruit Growers Ltd, a cooperative representing 95% of the processing raspberry production.

75 years of pioneering research September 6 was the 75th anniversary of the origins of the Institute, which date back to September 6, 1920, when the Directors of the new Scottish Society for Research in Plant Breeding (SSRPB) met for the first time. The aim of the Society was to "establish a thoroughly equipped station for the improvement of agricultural plants ... possessing in the highest degree those qualities which will make them most profitable under Scottish conditions". The members of the Society, who were primarily farmer and landowner members of the Highland and Agricultural Society of Scotland, launched a public appeal to set up a research station. The appeal raised £22,363, which was matched by the Government of the day to give a total fund of £44,726, the equivalent at today's prices of c. £1m.

The first site of the then Scottish Plant Breeding Station (SPBS) was at East Craigs, Corstorphine, now the home of the Scottish Agricultural Science Agency. By 1945 further impetus to the development of agriculture and the attainment of self-sufficiency in basic foodstuffs was given by the emergence from the 1939-

Lord Lindsay; D.C. Bickerstaf, SAC and P. Rankin, SOAEFD (l - r). (Copyright Photo Express)

1945 war years. As a consequence, additional staff were appointed and the staff moved, in 1954, to new and larger premises at Pentlandfield, Midlothian. SPBS was vested in the Scottish Horticultural Research Institute (which changed its name to SCRI) on 5 August 1980, and the SSRPB and the Scottish Horticultural Research Association were united to form the Scottish Society for Crop Research. The gradual development of the two initial stations, and the considerable expansion at Invergowrie following the move to a single site, represent a considerable investment by DAFS / SOAEFD, whose role throughout the history of both organisations is gratefully acknowledged.

Modern agriculture owes much to the vision of those who founded the Society, and similar groups such as the ADRA at the Moredun Research Institute. By 1970, some 30 named potato varieties had been produced by the SPBS in its first 50 years, of which three have had significant impact on UK potato cultivation, and one on the production of the crop overseas. Together the Craigs, Pentland, and Roslin series of potatoes were phenomenally successful. At their height, in the 1970's, Pentland Dell and Pentland Crown together occupied nearly 40% of the maincrop acreage of the country, and were estimated to be worth, at today's prices, c. £26m a year to British agriculture. To date, 41 varieties have been bred by SPBS/SCRI and released in the UK, with an additional 12 bred and released in Africa, especially Malawi and Kenya, and India.

SCRI at SRT: the ethics of genetic engineering of non-human species

T. Michael A. Wilson

In December 1993, the Society, Religion and Technology (SRT) Project of the Board of National Mission of the Church of Scotland set up a multi-disciplinary working group to debate ethical issues raised by rapid developments in the genetic engineering of non-human species, and by the prospect of having genetically manipulated organisms (GMOs) in free circulation.

A group of ten experts in relevant fields (bacterial, plant and animal genetics; animal welfare; theology; sociology; environmental risk and public perception; Third World issues and ethics) which included GMO enthusiasts and sceptics, congregated at monthly intervals in John Knox House, Edinburgh. Our unique, multidisciplinary group quickly established an empathy and a rapport, despite adopting a 'no-holds-barred' and frank approach to the debates, which everyone found thought-provoking and constructive. We reviewed recent developments and relevant literature, considered complex issues, expressed and listened to widely differing viewpoints, and finally distilled a vast number of notes, scientific papers, articles, case studies and major issues debates into a single text (of approx. 200 pages), soon to be published under the SRT banner.

Through our convenor and director, Dr Donald Bruce, the group was also invited to make significant and acknowledged contributions to the Nuffield Council on Bioethics, on Xenografts; to the MAFF (alias Banner) Committee, on Ethical Implications of Emerging Technologies in the Breeding of Farm Animals; and to the Department of Health Advisory Group, on the Ethics of Xenotransplantation.

Such a non-partisan group, holding detailed and extensive ethical debates, providing balanced, unequivocal and unambiguous guidance, can have unique appeal and trustworthiness to the public-at-large. This is particularly the case as the motives and advice of politicians, industrial leaders, company scientists, and, regrettably, even academic research scientists, are no longer seen by the media or general public as being wholly unbiased, reliable, or objective.

Following a technical introduction to the fundamental principles of microbial, plant and animal genetic engineering (presented in a matter-of-fact, unbiased and non-judgmental manner) the SRT text then describes ten specific Case Studies. These are used to introduce the reader to particular ethical and moral issues, which are discussed later and in greater detail in seven major 'issues' chapters.

The Case Studies include topical and media-controversial examples such as: the slow-ripening Flavr Savr^{TM} tomato; the insect super-killer virus armed with a scorpion toxin gene; the use of bacterially produced cow hormone (BST) for increased milk yield; 'Tracy' the genetically engineered sheep whose milk contains a valuable human therapeutic medicine; the xenografting of pig hearts into humans; the new SCRI plant virus 'overcoat' protein production system for vaccines, therapeutics or industrial enzymes (see p. 135, in this Annual Report); and the first patented, genetically engineered mammal, the so-called Harvard oncomouse.

Each of these Cases raised a unique subset of ethical issues for later discussion including (but not exclusively limited to): animal welfare, dignity and rights; cost-benefit analyses in comparison with 'accepted' current practices such as selective breeding and intensive agriculture; transgenic foods for human (or animal) consumption; the patenting of living organisms (a 'commodification of life'); societal attitudes to GMOs; gradualism in public attitudes and acceptability; the influence of commercial drivers; environmental impacts; risks to humans and the distribution of risk to various sectors of society; effects on Third World economies and peoples; and, underlying (intrinsic, deontological or consequentialist) ethical and theological issues.

For scientists, compliance with ethical norms is a prerequisite for the conduct of research. Thus, in addition to our basic tenets such as objectivity and honesty, there is a need for communal awareness (sharing of results), universalism (concern with fundamental principles, not parochial issues), altruistic disinterest (having no vested interest in the outcome of

research) and organised scepticism (subjecting scientific hypotheses to rigorous examination). Without these norms, the validity of scientific knowledge is constrained and compromised, as can occur when the drive for commercialisation assumes dominance.

One feature of scientific research which often unsettles the public, the media, and political decision-makers, is that scientific knowledge is always provisional. Yet planes fly, bridges stand, antibiotics work, crops grow and Green Revolutions happen! Nevertheless, scientific theories/hypotheses can be refuted and discarded. Rarely, if ever, will a scientist give an unreserved, unequivocal 'yes' or 'no' answer, especially on issues concerned with biological systems. Confidence limits, statistical analyses and probabilities abound, and can be interpreted by different self-interest groups to support their own particular agenda.

To paraphrase[1] the Scottish philosopher, David Hume (1711 - 1776): 'Science is based on logic, while religion is founded on faith. The two are inherently incommensurable'. Thus one could argue that it is futile and hopeless to seek a consensus, in society, or even among members of the SRT group! Yet mutual respect, new perspectives and better understanding can be achieved.

Those with deeply held religious beliefs may have a clearer, but not more rational view on genetic engineering than those with intrinsic but nonetheless compelling, intuitive feelings. Nevertheless, scientists should recognise that perspectives and debates in these areas cannot simply be dismissed as 'rational scientists' versus 'irrational lay-people'. Science and research do not exist in a social vacuum. Moreover, in a plural society in the real world, where issues and problems are contextualised in different ways, there exists a variety of competing rationalities. Despite its cognitive power, the role of scientific knowledge and how it should be used are not straightforward. It has to be interpreted and evaluated within extra-scientific frameworks, typically with economic, political and ethical dimensions.

For each application of biotechnology, or genetic engineering, one can identify ethical issues and try to 'best-fit' them to a scheme amenable to rational analysis. Few people actually subject their ethical or moral beliefs to rational analysis, yet the importance of this is clear in the norms and institutions of so-called civilised societies (e.g. laws which seek to establish racial and sexual equality). Individual ethical judgements or moral beliefs are based on ethical principles and theories, which fall into either or both of two categories: deontological and consequentialist. Deontological theories are based on the concept of duty, i.e. the belief that actions are intrinsically right or wrong and independent of their consequences. Some of these deeply held beliefs have a religious motive, others are based only on intuition (the 'categorical imperatives' of Kant). Deontological forms of ethical reasoning are used widely. For example, various Declarations of Human Rights assert the innate rights of individuals, irrespective of gender, age, race or religion.

Consequentialism is the opposite of deontology, and teaches that the rightness or wrongness of an action depends only on whether it is likely to produce more benefits than problems. Utilitarianism is a branch of consequentialism which espouses the doctrine that actions are right because they are useful, or provide the greatest happiness for the greatest number of people. To apply these theories requires one to make rigorous cost-benefit analyses (e.g. that a gain to medicine outweighs the pain/harm, the argument used by biomedical researchers to justify animal experimentation). Utilitarianism is commonly used by legislative or governmental bodies to reach acceptable decisions on issues of public concern and, as such, involves deontological factors because offending a person's intrinsic beliefs can contribute to the cost-benefit analysis. Clearly, few people rely exclusively or stringently on either deontological or consequentialist ethics in their everyday decision-making. We try instead to match our ethical principles with our own moral experiences, and extrapolate from these to a universal code of morals and ethics. The bioethicist tries to analyse moral concerns in a rational way, then to clarify ethical dilemmas and suggest actions to ameliorate or resolve them.

Deontological or consequentialist theories of ethics are applied to the four principles of ethics: freedom, fairness, harmlessness and kindness. Such debates or analyses are common in medical ethics, but relatively rare in agricultural biotechnology. Yet, to adopt our new technologies in humane, publicly acceptable, and effective ways, efforts must be made in this direction. The SRT project is a start, but we must not abandon public scientific 'education' to the single-issue pressure groups. Luddite, anti-technology groups, and overly precautious risk assessment and regulation, have largely created a self-fulfilling prophecy, namely that the promises made by biotechnologists in the 1980's would not be delivered[2].

In the short-to-medium term, public attitudes towards genetic engineering are likely to influence both the future direction of the technology and consumer acceptance of specific applications, such as food products from GMOs. Ethical objections tend to focus on genetic engineering involving animals or human genes, rather than on plants or micro-organisms. The most important determinant of public acceptance is likely to be the perceived need for the technology, and the tangible benefits arising from it (i.e. some form of intuitive cost-benefit analysis).

As yet, there have been no clear steers or conclusions to inform or guide public opinion. Media-controlled debates are 'set-up' emotively with prelabelled 'good' and 'bad' guys, for good TV or radio ratings or to sell newspapers, not to address the issues in a balanced way. Nevertheless, this is probably the most effective medium by which to reach most people.

To make real progress in this area, to break out of the perennial and emotional 'what-if' cycle of objections and counter assurances, is critical for the future competitiveness of UK biotechnology research and development, and to future 'high-tech' employment opportunities, in order to prevent the UK (and EU or even the western hemisphere) becoming the low-paid industrial sweat-shop and food basket for the emerging tiger economies of the Asia-Pacific zone. Predictably, the USA and Japan are ahead of us in almost all biotechnology indicators.

To our political and financial decision-makers, the nation's economic survival and stability in the next millennium depends largely on the understanding, perceptions and concerns of the general public (and our media image-makers) on biotechnology. These issues must be addressed promptly and in a clear and articulate fashion. We already live in a far-from-natural world, with 5.5+ bn neighbours (rising to 8+ bn by 2050AD), where romantic notions of self-sufficiency, organic farming, or returning the bulk of the world's population to some stress-free, hunter-gatherer status are simply untenable, unrealistic and unacceptable to the majority. Global demands for food and health security, and perennial expectations for an improved quality of life (often modelled on Western, US, media-values) and annually higher living standards are driving much of the technology. To reverse the trend would likely result in social turmoil, instability, and even mass revolution.

Without clear conclusions, the focus, direction and applications of biotechnology become lost in a fog enhanced by religious beliefs, public perceptions, over-zealous claims, and speculative risks and benefits. Different ethical and practical issues of biotechnology elicit different responses in sectors of the public of differing age, race, religion or gender. How can one address this? Our perceptions are modified and manipulated by media, marketing agents, and political persuasion. Presentation is paramount.

SCRI seeks to play its full and constructive part in raising the public understanding of, and appreciation for, modern science. This will assist people to carry out properly informed, detached and clinical analyses of the real issues, impacts and consequences of the new technology, as it affects the fundamentals of human society and our interactions with the living world, for which all the world's religious texts teach that we were given a duty of care and control.

Common Ethical Dilemmas

1. Some Issues of Principle

Is genetic engineering unnatural?
Is genetic engineering 'playing at God?'
Can one patent lifeforms?
Does this encourage a utilitarian view of Creation?
Animal welfare and intrinsic value issues?

2. Some Issues of Consequence

Are there any special risks associated with genetic engineering?
What are the socio-economic effects in the Developed and Developing Worlds?
Is there a loss of biodiversity?
Are commercial interests driving biotechnology solely for profit?
Are ethical/moral values being eroded (societal gradualism)?

References

[1]Hume, V. (1748). An Enquiry Concerning Human Understanding. sect. 12., pt. 3. "If we take in our hand any volume; of divinity or school metaphysics, for instance, let us ask, *Does it contain any abstract reasoning concerning quantity or number?* No. *Does it contain any experimental reasoning, concerning matter of fact or existence?* No. Commit it then to the flames; for it can contain nothing but sophistry and illusion".

[2]Hillman, J.R. & Wilson, T.M.A. (1995). Delivering the Promise - Plant Biotechnology. *Science in Parliament* 52, 7 - 12.

Plant genetics

John E. Bradshaw & Ronnie J. McNicol

Crop improvement by scientific breeding methods requires a sound knowledge of the inheritance of economically important traits. Plant Genetics research at SCRI aims to acquire such knowledge and use it to develop faster, more efficient, and novel breeding methods based on genotypic selection. Equal importance is attached to demonstrating that new methods work in practice and to releasing new cultivars so that growers and consumers can benefit from improvements in yield, quality and resistance to pests and diseases.

In September 1995, the EAPR pathology section held a conference in Dublin to mark the 150th anniversary of the first record of late blight in Ireland. One of the SCRI contributions reviewed progress over the last 75 years in breeding for resistance to late blight, first at the Scottish Plant Breeding Station (SPBS) and latterly at SCRI. Likewise, the Potato Cyst Nematode Review Meeting organised by SOAEFD in Edinburgh in February 1996 prompted a review of 45 years of progress in breeding for resistance, a shortened version of which is given on p. 30. Both reviews provided examples of the importance of the Commonwealth Potato Collection (CPC) as a source of resistances lacking in European potato cultivars, of the contribution of SCRI in providing resistant cultivars, and of the likely future need for new resistances as the durability of existing ones can not be guaranteed. The CPC is held at SCRI. Dr Gavin Ramsay was appointed as its new curator in September 1995, just in time to welcome Professor J.G. Hawkes, one of its initiators, on a visit to check accessions. The 1994 Inventory of the Collection is now available on the World Wide Web (http://www.scri.sari.ac.uk). The CPC also provided the raw materials for a programme to adapt primitive cultivated South American species of group *Phureja/Stenotomum* to long day north European conditions. The products of this research have potential as alternative food crops for the UK, as described on p. 34, as well as for widening the genetic base of European potato cultivars with respect to cooking quality and possibly soft rot and powdery scab resistance.

It was also clear from the disease reviews that the history of breeding for resistance has been very much one of transferring resistance genes from wild and primitive cultivated species into clones with high marketable yield and table or processing quality. Whilst these transfers were done quickly in terms of number of generations, they did sometimes take up to thirty years. In future, it should be possible to locate and monitor the transfer of resistance genes through their linkage to molecular markers, and to accelerate the whole process by selecting for markers associated with

resistance and against markers associated with undesirable traits from the wild species. It should also be possible to use genetic transformation to incorporate natural resistance genes from virtually any source directly into an otherwise commercially acceptable potato cultivar, as well as to engineer novel forms of durable resistance. In order to explore and develop these exciting possibilities, vacancies in Crop Genetics have been filled with new staff expert in molecular genetics and cytogenetics to complement the skills of existing staff in applying quantitative genetics and selection theory and tissue culture techniques to plant breeding. Indeed, recognition of the importance of these existing skills for effective and efficient potato improvement led to the secondment of the Head of Crop Genetics, Mr George Mackay, to the International Potato Centre (CIP) in Peru for a year from June 1995. Such skills also resulted in SCRI continuing to attract commercial funding for targeted potato breeding programmes.

An internal review of faba bean research at SCRI was completed by Dr Gavin Ramsay on 1 September 1995 and concluded that genetical linkage studies had the greatest potential for impact on breeding. This stimulated an increased effort on the faba bean genetic map, in collaboration with colleagues in Cellular and Molecular Genetics, and the results are presented on p. 38. In contrast, genetic transformation of *Vicia faba* remains an intractable problem.

Commercialisation of the products of the forage brassica breeding research done from 1977 to 1989 continued with the addition of the kale Caledonian, the forage rape Interval, and the turnip Massif, to the UK National List early in 1996. They are being marketed by Sharpes International Seeds Limited along with the swedes Brora and Invitation.

A Department of the Environment funded programme on the population dynamics of feral oilseed rape under present land management practices was completed in March 1996 and the results are presented on p. 40. They should prove valuable for further work aimed at assessing some of the perceived risks associated with the release of genetically modified oilseed rape.

The soft fruit breeding and genetics programmes continue to build on their long-standing past successes. Since 1969, the combined strawberry, raspberry, blackberry, hybridberry and blackcurrant programmes have released a total of 39 cultivars (Table 1). However, any assessment of the quality of these outputs should not be solely based on the numbers of cultivars released but on the value placed on them by industry. It is estimated that SCRI-bred cultivars account for more than 95% of the Scottish and 70% of the UK raspberry, 80% of the UK and in excess of 50% of the world blackcurrant, and 8% of the UK strawberry areas. This does not, however, take any account of the very considerable contribution that SCRI germplasm has made to the parentage of cultivar releases from other breeding programmes throughout the world in recent years.

The appropriately named Glen MARS series of raspberries (Glen Magna, Glen Ample, Glen Rosa and Glen Shee) released into commerce in 1994/95 are continuing to perform well, with demand for planting stock exceeding supply. The earliest commercial plantations of these cultivars are now starting to come into production and are fulfilling all the early promise shown in trials. Glen Ample has been attracting most interest, mainly because of its good eating quality,

Crop	Cultivar	Year of release	Where named
Red Raspberry	Glen Clova	1969	UK
	Glen Moy	1981	"
	Glen Prosen	1981	"
	Glen Lyon	1991	"
	Glen Garry	1990	"
	Glen Magna	1994	"
	Glen Shee	1994	"
	Glen Rosa	1994	"
	Glen Ample	1995	"
	Glen Yarra	1993	Australia
Purple Raspberry	Glencoe	1989	UK
Blackberry	Loch Ness	1988	UK
Hybridberry	Tayberry	1978	UK
	Tummelberry	1984	"
Blackcurrant	Ben Lomond	1975	UK
	Ben Nevis	1975	"
	Ben More	1979	"
	Ben Sarek	1983	"
	Ben Alder	1988	"
	Ben Tirran	1990	"
	Ben Connan	1993	"
	Ben Loyal	1994	"
	Ben Rua	1990	New Zealand
	Ben Ard	1990	"
	Ben Eder	1990	"
	Ben Lincoln	1990	"
	Ben Mac	1990	"
	Ben Mapua	1990	"
	Ben Nora	1990	"
	Ben Thomas	1990	"
	Ben Tron	1993	Scandinavia
Strawberry	Saladin	1977	Scandinavia
	Silver Jubilee	1977	"
	Tantallon	1977	"
	Troubadour	1977	UK
	Rhapsody	1988	"
	Melody	1992	"
	Symphony	1994	"
	69DK60	1992	Australia

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

exceptionally high yields, and very wide environmental adaptation, which resulted in it being the best cultivar in trials in Scotland, England, France, Canada and the USA (Washington State).

The 1995 fruit season was very difficult for strawberry growers, with low temperatures and wet conditions being experienced at flowering. This resulted in a large proportion of the crop being small fruited and suffering severely from malformation. Some growers sustained grading out losses in the region of 70% of their crop. However, the newest SCRI strawberry cultivar, Symphony, does not show tip malformation under such conditions. Nor does splitting of the skin around the neck of the fruit occur after rain as happens to Elsanta, which is the dominant cultivar within the UK at present. Symphony also received two honours during the year. The first was an Award of Garden Merit by the Royal Horticultural Society and second was that it was the first UK-bred cultivar to be given preliminary approval by the largest retailer of strawberries in the UK.

Two new blackcurrant selections, C1 and F4, were released to industry for large-scale commercial propagation and ultimate evaluation. They have both performed well in extensive trialling over a number of years, and have given consistent crops under a range of conditions. Both are highly suited to the juicing market, with strong colour and high levels of ascorbic acid. In addition, F4 is resistant to reversion disease, while C1 has reduced susceptibility to blackcurrant gall mite. For the first time in the release of a new blackcurrant, tissue culture has been used on a major scale to ensure that planting stocks reach commerce quickly.

Reorganisation within the Institute has seen the fruit team strengthened by the transfer of two mycologists, two virologists and four entomologists (two with molecular specialisms) to the Department, while being contained within a designated Research Unit of Fruit Pathology. The objectives are to further improve coordination of research projects and achieve efficiency savings in the use of resources. Another development has been the incorporation of an ADAS fruit technology officer within the Department. In combination with established links with advisory officers from SAC, these developments were designed to increase the two-way flow of information between farmer and scientist, and aid technology transfer.

Amongst many other notable achievements, the Institute planted out the first field trial in Europe of any genetically modified fruit crop. The trial contains plants of the SCRI strawberry cultivars Melody, Rhapsody and Symphony, which have been modified to contain the cowpea protease inhibitor gene (CpTi), using techniques pioneered at SCRI. This gene has been shown in our glasshouse trials to control vine weevil infestations in strawberry. The trial has been designed to monitor not only the effectiveness of the inserted gene in a field situation, but also to ascertain in a carefully controlled manner, any other effects, beneficial or harmful, that the technology may have on other organisms.

Breeding potatoes at SCRI for resistance to potato cyst nematodes

J.E. Bradshaw, M.F.B. Dale & M.S. Phillips

Introduction The European cultivated potato, *Solanum tuberosum* subsp. *tuberosum*, was derived from a narrow genetic base of two introductions of subsp. *andigena* from South America in the late 16th century, and possible further casual introductions in the 17th and 18th centuries. As a consequence, it lacked genes for adequate levels of resistance to a number of pests and pathogens which became problems once it had assumed its role of a staple food crop. Fortunately, by the time that the potato cyst nematode (PCN) was becoming a serious problem in the UK in the early 1950's, Central and South America had been recognised as the centres of origin and diversity of the tuber-bearing members of the genus *Solanum*, and hence the primary sources of genes for disease and pest resistances lacking in European cultivars. There were many collecting expeditions to these areas after the pioneering Russian visits in the 1920's, including those of Balls and Hawkes who, in 1939, initiated the Commonwealth Potato Collection (CPC). This unique germplasm collection is held at SCRI and has proved a valuable source of resistances to PCN.

As a successful potato cultivar must combine a high marketable yield with good quality, the history of breeding for PCN resistance has been very much one of transferring resistance genes from wild and primitive cultivated species into clones with good agronomic and table or processing quality.

Sources of resistance successfully used in breeding at SCRI The first source of resistance to be used successfully came from a CPC accession (CPC 1673) of *Solanum tuberosum* subsp. *andigena*. It proved to be a simply inherited dominant major gene which was named H1 and which was effective against what are now known as pathotypes Ro1 and Ro4 of *Globodera rostochiensis*. Following a cross between CPC 1673 and cultivar Kerr's Pink in 1952, it took three further backcrosses to the European cultivated potato, with selection for commercially desirable traits as well as for PCN resistance in nematode-infested soil, before Pentland Javelin was released from the Scottish Plant Breeding Station (SPBS) in 1967. The Plant Breeding Institute (PBI), Cambridge, had achieved the same feat a year earlier with Maris Piper. At SPBS, Pentland Javelin was followed by Pentland Lustre and Pentland Meteor in 1968 and 1970, respectively. Thus, it had taken four generations spread over 15 years to achieve a commercially successful cultivar with PCN resistance.

Having incorporated the H1 gene into a number of cultivars and breeding lines, these can be intercrossed in a breeding programme and offspring sought with two copies of the gene, through test crosses to a susceptible line. These duplex lines can, in turn, be intercrossed and offspring sought with three or four copies of the H1 gene. For example, clone 14981AC8, with three copies of H1, has just completed its second year in National List Trials. Whilst only one copy is required for resistance, such clones are extremely useful as parents in a breeding programme because all of their progeny are resistant even when the other parent is susceptible, thus avoiding the need to screen the progeny for resistance or waste resources on raising susceptible seedlings (Fig. 1).

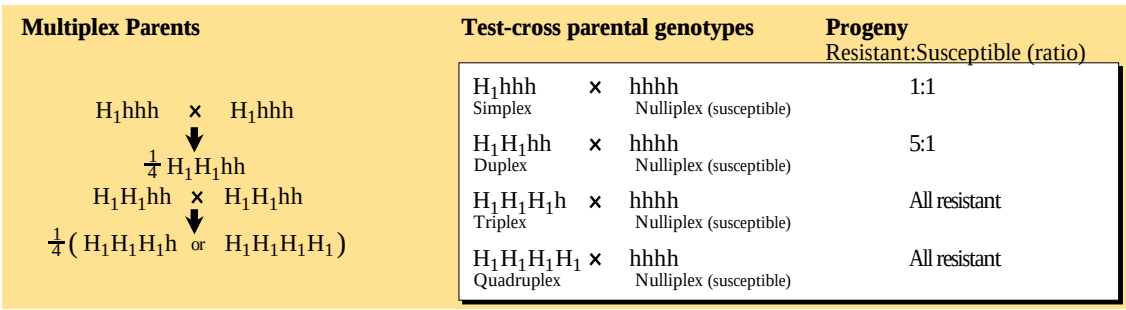

Figure 1 Procedure for producing multiplex resistant parents.

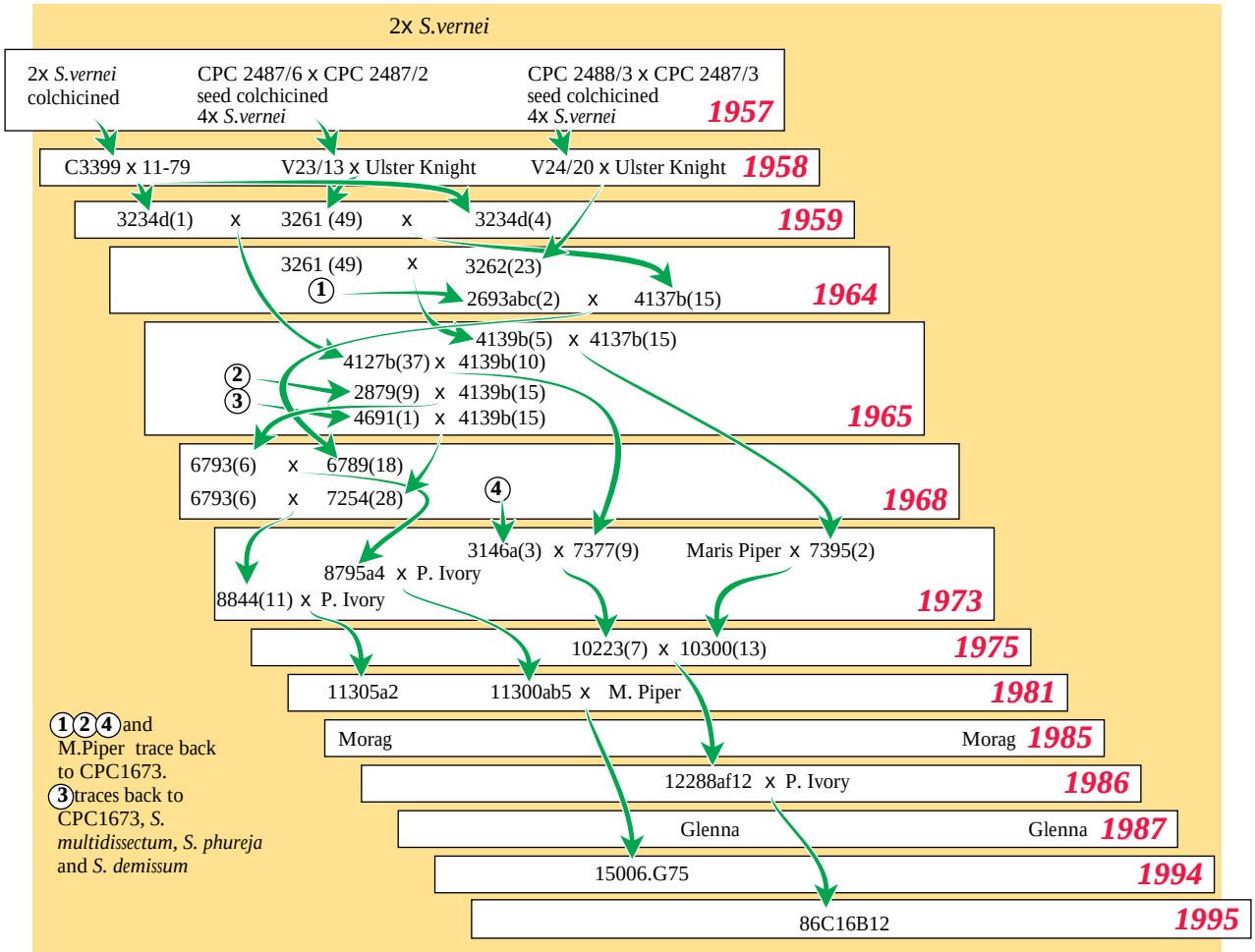

Figure 2 Pedigrees of SCRI cultivars with PCN resistance from *S. vernei*.

The H1 gene has provided an extremely high level of resistance which is effective at reducing the size of PCN populations. However, as PCN larvae are inhibited in potato roots at various stages of development prior to cyst formation, tolerance of root damage such as found in the Irish cultivar Cara is also required, and appears to be inherited independently of the H1 gene. The resistance has proved durable in the UK where Ro1 appears to be the only pathotype, but pathotypes such as Ro3, which can overcome H1, have been found on the Continent.

The population of PCN from Duddingston, near Edinburgh, which was found by Dr J.M. Dunnett in 1956 to overcome the H1 gene, subsequently became known as pathotype Pa1 of *G. pallida*. Dr Dunnett quickly found a major gene (H2) which confers resistance to Pa1 (but not Ro1) in the diploid wild species *S. multidissectum*, and showed that it could be transferred to *S. tuberosum* through unreduced female gametes. The H2 gene has not been incorporated into any SPBS/SCRI cultivars, and cultivars like Corsair from PBI have not been widely grown. Anyway, PCN populations which could overcome the H1 and H2 genes were soon found, and proved to be what is now known as pathotype Pa2/3 of *G. pallida*. As a result, the breeding effort turned to another species for resistance, namely *S. vernei*. This diploid wild species from South America was confirmed as having quantitative resistance to both *pallida* and *rostochiensis* by Dr Dunnett during 1957 and 1958.

Colchicine treatment produced tetraploid plants of *S. vernei* which were crossed with *S. tuberosum* subsp. *tuberosum* in 1957 and 1958. As the resistance was not simply inherited, the resulting hybrids were intercrossed (to bring different resistance genes together) and also outcrossed to other cultivars and clones for four generations with selection for PCN resistance and other desirable traits, before cultivars Morag and Glenna were released in 1985 and 1987, respectively. Overall, it took five generations spread over 28 to 30

years to produce commercially acceptable clones. SCRI's most recent NL submissions with quantitative resistance from *S. vernei* were 15006.G75 in 1994, from a cross between a close relative of Morag and M. Piper, and 86C16B12 in 1995, from a cross between Glenna and P. Ivory. Santé from the Netherlands and Nadine from Caithness Breeders are other examples of cultivars with PCN resistance from *S. vernei* (Fig. 2).

The level of partial or quantitative resistance now being achieved is around the 95% effective level required to halt an increase in PCN populations with a twenty-fold multiplication rate, but aggressive populations, such as one from Luffness near Edinburgh, are known, to which cvs Morag and Santé, but not Glenna, are susceptible. Furthermore, tolerance such as occurs in cv. Glenna is also required and appears to be inherited independently of resistance.

The third main source of resistance to be successfully incorporated into the European potato was quantitative resistance to *G. pallida*, but not to *G. rostochiensis*, from *S. tuberosum* subsp. *andigena* (CPC2775 and CPC2802). This source is known as H3 because it was incorrectly thought to be due to a single major gene. Starting in 1969, it took just two generations, but 22 years as part of a routine breeding programme, to obtain the commercial cultivar Eden. Breeding line 12601ab1 has even better resistance than Eden, with cyst numbers in the range 3-7% of susceptible control cultivar Désirée with both Pa2/3 and the Luffness population. It is also tolerant to PCN. Therefore, when H3 is combined with the H1 gene, as in Eden and 12601ab1, a high level of resistance to both *G. pallida* and *G. rostochiensis* is obtained.

Combining resistances already available with other desirable traits In the immediate future at SCRI, the aim is to combine the resistances which are already available in breeding lines and cultivars of *S. tuberosum* with other desirable traits. As already implied, the H1 gene can be incorporated readily into new cultivars. Indeed, the success of such cultivars in controlling *G. rostochiensis* has probably encouraged the spread of *G. pallida*. Hence, recent attention has been focused on combining quantitative resistance to *G. pallida* as quickly as possible with quantitative field resistance to late blight and commercially acceptable tuber yields and quality. At the start of a new breeding programme in 1991, it was possible to confirm that prospects were good for achieving these objectives. Use was made of seedling progeny tests which had been developed and validated at SCRI for PCN, foliage and tuber blight, and visual assessment of the commercial potential of tubers (Ann. Rep. 1991, 13-16). These tests were done within a year of making crosses from a mating scheme which gave genetical knowledge of direct relevance to the programme (Fig. 3). Large amounts of variation due to differences in parental general combining abilities (GCAs) were found for PCN and the other traits, and this must be indicative of much additive genetic variance in the population. Furthermore, no adverse correlations were found between traits. Hence, continued progress can be expected over a number of generations of multitrait genotypic recurrent selection, which has been shown can be operated on a three year cycle (Ann. Rep. 1994, 36-39), so as to include tuber progeny selection for resistance to after cooking blackening and to low temperature sweetening. Each cycle, new cultivars can be sought over a number of clonal generations from the clones evaluated as tuber progenies at the Institute's seed farm, and from resowings of more true seed of the very best progenies.

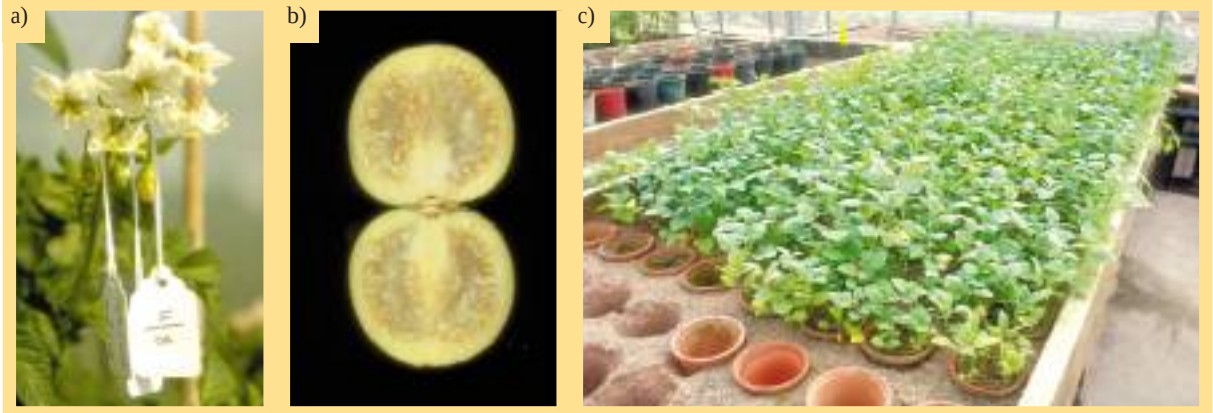

Figure 3 PCN seedling progeny test a) Crossing, b) Seed in berry, c) The test.

Figure 4 Container test for resistance to PCN.

Clonal tests for PCN resistance, developed at SCRI by Phillips, Forrest and Farrer, allow the rapid and reliable assessment of large numbers of individual potato genotypes in clear closed containers. Replicate tubers of each genotype are planted in separate 60 ml Clearopac containers containing soil and potato cyst nematodes (*G. rostochiensis* or *G. pallida*). The numbers of cysts visible through the transparent walls of the containers are counted after 7 weeks to determine the level of resistance (Fig. 4). Clonal material can be appraised at any stage of a breeding programme. At SCRI, the test has been adapted for screening tubers from the glasshouse-grown seedlings during the first year of multiplication, thus allowing breeders to shift the level of resistance in the population very quickly and efficiently. Individual genotypes then have their resistance confirmed in later clonal generations, usually two or three years after the initial test.

In due course, those clones with the greatest potential will be evaluated more extensively in collaboration with commercial partners, who will then develop the best as finished cultivars. Overall, this is a very efficient way of combining the short term need for new cultivars with continued longer term progress.

Clones with good GCA have been identified from this work for use as parents in further breeding, and such parents can and are being made available for a range of commercially-funded breeding work through Mylnefield Research Services (MRS) Ltd, the commercial wing of SCRI. Clone 12601ab1 has very good GCA for resistance to *G. pallida*, just as cv. Stirling has very good GCA for quantitative resistance to late blight. The cross between Stirling and 12601ab1 has, therefore, been chosen for a more detailed genetical study, funded by the PMB and in collaboration with colleagues in Cellular and Molecular Genetics, in which the genes for PCN and for late blight resistance are being sought through their linkage to molecular markers. This should allow their more efficient manipulation in a breeding programme, and lead to pathologists finding out more about what the genes do, and hence the likelihood of durable resistance.

Seeking and utilising new sources of resistance

Although the H1 gene for resistance to *G. rostochiensis* and the quantitative resistances to *G. pallida* should remain useful in the UK for the immediate future, in the longer term new sources of more durable resistance are almost certainly going to be required. Work described elsewhere in this Annual Report is in progress at SCRI studying the variation in virulence between and within PCN populations from Europe and South America, so that the most appropriate PCN populations can be used in the search for resistances. Further screening of the CPC has already identified new resistances in the following diploid species from S. America (*S. boliviense*, *S. chacoense*, *S. kurtzianum*, *S. megistacrolobum*, *S. sanctae-rosae*, *S. sparsipilum*). Screening of the recently incorporated Birmingham Collection of Professor Hawkes is planned.

The usual first step in the transfer (i.e. introgression) of resistance genes from a wild species is its successful hybridisation with the cultivated potato. The commonest barrier to crosses between tetraploid potato cultivars and closely related wild species is embryo-abortion following endosperm collapse, a phenomenon associated with differences in effective ploidy, called endosperm balance number (EBN), rather than in ploidy *per se*. This has been overcome by bridging crosses, by embryo rescue, and by equalising the parental EBNs through manipulating ploidy levels in various ways. However, tetraploid cultivars and diploid wild species can be somatically hybridised by protoplast fusion. A range of somatic hybrids between *S. megistacrolobum*, *S. sanctae-rosae* and *S. sparsipilum* and cultivar Brodick have been secured by electrofusion in a recently completed PMB-funded research project (Fig. 5). Hybrid status has been confirmed by a combination of molecular, morphological and cytological analyses. The range of chromosome numbers in the three regenerant populations was consistent with extensive chromosome elimination during the regeneration process, resulting in a range of aneuploid regenerants (2n = 52 - 71). Their morphological affinity to cv. Brodick suggests that the wild species chromosomes were lost preferentially to those of *S. tuberosum*. A further smaller sub-group of regen-

Figure 5 Somatic hybrids between *S. tuberosum* cv. Brodick and (l to r) *S. megistacrolubum*, *S. sanctae-rosae* and *S. sparsipilum*.

erant hybrids consisted of tetraploid plants. Although a proportion of these may be cv. Brodick affected by somaclonal variation due to the tissue culture procedures used, preliminary molecular analyses indicated that some of these regenerants still contain DNA from the wild species. Fourteen of these tetraploid plants exhibited varying levels of resistance to *G. pallida* (Pa2/3) in preliminary tests. Whilst protoplast fusions may not lead directly to improved cultivars, they should allow the more rapid incorporation of new sources of resistance into the *S. tuberosum* gene pool. Furthermore, once the introgression of resistance genes can be monitored through their linkage to molecular markers, the whole process can be further accelerated by selecting for markers tightly associated with PCN resistance and against those markers associated with undesirable traits from wild species. This will be particularly useful for polygenic resistance.

A major way ahead for breeding is the use of genetic transformation to incorporate natural or even synthetic resistance genes from virtually any source directly into an otherwise commercially acceptable cultivar. The use of *Agrobacterium tumefaciens* Ti plasmid-mediated transformation is available in potato and has been used to demonstrate that transgenic plant resistance to viruses works. It may, however, prove more difficult with PCN to isolate natural genes or to engineer novel forms of resistance which confer durable resistance to PCN. Nevertheless, these approaches are being given serious consideration at SCRI, as are the risk assessment experiments which will be required before the full commercial exploitation of transgenic material can take place.

The adaptation and use of primitive cultivated diploid potato species

M.J. De,Maine

Breeders frequently use primitive relatives of modern crop plants to introduce genes for resistance to pests and diseases. It is unusual to introduce one of these relatives as a new crop plant in its own right. The golden potato, *Solanum phureja*, from South America has already been a source of genes for modern potato cultivars but could be about to make its mark as a speciality vegetable.

The species is cultivated in the eastern lower Andean regions of Colombia, Bolivia, Venezuela, Peru and Ecuador. In Colombia it is known as 'Yema de Heuvo' (Egg Yolk) because of its deep yellow flesh colour. Unlike the potato grown in Western Europe and North America, it is cultivated as a mixture of clones and not as a single clonal cultivar. In the capital, Bogota, it is sold fried in pieces as the delicacy 'papa criolla'. The skin can be a variety of different colours including red, purple, yellow and white, with patterns common such as spectacles (presence or absence of pigment restricted to around the eyes), pink or lilac flushes and colour splashes (Fig. 1). It

Figure 1 Tubers of *Solanum phureja* clone showing white spectacles on red background.

has a stronger potato flavour than tubers of the common potato species *S. tuberosum* and the cooked flesh is soft, dry and floury[1]. Dry matter content can be high (about 27% compared with 20% for common potatoes) although lower dry matter clones also have the floury flesh texture. Flesh colours range from the common yellow to whites, reds and purples (Fig. 2). The stability of the red and purple-fleshed types varies on cooking, however, and the pigment may degenerate to an unappetising grey. Tubers are smaller than those of *S. tuberosum* and can be a variety of shapes from round to long and sausage-like. They are usually rougher and have deeper eyes. As its native growing season is equatorial, the life cycle of *S. phureja* is adapted to short daylengths of approximately twelve hours duration. It will only produce tubers, therefore, under short day conditions.

In the 1950s, Prof. Norman Simmonds, then working at the John Innes Research Institute, was interested in the modification of wild and primitive cultivated species using mass selection techniques. By studying this, he hoped to learn how such species could be adapted to produce crop plants useful to modern man and, at the same time, increase the gene pool available to breeders of their modern derivatives. Mass selection techniques have been used by man ever since the beginning of agriculture. From amongst a wild population of plants, the highest yielding (and least toxic) would be selected. Some of the produce would be eaten and what was left would be planted near the homestead. The plants would grow, flower and set seed. Seed would be sown and the best from the new population would be replanted and so on. Gradually, because the parents used to produce the next generation improved with each cycle, the overall yield and quality of produce would increase year by year. It was a simple, benign method of breeding with the advantage that it retained a large amount of genetic variation within the population. Simmonds was concerned that pedigree breeding methods, whereby families are bred from a few selected lines, could lead to a depletion of the gene pool available to modern breeders. The repercussions of this would only be felt in the future, as resistances to diseases and pests broke down and replacement genes were unavailable because they had been lost from the population.

Figure 2 *Solanum phureja* clones showing a range of skin and flesh colours.

Another use of such populations was as a direct source of material for other breeding strategies. At any stage, individuals could be drawn from a population and used, in pedigree breeding for example, while retaining the original gene bank. The population was being continuously improved by mass-selection and so became a pool of increasingly 'tamed' germplasm. The constituent genotypes acquired more agronomically useful characteristics, which meant that intercrossing them with modern cultivated forms produced offspring with reduced wild characteristics. This would be an advantage when the species was used as a

source of genes for plant breeding purposes because it would mean that less breeding out of unwanted wild characters would be required. Usually, such breeding to expunge wild traits causes significant delays in introducing traits from wild and primitive cultivated species into commercially useful lines.

The original population of *S. phureja* was raised from seed obtained from the Commonwealth Potato Collection. Because the species was adapted to short daylengths, very few seedlings produced tubers. Tubers were taken from all those that did and planted in the field to allow random pollination by bumble bees to take place. Berries were harvested, seed extracted and the following year another seedling generation was raised from a sample of the seed. C.P. Carroll[2] took over the running of the experiment in the late 1960s. At this stage, only fifty per cent of the seedlings tuberised in the long days of the Scottish growing season. Two sub-populations were produced based on selections made on performance in the seedling and first tuber years respectively. Selection criteria were good tuber yield, size, regularity, shallow eye depth, oval tuber shape and reduced sprouting in store. In order to bias the germplasm in favour of improvements in these characters without losing too much variation, a three grade weighting was introduced into selection. Twenty to twenty-five per cent of the clones in each population were selected. Three tubers were taken from each clone in the best third of the selections, two from those in the middle third and one from each in the poorest third. All the tubers were then combined, randomised and planted out to form a new population, which would be allowed to open-pollinate in an isolation plot and be subjected to further selection at tuber harvest. Referring to Figure 3, 'seedling population 1' was the origin of tubers selected from plants in their seedling year. Those tubers were planted in the field the following year and the plants produced from them allowed to inter-pollinate. True seed was harvested from the plot and the selection of tubers, this time from material grown in its first clonal year, again carried out. The true seed was sown to produce 'seedling population 2' the following year and the selected tubers planted to form the 'ongoing tuber population'. Plants produced from this, more highly selected tuber population, were allowed to inter-pollinate and the seed harvested sown to produce another 'seedling population 1'. Tubers selected from seedling populations 1 and 2 were brought together and planted to form the 'combined first (clonal) year tuber population' to complete the cycle. Using this method, it was found that the percentage of seedlings producing tubers under long days had reached 90% after two full cycles.

One of the characteristics of unadapted *S. phureja*, even that cultivated in South America today, is that the tubers sprout at harvest. This low dormancy was originally selected for by the Indians as it meant that, in their climate, two or three crops could be grown per year. At SCRI, sprouting at harvest was selected against and material which is only moderately worse than some of the early sprouting *S. tuberosum* cultivars for dormancy break in store can be found in the population. Tuber yield and mean tuber weight is less than for *S. tuberosum* with yields at 50 to 60% although tubers are substantially larger than the golf ball sizes produced by crops grown in S. America. The lower yield of *S. phureja* could be ameliorated by growing more than one crop per year in the warmer parts of the UK under polythene.

The *S. phureja* mass selection scheme has continued to the present day, with a break between 1987 and 1994. Almost all seedlings raised now produce tubers under long days. The species is a source of resistance to potato viruses Y and Leaf Roll, Common Scab (*Streptomyces scabies*) and possibly Soft Rot (*Erwinia* spp.). As well as being a source of genes for incorporation into *S. tuberosum*, *S. phureja* has also aroused interest from commerce as a speciality vegetable in its own right. Three selection programmes are currently underway involving partnerships with potato growers and wholesalers, supermarkets and seed suppliers.

S. phureja is not a potato which can be treated like any other. Because of its soft flesh it may break down when boiled or steamed. In South America, this char-

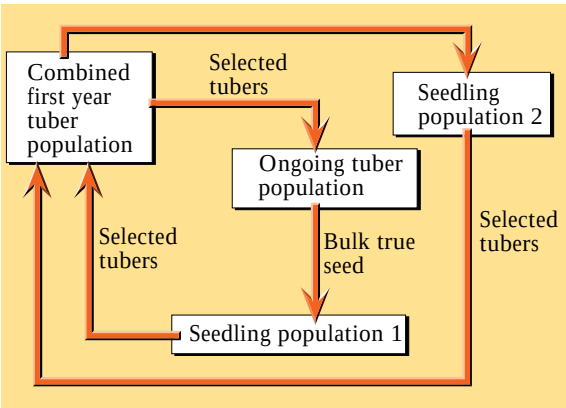

Figure 3 Selection cycles in the diploid mass-selection scheme.

acteristic is exploited by using it in soups and stews. Also, because of early dormancy break, it must be used soon after harvest. These characteristics suggest that it is probably best suited to processing prior to offering it to the housewife at the point of sale. Experiments have been carried out at the Duncan of Jordanstone College, Dundee to look at different possible processing methods. *S. phureja* was found to produce good fried products such as French Fries and crisps. It also baked well in a conventional dry oven or a microwave. Possible options for processing, therefore, are frozen par-cooked French Fries or whole-baked. Consumer acceptance trials carried out in the Jordanstone training restaurant with 26 members of the public found that *S. phureja* chips were preferred to a proprietary brand of *S. tuberosum* frozen chip by a ratio of almost three to one. Also, ninety per cent of fifty school children visiting the College for an open day preferred *S. phureja* crisps to those produced from a *S. tuberosum* cultivar (Pentland Dell).

There are nine edible potato species and sub-species, of which only one (*S. tuberosum* ssp. *tuberosum*) is cultivated in Western Europe and North America. Besides the common potato, all these are cultivated by peasant farmers in South America and would not normally be available to western consumers. In addition, the South American Indians cultivate varieties of some wild potato species and all are regarded as delicacies eaten to introduce different tastes and textures into the diet. Clearly, some of these could be of interest to Europeans. As well as the *S. phureja* population, therefore, small populations of two other diploid cultivated species have been raised from seed from the Commonwealth Potato Collection. They are *S. goniocalyx* ('the original golden potato of Peru') and *S. ajanhuiri*. *S. goniocalyx* produced very small tubers from only ten per cent of seedlings whereas *S. ajanhuiri* produced tubers from seventy per cent. *S. ajanhuiri* seedlings usually produced moderate yields of tubers which were often surprisingly large. They were generally round, showed chain growth (i.e. a series of tubers on the same stolon), were rough and irregular in shape and many were sprouting at harvest. Cooking tests will be carried out on material from subsequent generations when tuber characteristics have been improved.

Of the nine taxa of potato which are cultivated, four are diploid and the remainder polyploid. The diploids are *S. phureja*, *S. stenotomum*, *S. goniocalyx* and *S. ajanhuiri*. These are sexually self-incompatible whereas tetraploids, including *S. tuberosum*, are self-compatible. Diploids can be allowed to cross-pollinate naturally, mainly by bumble bees, whereas selfing would occur in tetraploids such as *S. tuberosum* and could lead to a reduction in vigour of the population. Diploid potatoes, therefore, are likely to be more amenable to the maintenance and gradual improvement of a population by the mass selection techniques described. They are also able to produce hybrid true (botanical) potato seed (tps) without the emasculation and hand-pollination required with tetraploids such as the common potato. A mixed planting of two selected parent clones will produce F1 hybrid seed by natural means, again with bumble bees as the main pollinating agents. Berries could be harvested from the whole plot and pulped to extract the seed by sedimentation in water. It is often said of the produce of 'tps' that variation in cooking quality, in particular, would make it unacceptable to European and North American consumers. Tests found that tubers of some true seed progenies of *S. phureja* gave no greater variation in cooked flesh hardness than tubers from clonally propagated *S. tuberosum* cultivars.

By careful selection of the parents of a 'tps' family, it would be possible to produce tubers with low variation in cooking quality but which had an assortment of skin colours. These could be of interest to the consumer and underline the novelty aspect of the crop. The peasant farmers of South America have always grown mixtures of potatoes in their plots and market stalls are piled with tubers of a wide variety of shapes, sizes and colours.

The use of primitive cultivated and wild potato species could, therefore, be developed further than acting as a source of useful genes for the common W. European crop plant. There is potential for introducing a number of niche crops. How those crops are presented to the public at point of sale is yet to be resolved but it is likely that some form of processing will be required to overcome problems of storage and cooking characteristics.

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[2]Carroll, C.P. (1982). *Journal of Agricultural Science, Cambridge* 99, 631-640.

Dissecting the *Vicia faba* genome

G. Ramsay, D.W. Griffiths, R. Waugh & W. Powell.

Faba beans (*Vicia faba* L.) are an important crop to the UK. Currently grown on about 120,000 ha, they cover a greater area than peas, the other major pulse in the UK, and are worth about £80m at the farm gate. The crop is primarily used for protein for animal feed, but large seeded types, known as broad beans, are also an important vegetable for freezing and garden use. The UK is the second largest producer, exceeded only by China, and produces about 10% of the world's faba beans.

Despite its importance to UK agriculture, and its long use as a favourite experimental organism, knowledge of genetics and genetic linkage in this species lags behind that for many other important crops. Studies on genetic linkage are very useful for determining the number of, and relationships between, genes controlling a wide range of traits. The information generated can guide the efficient selection of traits of interest to the breeder by i) clarifying the genetic control, including the number of loci with an influence on the trait, ii) helping identify the types of interactions between different traits, and iii) detecting genetic linkage which could either render the selection of desired genotypes more difficult or provide the breeder with tools to make selection more efficient.

Two types of faba bean population, a backcross population (BC) and a set of recombinant inbred lines (RILs) from the cross between an Afghanistan landrace (*Vicia faba* var. *paucijuga* '172') and cv. Optica (*V. faba* var. *major)* were developed and used for mapping (Fig. 1). Each population type has its advantages and drawbacks. The BC population has the advantage of only one round of recombination (in the F1 plants, on the paternal side in this case). This type of population, genetically identical at the BCF1 to a doubled haploid, can be used to detect distant linkages sensitively, but has the disadvantages of the difficulty of scoring markers which are recessive in the allelic form from the recurrent parent, and segregation in further generations which raises problems of maintenance and exploitation. RILs, on the other hand, are almost completely genetically fixed and can be propagated indefinitely and used in replicated experiments. The paucity of heterozygotes allows dominant alleles of all types to be used and renders complex traits more easily studied by eliminating intermediates. Although the increased recombination occurring in the generation of RILs (approximately four-fold compared to a BCF1) permits greater accuracy in the placing of closely linked markers, it also has the deleterious effect of reducing the ability to discern the linkage between distant markers.

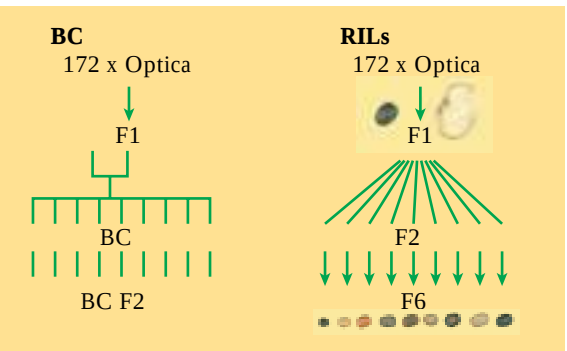

Figure 1 Scheme of construction of segregating populations.

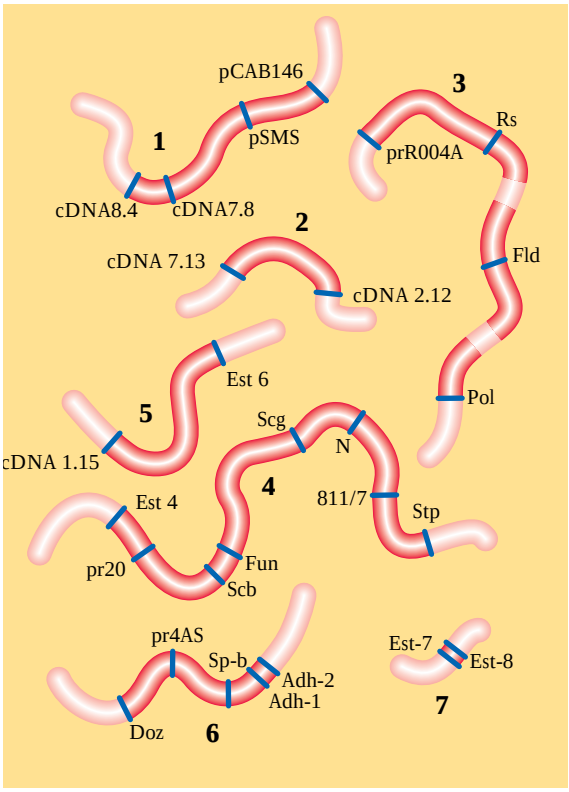

Figure 2 A partial linkage map for faba bean.

Genetic marker scores have been obtained from both types of population. In the backcross population, pools of samples were made in families from 100 BCF1 plants. Thirty nine markers were scored on 100 BC families and of these 22 were linked. In addition, some extra morphological and isozyme markers were scored on the RILs and a few of them are included in the partial map presented here (Fig. 2). This linkage map is over 300cM. Estimates of the expected size of the complete linkage map from knowledge of chiasma frequency would suggest that this covers less than a half of the genome. However, a much more detailed map using highly informative assays such as anchored microsatellite primer PCR and AFLPs is in preparation. The markers used to create the map shown in Figure 2 are RFLPs, RAPDs, isozymes and others including morphological markers. Among the latter category are a major gene affecting flowering date (*Fld*) , one controlling seed lustre *(Pol, polished*), one controlling dormancy (*Doz, dozy)* and five controlling seed or pod pigmentation, brown testa pigment, *Scb*, funicle colour, *Fun*, greyish pigments in the testa, *Scg*, hilum colour, *N*, and pod stripe, *Stp*. The last four of these genes control the distribution of melanin-like pigments derived from L-DOPA. Surprisingly, all five seed and pod colour loci are linked, indicating a clustering of genes with similar function. Although the pea genetic map is relatively detailed and genes are known which control seed lustre, seed colour and hilum colour, no obvious parallels between pea and faba bean linkage maps were detected for these characters.

Thirteen quantitative traits were scored for each BC family at the F2. This data was used in conjunction with Mapmaker/QTL to scan linkage groups for QTLs and a marker means method was used to test markers not already on linkage groups. Where a QTL influences a trait in the direction expected from the parental allele, this is shown above the line in Figure 3. These traits were of diverse types such as seed content of the antinutritional factors vicine and convicine, seed weight, yield, and the numbers and sizes of various plant parts. All linkage groups except one comprising Est-7 and Est-8 only were found to hold QTLs. All quantitative traits were associated with at least one marker; the 9 traits which were associated with linkage groups are shown in Figure 3.

The numbers of QTLs detected for each trait varied widely. Leaf width was controlled by 5 QTLs and the numbers of flowers per node by 4 QTLs. In contrast vicine:convicine ratio was almost completely controlled by one locus.

For each character, the proportion of the total variance explained by all QTLs differed. Some characters, however, were largely explained by the QTLs found. More than 60% of the variance was explained for leaf width, mean seed weight, seed yield and vicine and convicine content. The most important QTLs for these traits are clearly on this map, despite its incomplete nature.

Some QTLs may have multiple effects. Some plant parts appear to have dimensions controlled by genes at the same position as those controlling other plant parts. Leaf and pod width and length or depth, for example, can show this type of control with some sites affecting several of these traits together. These may represent single QTLs with multiple effects or alternatively may be a linked cluster of QTLs affecting related traits. There also appear to be QTLs without such multiple effects. For example, there are QTLs influencing leaf width which have no perceptible effect on leaf length.

A QTL for the depth of the pod is the only one found in these data sets to show an effect opposing that

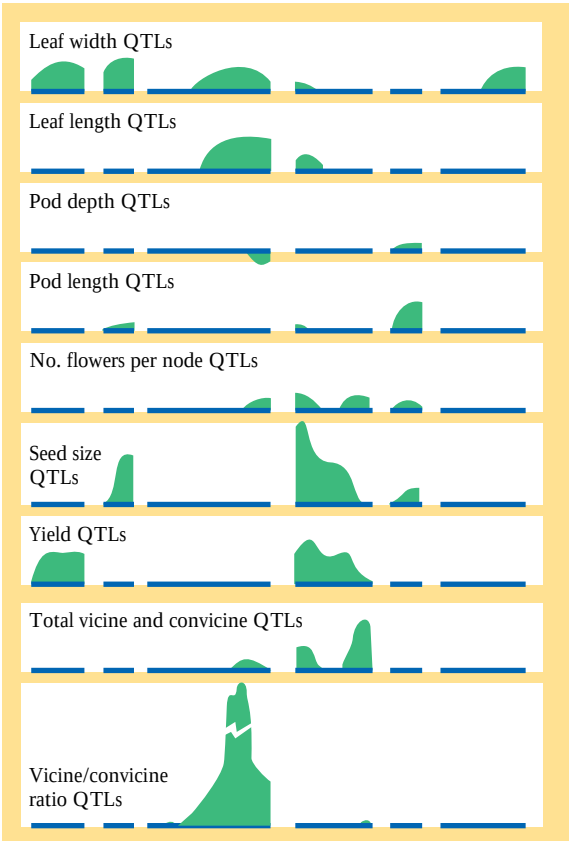

Figure 3 LOD-likelihood profiles of 9 traits in faba beans.

expected from the value of the parental line. It seems that sites influencing pod dimension tend to be more important for seed weight than leaf dimensions. This effect could be pleiotropic, with the pod directly influencing the growth of seeds within it. Alternative explanations are possible, such as clustering of linked loci as the uncoupling of the control of seed and pod sizes in segregating populations could be deleterious.

The relationship between seed weight and yield is of special interest in a breeding context. Seed weight has an extended plateau on linkage group 4, indicating that there may be several QTLs for this character on this linkage group. Interestingly, this linkage group contains genes for seed coat colour, mirroring the first report of linkage to a QTL, that between seed size and seed colour in *Phaseolus*. The profile for mean seed weight on group 4 is mirrored by a similar profile for seed yield. This effect is not surprising as it appears to be difficult to maximise yield in the smallest-seeded genotypes. Large effects on yield are also seen on group 1, showing that yield is only partially explained by seed size. The potential value to breeders of these methods is clear, permitting the efficient and controlled blending of the best QTLs.

The levels of vicine and convicine were influenced by genes at three positions in the genome. One of these has a very large effect on vicine/convicine ratio. This may represent the gene *vcr*, described from previous work on other crosses where the segregation was clear. Two further QTLs affected total amounts of vicine and convicine. All three QTLs might represent regulatory genes or genes coding for enzymes for the biosynthesis of these compounds. Mutagenesis studies have shown that there is one locus at which mutations lead to a large reduction in vicine and convicine contents. One of the two QTLs for total content of vicine and convicine appears to be the same locus, as both QTL and mutated locus are close to the gene for hilum colour, *N*. The implications of this result are that QTL studies, which by their nature are usually performed intensively in one cross, may be used to predict which markers can be used in other crosses where alleles of a more extreme type are of direct use to breeders.

Investigation of feral oilseed rape populations

Y.M. Charters, A. Robertson, J. Crawford & G. Squire

Advances in biotechnology during the 1980s have resulted in the production of genetically modified crops through the stable integration of foreign DNA into host plants. Oilseed rape is particularly amenable to such modifications and traits conferring herbicide and disease resistance, and altering the protein and oil quality, have been introduced. There are perceived to be risks and uncertainties associated with the release of genetically modified organisms and, therefore, field trials of these new cultivars are strictly controlled by national and international legislation. A principal concern is that transgenes will 'escape' the confines of agriculture and cause undesirable biological and environmental changes. Possible routes of escape include hybridisation following the movement of pollen from fields of genetically modified oilseed rape to feral oilseed rape populations.

The environmental significance of transgene movement depends on the nature of the transgene itself and on whether it can influence the factors currently limiting the size, abundance and persistence of feral populations. An understanding of the population dynamics of feral oilseed rape under present land management practices is, therefore, a pre-requisite for any study aiming to assess the impact of a transgene outside agriculture. The aim of the project was to survey the distribution of cultivated, feral and volunteer populations of oilseed rape and to investigate the structure and dynamics of feral populations, with a view to establishing their age and origins, whether they are self-sustaining, and hence whether they are likely to receive transgenes from nearby crops.

The estimation of feral population longevity, however, is made difficult by several factors. For example, populations cannot be considered extinct if they fail to produce visible plants in one year, since a potential population may still be present as dormant but living seed in the soil. Also, populations observed to re-

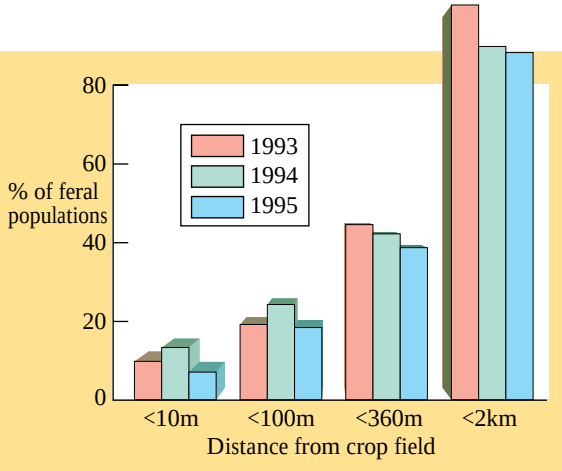

Figure 1 Distribution of data collected over a three-year period shows a substantial proportion of oilseed rape populations occur within 360 m of a cultivated oilseed rape field simultaneously in flower. Oilseed rape pollen has previously been shown to effect hybridisation over such distances.

occur at a site might be derived from quite independent sources such as pre- or post-harvest seed spillage, or from the import of soil containing oilseed rape seed. To determine whether a feral population is potentially related to one occurring previously at a site, requires the identification of the cultivars represented. Therefore, a multidisciplinary approach was adopted, combining the use of site observation (plant number and location over time), research into site history (e.g. cultivars grown in the vicinity of feral populations), and DNA fingerprinting (to assess the relatedness of populations re-occurring at a site over time).

Feral population survey A ground survey covering more than 500 km^2 of Eastern Scotland (Tayside Region) was conducted on eight occasions between 1993-1995 in order to study the distribution, size and site occupancy of feral oilseed rape populations. The following are the main conclusions. The populations were widespread with between 88 and 166 being mapped in each year. The flowering window of the ferals as a whole overlapped that of both spring and winter oilseed rape cultivations. Feral stands predominantly occurred in three habitat types: roadside verges (including embankments, central reservations and roadside herbaceous borders), field margins (including hedgerow habitats) and soil-tips and waste ground. Despite the range of land management practices employed on these sites, a substantial number of populations survived to produce viable seed. Some populations were observed to flower in three consecutive years, whilst others appeared transient or re-appeared after a year's absence. The majority of 1995 populations appeared to originate from either seed spillage, soil import or from the persistence of a viable seed bank. Many feral populations flowered within range of a source of oilseed rape pollen from a crop (Fig. 1).

Molecular analyses The identification of oilseed rape cultivars occurring at a site over time would help to distinguish between populations which have re-occurred through seed recruitment and populations which are progenitors of plants previously occurring at the site. This information can then be combined with survey and other data to assess persistence and self-sustainability of a population. Unfortunately, cultivars of oilseed rape are difficult (and individual plants impossible) to distinguish on the basis of their appearance (i.e. using morphological markers) or performance traits (such as seed yield). A molecular marker technique was, therefore, developed in order to distinguish between cultivars on the basis of their DNA fin-

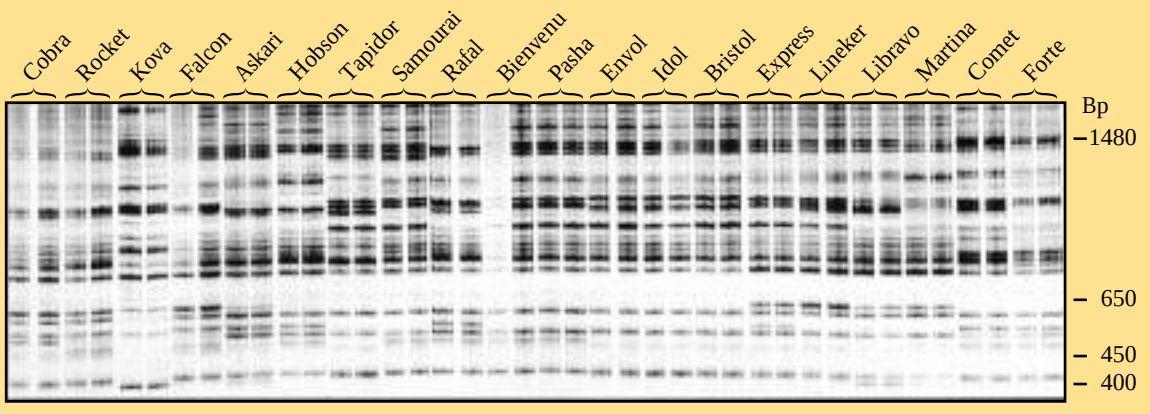

Figure 2 Between-cultivar variation shown by banding profiles from DNA samples of each of 20 cultivars using primer 888. Lane brackets indicate profiles produced by two independent PCRs.

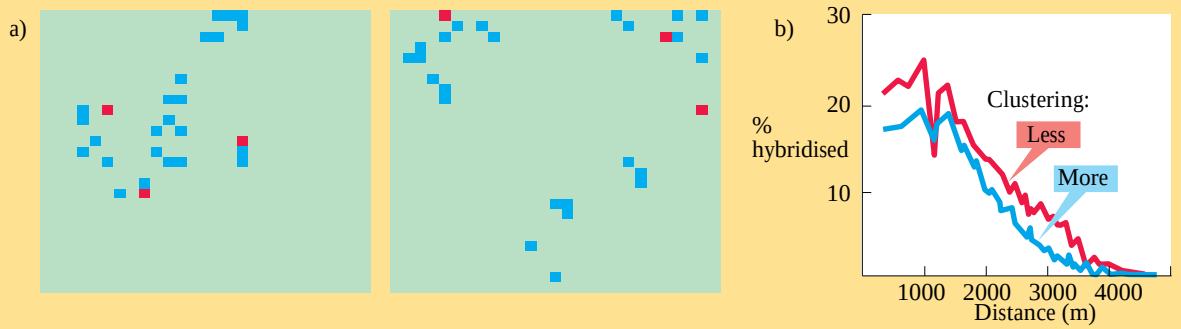

Figure 3 Simulation of transgenic seed set in feral populations. a) shows arrangements of transgenic (red) and non-transgenic (blue) fields in a landscape, the one on the left being more clumped, less dispersed; feral colonies (not shown) are randomly scattered in the background at mean field-to-feral distances as in Figure 1. b) shows the simulated average proportion of transgenic seed set in feral populations as a function of distance from the nearest transgenic field (red curve the result when less clustered, blue curve more clustered).

gerprints. The technique exploits the polymerase chain reaction (PCR) and its success partly relies on the design of the primers involved. These are complementary to simple sequence repeat (SSR) regions in the plant genome and are preceded by a short anchor sequence at their 5' end. The primers so designed target regions of the genome which are numerous and highly variable and, therefore, increase the potential to produce distinguishing fingerprints for the different cultivars.

Developmental work was conducted on 20 cultivars, the majority of which had been or still were grown in the survey area. Pooled DNA samples were extracted from 20 plants for each cultivar. These samples were investigated with two primers and the PCR products resolved into banding patterns (fingerprints) using polyacrylamide gels and a discontinuous buffer system and visualised with highly sensitive silver nitrate staining (Fig. 2). The use of one or other primer allowed 16 of the 20 cultivars to be distinguished on the basis of banding pattern differences. The combined use of both fingerprint sets allowed the distinction between all 20 cultivars using a taxonomic key. Further, more detailed, studies provided marker information that would enable identification of the cultivar to which any individual plant belonged.

This DNA fingerprinting system is now being applied to determine the cultivar-types that make up a range of feral oilseed rape populations. Initial results showed most feral populations consist of more than one cultivar, and indicate that some colonies contained plants that were last grown in the area more than five years previously.

Modelling pollen flow and hybridisation between fields and feral colonies Given that feral populations persist, it was necessary to investigate how many of them were likely to receive pollen from crops. Advanced mathematical techniques were used to construct models of the way pollen plumes from oilseed rape fields would spread and disperse over an agricultural region containing a mosaic of transgenic crops, non-transgenic crops and feral populations. The model was based on actual data of pollen density and hybridisation collected at SCRI which showed that pollen travelled up to 2 km from a source. The simulations revealed that the spread of pollen from a transgenic crop, and subsequent gene flow from crops to ferals, should depend on factors such as the period for which pollen is released each day and the distance of a feral colony from a field (eg data as in Fig. 1). In the study area in Tayside, the models predicted that all but a few feral colonies would receive pollen from agricultural fields. Moreover, the extent of gene flow from transgenic crops to feral colonies would be influenced by the arrangement of the two types of crop in the region. Less gene flow would occur to ferals from transgenic fields if the transgenic pollen was diluted by a high density of surrounding non-transgenic fields (Fig. 3).

Acknowledgements This survey and DNA analysis was financially supported by the GMO research programme of the Department of the Environment under contract PECD 7/8/237, and the modelling by the Scottish Office Agriculture, Environment and Fisheries Department.

New Blackcurrant Releases - Ben Connan and Ben Loyal

Ben Connan (P10/18/116) is derived from a cross between Ben Sarek and Ben Lomond, and provides a combination of regular heavy cropping and large fruit size. It is resistant to American gooseberry mildew and leaf spot, and also to leaf-curling midge. This high level of pest and disease resistance makes Ben Connan very suitable for cultivation with reduced agrochemical requirements. Harvest date is earlier than Ben Lomond by around 4 days.

Ben Connan

Ben Loyal (P10/18/121) is a sister seedling of Ben Connan, but crops significantly earlier in most areas. It too has outyielded Ben Lomond in trials, with heavy crops of large berries borne on very short strigs. Pest and disease resistance is again high, although leaf midge resistance is not as strong as in Ben Connan.

Fruit setting in both cultivars is excellent in most localities and climatic conditions, resulting in consistently high yields. The bushes have a very compact growth habit while retaining good vigour, enabling close spacing of bushes. Both cultivars have been successfully machine-harvested.

Ben Loyal

It is anticipated that these cultivars will be particularly suitable for the non-juicing, PYO and amateur markets.

Molecular biology

Wayne Powell

Science based research organisations must be able to respond to new technological developments and provide an infrastructure which is responsive to changes in research direction. In addition, the management of complex, multi-component projects poses many challenges where the need for planning, co-ordination, communication and focus is paramount. My task has been made much easier and indeed rewarding by the positive attitude of my colleagues in responding magnificently to changes in research priorities. Today's scientific culture demands that we access more funding from a wider range of external sources, communicate the relevance of our research findings to a diverse audience, protect and patent commercially sensitive discoveries, and participate actively in wealth creation. In all of these areas my colleagues have been outstandingly successful and these achievements provide a platform for further advancement of the department and institute.

The most significant strategic development during the past 12 months has been the successful integration of the Cereal Genetics Group into the department. This re-alignment of resources provides new and exciting opportunities to strengthen our cereal science research and attain a critical mass of scientists working on a major agricultural crop. Future developments in plant biotechnology are dependent on integration of different scientific disciplines such as biochemistry, genetics, computational biology and molecular and cellular genetics. The Cereal Genetics Group provides an extra dimension to both our conceptual thinking and practical endeavours, providing competitive germplasm and superb experimental systems for our molecular and cell biology research programmes. Of course, the cereal genetics and breeding programmes benefit from access to powerful enabling technologies to facilitate barley improvement. Indeed the activities of the department now span the 'resolution gap' between the molecule and whole organism biology. This research portfolio is summarised in Table 1 and

- DNA - genome analysis
- RNA Post-transcriptional processing
- Protein - transgenic biology
- Phenotype - plant breeding

Molecule → Whole organism

Table 1 Cell & molecular genetics.

provides a balance between fundamental and strategic research. Furthermore, the implementation of these activities provides a transparent, practical forum for interaction between Government and commercially funded research organisations.

In order to effectively manage, monitor and co-ordinate our research we have introduced a new departmental structure which is shown in Figure 1. This is designed to maximise our ability to have a major impact in six thematic areas. The unit structure embraces the principle of being flexible and responsive to both technological developments and the needs of Government, commerce and the scientific community. The organisational structure cannot reflect the amount of collaboration, co-operation and goodwill that exists within the department. However, these are vital ingredients for our continued advancement and must be carefully nurtured and encouraged. In this brief departmental overview I will attempt to highlight new developments and, where appropriate, identify future research directions.

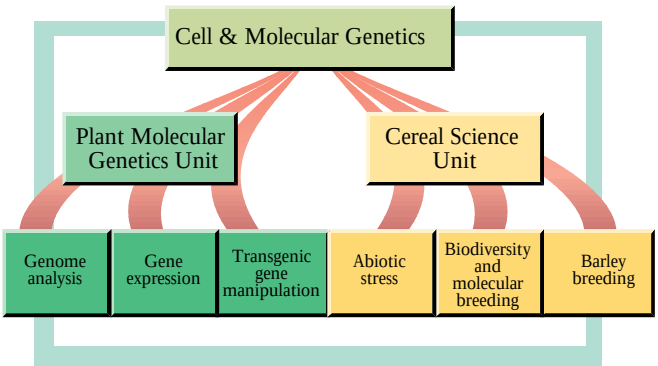

Figure 1 Cell & Molecular Genetics Department.

Amar Kumar and collaborators at the University of Dundee (Andy Flavell) and at the John Innes Centre (J.S.(Pat) Heslop-Harrison) have significantly enhanced our understanding of retrotransposons in plants. Retrotransposons are a particular class of mobile genetic elements that are distributed throughout the plant kingdom. In addition to the elegant technical approaches adopted in these studies, this work illustrates a unifying thread which I believe will permeate our research into the next millennium. Hierarchical studies of genome organisation, population biology, gene expression and how they impinge on evolutionary processes and whole organism phenotype are paramount to a conceptual and technical merging of complementary disciplines. In addition, the *Ty1*-copia group of retrotransposons may have significant practical relevance in providing abundant genetic markers for studying biodiversity and gene tagging.

Good examples of national and international collaboration are provided by new research findings emerging from John Brown's laboratory. The human spliceosomal proteins U1A and U2B" represent important models of how RNP motif-containing proteins execute sequence-specific RNA binding. Genes encoding these proteins have been isolated from potato and represent the only full length genes encoding plant spliceosomal proteins to have been cloned and characterised. As such they provide a valuable tool for the analysis of pre-mRNA processing in higher plants. Collaboration with Peter Shaw of the John Innes Centre, Norwich has focused on the organisation of the spliceosomal snRNPs in plant nuclei. Based on the known function of snoRNA genes from animals and yeast, *in situ* hybridisation with cloned plant snoRNAs and immunofluorescence have been used to describe the distribution of spliceosomal components in plant nuclei. These studies have shown that the distribution of spliceosomal components is conserved across a wide phylogenetic range of plant species. Furthermore, this research provides an experimental framework for the characterisation of novel factors involved in plant intron recognition.

Work on the improvement of potato by genetic manipulation continues in Gordon Machray's laboratory. A range of novel constructs has been generated for the manipulation of the low temperature sweetening phenotype. These are being transformed into a commercial crisping cultivar in a second developmental phase of this project which is funded by industry and involves an extensive cross-departmental effort. This work will provide material for the most extensive field-trialling of potato to be undertaken in Europe. Further work on the manipulation of protein quality in potato targets heterologous gene expression and protein stability as a means to remedy deficiencies in the essential amino acid content of potato protein. This is an increasingly important topic in the 'developing world' where potato production and consumption has increased dramatically and reliance is often placed on a limited range of foodstuffs to meet everyday nutritional requirements. Fundamental studies of gene expression in potato, which centre around studies of transcriptional and post-transcriptional control of invertase gene expression, underpin the applied work. A significant new finding from this research is the induction of alternative splicing of the invertase mini-exon by cold stress - an occurrence which may be exploited to unravel the signals governing invertase pre-mRNA splicing.

	AFLP *	Other markers	Total
Blenheim x E224/3	329	144	473
Dicktoo x Morex	249	78	327
Igri x Franka	202	469	671
Lina x *H. spontaneum*	580	460	1040

Table 2 Number of markers used to construct genetic maps in four barley doubled haploid populations.
* Amplification fragment length polymorphism. The AFLP data has been generated during 1995.

Trans-departmental, inter-disciplinary research is a very strong feature of the department's activities. For example, Brian Forster and Linda Handley are attempting to unravel the genetics of abiotic stress tolerance in barley. The approach is based on the use of naturally occurring ratios of stable isotope pairs $^{13/12}C$. Carbon isotope discrimination (measured as $\delta^{13}C$) provides an integrated record of the photosynthetic and water use history of the tissues sampled. It may therefore provide a good indicator of adaptation and this hypothesis is being tested in *Hordeum spontaneum* populations originating from the Middle East. The extent of ecogeographic differentiation between populations occupying strikingly different ecological habitats is being correlated with $\delta^{13}C$ data. A second feature of this research programme is the use of various molecular assays to examine the distribution of allelic variability in a diverse array of *H. spontaneum* populations that have also been evaluated for $\delta^{13}C$ under controlled conditions. Such an approach may reveal 'gametic associations' between polymorphic molecular markers and $\delta^{13}C$. A complementary approach is based on considering $\delta^{13}C$ as a quantitative trait and exploiting detailed linkage maps of barley to localise genes controlling the expression of $\delta^{13}C$ to specific linkage groups.

The Plant Genomics Group provides underpinning technology for plant breeding research and studies of biodiversity. Significant progress has been made in creating genomic libraries enriched for simple sequence repeats (SSRs). This pre-cloning enrichment technique has been used successfully to identify SSRs in barley, potato and mahogany. As part of this large scale sequencing activity, considerable effort has been devoted to automated reaction assembly using a Beckman Biomek 2000 automated laboratory workstation. Furthermore, the utility of microsatellite detection has been enhanced by the use of automated fluorescence detection on an ABI DNA sequencer using Genescan and Genotyper software. The main

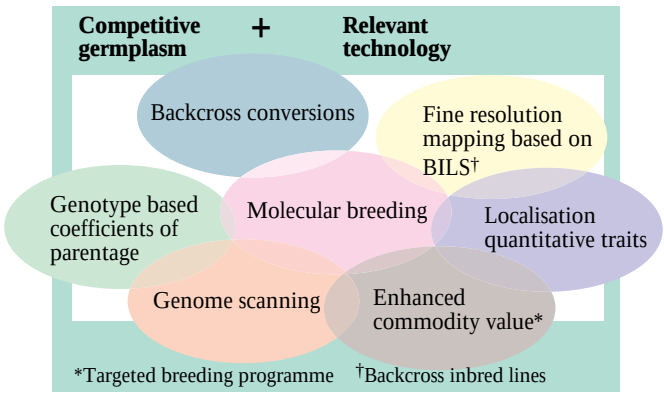

Figure 2 Current activities in the molecular breeding laboratory.

advantages of this approach include digitised data analysis and 'high throughput' attained by multiplexing microsatellite markers. Currently this technology is being applied to genotype potato cultivars to produce a DNA 'fingerprint' database encompassing all recognised potato cultivars. Robbie Waugh and colleagues are undertaking a particularly challenging task of creating a linkage map based on the segregation of alleles from tetraploid potatoes. This is a collaborative project involving John Bradshaw (Crop Genetics) and Chris Hackett (BioSS), bringing together complementary expertise in genetics, molecular biology, statistics and plant breeding. Both qualitative and quantitative traits are being studied with the dual goal of being able to locate traits to linkage groups and understanding the inheritance of complex traits in tetraploids.

A major effort has been devoted towards creating linkage maps in barley. The substantial progress attained in this area is summarised in Table 2 and these linkage maps will provide important tools for genetic analysis and manipulation. AFLP technology has not only increased the number and density of markers on each linkage group but has allowed the identification of polymorphic alleles that 'anchor' regions of the genome across four independent crosses. Further effort can therefore tackle the question of how robust our estimates of the location of quantitative traits are in different genetic backgrounds and environments. This principle may be extended to consider homology and synteny between barley and rice and this feature of cereal genome organisation will play a prominent role in future activities of the Genomics Group. Within monocotyledons, many examples of conserved gene order and function exist. These observations may facilitate the identification, mapping and eventual cloning of desirable genes in barley.

The Tree Genetics Group is sustained exclusively by external funding and continues to contribute substantially to our science base. The first genetic linkage map of tea (*Camellia sinensis* L.) has been constructed with more than 200 markers being located on specific linkage groups. Already, both qualitative and quantitative traits have been localised on the tea genome and provide new opportunities to manipulate these traits in breeding programmes. A comparison of genetic similarity estimates obtained with AFLP and RAPD marker systems has been undertaken in tea. These estimates are highly correlated indicating congruence between these two assays. Gene flow is a major determinant of population genetic structure and despite its potential importance, direct studies of pollen-mediated gene flow have not been widely reported. Simple sequence repeats (SSRs) have been used to measure pollen-mediated gene transfer in an insect-pollinated leguminous tree, *Gliricidia sepium* (see article by I.K. Dawson *et al.* on p.55). This information is vital for the implementation of scientifically based conservation programmes for tropical tree species. Future work will focus on the genetic consequences of forest fragmentation in both tropical and temperate tree species. For this purpose the chloroplast, mitochondrial and nuclear genomes will be evaluated to provide a comprehensive understanding of population differentiation. Many opportunities exist for the application of biotechnology to industrial tree species. The department is well placed to participate and indeed take the lead in the biotechnology programmes and priority will be given to forging stronger links with the Forestry Industry.

The newly formed molecular breeding laboratory is charged with the responsibility of developing molecular tools for plant breeding programmes. This is a complex and challenging task requiring the active participation of molecular biologists and plant breeders. Our current and planned endeavours in the molecular breeding laboratory are shown in Figure 2. Priority has been given to two activities: a barley germplasm database and backcross conversion. The allele composition of selected barley cultivars is determined following germplasm surveys and allele frequencies can be correlated with biochemical or phenotypic data to identify loci that contribute to a particular trait. The goal of the backcross-conversion programme is to identify recombinant progeny in a backcross that has inherited the gene of interest from the donor but still retains the maximum genetic background from the elite or recurrent parent. For this purpose both single and multi-locus molecular assays are being deployed.

Most barley cultivars have been developed through the pedigree breeding method. This means that detailed records of genealogy and agronomic trial data exist for numerous inbred varieties. Eileen Baird and Roger Ellis are using molecular markers to trace the flow of alleles through selected pedigrees. In this manner they hope to establish associations between molecular marker data and performance by identifying the co-segregation of marker alleles with traits that have persisted through the course of breeding programmes.

In addition to the scientific accomplishments of my colleagues, it is a great pleasure to record that Bill Thomas and colleagues have generated a new spring barley cultivar, Livet, which is entering NL2. It is the highest yielding entry tested in Scotland and has excellent resistance to mildew, *Rhynchosporium* and the rusts. Livet also combines high levels of hot water extract with good fermentability and is likely to be of considerable interest to the malt whisky distilling industry. In this context, Stuart Swanston is already screening progeny for high fermentability as part of an ongoing molecular breeding programme to produce barley genotypes with enhanced commodity value. Livet, together with other proprietary germplasm, has been uniquely genotyped by the use of microsatellite markers. While this is just the beginning of an exciting era in barley breeding, it also demonstrates our collective long-term commitment and vision to dynamically integrate biotechnology with crop improvement programmes.

Evidence for branchpoint involvement in plant intron splicing

C.G. Simpson, J. Lyon, P. Smith & J.W.S. Brown

The majority of plant protein-coding genes contain non-coding intron sequences which must be removed from the precursor messenger RNA (pre-mRNA) transcripts to produce mRNAs which can be translated into functional peptides. Splicing, the process by which introns are removed, occurs in a large RNA/protein complex (the spliceosome), the major components of which are five small nuclear ribonucleoprotein particles (snRNPs). During spliceosome formation, the snRNA components of each snRNP form a series of base pairing interactions with other snRNAs and the pre-mRNA itself. As the active spliceosome forms, these base pairing interactions are disrupted and unwound leading to new base pairing interactions and the continuation of the splicing reaction. Through this highly ordered biochemical pathway, the important pre-mRNA splicing signals are held in such a way that accurate and efficient excision of the intron and ligation of the protein coding exon sequences occurs (*Ann. Rep. 1993, 44*). In mammalian splicing, the branchpoint interaction with U2snRNA is one of the first key interactions in spliceosome assembly (Fig. 1).

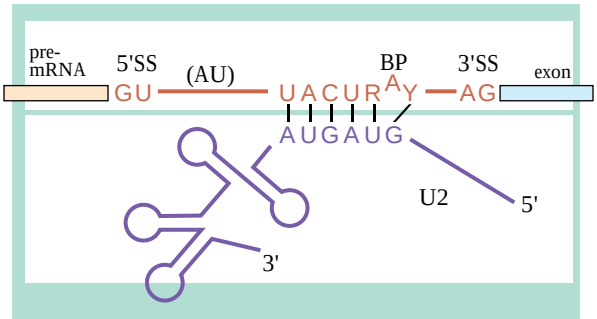

Figure 1 Schematic diagram of U2snRNA/intron branchpoint base pairing interaction showing the main features of plant introns (5' and 3' splice sites, branchpoint, AU-richness) and the secondary structure of U2snRNA.

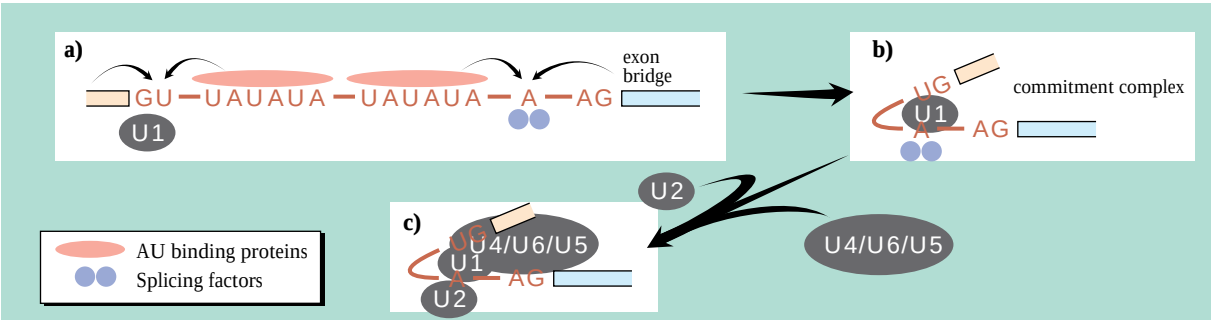

Figure 2 Schematic model of spliceosome assembly. (a) The 5' splice site and branchpoint region may be defined through interactions with factors bound to AU-rich sequences, exon sequences or adjacent splice sites (exon bridging). (b) Interactions between U1snRNP and factors recognising the branchpoint region interact to commit the intron to splicing (commitment complex). (c) Addition of U2 and U4/U6-U5 snRNPs lead to active spliceosome formation.

In plant splicing, specific branchpoint sequences were thought previously to be of secondary importance in comparison to AU-rich sequences (*Ann. Rep. 1992, 36*) and the 5' and 3' splice sites at the ends of the intron. Recently, we have identified a plant branchpoint consensus (YURAU) which matches closely the vertebrate branchpoint consensus (CURAY) (Y = U or C, R = A or G, branchpoint adenosine underlined). We have now demonstrated that plant introns contain preferred or optimal branchpoints by mutating the putative branchpoint adenosine in four plant introns. This led to dramatic reductions (5-10 fold) in splicing in plant cells which suggests that plant introns contain branchpoints required for optimal splicing. In addition, mutation of the 3' splice sites in these four introns led to use of downstream cryptic 3' splice sites in three of the four introns with a 2-5 fold reduction in splicing efficiency. These results support the vertebrate 3' splice site scanning model which proposes that the spliceosome scans from the branchpoint and usually selects the first 3' splice site downstream.

The involvement of a branchpoint in plant splicing requires some mechanism(s)/factor(s) by which the branchpoint is defined. In vertebrate systems splicing factors bind to a polypyrimidine sequence located between the branchpoint and 3' splice site. This sequence is often absent in plant introns. Branchpoint definition may therefore rely on factors, that remain to be defined, which bind either intron AU-rich sequence or factors which bridge the downstream exon. However the branchpoint is defined, it is likely, from our results, that an early step in plant intron splicing involves formation of commitment complexes between the 5' splice site and branchpoint region as shown for vertebrate and yeast systems (Fig. 2).

snoRNAs and pre-rRNA processing

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

Ribosomal RNA (rRNA) genes encode three of the four rRNA constituents of the ribosome. rRNA genes are organised in large tandem arrays. Each gene unit encodes the 18S, 5.8S and 28S rRNAs and is transcribed as a single precursor transcript from a promoter located upstream of the 18S rRNA. The precursor transcript contains the 5' external transcribed spacer (ETS), 18S rRNA, internal transcribed spacer region 1 (ITS1), 5.8S rRNA, internal transcribed spacer 2 (ITS2), 28S rRNA and a 3' extension (Fig. 1). By a series of cleavage steps in the ETS and ITS regions, mature rRNA subunits are produced for ribosome assembly. In vertebrate and yeast systems, these cleavages have been shown to involve numerous pro-

[1] John Innes Institute

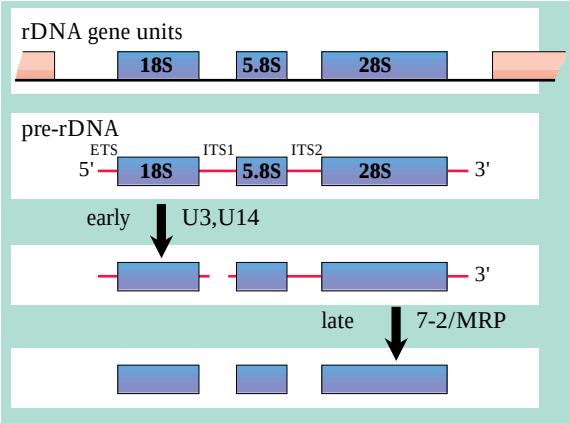

Figure 1 Schematic diagram of rDNA gene unit, pre-rRNA transcript and summary of cleavage steps involving U3, U14 and 7-2/MRP snoRNAs.

teins and small nucleolar RNAs (snoRNAs). We have now cloned plant U3, U14 (*Ann. Rep. 1994, 70*) and a number of other snoRNA genes and are using these sequences to analyse the distribution of snoRNAs and rRNA processing in the nucleolus of plants.

Transcription and processing of the rRNAs occurs in the nucleolus. The nucleolus consists of three cytologically defined regions: the fibrillar centre (FC), the dense fibrillar component (DFC) and the granular component (GC). The exceptional resolution obtained with pea root tip nucleoli has enabled a detailed analysis of the spatial distribution of transcription and processing to be made using *in situ* hybridisation and immunofluorescence. Studies in Dr Shaw's lab (JIC) have shown transcription to occur in the FCs and ETS-containing transcripts to be largely confined to the DFC region[1]. Localisation of *in situ* probes to ETS, ITS1, and the snoRNAs (U3, U14 and 7-2/MRP) clearly indicate that the processing steps necessary to produce 18S rRNA occur in the DFC region and later processing steps occur in the GC (Fig. 2). The results are consistent with the known functions of the snoRNAs under study: U3 and U14 are required for 18S processing and 7-2/MRP is needed for cleavage in ITS1 to give rise to a precursor for 28S rRNA. In addition, all of the snoRNAs accumulate in the nucleolar vacuole and may reflect storage or processing (*Ann. Rep. 1994, 70*) of snoRNAs.

Reference
[1]Shaw, P.J., Highett, M.I., Beven, A.F. & Jordan, E.G. (1995) *EMBO Journal* 14, 2896-2906.

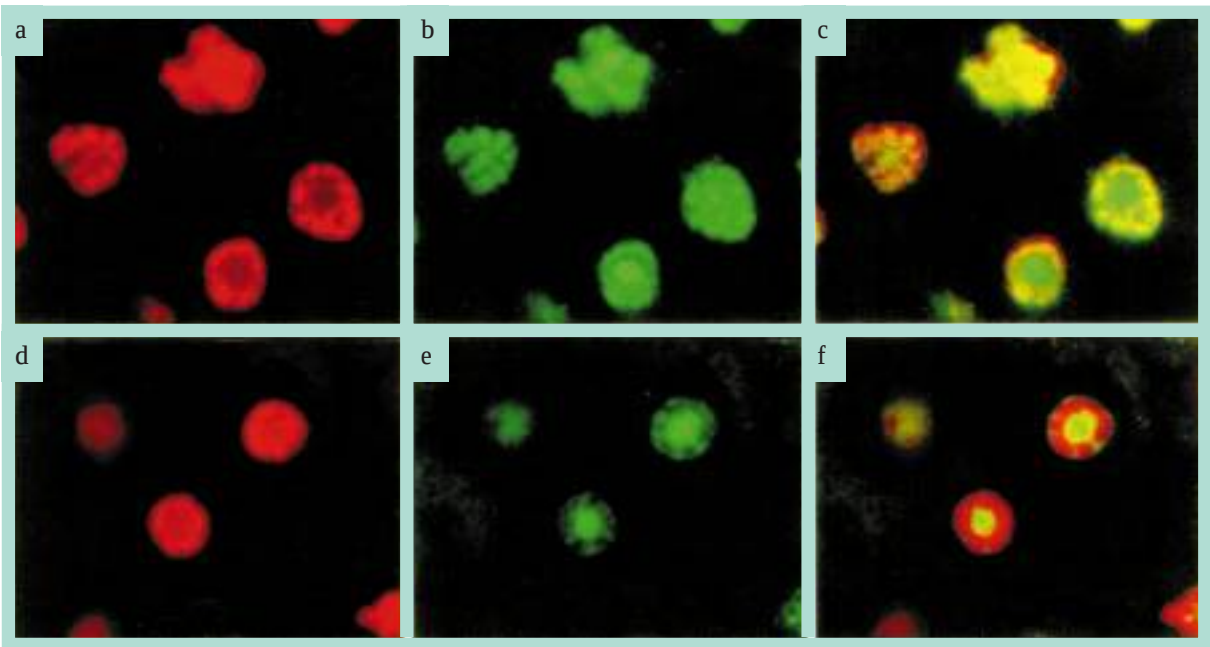

Figure 2 *In situ* hybridisation of ETS (red) (a and d) and the snoRNAs, U14 (green)(b) and 7-2/MRP (green) (e). Colocalisation of signal in the superimposed images (c and f) appear yellow.

Molecular characterisation of plant PRP8 genes

J.I. Hamilton & J.W.S. Brown

The spliceosome consists of four small nuclear ribonucleoprotein particles (snRNPs) and numerous non-snRNP protein factors. One of the most important proteins in the spliceosome is the U5snRNP-specific protein, PRP8. A *PRP8* gene was first isolated from yeast where the protein, PRP8, was shown to be an essential component of the splicing mechanism. Due to its exceptionally large size (>200 kDa), PRP8 is thought to act as a scaffold on which the spliceosome is assembled and structural integrity maintained. PRP8 has also been shown to contact the 5' and 3' splice sites and the branchpoint of the pre-mRNA at different times during spliceosome assembly and is involved in other protein-protein interactions in the spliceosome.

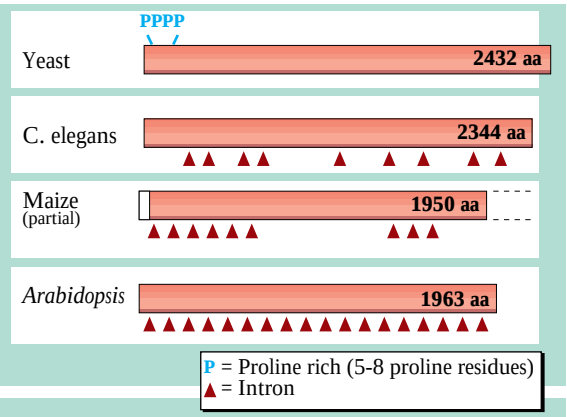

Figure 1 Comparison of gene structures of yeast, *C. elegans*, maize and *Arabidopis PRP8* genes showing intron number and position.

We have shown the plant PRP8 is highly conserved in size and epitopes to that of yeast Prp8 (Kulesza *et al.*, 1993). We have now isolated a *PRP8* genomic clone from maize and Drs Brian Schwartz and David Meinke (Oklahoma State University) have isolated *Arabidopsis PRP8* gene sequences. The two plant *PRP8* genes are very similar in their coding sequence with the translated proteins being >90% identical at the amino acid level. The importance of this protein is underlined by the high degree of sequence conservation across eukaryotes. The plant PRP8 protein sequence is >80% identical to that of the nematode *Caenorhabiditis elegans*. The structure of the genomic clones of the two plant genes is extremely interesting due to the different exon/intron organisation. *Arabidopsis* is an important model plant species because of its small genome size, short life cycle and the ability to obtain mutants. It is generally accepted that *Arabidopsis* genes will be more simply organised with, for example, fewer and smaller intron sequences. However, comparison of the maize and *Arabidopsis* gene structures shows that the latter gene has many more introns and that the intron positions of the genes are not conserved (Fig. 1). The existence and distribution of introns in homologous genes from different organisms has been used to analyse the evolution of gene structure. For example, conservation of intron position between distantly related species suggests that the introns were present in the ancestral gene before the species diverged. The surprisingly poor conservation of intron number and position between these two plant species suggests that since the divergence of monocotyledonous and dicotyledonous plants (2.8 billion years ago), either substantial loss or gain of introns has occurred in maize and *Arabidopsis* respectively. The *Arabidopsis* gene was isolated as a single T-DNA insertion mutant (*sus2-1*) where the T-DNA insertion occurred within a *PRP8* gene. The *sus2-1* mutant (which affects the suspensor - a column of cells to which the embryo is attached) was embryo lethal. However, mutant embryos could be dissected and survived in tissue culture suggesting the existence of more than one *PRP8* gene in *Arabidopsis*, with differential expression at least in embryonic tissues. We are utilising the *sus2-1* mutant, yeast *prp8* mutants, and the maize and *Arabidopsis* genes to establish complementation systems to address the function of PRP8 and investigate the expression of *PRP8* alleles in early embryogenesis.

Regulation of invertase gene expression in potato

A. Maddison, A-S. Bournay, R. Meyer, P. Hedley & G.C. Machray

Invertase cleaves sucrose to the hexose sugars, glucose and fructose, a conversion central to plant carbohydrate metabolism. Throughout the plant, this reaction is catalysed by a range of related invertase enzymes encoded by a family of genes. In potato, we have characterised a family of four genes which encode enzymes active in the cell wall while a further two genes encoding invertases targeted to the vacuole have also been described. A similar complexity of invertase genes has been elucidated in carrot, *Arabidopsis* and cereals. In concert these enzymes are involved in carbohydrate partitioning in the whole plant and at cellular level with highly specific tissue and developmental expression patterns. Experiments with transgenics in which invertase levels have been modified, demonstrate profound effects on photosynthesis and source-sink interactions. Highly co-ordinated regulation of invertases may underpin versatile partitioning of photoassimilate, and knowledge of the regulatory signals governing expression of invertase genes is critical to an understanding of how this may be achieved.

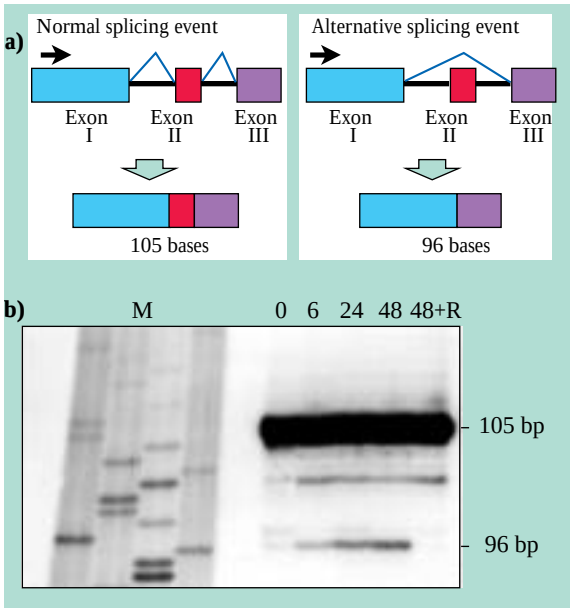

Figure 1 (a) Normal splicing and exon-skipping of the invertase mini-exon. (b) Induction of alternative splicing by cold stress: RT-PCR from leaf after exposure of plantlets to 4°C for indicated time (h) and after a recovery period of 24 h at 20°C (+R); M - DNA size markers.

We have examined aspects of both transcriptional and post-transcriptional events in the expression of invertase genes. Two of the genes we have cloned are linked in tandem in the potato genome with the promoter of the downstream gene being defined by the intergenic sequence. DNA sequencing of this region has revealed several motifs characteristic of promoters whose expression is regulated in a tissue- and developmental-specific manner and others which are involved in sucrose regulation of gene expression. The intact promoter and derivatives generated from it by deletion have been fused to a reporter gene which encodes ß-glucuronidase. Several series of transgenic potato lines have been generated from these constructs to allow analysis of the regulation of expression from this promoter, which has also been assayed by transient expression in tobacco protoplasts. Examination of tissue-specific expression from invertase promoters will be aided by the cloning of further invertase gene promoters which is also in progress.

The invertase genes we have cloned from potato, like most other plant invertase genes, include a very short exon of 9 bp which encodes the central three amino acids of a motif highly conserved in invertases of diverse origin. This mini-exon is one of the smallest known in plants and pre-mRNA from these genes may be susceptible to alternative splicing, because of a potential requirement for specialised interaction with the splicing machinery to ensure correct processing for the production of a mature mRNA. No evidence of aberrant post-transcriptional processing was observed during normal invertase gene expression in potato. The fidelity of post-transcriptional processing of the pre-mRNA from one of the genes was perturbed by cold stress, resulting in deletion of the mini-exon from some transcripts (Fig. 1). This alternative splicing event occurred under cold stress in both leaf and stem, but was not induced by wounding. This adds an example of exon skipping and the induction of alternative splicing by cold stress to the small number of transcripts which have been shown to exhibit alternative splicing in plants. The differential sensitivity of post-transcriptional processing to cold stress for the two transcripts examined will permit further dissection of the nucleotide sequence requirements for their accurate splicing.

Expression of heterologous protein in potato

G. Randhawa, D. Davidson, J. Lyon, P. Hedley & G.C. Machray

Potato, the fourth major food crop after wheat, rice and maize, has undergone a major transition - 30% of global potato production now comes from developing countries which produced only 11% in the 1960s. In Asia, the area planted to potato has more than doubled over the same period to reach five million hectares because the potato, with a short vegetative cycle, has been exploited as a suitable alternative crop within cereal-dominated farming systems. A prime source of carbohydrate, vitamins and minerals, the potato also supplies valuable protein to the human diet. Potato protein contains a higher proportion of the essential amino acid lysine than most cereal protein, but is deficient in the sulphur-containing amino acids, methionine and cysteine. The essential amino acid composition of plant foodstuffs is an important aspect of human diet in developing countries where nutritional reliance is often on plant protein from a single source. In this situation the improvement of plant products to yield protein with a balanced amino acid content is beneficial. Both conventional breeding and the techniques of genetic engineering have been applied to improve the quality of seed storage protein. Genetic manipulation is an attractive approach for the modification of potato tuber protein quality. Specific changes can be effected to existing favoured cultivars by the addition of a single gene (Fig. 1) without the reassortment of genes which occurs in breeding of cultivars exhibiting tetrasomic inheritance.

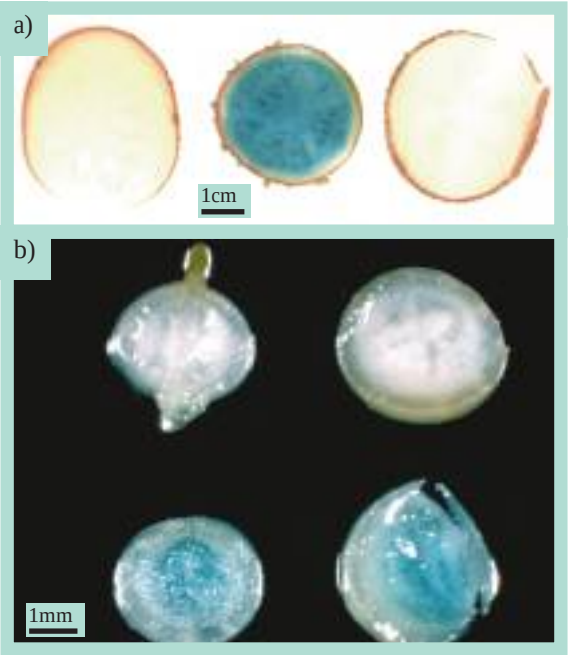

Figure 1 a) Mature cold stored potato tubers, transformed and control, stained for GUS activity. b) *In vitro*-grown microtubers, transformed and control, stained for GUS activity.

Modification of seed storage protein quality has been achieved by genetic manipulation to engineer expression of heterologous genes encoding proteins with a high content of sulphur-containing amino acids in seeds. An optimal application of this approach in potato requires high-level and tuber-specific expression from such genes to produce protein which is stable and can be stored in the vacuole. The patatin class I promoter, which is strongly expressed in tubers, has been chosen to ensure heterologous gene expression. Transgenic potato lines have been generated in which this promoter drives expression from two genes which encode methionine-rich proteins, from Brazil nut (*Bertholletia excelsa*), and maize (*Zea mays*), and a further gene encoding the storage protein, sporamin, from sweet potato (*Ipomoea batatas*). Transformants with the latter construct allow assessment of the ability of the tuber, with a high free amino acid content, to sustain production of additional storage protein; the ability of the tuber to incorporate more sulphur-containing amino acids into protein is challenged by the former constructs which encode proteins of 18% methionine and 8% cysteine, and 22.5% methionine and 4% cysteine respectively. Storage proteins are normally sequestered in the plant vacuole. To achieve vacuolar targeting, and consequent stability of the recombinant protein in these transgenics, we are investigating the use of a possible vacuolar signal sequence consisting of NH_4-terminal amino acid sequences from acid invertase, an enzyme normally targeted to the vacuole. Recombinant proteins have also been engineered to include an epitope tag to aid their identification and quantification where specific antibodies are not available. A rapid *in vitro* tuberisation protocol applied to a series of transgenic lines, generated using these constructs, allows determination of their effect on free amino acid pools and protein quality in microtubers.

Isolation, characterisation and use of SSRs as genetic markers

M. Macaulay, J. Provan, J. Russell, J. Fuller, H. Dewar, D. Milbourne, R. Meyer, A. Collins, G. White, W. Powell & R. Waugh

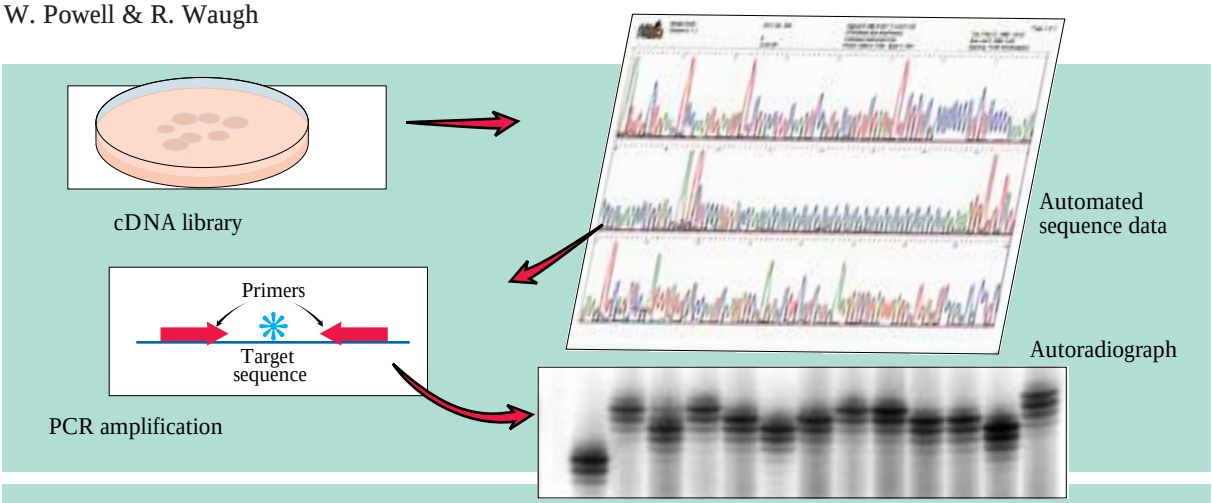

Figure 1 SSR isolation and analysis procedures.

The value of a genetic marker is determined by its informativeness, its abundance and its ease of use. Using these criteria, simple sequence repeats (SSRs or microsatellites) can be classed as ideal genetic markers. SSRs are tandemly repeated mono-, di-, tri-, tetra-, etc nucleotide motifs. Polymorphism occurs as a consequence of variation in the number of repeats in a specific SSR. This is thought to occur as a result of strand slippage during DNA replication. In the Department of Cell and Molecular Genetics, genetical studies on a number of crop species including barley and potato have been enhanced with the use of SSRs, with greater potential still to be realised.

SSRs can be isolated by various approaches: (1) Searching DNA sequence databases, including EMBL and Genbank, has proved to be productive in revealing sequences that include SSRs but there is a limit to the number of sequences that are available to analyse. (2) Screening libraries of cloned DNA sequences for the presence of particular classes of SSRs. (3) Construction of DNA libraries enriched for particular repeat motifs.

To obtain a large number of SSR sequences, we have focused on the last approach. After identifying clones that appear to contain an SSR, full characterisation is achieved via automated fluorescent sequencing. The resulting sequence data are then used to design oligonucleotide primers which flank the repeat motif and a simple PCR reaction carried out on total genomic DNA. After electrophoretic separation, visualisation and analysis of the different sized amplification products is either by autoradiography or by fluorescence on an ABI automated DNA sequencer with parallel data scoring and analysis using ABI Genescan and Genotyper software (Fig. 1). The potential for automating SSR analysis is a significant feature of this marker system.

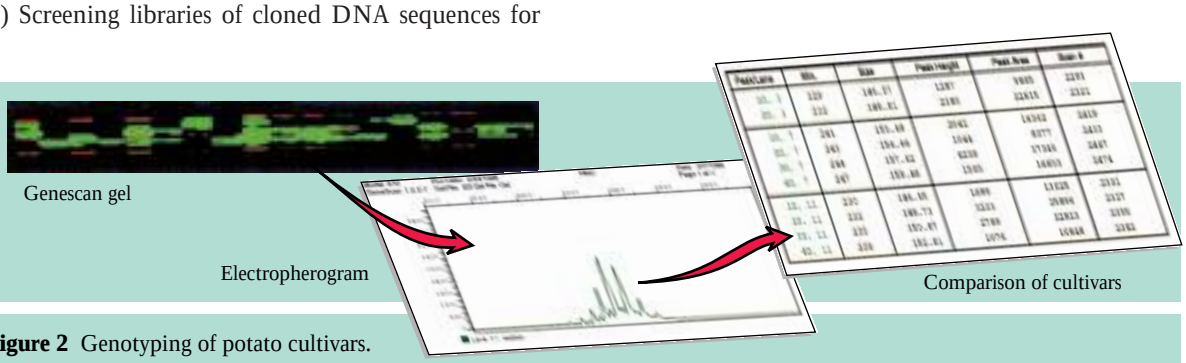

Figure 2 Genotyping of potato cultivars.

Other genetic markers, such as RFLPs and RAPDs, have been used across a wide range of species in the creation of linkage maps, diversity studies and population genetics. SSRs have enhanced this work due to their high information content. For example, we are currently assembling a DNA fingerprint database of all current UK listed potato cultivars using SSRs as a diagnostic assay (Fig. 2). We have also isolated and characterised over 300 SSR-containing sequences from barley and these are being used to assess the extent and distribution of genetic variation in wild barley populations, to genotype barley cultivars, to construct genetic linkage maps, and to examine evolutionary transportability across the Triticeae. Finally, because of their co-dominant, highly polymorphic nature, SSRs are particularly appropriate for detailed analyses of population genetics and gene flow. We have characterised around 70 SSR containing sequences from mahogany (*Swietenia* sp.) which are now being used to examine the effect of population fragmentation and genetic erosion on population vitality. The results from these studies will be of direct relevance to the conservation strategies being proposed for the maintenance of genetic variation.

Simple sequence repeats provide an exact indicator of pollen-mediated gene flow in the leguminous tropical tree species *Gliricidia sepium*

I.K. Dawson, R. Waugh, A.J. Simons[1] & W. Powell

Information on the extent of gene flow within and between plant populations has important practical relevance for the conservation and utilisation of plant genetic resources. The pattern and magnitude of gene flow can influence the genetic structure of populations, their effective size and the extent to which they may become genetically sub-divided. Despite its importance, however, actual measurements of gene flow in plants are few. In particular, the pollen component of gene flow in insect-pollinated plants has received little attention, and has been based on indirect methods associated with pollinator movement. Levels of gene flow can be difficult to infer from pollinator movements alone since pollen may be transferred across several plants before final deposition on the stigma. As a result, actual measures of gene flow by pollen may be underestimated from observations of pollinator movements.

Molecular markers provide great potential for assessing pollen-mediated gene flow because they provide a direct means of parentage determination in progeny arrays. With the advent of PCR, new approaches

Figure 1 Ian Dawson collecting seed samples of *Gliricidia sepium* in Guatemala.

[1] Oxford Forestry Institute, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB

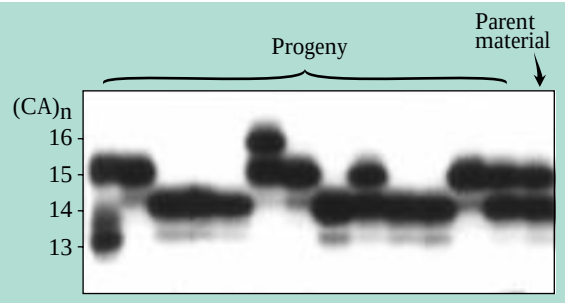

Figure 2 Allelic variation revealed at a single SSR locus, GsCA56, in a subset of 13 progeny sampled from a single tree of *Gliricidia sepium* at Retalhuleu, Guatemala (number 20; see Fig. 1). Alleles not present in the maternal parent (in this case CA16 and CA13) can be assigned minimum paternity exclusion distances of 11 and 32 metres, respectively.

have become available for parentage analysis. Foremost amongst these is the deployment of simple sequence repeat (SSR) analysis. Allelic variation at individual SSR loci is often extremely high and products are normally inherited in a co-dominant manner. The approach could therefore prove ideal for exclusion analysis in pollen-mediated gene flow studies in natural plant populations.

Gliricidia sepium is an important, multi-purpose leguminous tree native to Meso-America, which is now cultivated throughout the tropics (Fig. 1). It is an obligate outbreeder, the primary pollinator being the solitary bee, *Xylocopa fimbriata*. Due to its importance in sustainable agricultural systems, extensive seed collection missions have been undertaken together with agronomic trials to identify superior provenances. These trials have identified the Retalhuleu provenance of *G. sepium*, located close to the Pacific coast of Guatemala, as an important source of germplasm. This population of *G. sepium* is severely threatened due to its limited size and extensive environmental degradation. An understanding of the factors which determine genetic structure such as gene flow is particularly important for optimising *in situ* conservation programs. The purpose of this work was therefore to develop and deploy SSR markers to obtain direct estimates of pollen-mediated gene flow in this population.

Six amplifying alleles were detected in the Retalhuleu population (n = 138) using primers flanking the GsCA56 SSR locus. Based on individual SSR profiles, 23 trees whose progeny were likely to be informative for an assessment of pollen-mediated gene flow were chosen. In each case, approximately 20 progeny

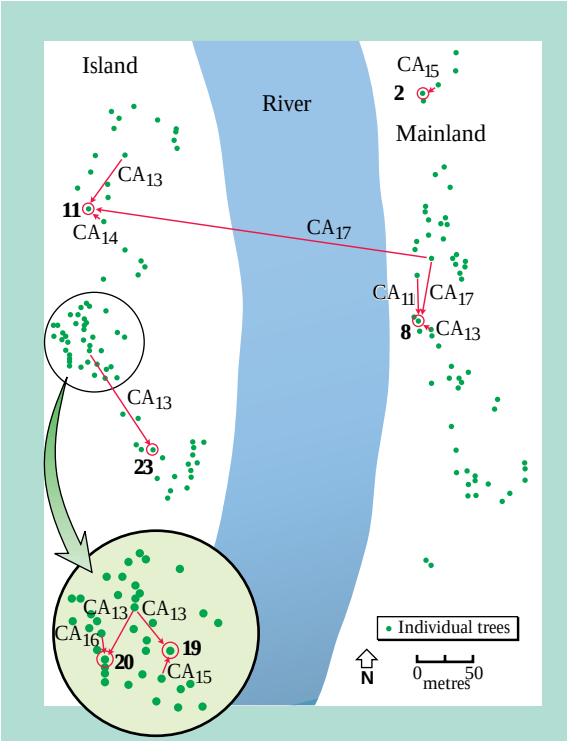

Figure 3 Geographic distribution of trees in a single population of *Gliricidia sepium* sampled from Retalhuleu, Guatemala. Part of the island subpopulation, where trees were in close proximity, is shown enlarged. Likely pollen-mediated gene flow events identified by a single SSR locus, GsCA56, in a subset of six progeny arrays are also shown. In each case, arrows point from the nearest potential donor of an allele detected in a particular array, which is therefore most likely to be the paternal parent. No account is taken here of intermediate pollinator movements.

were genotyped with GsCA56. Polymorphism revealed in a subset of individuals from a single array is shown in Figure 2.

In order to assess pollen-mediated gene flow, progeny array genotypes were compared with the entire parental population. For every array, distances to the nearest trees containing each of the six SSR products present in the population were calculated. Occurrence of particular non-maternal alleles in arrays therefore allowed the assignment of minimum gene flow distances by excluding as pollen donors all trees within the defined radii. Figure 3 shows likely gene flow events which were identified for a subset of six progeny arrays. For two of these arrays, a minimum of three paternal parents were likely to be involved in fertilisation events. In one case, data indicated pollen transfer between mainland and island subpopulations. Overall, combined data indicated that most pollen

movement could be attributed to within 75 m of the mother tree; 3.0% of observable events, however, occurred over a greater distance. The average distance observed per event was 17.8 m, and did not differ significantly between mainland (17.9 m) and island (17.7 m) sites.

An evolutionary consequence of relatively long distance pollen-mediated gene flow should be to reduce the amount of genetic substructuring in populations. Observation of pollen movement between island and mainland sites clearly demonstrates the homogenising effect of gene flow in determining the spatial structure of genetic variation in this fragmented population, with important implications for genetic resource management.

To our knowledge, data presented here represent the first application of SSR analysis to provide exact measures of pollen flow in natural stands of any species. These direct estimates of gene flow are sufficiently high to influence the evolution of small populations of insect pollinated tropical tree species. Apart from indicating the important role pollen transfer may play in preventing the fragmentation of endangered populations, data has implications for seed orchard isolation and the interaction of provenances in exotic locations. The high allelic variation detected with SSR, together with co-dominant inheritance, indicates that the microsatellites will have a major role in determining the pattern and nature of gene flow in tropical tree species.

Chloroplast simple sequence repeats: genetic markers for population, ecological and evolutionary genetics

W. Powell, J. Provan, G.C. Machray, J.W. McNicol & R. Waugh

The chloroplast is the most extensively studied plant genome for research into molecular evolution and systematics. The reasons for this include its small genome size (approximately 150 kb), high copy number per cell, ease of analysis, perceived conservative rate of evolution and mainly uniparental inheritance. Most studies have focused on interspecific and intergeneric comparisons with analyses based on restriction site variation predominating. Chloroplast DNA variation has not been used extensively to examine intraspecific variability and there is a need to develop methods that render the analysis of chloroplast DNA variation useful below the species level.

Do SSRs occur in the chloroplast ?
Can we detect length variation ?
What is the moleular basis for the polymorphism ?
How polymorphic are chloroplast SSRs ?
Are they useful genetic markers ?

Table 1 Questions posed.

Simple sequence repeats (SSRs), also known as microsatellites, are a powerful new class of genetic marker that are being used extensively in human forensic analysis and genetic analysis (see article by Macaulay *et al.* on p. 54). The advantages of SSRs arise from their relative abundance in eukaryote genomes, high levels of allelic diversity, co-dominance and ease of analysis by PCR. Until recently the exploitation of PCR based SSR length assays has been restricted to the nuclear genome. The primary objective of our research is to develop a convenient molecular assay that would take advantage of highly variable regions within the otherwise conservative chloroplast genome. For this purpose we have focused on short mononucleotide repeats that have been identified in chloroplast genomes and are analogous to nuclear microsatellites. The specific goals of our work are summarised in Table 1.

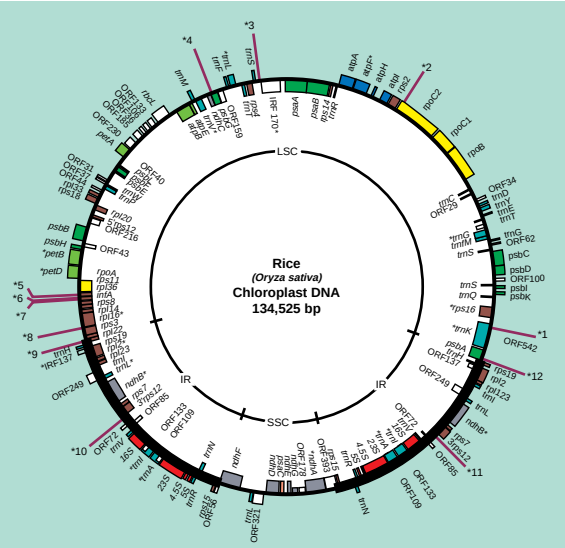

Figure 1 Distribution of SSRs in the rice chloroplast.

Results of computer database searches to detect chloroplast SSRs (cpSSRs) are given in Figure 1. Single nucleotide repeats predominate with A/T mononucleotide repeats almost exclusively being detected. The number of A/T repeats observed is higher than expected and decreases exponentially when the length of the repeats increases. Dinucleotide $[d(A\text{-}T)]_n$ and $[d(T\text{-}A)]_n$ repeats were only found in the chloroplast of liverwort (*Marchantia polymorpha*), maize (*Zea mays*), the non-photosynthetic green plant *Epifagus virginiana* and pea (*Pisum sativum*, CHPS GPA1, GPA1 gene for subunit A of chloroplast glyceraldehyde-3-phosphate dehydrogenase). A total of 466 cpSSRs were identified with repeat motifs being greater than 10 repeat units.

Experimental evidence that simple mononucleotide repeats in the chloroplast genome exhibit length variation was first obtained from soybean. Primers flanking the mononucleotide repeat motif (T_{13} G_{10}) in the chloroplast $tRNA^{Met}$ gene were used to amplify soybean DNA from the subgenera *Soja* and *Glycine* by PCR. Considerable polymorphism in the form of length variation was detected. In order to confirm that the length variation observed was due to changes

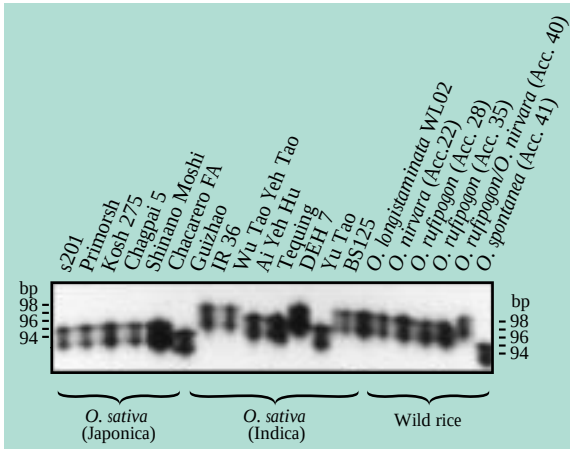

Figure 2. Variants identified in a sample of rice accessions with Oscp 134,510.

in the repeat region, the PCR products generated in the amplification reaction were sequenced. This demonstrated that the observed length variation was due to changes in the repeat region rather than in the sequences flanking the repeat motifs. Thus, hypervariable regions of the chloroplast genome can be targeted to detect both intra- and interspecific variability in the chloroplast genome.

The chloroplast genomes of five species (rice, tobacco, black pine, liverwort and maize) have now been fully sequenced. This provides new and unique opportunities to explore the potential of chloroplast mononucleotide repeats to generate informative intraspecific assays. Rice is considered to be a model plant for cereal genome research and is the world's most important food crop. We have therefore examined the distribution of mononucleotide repeats in the rice chloroplast genome and determined the levels of polymorphism associated with these cpSSRs. The distribution and location of cpSSR sites with a minimum of 10 uninterrupted mononucleotide repeats is shown in Figure 1. Twelve such regions were identified of which six were polymorphic when tested on 20 rice accessions. Variants detected at one such region (Oscp134,510) are also shown in Figure 2 together with the primers used to amplify rice genomic DNA. The number of variants identified and diversity

	Oscp3,536	Oscp75,969	Oscp76,221	Oscp78,412	Oscp80,599	Oscp134,510
Number of variants	2	3	2	4	5	5
Diversity index	0.070	0.397	0.375	0.540	0.570	0.720

Table 2 Number of variants identified and diversity indices obtained for six rice chloroplast microsatellites evaluated on 20 rice accessions.

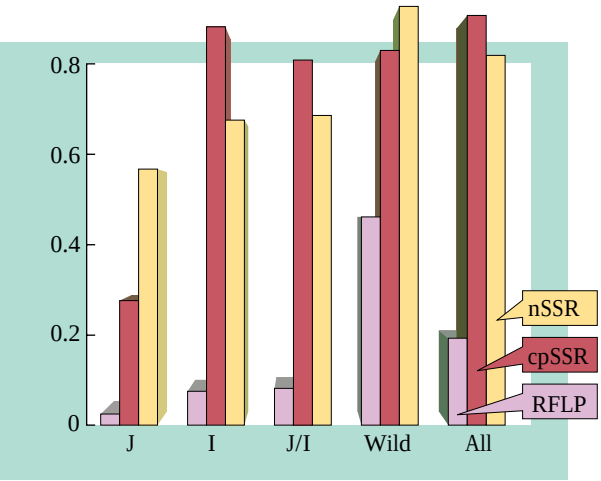

Figure 3 Diversity indices obtained with RFLP, nuclear and chloroplast simple sequence repeat on 20 different rice accessions. J=*japonica*, I=*indica*, W=wild rice species.

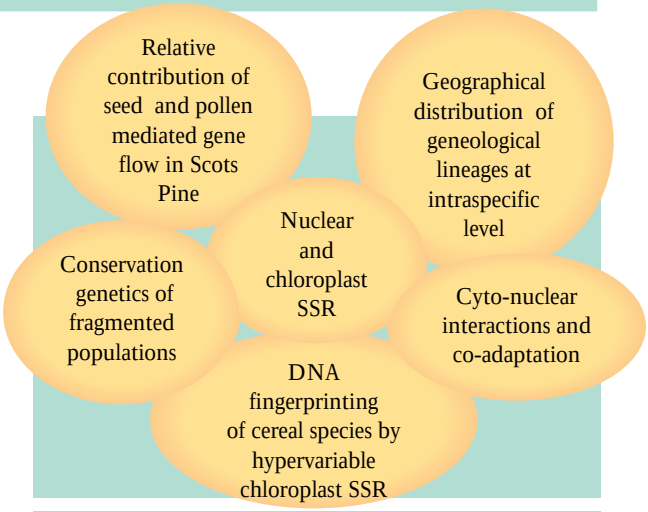

Figure 4 Current and future research opportunities based on chloroplast and nuclear simple sequence repeats (SSRs).

indices for six chloroplast microsatellites are shown in Table 2. Between two and five variants were detected at each polymorphic microsatellite locus and diversity values ranged from 0.07 to 0.72. Since genotypes for chloroplast DNA represent non-recombining characters, information from size variants may be combined to produce haplotype data. An examination of the joint distribution of variants at the polymorphic cpSSR loci generates 15 unique haplotypes for the 20 rice accessions studied. These 20 accessions were also genotyped with nuclear RFLP and microsatellite markers allowing a comparison between the informativeness of cpSSRs with the nuclear assays. The diversity indices obtained for cpSSRs with the rice accessions are shown in comparison with values for nuclear SSRs and RFLPs in Figure 3. When all the accessions are considered, the diversity levels obtained with the combined chloroplast microsatellites are greater than the average values obtained with RFLPs or nuclear microsatellites.

In conclusion, we have developed a generic, high resolution PCR based assay for the chloroplast genome. In addition, for gymnosperms where chloroplasts are mainly paternally transmitted, chloroplast microsatellites provide pollen specific markers. Deployment of this assay will provide new insights into a number of macro- and microevolutionary processes operating in plants. Some of our current and planned research activities based on cpSSRs are summarised in Figure 4 and will provide new opportunities to study plant population structure, differentiation and diversity.

Detection by AFLP analysis of major and minor effects controlling the genetics of resistance to scald (*Rhynchosporium secalis*) in barley

W.T.B. Thomas, W. Powell, R. Waugh, B. Harrower, E. Baird, A. Booth & P. Lawrence

Scald (*Rhynchosporium secalis*) (Fig. 1) is a major fungal pathogen of barley throughout the world. A number of major resistance genes to the pathogen exist and some cultivars are also thought to possess partial resistance to the disease. Many of the resistances deployed in commercial cultivars are alleles at a complex locus on barley chromosome 3, which has been located on morphological genetic maps. In a previous article, we described the intra-chromosomal location of a major-gene resistance locus for *Rhynchosporium secalis* in barley (*Ann. Rep. 1992, 28-30*). This gene was present in the SCRI breeding line E224/3, derived from cv. Sergeant, and is similar to the resistance present in the SCRI NLT2 spring barley Livet (B88-71/16). A doubled haploid population between Blenheim and E224/3 was used to locate the gene on chromosome 3. The classification of the DH lines was based upon a comparison of parental and

Figure 1 Typical symptoms of scald *(Rhynchosporium secalis)* infection of barley.

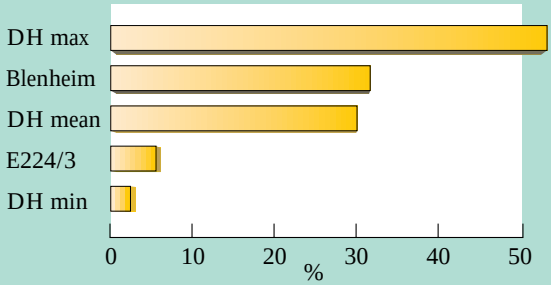

Figure 2 Mean percentage leaf area infected by scald *(Rhynchosporium secalis)* in disease nurseries grown in 1991 and 1992.

doubled haploid progeny scores of percentage leaf area infected with the disease in naturally infected field nurseries. Whilst this enabled the gene to be mapped, a number of DH lines were considerably more susceptible than Blenheim and there were some lines that were more resistant than E224/3, indicating transgressive segregation (Fig. 2). This indicates that there was also some quantitative variation in the cross which could be located by QTL mapping.

The genetic map of Blenheim x E224/3 (*Ann. Rep. 1994, 61*) has been extended through the use of

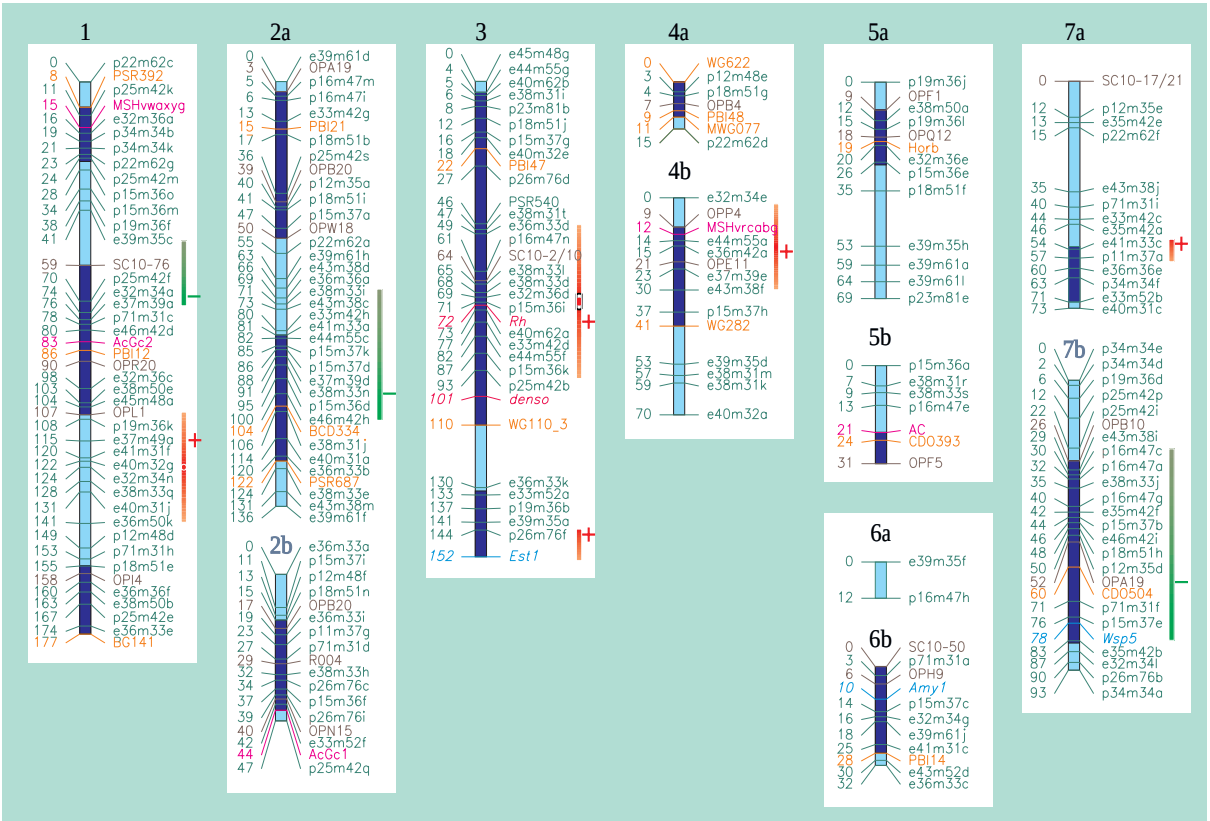

Figure 3 Genetic maps of the spring barley cross Blenheim x E224/3. For clarity, markers mapping within 1 cM or less of another have been omitted and the figure contains 222 of the mapped markers. AFLPs are coloured green, microsatellites magenta, morphological and disease resistance red, proteins and isozymes blue, RAPDs brown and RFLPs orange. Dark blue segments indicate regions mapped prior to the addition of AFLPs. Thick green and red lines indicate the position of scald resistant QTL peaks with '+' and '-' indicating the effect of Blenheim alleles upon % leaf area infected.

Amplified Fragment Length Polymorphism (AFLP) markers. This class of marker can generate a large number of polymorphic products in a short period of time and thus can greatly speed up the creation of genetic maps. Two enzyme combinations were used to digest the DNA from the Blenheim x E224/3 DH lines and a range of different primers was used to create AFLPs from the enzyme combinations. Twenty-eight primer combinations were used to amplify products from *Eco*RI/*Mse*I digests, producing an average of 8.4 AFLPs for each primer combination. Eight primer combinations were used for *Pst*I/*Mse*I digests, which produced an average of 3.2 more AFLPs than the *Eco*RI/*Mse*I digests. This gave a total of 329 AFLPs which were produced in a matter of months compared to the years taken to produce the 144 markers used to produce an earlier version of the genetic map of Blenheim x E224/3 (*Ann. Rep. 1994, 61*).

The inclusion of the AFLP data for the Blenheim x E224/3 population has a dramatic effect and results in a large increase in the mapped portion of the genome to over 900 cM (Fig. 3) compared to the previous map (*Ann. Rep. 1994, 61*). This is not a reflection of an increase in the map length irrespective of linkage relationships, a phenomenon often referred to as 'map extension'. The fact that the lengths of the previously mapped segments are virtually the same with or without the inclusion of AFLPs provides a strong indication that 'map extension' is not a feature of AFLPs in this data set. Instead the increase in mapped length is through the bridging of gaps between previously discrete segments such as chromosome 1 and extensions of other segments such as the second segment of chromosome 4 (Fig. 3). The map now provides good coverage of the barley chromosomes although the long arms of chromosomes 4 and 5 and the short arm of 6 are still poorly covered. This may, however, represent a general lack of polymorphism in these regions of the genome. Given the large increase in map coverage compared to last year's map, we have initiated further quantitative trait analyses.

The scald scores used to derive the means shown in Figure 1 were derived from experiments grown in replicated field nurseries at SCRI in 1991 and 1992. Each year, the nursery was scored twice, once at or just after heading and the second two weeks later during grain fill, providing four replicated measures of resistance for each line. As the scores were percentages of leaf area infected with the disease and many scores were either very low or very high, an angular transformation was applied to the data. Analysis of variance

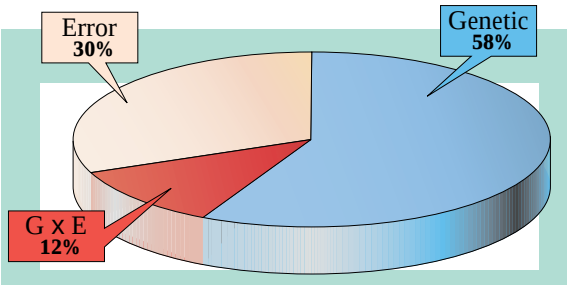

Figure 4 Proportions of genetic, environmental and genotype x environment components of variance for scald resistance.

of the transformed data revealed significant genetic variation and significant genotype x environment interactions but the genetic effect was the largest (Fig. 4). The proportion of the phenotypic variation due to genetic effects (heritability) was very high (66%), indicative of major-gene control of resistance but the presence of transgressive segregation (Fig. 2) suggests that more than one gene may be involved in the control of resistance.

The mean scores from each of the four measures of resistance were used to search for QTLs using the package MQTL[1]. MQTL has a number of advantages over other packages used to search for QTLs. One is permutation[2], in which the data is randomly mixed and examined a number of times to determine the appropriate statistical thresholds to test for QTLs, compared to arbitrary thresholds applied when using packages such as MAPMAKER/QTL. These packages simply take into account variation at the interval under study when searching for QTLs - a method known as simple interval mapping. MQTL carries out this method of analysis but also uses background markers to account for variation in intervals other than that under study in an approach termed simplified compound interval mapping (sCIM)[3], which leads to greater refinement in the location of QTLs. A third advantage of MQTL is that it can fit effects to data obtained from several environments[3] which enables QTL x environment interactions to be detected as well as the detection of QTLs of small but consistent effect which would not reach significance in analyses of individual environments.

MQTL revealed a highly significant peak at the *Rh* locus on chromosome 3 affecting scald resistance (Fig. 3). This QTL main effect accounted for 59% of the phenotypic variation and the difference between the resistant and susceptible alleles represents a range of 16.3% leaf area infection in the DH progeny. Whilst this locus clearly played a major role in the control of

resistance to the disease, some 7% of the main effect variation was unaccounted for and the observed range between the extreme DH lines was 49.6 %. This provides further evidence for additional genes controlling resistance to the disease. Inspection of the MQTL scans reveals a second peak in the region of the e41m33c locus on chromosome 7a (Fig. 3) and inclusion of this as a main effect increases the portion of the genetic variation to 60% and the DH range to 20.7% leaf area infected. This suggests further genes are involved in the control of the disease and examination of the MQTL scans reveals six QTL x environment interactions. Including all six QTL x environment interactions in the model accounts for 65% of the phenotypic variation as main effects and 77% as main effects and interactions so all the genetic and interaction variance appears to be accounted for by this model. Summing up the additive effects of the QTLs produces an expected DH range of 51.7% leaf area infection (Table 4), which is much closer to the observed value. The additive effects of the QTLs from each parent results in a parental difference of 23.8% (Table 4) leaf area infected, comparable to the observed value of 25.9% (Fig. 2). Apart from the effect at the *Rh* locus, all the QTL effects were only revealed by the sCIM analysis but each fitted effect was significant in at least one environment.

Two of the QTL x environment interaction QTLs were located on chromosome 1 (Fig. 3). The remaining four were found on chromosomes 2, 3, 4, 5 and 7b. The two on chromosome 1 were QTLs linked in repulsion and so may escape detection by simple interval mapping, although the resistant allele from E224/3 exerted a greater effect than the resistant allele from Blenheim (Table 1). All the QTL x environment interaction QTLs were located in regions where QTLs controlling resistance to other barley foliar pathogens were also located in the Blenheim x E224/3 population, with the exception of the interaction QTL on chromosome 2. The Blenheim resistant allele on chromosome 1 was located in the same place as a QTL x environment interaction affecting mildew resistance and the QTL x environment interactions on chromosomes 4, 6 and 7b were located in regions where main effect QTLs for Yellow Rust resistance were also located. In addition, Brown Rust resistance main effect QTLs were located in the same, or adjacent regions as the QTLs for scald resistance on chromosome segments 7a and 7b. All the main effect QTLs for rust resistance have a large effect whereas the effects on scald resistance were small. In addition, there was no Yellow Rust or Brown Rust infection in the nurseries so the effects are not due to mis-scoring of disease symptoms. The scald results suggest that certain major resistance genes appear to exert some partial resistance upon another disease but further investigation is required to determine whether the observed effects are genuine or not.

Chromosome	Position (cM)	Nearest marker	Effect (Blenheim-E224/3 QTL alleles)
1	75.8	e32m34a	-1.6
1	115.0	e37m49a	3.0
2a	90.8	e38m33n	-2.0
3	71.7	Rh	* 24.4
3	143.7	p26m76f	3.8
4	15.4	e36m42a	2.3
7a	53.8	e41m33c	* 4.1
7b	52.8	OPA19	-4.7

Table 1 Mean effect (angular transformation) and location of QTL main effects and QTL x environment interactions for scald resistance. * QTL main effect - the remainder are QTL x environment interactions.

All but two of the effects shown in Table 1 are located at or close to AFLP markers, demonstrating that the addition of AFLP markers to the data set has helped to refine QTL location. This is particularly so for the QTLs on chromosome 1 as one of the loci would not have been detected without the AFLP data since it was located in a previously unmapped region of the genome (Fig. 3). This would probably mean that the other locus on chromosome 1, where the Blenheim allele was resistant, would not have been detected either because only the net effect of the two QTLs would be apparent, which would probably not reach statistical significance.

In summary, AFLPs are a rapid means of generating large numbers of markers that can be used to create or augment genetic maps. These maps can be used to reveal major and minor gene control of a character and, in this case have highlighted some usable partial resistance to a major plant pathogen with even the apparently susceptible parent contributing resistant alleles.

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Genetic variation in barley starch

R.P. Ellis & J.S. Swanston

Concern for the natural environment will favour materials which are derived from renewable sources and which are bio-degradable. For these reasons, starch is likely to find greater use in the plastics industry along with current utilisation in areas such as paper making, textile finishing, and adhesives (Fig. 1). Starch is the main constituent of cereal grains and potato tubers and both are used as sources for industrial processes.

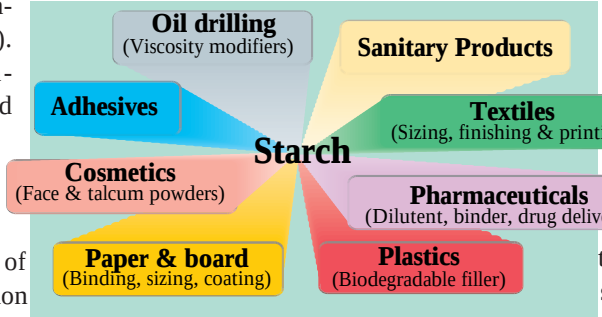

Figure 1 Some existing and potential industrial uses of starch.

Starch occurs in the form of granules and is a combination of two carbohydrates, amylose (AM) which is a straight chain and amylopectin (AP) which is highly branched. Granules can vary in size and shape, both between and within species and may contain small quantities of other constituents such as lipid and protein. Barley starch is of particular interest as there is a considerable degree of genetic variation which affects granule composition and size distribution, and such factors will determine the usefulness of starch for a range of industrial processes.

Starch, in the barley grain, must be present, however, in maximum quantity and also be readily accessible to amylases, to optimise malting quality. As malting remains the main industrial outlet for barley, changes to starch granules, which reduce starch content, or increase the barriers to degradation, are unlikely to have widespread acceptance. For that reason, research at SCRI has focused not only on the chemistry of barley starch, but also on starch as an integral component of the barley grain.

Much has been learned about starch, by comparing normal (wild type) barley with spontaneous or induced mutants. There are two mutations which affect the ratio of AM to AP. The waxy (*wx*) gene reduces the amylose proportion from around 75% in wild type barley to less than 10%, by eliminating one of the enzymes responsible for starch synthesis in the developing grain. Waxy starch absorbs water more readily than normal starch, a factor which could be utilised in non-food or industrial applications e.g. in sanitary products. However, the waxy gene is associated with a greater resistance of cell walls to either mechanical or enzymic breakdown. This may make the starch more difficult to extract and precludes the use of waxy barleys in malting and brewing.

The high amylose gene (*amol*) increases amylose content to around 45% and also affects the relative sizes and proportions of large (or A-type) and small (B-type) starch granules. High amylose starch may be of particular use in adhesives, but lines carrying this gene are invariably of poor malting quality, tending to have increased levels of storage protein forming a highly resistant matrix around the starch granules. During malting, protein surrounding starch granules is generally degraded, but scanning electron microscopy of malted high amylose grain (Fig. 2) shows the starch still obscured by protein and cell wall material.

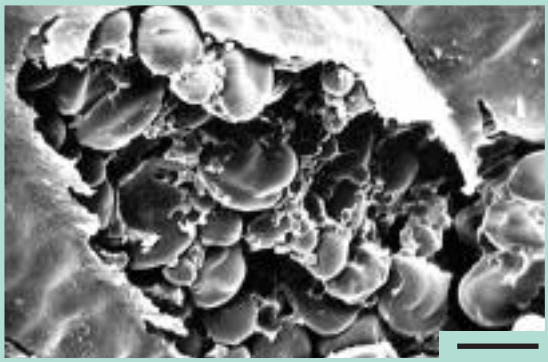

Figure 2 Scanning electron micrograph of malted high amylose grain - longitudinal section through the centre of the endosperm close to the scutellum. (Bar = 10 microns).

Changes in starch composition are likely due to mutations affecting enzymes of starch synthesis. However, granule structure or size distribution may be altered by mutations which have their primary effect on grain components other than starch. Two examples, which

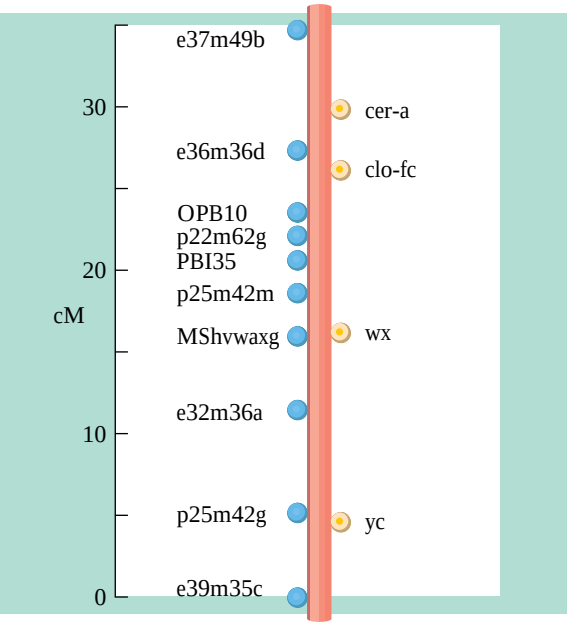

Figure 3 Section of the long arm of chromosome 1, illustrating correspondence of molecular markers (left) with location of genes for seedling pigmentation (clo-fc, yc) and epidermal wax (cer-a), around the wx locus which codes for starch granule bound starch synthase. The frequency of molecular, compared to physical, markers, is clearly demonstrated.

are both characterised by a reduction in number of small B-type granules are the genotypes Riso 56 and Chalky Glenn. Riso 56 was produced by a mutation at one of the storage protein loci, while Chalky Glenn has a reduced β-glucan content in its cell walls. Inbred lines, from a cross between Chalky Glenn and a waxy barley, have included several in which the apparent association between the waxy gene and poor cell wall modification during malting has been broken. These lines were generally of low thousand corn weight and, hence, would have lower starch content in the grain. This was probably due to a genetic factor, affecting grain size, known to be on the same chromosome as the waxy gene. It is, however, sufficiently distant to permit a substantial degree of recombination, so further crossing should enable production of waxy lines with desirable levels of both grain size and cell wall modification.

The existence of a wide range of mutations has made it possible to determine the chromosomal location of some of the genes affecting starch structure, composition or synthetic enzymes. Molecular techniques, such as RFLP have greatly enhanced the number of reference points, enabling location, for example, of the *amol* (high amylose) and ADP-glucose pyrophosphorylase genes. Newer techniques such as AFLP and microsatellites (SSR) enable identification of many more loci, greatly enhancing the accuracy of the gene mapping process, but they may also offer further advantages. For example, the microsatellite at the waxy locus (Fig. 3) makes it possible to follow the gene through cycles of crossing and selfing, without the need for starch testing of progeny. The ability to select for specific genes may also make it possible to determine if the associated effects of starch mutations are due to close linkage or pleiotropy or whether they are a function of physical changes in the way in which grain structural components pack together.

A molecular approach to study the role of polyamines in plant development

A. Kumar, M. Taylor, S. Mad Arif, R. Wheatley & H.V. Davies

Major questions are currently being addressed in many biochemical systems concerning the role of a class of aliphatic amines known as polyamines. Polyamines are ubiquitous in living organisms and have been implicated in playing an important role in a wide range of biological systems, including plant growth and development. There is direct evidence that they are essential for the growth and development of prokaryotes and eukaryotes. For example, a mutation in the SAMDC gene of yeast (*Saccharomyces cerevisiae*) results in severe inhibition of its growth and development. Although extensively studied in plants, the role of polyamines in plant growth and development, based on previous physiological and genetic studies, remains to be fully clarified. Therefore, we have taken a cell and molecular biological approach to address this question.

S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50) is a key enzyme in the biosynthesis of the

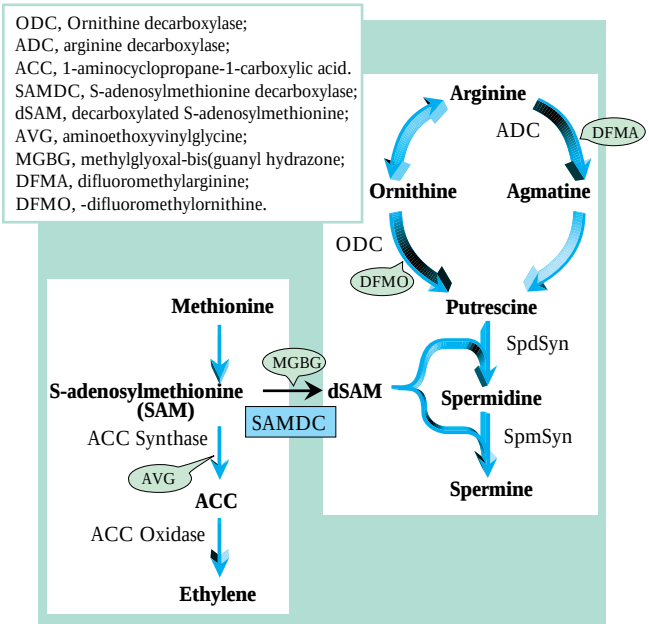

Figure 1 Polyamine and ethylene biosynthesis pathways and their inter-relationships.

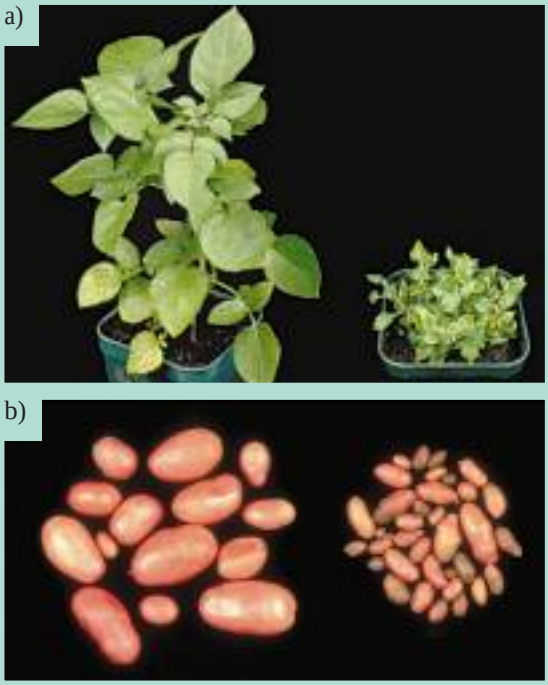

Figure 3 Phenotypic comparison between the SAMDC antisense transgenic and the control plant. a) Phenotypes of the 35S SAMDC antisense potato plants (line A9)(on the right) grown for 5 weeks in the glasshouse showing a range of stunted growth characteristics with highly branched stems, small leaves, and early senescence. b) Tuber morphology of the antisense plants (r) and the control plants (l).

polyamines spermidine and spermine from putrescine and its activity has been shown to be rate limiting in this pathway (Fig. 1). SAMDC is also known to influence the rate of biosynthesis of ethylene. We have previously isolated and characterised a cDNA clone of the SAMDC gene from potato (*Ann. Rep. 1993, 36-38*). This cDNA clone has been used to construct engineered SAMDC genes in antisense and sense orientations under the control of the 35S CaMV promoter (Fig. 2a) to manipulate the expression of the endogenous S-adenosylmethionine decarboxylase (SAMDC) gene. Modulation in the expression of the SAMDC gene is likely to effect not only the biosynthesis of polyamines but also that of the plant growth regulator ethylene, since SAM is a precursor (Fig. 1) in both biosynthetic pathways. *Agrobacterium*-mediated transformation has been used to produce transgenic potato plants with the engineered antisense and sense SAMDC genes (Fig. 2a) in order to down-regulate or over-express the SAMDC transcript respectively. Decreases in the level of SAMDC transcript in the antisense plants were observed (Fig. 2b). Antisense transgenic plants which expressed the engineered SAMDC gene constitutively under the control of the two copies of 35S CaMV promoter showed a range of stunted phenotypes with highly branched stems, short internodes, small leaves and inhibited root growth (Fig. 3a). Additionally, these antisense

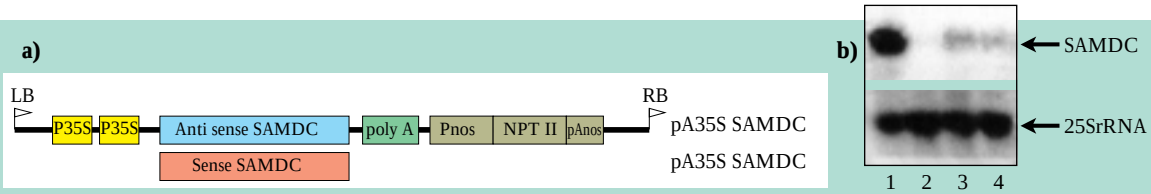

Figure 2 (a) Diagrammatic representation of antisense and sense SAMDC constructs; pA35SSAMDC, pS35SSAMDC. (b) Northern blot of total RNA extracted from whole *in vitro* grown potato plantlets transformed with a SAMDC antisense construct (pA35SSAMDC). Lane 1, control plants; lane 2, line A9; lane 3, line A5; lane 4, line A4. Top panel, blot probed with potato SAMDC cDNA. Lower panel, control blot in which the stripped blot from top panel was hybridised with a potato 25S rRNA probe.

Potato line	Transgenic phenotypes	SAMDC* activity (mmol CO_2/g FW/hr)	Free polyamine content* (µg/g FW) PUT	SPD	SPM	Rate of ethylene* biosynthesis (pmoles/g FW/hr)
Control	Normal	0.116	17.4	33.0	19.1	90.5
A4	S, SL	0.033	25.6	30.1	21.3	101.8
A5	S, SL, PR	0.017	8.6	18.2	6.9	273.9
A9	S, BS, SL, PR	0.012	2.8	6.3	6.2	4129.1

Table 1 Analysis of whole potato plantlets grown in tissue culture, transformed with the SAMDC antisense construct pA35S SAMDC. * Values are mean of three independent assays.
S - stunted; BS - branched stems; SL - small leaves; PR - poor root growth.

plants produced very small and elongated tubers compared with the control tubers (Fig. 3b). These abnormal characteristics of antisense plants correlated with the altered levels of SAMDC transcript, SAMDC activity, polyamine content and rate of ethylene evolution (Table 1). Attempts to produce sense transgenic plants with the 35S SAMDC sense construct were unsuccessful indicating that a constitutive over-expression of the engineered SAMDC is lethal to the plants.

In summary, we have shown that antisense and sense technology can be used to modulate the biosynthesis of polyamines and ethylene by targeting the SAMDC gene. Also, because this experimental approach is more precise than the other previously used approaches, we have been able to show a clear correlation between the phenotypes and altered levels of SAMDC transcript, SAMDC activity, polyamine content and ethylene rate of evolution. However, further work is required to fully understand the precise role of polyamines and ethylene in plant development. It may be that some of the developmental processes (i.e. ripening of fruits, senescence of leaves and root growth) in plants are controlled by the interdependent regulation of polyamine and ethylene biosynthesis. This hypothesis could now be tested using antisense and sense approaches since most of the key genes involved in the biosynthesis of polyamines and ethylene have been isolated.

Reference

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A molecular approach to clone a wide spectrum nematode resistance gene (the *Hero*) of tomato

A. Kumar, M.S. Phillips & M. Ganal[1]

The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are major pests of potato throughout Europe. Control of this pest is by rotation, use of nematicide and resistant cultivars. There are few natural resistance genes to PCN. The H1 gene confers resistance to two of the *G. rostochiensis* pathotypes (Ro1 and Ro4) and the Gro1 gene to Ro1 and Ro5. There is only one effective major gene (H2) against the *G. pallida* Pa1 pathotype which is found mainly in Northern Ireland. There is no qualitative resistance to the remaining pathotypes of both species (Ro2, 3 and Pa2, 3). Partial resistance is available, but due to its polygenic nature and its origin in wild species, it is difficult to breed for both high levels of resistance and good agronomic characters.

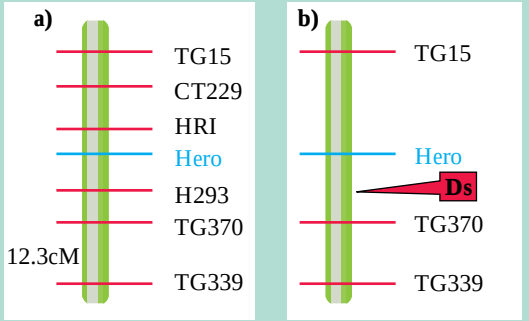

Figure 1 (a) high resolution genetic map of the *Hero* gene on chromosome 4. (b) Mapped position of the *Ds* element on chromosome 4. Estimated map distance between the *Hero* gene and the *Ds* element is approximately 3-5 cM.

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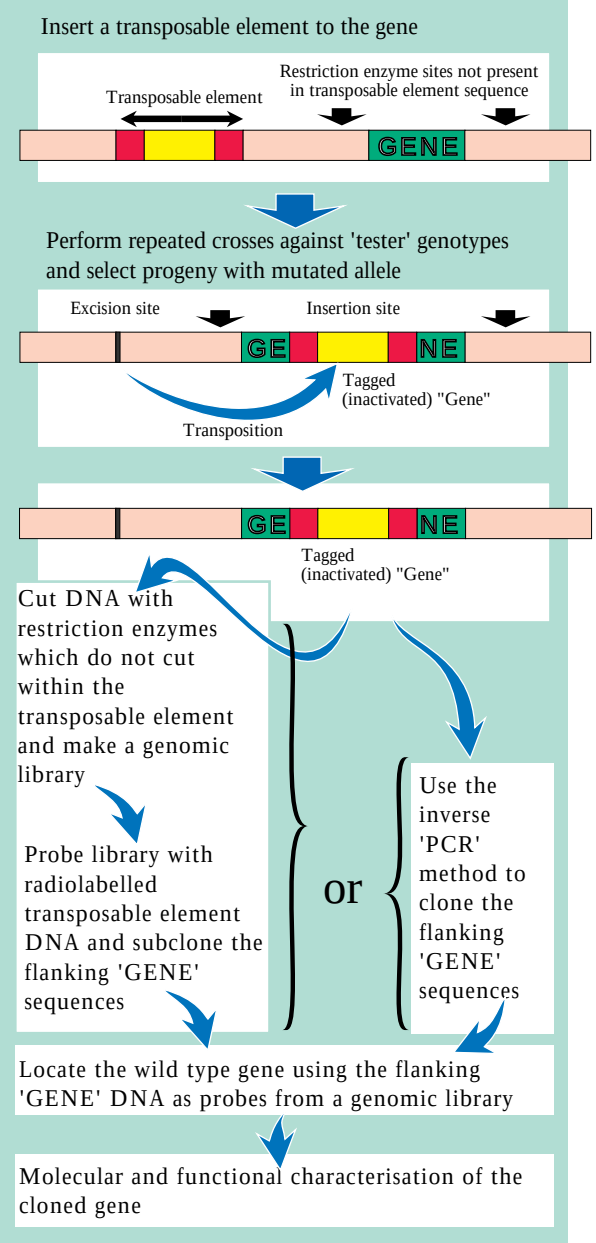

Figure 2 Diagrammatic representation of the transposon tagging cloning strategy for the *Hero* gene.

This article describes progress towards isolation and characterisation of a major resistance gene in tomato (*Lycopersicon esculentum*), known as the *Hero* gene, which is effective against all pathotypes of *G. rostochiensis*, Ro1-5 and confers some resistance to *G. pallida*. This gene has been introgressed from the wild tomato species *L. pimpinellifolium* into the cultivated tomato. We have used RFLP and RAPD analysis to identify the *L. pimpinellifolium* chromosome segment which contains the gene and have found that the resistant line contains a single introgressed segment on chromosome 4. This can be characterised by three RFLP markers from the high-density RFLP map of tomato[1]. The map position of the *Hero* gene is not equivalent to any of the previously mapped *G. rostochiensis* resistance genes in potato. For the fine mapping of the *Hero* gene in large populations, four additional RAPD markers were identified in the introgressed region. After analysing more than 800 gametes for recombination, we found that one marker is only 0.4 cM away from the *Hero* gene[1] (Fig. 1a).

No clear route exists for isolating this gene on the basis of its biochemical properties, since nothing is known about what it encodes. Therefore, cloning methods based upon genetic properties are needed. Transposon tagging combined with genetic map analysis, is one of the most promising method available and is most applicable to tomato (Fig. 2). Recently, several groups have demonstrated the successful use of the *Ac*-based transposon tagging method for isolating genes from heterologous plants such as Petunia, tobacco and tomato plants[2]. They were successful largely due to (i) the knowledge of chromosomal locations of the target genes, (ii) the insertion of the engineered *Ac* or *Ds* elements close to the target genes and (iii) development of efficient screening protocols for the identification of insertional mutant phenotypes.

The conditions required for applying transposon tagging technique for isolation of the *Hero* gene of tomato have already been established at SCRI. The *Hero* gene location on the short arm of chromosome 4 has been mapped (Fig. 1a). Dr J. Jones's group in Norwich, has identified and provided us with a

Figure 3 Easily identifiable golden females of *Globodera rostochiensis* Ro1 pathotype on roots of a susceptible tomato line. Approximately 30-100 female cysts are normally present on roots of the susceptible tomato line whereas only 0-10 female cysts are present on resistant roots.

tomato line which contains a *Ds* element also on the short arm of chromosome 4 (Fig. 1b). The *Ds* element is located at approximately 3-5 cM map distance from the *Hero* gene (Fig. 1a and b). The tomato line containing the *Ds* element has been used to make a series of crosses with the homozygous *Hero* tomato lines and a few lines that are homozygous for the *Hero* and *Ds* have been identified. The homozygous *Hero* and *Ds* lines have been crossed with the homozygous *Hero* lines containing a stabilised *Ac* element in order to trans-activate the *Ds* element in the hybrid lines. We have established a highly reliable and efficient PCN screening protocol for the identification of insertional mutants which phenotypically appear as susceptible plants (Fig. 3). We are in the process of identifying the rare insertional mutant lines. After confirming that the mutant phenotypes co-segregate with the *Ds* element the standard molecular techniques will be used to clone the *Hero* gene as described in Figure 2.

Isolation and characterisation of a broad spectrum nematode resistance gene such as the *Hero* gene of tomato would be of great value for studying the mechanisms involved in resistance against a major pest. Introduction of the *Hero* gene into susceptible potato genotypes would greatly enhance the control against this pest with a consequent reduction in the reliance on toxic nematicides.

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Linkage analysis in tetraploid potatoes using 'single dose' PCR-based markers

R. Waugh, D. Milbourne, J. Bradshaw, C. Hackett, W. Powell & R. Meyer

Almost all reported genome-based research in potatoes has been carried out at the diploid level in order to overcome the inherent difficulties associated with tetrasomic inheritance. While this has been a particularly valid approach for the construction of genetic linkage maps and the identification of markers linked to both major genes and the components of polygenic characters, it remains one step removed from most potato breeding programmes. This is largely because potato varieties are tetraploid and traditional breeding is carried out at the tetraploid level, a situation which is likely to remain for the foreseeable future. At SCRI, we have a strong commitment to the development of new and improved potato varieties, including the incorporation of disease and pest resistances from wild and primitive cultivated species. It has often taken up to 30 years, but maybe only four or five generations, to produce an acceptable potato variety which even then may ultimately fail in the market place for apparently trivial reasons. While new breeding methodologies based on progeny testing are currently being devised to accelerate the identification of superior genotypes for further improvement cycles, marker assisted breeding should help to improve the speed and precision of the overall scheme.

Tetrasomic inheritance, a feature of tetraploids in which random pairing of homologous chromosomes occurs at meiosis, imposes a number of problems on linkage studies which are absent at the diploid level. For example, the possible number of allelic combinations in a segregating population is significantly higher (Fig. 1b). Nevertheless, linkage mapping at the tetraploid level should be possible using the 'single dose marker' approach[1] successfully used in *Saccharum*[2]. We have been examining a tetraploid F1 population derived from a cross between the variety Stirling and the advanced breeding line 12601ab1 to gain an insight into the genetics of tetrasomic inheritance. The parents were chosen because they express complementary quantitative resistance to late blight

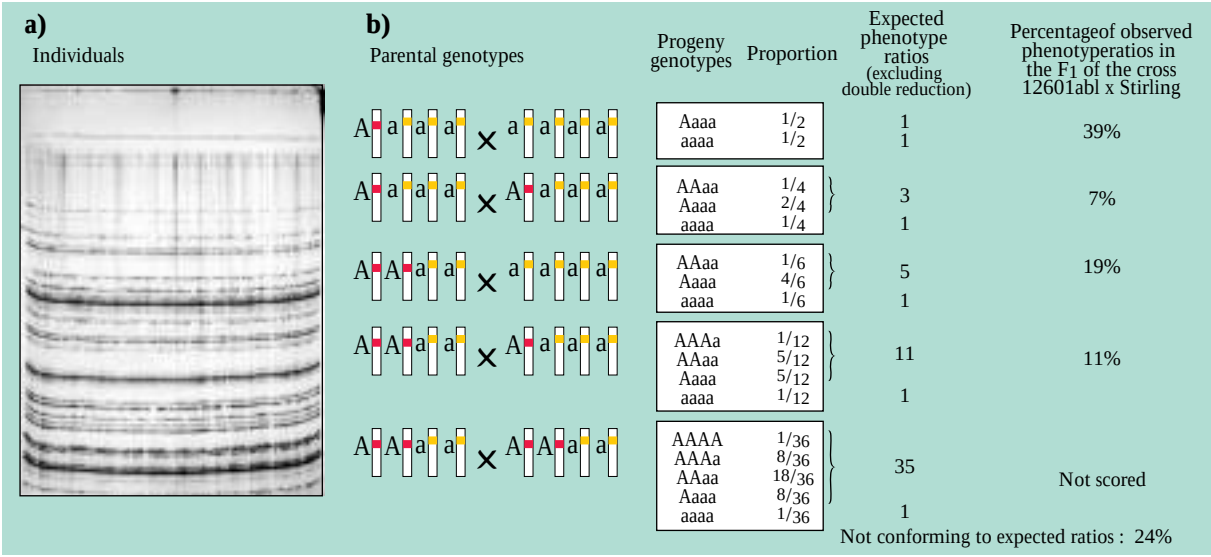

Figure 1 Segregation of dominant PCR based markers in a tetraploid potato cross. Figure 1a shows a typical AFLP autoradiograph of 94 individuals and the two parents of the tetraploid potato cross 12601ab1 x Stirling. Figure 1b shows parental genotypes which produce these classes. AFLP is a dominant marker system, thus the allele A represents the presence of a marker, whereas the recessive allele, a, is characterised by the absence of a marker. The genotypes of the resulting F_1 progeny for the allelic combinations shown are illustrated in Figure 1b, and the frequencies of the resulting phenotype (i.e. band present or absent) in the progeny. The final column shows the percentage of each segregation type occurring in the 12601ab1 x Stirling cross for the approximately 500 segregating markers scored to date. As can be seen, 24% of these markers do not segregate in the expected ratios.

and the white potato cyst nematode (*Globodera pallida* Pa2/3). The breeding line 12601ab1 also possesses the H1 gene for resistance to the golden potato cyst nematode (*G. rostochiensis*) and is resistant to low temperature sweetening, an important processing characteristic. Progeny from this cross have been evaluated under replicated conditions for late blight and also screened for PCN resistance. A subset of 94 individuals from the population was chosen for our mapping studies which began in autumn 1995. Our initial hypothesis was that we should be able to use markers with expected segregation ratios of 1:1 (simplex x nulliplex), 5:1 (duplex x nulliplex) and 3:1 (simplex x simplex) as these marker classes could be easily distinguished at the 98% confidence level. We decided to use two molecular assays, SSRs and AFLPs. These provide complementary information. AFLP allows a large number of segregating markers to be followed (Fig. 1a). SSRs allow the detection of multiple alleles at a single locus. Our hope is that the SSRs will allow us to bridge the different linkage maps derived from each of the individual linkage groups. Using only single dose markers, we would expect to obtain up to 96 linkage groups (four for each of the 12 linkage groups from each parent) in this population. To date we have surveyed approximately 3000 loci in this population with AFLP. Over 500 markers segregate in the population. Thirty-nine percent fit the expected segregation ratio of simplex x nulliplex, 19% duplex x nulliplex, 18% simplex or duplex x simplex but 24% do not conform (at the 98% level) to any expected ratio (Fig. 1b). Analysis of the segregating 'single dose' markers revealed that *c.* 60% originate from maternal parent 12601ab1. The markers can be assembled into 19 (Stirling) and 29 (12601ab1) parental linkage groups (defined as at least a pair of linked markers) with *c.* 24% remaining unlinked. Inverting and appending the datasets for each parent to the original data file failed to detect any disomic type repulsion phase linkages indicating that the parental clones behave as the autotetraploids (in diploid, population repulsion phase linkages can be detected with the same precision as coupling phase linkages).

Most of the desirable traits in potato improvement programmes are polygenic or quantitative characters which, in a population, exhibit continuous variation. With a potential maximum of eight alleles segregating at a single locus (and hence in the absence of double reduction, 36 potential genotypes at one locus in a population from a cross between two parents), precise QTL analysis will undoubtedly be difficult. Nevertheless, the entire dataset has been examined for

potential linkages between markers and components of blight resistance using one way ANOVA. Approximately 20% of the markers showed some association, accounting for between 3.5-15% (1:1 markers) and 3.6-22% (5:1 markers) of the total variation for the trait. In all cases where an association was detected, the resistance originated from the resistant parent Stirling. Although QTL analysis at the tetraploid level will undoubtedly be complicated and potentially lack the resolution of similar studies at the diploid level, it will be possible to compare the location of QTL at the two ploidy levels. Thus, for the first time detailed genetical studies are being carried out on tetraploid potatoes which have to date been recalcitrant to such analyses.

This work was in part funded by the Potato Marketing Board, the European Union and the International Potato Center, Peru/United States Department of Agriculture.

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Cellular and environmental physiology

Howard V. Davies

To create critical mass in key areas and to improve the fluency and effectiveness of science management, the Institute has adopted the concept of Research Units. Five Units have been structured within the Department of Cellular & Environmental Physiology - Cell Physiology, Plant Transport Processes, Industrial Crops, Integrative Bioscience, and Stable Isotopes. Research articles from members of each of these Units accompany this overview and continue to highlight the skills and breadth of knowledge, not only of the core staff, but also of the students, postdoctoral fellows and visiting workers attracted to the department by its international reputation in several areas of high profile research.

The Unit of Integrative Bioscience comprises a widely skilled team dedicated to the study of complex biological systems ranging in scale from biochemical pathways to ecosystems. The major challenge is to obtain a mechanistic understanding of how the large scale features of such systems arise from the interaction of their components. This is being achieved through a fusion of innovative theories with pioneering experimental studies. Work is focusing on the interactions in the soil/plant/microbe system with a view to improved and sustainable management of agricultural and semi-natural ecosystems. In the vegetation dynamics programme, the first major species interaction ecological experiment was established at several sites with the aim of quantifying the impact of gene-flow on functional trait diversity and the dynamics of complex eco-systems. The work has gained significant external funding from the SOAEFD Flexible Funding initiative, MAFF and NERC. Mathematical models of pollen movement, geneflow and establishment of feral and oilseed rape have been constructed, tested and applied to policy and management issues e.g. isolation distance, spread of herbicide resistant transgenics. Models indicate that the unpredictability of the arrangement of fields across an agricultural region is more important than uncertainty in pollen movement

for assessing spread of introduced genes to the environment. Although management practices can reduce the volunteer weed problem resulting from the use of herbicide - resistant oilseed rape, uncertainty in the competition between the crop and weed at high weed density currently limits our ability to predict risk. It has also been found that rate-temperature relations for germination times in some cultivars of oilseed rape are genetically determined and linked to certain DNA markers. This relates to progress in developing a theoretical framework for within-population variability on the dynamics of species rich communities. There are marked differences in the predicted behaviour of systems, compared with approaches which average out this variability. Another area of the Unit's research with important ecological ramifications (recognised via two mentions in BBC's Tomorrow's World programme) is the mapping of the distribution of New Zealand flatworm populations. The survey associated with this programme also showed the Australian flatworm to be far more widespread than was initially thought.

In microbial community dynamics, techniques are being applied which enable the broad-scale measurement of microbial community structure, using community DNA profiling. The impact of elevated CO_2, and thence rhizodeposition, upon rhizosphere community structure has been studied. The G-C content of the community is altered slightly by increasing rhizodeposition through elevated CO_2, and cross-hybridisation studies indicate a 20-40% change in similarity between communities derived from rhizospheres generated under ambient and elevated CO_2. These techniques are also being applied to study the coherence of microbial communities below characteristic upland grasslands, to test the hypothesis that vascular and microbial communities are coupled. This latter work is part of the SOAEFD Micronet initiative, a co-ordinated programme of research involving six research institutions throughout the UK.

Nitrification rates in soils are limited by substrate levels and by constraints imposed by soil structure on flow rates. Even after these constraints have been removed in the laboratory, temporal variations in rate still persist.

The regulatory role of specific volatile organic compounds (VOCs) produced during the mineralisation of organic matter incorporated into soil is being investigated. A European framework has been established to investigate the interactions between carbon flows (including VOCs) and nitrogen transformations (particularly the production and uptake of gaseous nitrogen oxides) in sustainable agricultural systems.

In soil biophysics theoretical work has successfully related the structure of soil to extraction of moisture. This is the first time that a direct quantification of structure has been related to water properties. The same framework can be used to further our understanding of the effects of structure on microbial mobility (see p.86) and therefore further analysis of the complex interaction between structure moisture and microbial activity is possible. This unique capability has attracted attention from industry and a sig-

nificant contract has been awarded to improve the efficiency of nematicides.

In Cell Physiology, a combined $^{14}C/^{13}C$ approach has been developed and used to provide quantitative estimates of carbohydrate metabolism *in vivo* in developing tuber tissues. The studies indicate that the enzyme fructokinase catalyses a rate limiting reaction *in vivo* during conversion of sucrose to starch. In collaboration with theoretical biologists, a new mathematical framework has been developed to study the dynamics of biochemical pathways. The approach integrates non-linear mathematics with non-equilibrium thermodynamics to study the effect of specific enzymes, or groups of enzymes, on the stability and thermodynamic efficiency of complex biochemical systems. Current research focuses on the central processes in higher plant glycolysis, and has identified the major sources of efficiency and stability.

This year has seen the initiation of a major programme focusing on the molecular control of starch biosynthesis in potato tubers. The effects of introducing heterologous genes involved in starch biosynthesis from other plants, fungi and bacteria, into potato are currently under investigation. The effects on starch structure will be determined using a range of physical chemistry techniques. Other genes that may be involved in controlling the flux of carbon into starch are also being studied. Most notable amongst these is a novel alpha-glucosidase gene that has recently been cloned from potato tubers. A preliminary intellectual property recordal detailing the sequence and potential uses of the gene product has been filed. This year has also seen the first field release of transgenics with modified expression of genes encoding an array of carbohydrate handling enzymes. The work, supported by the ECSA Research Ltd, is aimed at minimising reducing sugar accumulation in cold stored potato tubers with a view to improving processing quality. The programme, involving the Departments of Cellular & Environmental Physiology, Cell & Molecular Genetics and Crop Genetics, will span three years and will test the efficacy of *c.* 20 genetic constructs on sugar metabolism.

The theme of gene expression during tuber formation and development has been maintained in an EC funded tuberisation project. The cDNA clones of two novel genes that are differentially expressed during tuberisation have now been fully characterised. The expression level of these genes is being manipulated by the generation of antisense mutants. In addition, the effects of manipulating polyamine levels in the tuberising stolon are being investigated. The ability to manipulate polyamine levels in plants by changing the expression level of the S-adenosylmethionine decarboxylase (SAMDC) gene has recently been demonstrated for the first time at the SCRI. Using a tuber-specific-promoter, it is possible to design transgenic plants in which the changes in polyamine levels are confined to the tuber. It will then be possible to pinpoint more accurately the role of these compounds in tuberisation and storage organ metabolism.

Studies into the molecular mechanism of fruit ripening continue to gather momentum in several externally funded programmes. The promoter of a blackcurrant gene that has a fruit-specific expression pattern has been isolated and is being characterised. The transcription start site of the gene has been determined and promoter-reporter gene constructs have been made. These will be assessed in a transient assay system being developed for the biolistic transformation of blackcurrant. In a related project, genes that determine the post-harvest quality of raspberry fruit are being targeted. Already cDNA libraries have been constructed and screening is underway.

In the Unit of Plant Transport Processes, work continues on the structure/function of higher plant plasmodesmata. New molecular biology approaches, coupled to novel imaging methods utilising confocal laser scanning microscopy (CLSM), are giving new insights into the ways in which plant cells communicate with one another. Specifically, the modifications of plasmodesmata induced by viral genes are now beginning to be unravelled in detail using virus constructs which express the gene for the green fluorescent protein (GFP) from the jellyfish *Aqueorea*. Additional work on the role of plasmodesmata in phloem unloading pathways is exploiting a range of fluorescent xenobiotics to produce predictive structure activity relations (SAR) models for phloem transport. This work is being carried out in collaboration with Dr R. Horobin (Sheffield) and Dow-Elanco (Indianapolis) and is leading to a better understanding of the factors which predict the phloem mobility of agrochemicals such as herbicides and fungicides.

Studies of the transport pathways in the barley caryopsis have shown that cell-cell communication is both spatially and temporally regulated during the early stages of grain filling. This developmental orchestration of plasmodesmata leads to a highly organised delivery of solutes to different developing tissues fol-

lowing anthesis, culminating in a strictly channelled supply of assimilates to the developing endosperm. This work, coupled to continued work on the *Arabidopsis* root tip, is providing new insights into the role of plasmodesmata in plant developmental processes.

In the Unit of Industrial Crops, applications of the use of the FT-IR spectrometer and microscope to the non-destructive characterisation of plant materials and products have increased. The quality of previously published work has resulted in a number of collaborations being initiated, especially in relation to plant cell walls. In particular, the interpretation of spectra of lignin-containing materials has been made for groups involved in modifying lignin structure by the genetic manipulation of enzymes of lignin biosynthesis. This is an area of great potential commercial value.

The use of the DRIFT attachment facilitated funding for work on the storage of fibrous materials under the LINK Crops for Industrial Use programme and the assessment of Reed Canary Grass. The latter is funded by the EU FAIR programme. The former has shown that the changes in fibre composition and, therefore, quality are dependent on the methods and conditions of storage. Indeed, there is evidence of changes not only of the carbohydrates but also the lignin components under certain storage conditions. Plots of Reed Canary Grass have been established at SCRI for the latter project and SCRI will be responsible for the analysis of samples from a number of sites in Northern Europe. The use of peracids in the delignification/bleaching of plant fibres has been extended to cover a wider range of oilseed rape materials and other fibre sources such as jute, sisal and manilla.

The work on the laccase-type phenol oxidases which are present in lignifying tissues has been extended to tobacco. The ionically-bound oxidases are able to oxidise coniferyl alcohol. A number of different isoforms occur and the set of isoforms from younger, apical stem tissue is different to that from older basal stem tissue. This suggests that they are expressed differently during xylem maturation. One polypeptide, of *c.* 100 kDa, has similar properties to a polypeptide of similar size previously identified in Sitka spruce. Investigations on glucomannan synthases (GMS) now concentrate on peas due to the higher levels of enzyme activity relative to flax. Membrane-bound enzymes have been prepared by a modified digitonin method and purified by affinity chromatography. The GMS activity does not bind to a mannose-specific ligand but does to a glucose-specific one. Eight major polypeptides have been identified and these are being characterised to determine their specific functions.

The Stable Isotopes Unit has used ^{18}O and wheat to indicate the presence of various water pools in leaf and stem tissues. A collaborative project between SCRI and the Universities of Dundee and York has found evidence for possible feedbacks between elevated levels of CO_2 and the capacity of wheat to exploit soil nutrients when supplied with inadequate amounts of water. Despite the fact that plants grown in elevated CO_2 produce larger root systems, they are less able to take up nitrate available in the soil when watering is restricted. This does not happen when water supply is maintained. One explanation for this (which is now being investigated further in collaboration with the University of Western Sydney), is that, under elevated CO_2, C3 plants (such as wheat) use less water. Thus the supply of mobile nutrients such as nitrate by the mass flow of soil solution to the root surface is smaller. This has little effect in moist soil where the rapid diffusion of nitrate can compensate for the smaller mass flow. In a dry soil, however, ion diffusion is impeded and so uptake is restricted. This implies a limited response of C3 crops to future CO_2 concentrations in a changed climate in which rainfall may also be less predictable.

A new, externally funded project aims to assess the extent to which the supply of nutrients from decomposing organic matter is coupled to the activities of soil microbes. It will use SCRI's state-of-the-art stable isotope facilities to monitor, simultaneously, the supply of nitrogen to and its uptake by plants, and the release of carbon from decomposing substrates.

Other research highlights include a major review on the current understanding of the interpretations of $\delta^{15}N$ values in natural systems. This includes original data on a Scottish lowland system which emphasises the complexity and range of values. Data substantiates the growing body of literature which suggests that plants occupy nitrogen niches so that competition for the scarce resource is frequently avoided in nature. Other studies have revealed gender-linked differences in the use of nitrogen and in carbon isotope discrimination within a single population of Juniper across water availability gradients. These data have conservation management implications and suggest that further ecophysiologcal research is required for best management. Other projects are demonstrating trophic patterns in below-ground food

webs, similar to those seen previously in aquatic ecosystems. A recently completed field study on the role of ectomycorrhizas in N cycling has shown that the $\delta^{15}N$ patterns consistently observed among ectomycorrhizal fungi and their hosts are largely due to internal reworking and loss of fungal N to the growing medium or soil and not to the widely postulated translocation of N to host trees or to the initial value of the source soil N. This has immediate application in our understanding of N cycling in natural systems.

Two new SOAEFD programmes involving stable isotopes have been initiated through the Flexible Funding scheme. The first involves collaboration with Cell & Molecular Genetics in a dual approach involving DNA markers and $\delta^{13}C$/gas exchange to assess the remaining genetic diversity in Scots Pine. The Forestry Commission, Royal Botanic Gardens Edinburgh, Scottish Natural Heritage and Newcastle University are also involved. The second is a pilot study to determine whether the monitoring of multiple stable isotopes can be used to describe foodwebs and the impact of suspected nitrate pollution in nitrate-sensitive regions. This study is integrated with complementary studies conducted by Aberdeen University and the MLURI.

Effects of elevated atmospheric carbon dioxide levels on below-ground processes

M.M.I. van Vuuren[1], D. Robinson, J.A. Raven[1] & A.H. Fitter[2]

Our ever-increasing consumption of fossil fuels is releasing carbon dioxide into the atmosphere faster than it can be absorbed by plants and by the oceans. As a result, the atmospheric concentration of carbon dioxide ($[CO_2]$) is increasing at an average rate of 0.35% per year. If this trend continues unchecked, the current $[CO_2]$ of 360 µmol mol^{-1} will double during the next century. To accurately forecast the consequences of this global change, we need to know more about the effects of elevated $[CO_2]$ on plants and soils.

Research on the effects of $[CO_2]$ on plants has focused on above-ground processes (e.g. photosynthesis, canopy development). Less is known about effects below-ground, on the functioning of roots or on microbial processes. Elevated $[CO_2]$ almost always increases plant biomass; this applies equally to crops and natural vegetation[1]. Such increases vary from 0% to 40%, depending on species. The effect of elevated $[CO_2]$ on plants also depends on environmental factors (e.g. temperature) or on the availability of other resources (e.g. nutrients and water).

We are investigating the effects of elevated $[CO_2]$ on above- and below-ground processes. We are also investigating the interactions between the effects of elevated $[CO_2]$ and the availability of nutrients and water. These interactions are complicated by the possible indirect effects of elevated $[CO_2]$ on nutrient uptake by plants.

Elevated $[CO_2]$ may affect nutrient uptake by plants in several contrasting ways: (1) A greater root mass and root length may improve the plants' capacity for exploiting the soil's available nutrients. (2) However, this may be countered by the greater loss of carbon compounds from the roots if inorganic nitrogen is immobilised by microbes using the carbon as a substrate for growth. (3) C_3 species such as wheat (in contrast to C_4 species like maize) use less water under elevated $[CO_2]$. The correspondingly smaller flow of water through the soil to the roots may reduce the supply of certain nutrients (e.g. nitrate) that are present largely in the soil solution. If water uptake is constrained by elevated $[CO_2]$, nutrient supply may be sustained by the rapid diffusion of ions through

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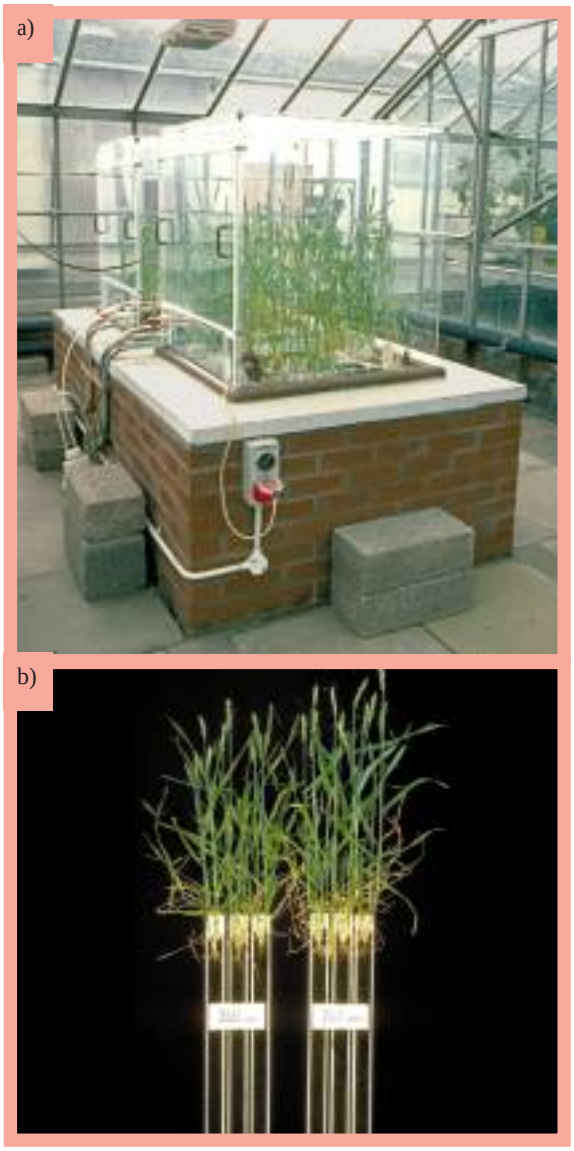

Figure 1 (a) Facility for growing plants at different CO_2 concentrations; containers filled with soil are surrounded by a cooling system in the brick base. (b) Soil containers (front cover removed) with spring wheat plants grown at 350 and 700 µmol CO_2 mol^{-1} for 87 days.

moist soil. But if water uptake is constrained by dry soil as well, diffusion is also hampered and uptake is reduced. Then, a greater root length will be advantageous to offset these constraints. (4) Other nutrients (e.g. phosphorus) are usually present in low concentrations in the soil solution. Their uptake may be reduced by the smaller water use by C_3 plants. However, a greater root length per unit volume of soil, induced by elevated [CO_2], may compensate for this reduction, and nutrient uptake at elevated [CO_2] may exceed uptake at ambient [CO_2].

We are investigating these possibilities using spring wheat (*Triticum aestivum* L. cv. Tonic). These plants are grown in 1.2 m long containers filled with soil. One of the broad sides of each container is removable, allowing repeated access to the soil and the growing root system. The containers fit in specially designed growth chambers (Fig. 1) located inside a glasshouse. In these chambers, CO_2 is maintained at different concentrations. At the same time, the soil and air are maintained at temperatures that resemble those in the field[2]. The plants can also be labelled with the stable isotopes ^{13}C or ^{15}N; this allows us to follow the fates of carbon and nitrogen as these elements are absorbed or released by plants and soil microbes.

Wheat grown at 700 µmol CO_2 mol^{-1} ('elevated CO_2') had a greater root biomass than when grown at 350 µmol CO_2 mol^{-1} ('ambient CO_2'). The difference was 10-35% for mature plants, i.e. after about four months of growth. The distribution of the root biomass over different soil layers was also affected: elevated CO_2 stimulated root growth more in the surface layers than deeper in the soil. Sequential video recordings of visible roots showed that the average total root length was greatest for the elevated CO_2 plants for most soil layers, but only when water supply was ample. There was no difference in root length between [CO_2] treatments when the plants were watered infrequently.

Root mortality occurs when entire roots are lost. The video recordings indicated that the number of whole, dead roots lost to the soil during the growing season was not affected by [CO_2]. If elevated [CO_2] does influence the input of carbon from plants to soil in this system, it occurs via losses from living roots or from the decomposition of roots following harvest (see below).

In an experiment with an ample supply of nitrogen (mainly nitrate), wheat grown at elevated [CO_2] contained more nitrogen and depleted inorganic nitrogen in the soil more than when grown at ambient [CO_2] (Fig. 2a). Elevated [CO_2] plants also contained more phosphorus (Fig. 2b). More nitrogen and phosphorus was obtained from the soil despite the smaller amounts of water used at elevated [CO_2] (Fig. 2c). This suggests a positive effect of the greater root biomass and length on nutrient uptake.

The amount of microbial carbon in the soil from this experiment was similar in the two [CO_2] treatments. We found no evidence of a greater sequestration of

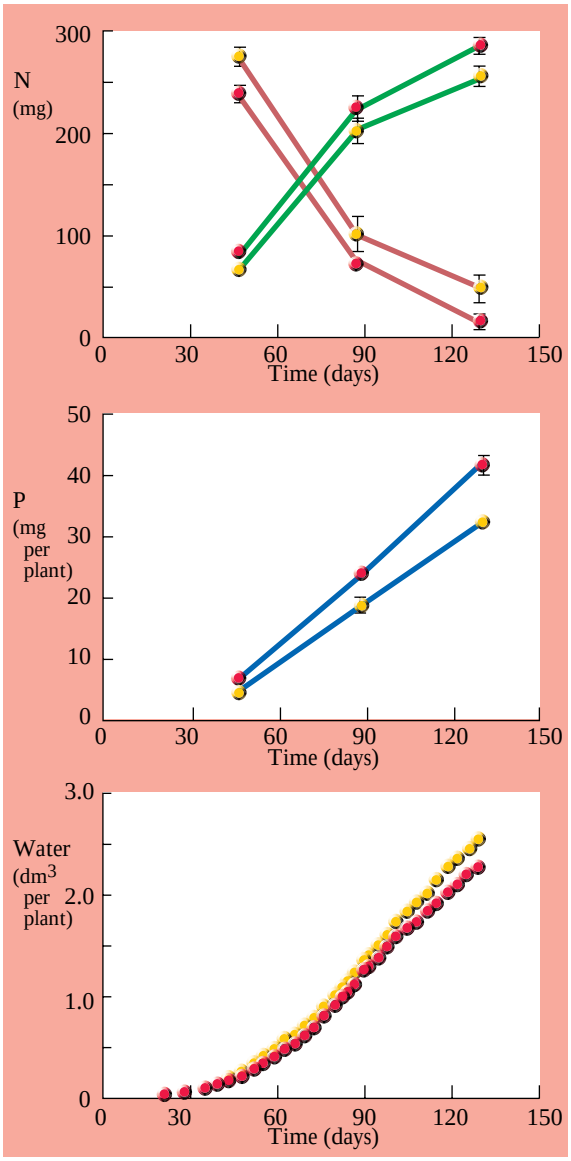

Figure 2 (a) Total plant nitrogen (—)and total soil inorganic nitrogen (—); (b) total plant phosphorus; and (c) plant water use for spring wheat plants grown at 350 µmol CO_2 mol^{-1} (●) and 700 µmol CO_2 mol^{-1} (●). Data are averages ± standard errors (n = 4).

^{15}N label in the elevated [CO_2] soil. In fact, we found a greater uptake of the label into plants grown at elevated [CO_2]. The greater allocation of carbon into the root system at elevated [CO_2] does not seem to be associated with a larger soil microbial biomass and the microbial immobilisation of inorganic nitrogen is probably no greater at elevated than at ambient [CO_2].

Finally, we are investigating the effects of elevated [CO_2] on the soil system after the plants' death. Since the plants are grown in an atmosphere depleted in ^{13}C, carbon derived from them can be detected as it is evolved by microbial respiration. After harvesting the above-ground parts of the plants, the amount of CO_2 evolved from the decomposing roots was, on average, 20% greater following growth at elevated [CO_2]. The abundance of ^{13}C in the CO_2 that evolved changed as the decomposition proceeded, reflecting the progressively smaller contribution of the wheat-derived carbon to the total production of CO_2. The greater evolution of CO_2 is probably the result of more decomposable root material in the elevated [CO_2] containers. However, this material may also have a faster decomposition rate, a possibility that we are currently testing.

Of the possible influences of elevated [CO_2] on nutrient uptake listed above, we have found that (1) and (4) are probably true for wheat, but (2) is not. Although we have some evidence for (3), we need to verify it.

What is clear from our work so far is that the impacts of elevated [CO_2] on plant-soil systems are unlikely to be straightforward, and we may expect many feedback effects to occur among the important variables. Accurate predictions of the responses of crops and natural vegetation to elevated [CO_2] (and other changes in the global environment) will require information about above-ground processes as well as those occurring in the soil.

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Exploiting the green fluorescent protein in plants - viruses carry the torch

K.J. Oparka, A.G. Roberts, P. Boevink, D.A.M. Prior & S. Santa Cruz

The jellyfish *Aequorea victoria* fluoresces when disturbed due to the presence of a green fluorescent protein (GFP), a 27 kDa protein that absorbs blue light and emits green light[1]. The natural function of GFP appears to be to convert the blue chemiluminescence of the Ca^{2+}-sensitive photoprotein aequorin into green emission through a mechanism that involves both radiative and non-radiative energy transfer.

A major breakthrough occurred recently when the gene for the green fluorescent protein was cloned[1] opening up enormous opportunities for the use of this protein as a marker for gene expression and protein targeting in living cells.

The advantages of GFP

The demonstration by Chalfie *et al.* (1994)[2] that expression of GFP in both *E. coli* and the nematode *Caenorhabditis elegans* resulted in the accumulation of fluorescent protein provided the first evidence that fluorescence of GFP requires no additional gene products from *A. victoria*, and that formation of the fluorophore is not species specific. In addition to its ability to be expressed in heterologous systems, the following properties make GFP an exceptionally powerful tool in plant-cell biology studies.

Non-invasive detection *in vivo* Unlike conventional gene reporters such as GUS and luciferase, GFP is intrinsically fluorescent and, with the aid of confocal laser scanning microscopy (CLSM), can be detected within living material at the subcellular level. Thus, imaging of the GFP requires no tissue preparation or staining and is completely non-invasive, opening up enormous opportunities for imaging gene expression in intact, developing systems.

No exogenous substrates or co-factors Several of the conventional gene reporter systems require the application to the tissue of either a substrate to produce a colour reaction (as in the GUS reaction) or the presence of a co-factor. Some of these assays are fraught with problems in that the reaction products are diffusible and may be localised at some distance from the site of the true reaction, leading to staining artifacts and subsequent misinterpretation of results. By contrast, GFP requires no substrates or co-factors and, following translation and maturation of the protein, the fluorophore is exceptionally stable and not subject to degradation.

No photobleaching Unlike several other fluorescent compounds, we have found the fluorescence of GFP to be exceptionally stable under the excitation wavelengths employed in the CLSM. This has allowed imaging of the GFP in living cells over extensive periods of time with no evidence of loss of fluorescence, or more importantly, loss of cell viability.

Fluoresces in fusion proteins An enormous advantage of GFP as a reporter system is its ability to be fused to a second protein without loss of fluorescence, and without interfering with the function of the protein to which it is fused. This property of the GFP allows for protein tagging experiments and for dynamic, *in vivo* studies of protein trafficking within cells, as will be shown below.

Virus vectors for the delivery of GFP

Despite its apparent promise in plants, initial attempts to stably transform *Arabidopsis* plants with the GFP gene were thwarted by aberrant splicing of the GFP mRNA. However, successful expression of the GFP was accomplished using a modified *gfp* sequence engineered to eliminate the cryptic splice sites[3]. The occurrence of GFP mRNA splicing in other species has yet to be determined. To date there have been very few reports of the transgenic expression of GFP in plants although this situation seems likely to change in the near future.

As a rapid alternative to transgenic expression, we have been exploring the potential of plant viruses as episomal vectors for the expression of GFP in plants. This strategy has distinct advantages over production of transgenic plants. First, because plant viruses can multiply to very high numbers, the levels of foreign gene expression can exceed those obtainable in transgenic plants. Second, in comparison with the procedures involved in stable transformation, the production of modified viruses and the infection of

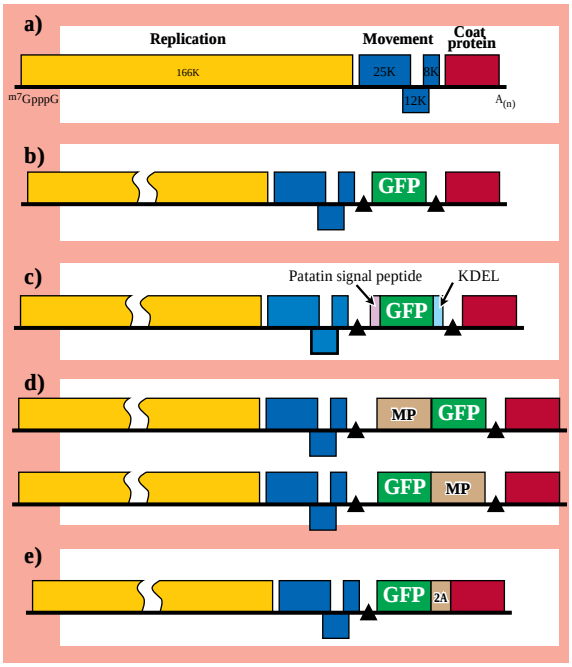

Figure 1 Schematic representation of potato virus X and modified viral genomes. (a) Wild-type PVX. (b) The gene for the GFP is inserted between the triple gene block and the coat protein. Expression of GFP is under transcriptional control of the coat protein subgenomic RNA promoter. (c) GFP-ER targeting construct. The patatin signal peptide and KDEL ER-retention signal are fused to either end of the GFP gene. (d) The gene encoding the movement protein of tobacco mosaic virus is fused to the GFP. (e) Fusion between GFP gene and the PVX coat protein gene to produce a fluorescent 'overcoat' virus.

plants are simple and rapid. This permits the particular protein expressed, or its precise amino acid sequence, to be changed easily and the effects of these changes to be analysed rapidly.

Potato virus X

PVX is a single-stranded RNA virus with a monopartite genome which is packaged into filamentous rods comprising a helical array of identical coat protein subunits. The genome of PVX is shown schematically in Figure. 1a and the known or predicted functions of the virus-encoded proteins are indicated. PVX has a wide experimental host range and accumulates to high levels in infected plant tissues.

Virus-mediated targeting of GFP to different cellular compartments

The cytosol Initial experiments to investigate virus-mediated expression of GFP in plants exploited a duplication of the viral coat protein subgenomic promoter sequence to direct transcription of mRNA

Figure 2 *Nicotiana benthemiana* plant infected with PVX carrying the GFP gene (see Fig. 1b). The inoculated leaf is shown to the right of the plant. Note that circular fluorescent lesions spread towards the leaf veins where the virus enters the phloem. Note, also, the lack of virus movement into mature 'source' leaves. Two of the leaves shown were undergoing the sink/source transition resulting in a lack of movement into the apical (source) region of the leaf.

encoding the GFP[4]. The genome organisation of the modified virus is shown in Figure. 1b. From this construct, the GFP is produced as free protein in the cytoplasm of virus-infected cells. Infection of plants with this viral construct resulted in the development of circular fluorescent lesions on inoculated leaves which were first detectable by eye under UV illumination within two days of inoculation (Fig. 2). Expression of the GFP was extremely high making this construct a particularly useful tracer for following virus movement in intact plants. CLSM images of a

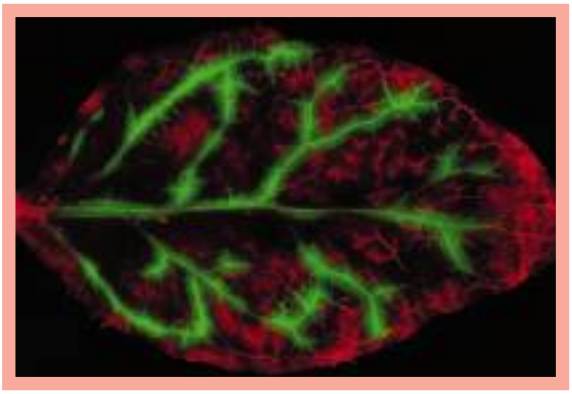

Figure 3 Immature, developing leaf systemically infected with PVX carrying the GFP gene. The virus has unloaded from vein classes I-III, but not from classes IV and V (the minor veins which will later be utilised in phloem loading of assimilates). To identify the veins the fluorescent probe texas red was introduced into the xylem of the infected leaf.

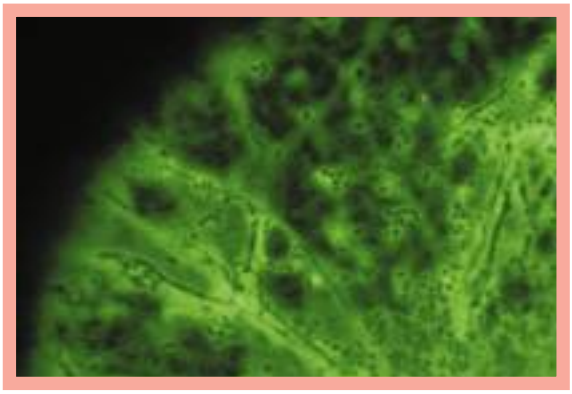

Figure 4 Cytoplasm of infected leaf cell showing GFP in the cytoplasm. Note the negative appearance of organelles against the GFP-containing cytosol.

growing (sink) leaf are shown in Figure 3. Here, the GFP-expressing virus can be seen to unload only from vein classes I, II and III (but not from classes IV and V, which are later used in phloem loading) and move subsequently from cell-to-cell into subtending tissues. At the single-cell level, the cytoplasm of individual, infected cells can be seen to be fluorescent due to the high levels of GFP produced (Fig. 4). Using the above virus construct, in which each infected cell fluoresces intensely, we have begun to make significant inroads into unravelling the mechanisms of both local and long-distance virus movement.

The endoplasmic reticulum The GFP has the potential, if targeted to the plant endomembrane system, to trace protein trafficking within cells *in vivo*. In a Leverhulme-funded project we again utilised the PVX vector and fused the ER-targeting signal peptide from the potato storage protein, patatin, to the GFP. We also found that it was necessary to introduce the ER-retention signal (KDEL, Fig. 1c) in order to retain the GFP within the endoplasmic reticulum. Figure 5 shows the appearance of the cortical ER in a living mesophyll cell after infection of the leaf with PVX carrying the appropriate GFP-ER targeting/retention sequences. Note that in comparison with Figure 4, in which the GFP is located in the cytoplasm, the GFP is now located exclusively in the lumen of the endoplasmic reticulum. Using this approach, the door is open to studying the movement and targeting of proteins in plant cells. From the endoplasmic reticulum, trafficking of storage proteins to the vacuole can be studied, as well as vesicle-mediated secretion of proteins into the cell wall. In collaboration with Dr C. Hawes (Oxford), we are using the above strategy to study the transfer of proteins from the endoplasmic reticulum to the Golgi apparatus. Also, by using the GFP-protein fusion strategy mentioned above, it will be possible to tag endogenous proteins, including enzymes, with the GFP in order to study their location and function within individual living cells.

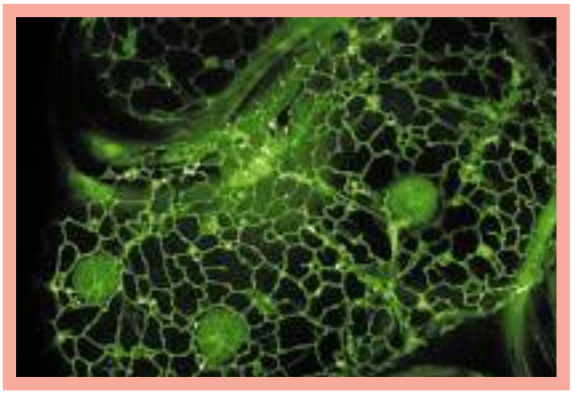

Figure 5 Targeting of the GFP to the endoplasmic reticulum (see Fig. 1c). The lumen of the cortical ER is now illuminated due to the presence of GFP. There is little or no background signal from the cytosol (cf. Fig. 4)

Plasmodesmata Our group has a long-standing interest in the structure/function relations of plasmodesmata, the small channels which interconnect higher plant cells (*see Ann. Rep. 1993, 50-53*). Plasmodesmata are the sole conduits for intercellular virus movement and there has been considerable interest in elucidating the mechanisms by which viruses modify these structures in order to move from cell-to-cell during systemic infection. It would appear that a number of virus groups utilise specific movement proteins (MPs) which target to plasmodesmata during virus infection. The most well characterised of these is the 30 kDa MP of tobacco mosaic virus, a single-stranded RNA binding protein which has been suggested to target to plasmodesmata and increase the molecular size exclusion limit of the pore to a size sufficient to permit the passage of the viral RNA[5]. To date, studies of the interaction of viral MPs with plasmodesmata have been hindered by the small size and inaccessibility of the latter and have necessitated EM-based immunological studies of MP antibody localisation.

Using the fusion strategy, we made both N- and C-terminal fusions of the MP of TMV to the GFP, again using PVX as the vector (Fig. 1d). When leaves were infected with this virus construct, punctate fluorescence appeared at discrete sites at the cell wall (Fig. 6), which we know from other forms of microscopy to be plasmodesmatal pit fields. Thus, the MP-GFP was successfully targeted to plasmodesmata, confirming

Figure 6 Targeting the GFP to plasmodesmata (see Fig. 1d). Bright spots on the cell walls represent plasmodesmatal pit fields illuminated during cell-to-cell virus spread..

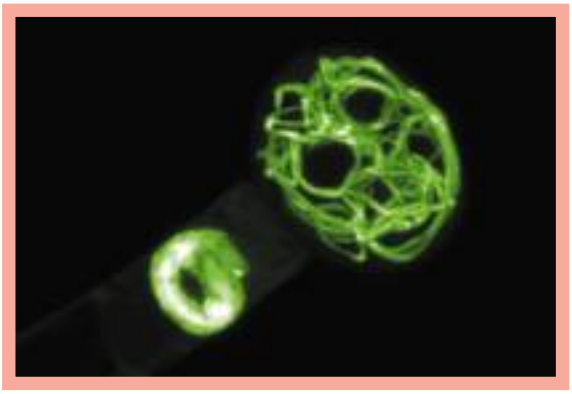

Figure 7 Fluorescent 'overcoat' virus. The GFP was fused to the viral coat protein (see Fig. 1e) resulting in the formation of fluorescent virus aggregates. Note the difference in appearance of the virus aggregates between the tip cell and neck cell of the trichome.

earlier reports that during virus infection the MP interacts with one or more of the constitutive proteins of the plasmodesmatal pore. Future studies will now be aimed at examining further the intimate relations between viral MPs and plasmodesmata.

Construction of a fluorescent virus

In order to study virus replication and movement in greater detail we sought a tag for the virus itself, rather than simply the presence of virus-infected cells. We did this by fusing the GFP to the coat protein gene of the virus (Fig. 1e) to produce a virus with a fluorescent 'overcoat'. The GFP-overcoat virus was extremely fluorescent and aggregates of virus were clearly visible in the cytoplasm of infected cells. In Figure 7 the tip cell of an infected leaf trichome is shown. Note in this case that the GFP is completely associated with the virus rather than any plant-cell constituent and that the virus aggregates adopt different morphologies in different cell types. The 'overcoat' fusion strategy has enormous potential in the production of foreign proteins in plant cells using virus vectors and is the subject of a separate report by Chapman *et al.* (p.135).

A virus-delivery service

The ability to image the cell-to-cell spread of viruses using GFP should begin to answer some of the unresolved questions concerning the infection of plant cells by viruses and, in particular, the complex interaction between viral MPs and plasmodesmata. In this respect, the GFP is already proving to be invaluable as an *in vivo* reporter of virus movement in plants. As the number of viruses into which the GFP gene can be inserted increases then the range of host plants into which GFP can be introduced using virus-based vectors will also expand. Thus, viruses may prove to be a valuable delivery system for the high-level expression of GFP in plants, avoiding the labour-intensive route of raising transgenic plants expressing the GFP gene.

In addition to exploiting the GFP gene in virus transport studies, we are beginning to have success in utilising virus vectors to deliver the GFP to different subcellular compartments in cell-biology studies. Theoretically, a wide range of plant proteins could be fused to the GFP and introduced into individual cells using virus-based vectors. Thus, in addition to the organelles reported here, GFP could be potentially introduced into chloroplasts, mitochondria and nuclei using the appropriate targeting sequences and, in this way, be used to study real-time changes in the structure/function activities of these organelles.

The development of GFP mutants with altered excitation and emission spectra promises to increase further the utility of fluorescent proteins as markers for gene expression and protein localisation. For example, one mutant has been produced which fluoresced bright blue under UV excitation (in contrast to the green of the wild-type protein) and has been termed blue fluorescent protein (BFP)[6]. The use of GFP and BFP, either simultaneously or in sequence, opens up enormous opportunities for the two-colour assessment of differential gene expression, developmental fate and protein trafficking. In addition to mutants with altered fluorescent properties, a mutant showing accelerated oxidation to produce the fluorophore has also been described, thus allowing more rapid detection of the protein following gene induction[7].

In short, GFP (and its variants) is quite literally poised to shed new light on a vast range of cell-biological processes.

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Unravelling the control of seed dormancy in forest species

S.B. Jarvis, M.A. Taylor & H.V. Davies

The phenomenon of seed dormancy is widespread in plant species, especially in those from temperate zones. Breaking of dormancy by exposure to specific environmental factors can ensure that germination is limited to times that are favourable for seedling establishment. In many forest tree species, dormancy-breakage of seeds requires exposure to cold, moist conditions (stratification) for periods ranging from a few weeks to several months. Hence dormancy prevents germination during the winter and only once the cold season has ended will germination occur. In addition, within any seed population, different individuals will exhibit various depths of dormancy, so that during the moist chilling treatment seeds do not lose their dormancy synchronously. Instead, they gradually become less dormant during the exposure. Even after prolonged stratification some seeds will remain dormant. In the field, it is estimated that approximately 30% of viable seeds of forest species are wasted because their dormancy has not been released. This failure of seeds to germinate not only represents lost revenue but also prevents the sowing of seed directly because it is not possible to predict seedling emergence. Instead, seedlings must be developed in the nursery and then transplanted as young trees. The scale and cost of this problem can only increase as Europe enters into extensive forestation programmes, both as an alternative to set-aside policies and as a means of reducing import bills for timber products.

Despite many years of research on tree seeds, the mechanisms underpinning dormancy-breakage during stratification remain unclear. This review demonstrates progress at SCRI within a current EU collaborative project funded through the AIR programme (1993-1996). The programme involves a multidisciplinary group of scientists researching tree seed dormancy from basic, molecular aspects, through to the problems encountered in the nursery. The work carried out at SCRI has centred on Douglas fir (*Pseudotsuga menziesii*), a coniferous species from North America which is grown in the UK for timber products. The work has concentrated on isolating and characterising genes that exhibit changes in expression during dormancy-breakage. This forms the basis of a targeted approach aimed at furthering our understanding of the cascade of events which accompany the transition from dormant to non-dormant states.

Dormancy in seeds of Douglas fir can be broken by a period of moist incubation at 4°C. Figure 1 shows the effect of stratification on germination of intact seeds of Douglas fir at 20°C. Both the rate of germination and the final percentage of seeds germinated

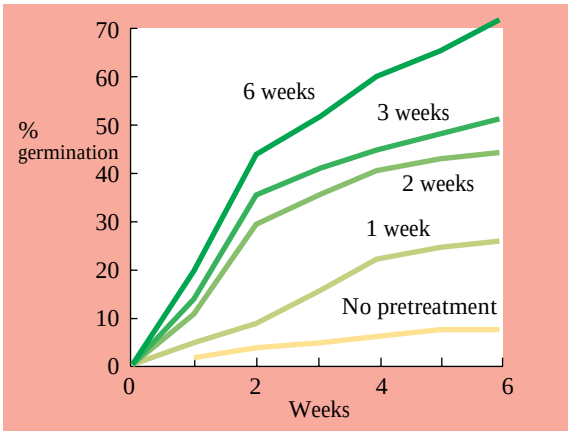

Figure 1 The percentage germination of seeds at 20°C following different lengths of stratification at 4°C.

are enhanced by increasing the period of cold temperature pre-treatment. During the chilling period the seeds became progressively less dormant. After 4 weeks of stratification 73% of seeds germinated following transfer to 20°C. Without the chilling period, the vast majority of seeds maintained at 20°C will not break dormancy (approximately 7% in most seed-lots will germinate without stratification). However, 27% of the viable seeds failed to germinate even after 6 weeks of stratification. In nature, this deep dormancy will protect the species from eradication in the event of adverse environmental conditions. However, for the grower this clearly represents a problem.

Dormancy is thought to be controlled by the regulation of specific genes in response to environmental cues so that germination is prevented early on in seed imbibition. Analysis, by 2D gel electrophoresis, of the products of *in vitro* translation of RNA extracted from seeds, has confirmed that changes in gene expression occur during stratification which are not apparent in seeds maintained at 20°C[1]. It also became apparent that changes in gene expression occur within 7 days of chilling. Some of these changes may be important in dormancy release, whereas others may contribute to the overall fitness of the seed for survival and/or may be involved in preparing the seed for subsequent germination. However genes exhibiting differential expression during the early stages of dormancy-release were thought more likely to be involved in dormancy-breakage *per se*. Genes of interest were predicted to be expressed in seeds stratified for just one week.

Isolation of genes induced during dormancy-breakage A PCR-based subtractive hybridisation technique was used to generate a library enriched with genes that are expressed more abundantly in seeds that have been imbibed and chilled for one week compared with those maintained at 20°C[2]. In this way genes specific to imbibition or involved in general maintenance of the seed should not be isolated. Essentially, biotin tagged cDNA from warm-incubated seeds is hybridised to untagged cDNA from cold-treated seeds. Tagged hybrids are removed using streptavidin-coated magnetic beads. Repeated rounds of subtraction are used to enrich the population of cDNAs for cold-induced genes. These are then amplified using a specific primer ligated during the cDNA synthesis and this DNA is then used to construct a library.

The library has been analysed, and the expression pattern of clones of interest determined by Northern blotting to identify genes showing differential expression during stratification. The steady-state expression of three genes has been studied in detail during dormancy-breakage at 4°C and in controls held at 20°C (Fig. 2). All three are expressed in the dry seed but within 1 h of imbibition transcripts could not be detected. Within 4-8 h of chilling, expression of all three genes could be detected, further increases in expression occurring with prolonged chilling. Transcript levels generally increased 3-4 fold. Conversely, at 20°C, one of the genes, DF65, could not be detected. Expression of DF6 and DF77 was detected after one week at 20°C but transcript levels subsequently fell by 90% and remained low for the following 5 weeks of treatment.

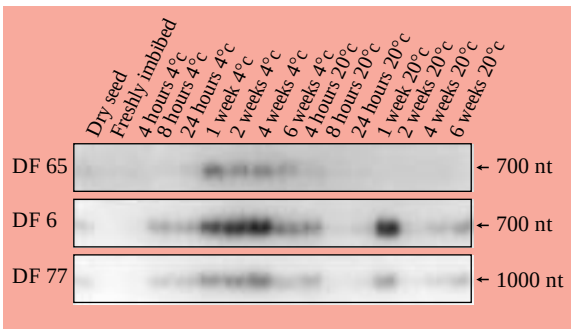

Figure 2 Expression pattern of three cDNA clones isolated from a subtractive library of one week chilled seeds. RNA was isolated from intact seeds incubated at either 4°C or 20°C.

The cDNAs obtained from the subtractive hybridisation were sequenced and compared with known sequences in the EMBL database. On the basis of sequence similarity, it was possible to identify DF6, DF65 and DF77 as members of a family of genes known as the Late Embryogenesis Abundant genes or

LEA genes. Each Douglas fir clone was representative of a different class of LEA, classes 1, 2 and 3 respectively for DF6, DF65 and DF77[3]. More detailed examination of a cDNA library prepared from seeds stratified for 2 weeks, revealed that DF65 and DF77 were members of multigene families. The amino acid composition of LEA proteins appears to be generally conserved between plants with characteristic repeating motifs and a high content of hydrophilic amino acids.

Role of LEAs in dormancy-breakage LEA proteins are typically synthesised during seed development and are most abundant just prior to the desiccation phase, as their name suggests. Once imbibition has occurred, the transcripts for LEA proteins are, usually, no longer expressed and the proteins themselves are not detected in the germinating seed. In dormant wheat caryopses, as in Douglas fir, LEA protein transcripts are maintained in the embryos of hydrated but not germinating seeds[4]. LEAs can also be found in mature plants which have been subjected to stresses such as cold, desiccation, salt or following the application of ABA.

No definitive role has been ascribed to LEA proteins. However, because of their highly conserved sequence motifs and high composition of hydrophilic amino acids, it is thought that they might play similar roles. The most likely function of these proteins is a role in determining internal hydration states. Recently a barley group 3 LEA, has been introduced into rice plants where it conferred increased tolerance to both salt and water stress[5]. In terms of the physiology of the propagule, they could provide desiccation tolerance to the seed after dispersal and then assist in regulating water uptake during imbibition. In the dormant seed, LEAs may protect against repeated wetting and drying cycles that the seed would be exposed to during the winter months on the forest floor. However the induction of DF65 solely in dormant seeds subjected to low temperature suggests a more direct role in dormancy-breakage. In this instance, as previously reported for the wheat caryopsis, the presence of LEA proteins may cause parts of the seed to be subjected to water stress by binding available water.

Factors controlling dormancy Douglas fir seeds, like those of wheat caryopses, exhibit a seed-coat imposed-dormancy. Embryos excised from the dormant seeds are capable of germination. In the intact seed, it is the tissues surrounding the embryo which prevent germination. Northern blot analysis was used to assess the distribution of LEA transcripts between the megagametophyte and embryo (Fig. 3). The expression of

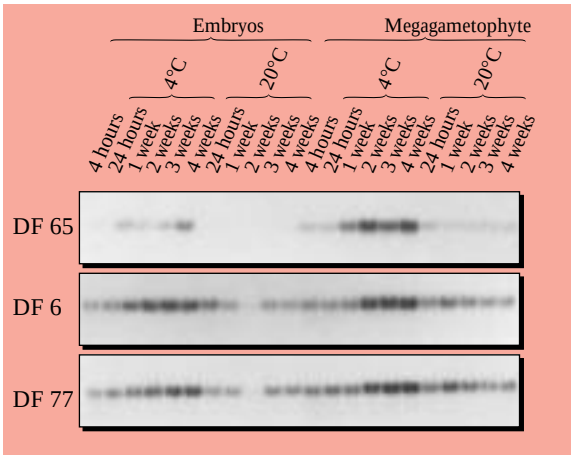

Figure 3 Expression pattern of the three LEA genes in megagametophytic tissue and embryos. RNA was isolated from dissected seeds which had been incubated at either 4°C or 20°C.

DF65 was observed both in chilled embryos and megagametophyte. However, the rate of induction of the DF65 transcript in the embryo was slower than that in the megagametophyte and the steady state level of transcript in the embryo increased during stratification. The expression patterns for DF6 and DF77 were identical for both embryo and megagametophyte tissues and chilling elevated transcript levels in both cases. The expression patterns for all three cDNAs indicate that both the embryo and megagametophyte react to the chilling stimulus with changes in gene expression. The pattern for DF65 suggests however, that the two tissues do not respond in exactly the same manner.

For many years plant hormones have been linked with dormancy. Although there is strong evidence that abscisic acid (ABA) is involved in initiating dormancy during seed development, the subsequent role that this hormone plays in maintenance and release of dormancy is less clear, partly because dormant and non-dormant seeds can have similar abscisic acid contents[6]. The mode of action of abscisic acid is unclear but it is believed to act directly at the level of gene transcription. Application of abscisic acid (100 µM) to non-dormant seeds of Douglas fir will prevent germination. This level of abscisic acid only induces expression of DF6 and DF77 in non-dormant seeds held at 25°C (Fig. 4). DF65 is not specifically induced by this treatment. These results are unexpected as other genes which are up-regulated during dormancy-breakage (in wheat and oats for example), including transcripts for LEA proteins, are all inducible by ABA in dormant seeds.

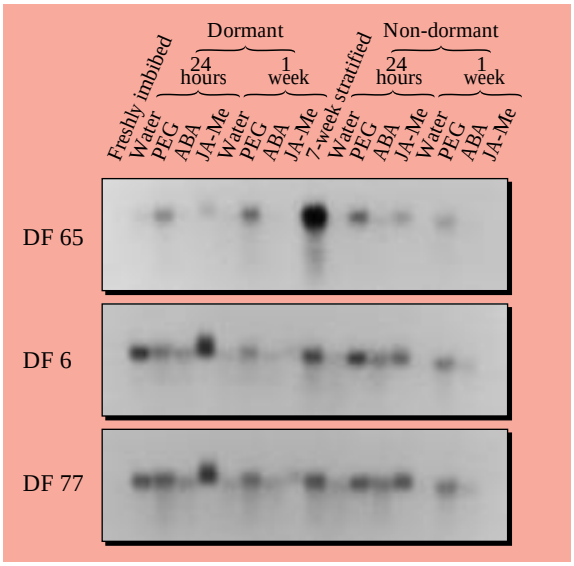

Figure 4 Expression pattern of the three LEA genes in intact seeds which had been subjected to different treatments. Dormant seeds and non-dormant (6 weeks stratified) were treated with methyl jasmonate (45 µM), ABA (100 µM) PEG (-2 MPa) or water were maintained at 25°C for one day or one week.

Jasmonic acid (JA) is a plant growth regulator discovered relatively recently. Workers on other systems have proposed roles for jasmonic acid and methyl-jasmonate in many developmental processes[7]. Jasmonates have been reported to break dormancy in sycamore and apple seeds but have also been reported to inhibit germination[8,9]. Application of methyl-jasmonate to dormant seeds of Douglas fir and Sitka spruce seeds resulted in significant levels of germination. 45 µM methyl-jasmonate was able to substitute for the equivalent of 3 weeks of stratification. However, within one week of germination the seedlings appeared abnormal (see Fig. 5 with Sitka spruce seedlings). Root growth was severely inhibited and the seedlings failed to establish once transplanted into soil. Treatment of dormant and non-dormant seeds with this level of methyl-jasmonate also led to the induction of all three LEAs within 24 h. However, during prolonged exposure (one week), the transcripts for DF6 and DF77 were greatly reduced and that for DF65 could not be detected. This loss of detectable transcript prior to germination is again consistent with the hypothesis that the expression of these genes is associated with the dormant state.

As mentioned above, LEA proteins may provide desiccation tolerance to developing and dormant, hydrated seeds. In addition, many of the stress factors which induce LEA transcripts in mature plants are mediated by water stress. Polyethylene glycol (PEG, -2 MPa) has been used to induce water stress in Douglas fir seed, the treatment preventing germination of non-dormant (6 week-stratified) seeds. Treatment of both non-dormant and dormant seeds with PEG led to the induction of all three transcripts. This is again consistent with the hypothesis that these seeds are under water stress during stratification.

Future The cloning of several genes by subtractive hybridisation and the tight correlation of their expression, in particular clone DF65, with dormancy breaking treatments, gives us a new insight into the developmental process in Douglas fir. However, many questions remain unanswered about the signal transduction pathway during dormancy-breakage and whether the same mechanisms are involved in seeds of other forest species requiring a stratification treatment. Do other gymnosperms show the same responses? Are seeds of broad-leaved species different again? Are additional mechanisms induced in seeds which have pronounced embryo dormancy? What is the actual role of LEAs in dormancy-breakage? What other genes are involved? By comparison with many other developmental processes, our basic understanding of dormancy is poor. Yet, in its widest sense, the induction and release of dormancy has major implications for both natural ecosystems and for crop production in the arable/horticultural sectors. Dormancy is important in cereal grain development, storage and utilisation, bud break in flowers and trees (forest, ornamental and fruit species) and for the storage and development of vegetatively propagated species such as potato. Induction and release of dormancy responds to and reflect the climate and the seasons. As such, climate change may be expected to modify this developmental process as much as any other.

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Soil structural heterogeneity, micro-organism movement and volatile diffusion

I.M. Young & A. Anderson

The role of chemotaxis in guiding organisms to food sources is well documented. However, the process within soil, through a heterogeneous architecture containing high concentrations of mineral particles, water and organic matter, has never been examined. Therefore a great deal of the research carried out on soil micro-organisms remains empirical with little effort being made to include structural heterogeneity quantitatively, even at a simple level.

The importance of structural heterogeneity in mediating all soil processes has been reinforced through recent quantitative studies at SCRI on gaseous flow, water movement and microbial predation dynamics. In the work presented here, experiments and theory are developed to take account of the effects of structure on the diffusion of volatiles from substrate, and reaction of micro-organisms, moving through the same structure, to volatile gradients. Nematodes are chosen as a model system, not only because they are relatively easy to observe, but also because they have a major impact on nutrient turnover and plant health. As a result they have significant import in plant development world-wide, through both advantageous and disadvantageous mechanisms, depending on whether phytophagous or saprophagous nematodes are present.

Four experimental conditions were examined. These consisted of a single nematode (*Caenorhabditis elegans*) placed on one side of a homogeneous nutrient agar in a Petri dish, with or without, on the opposite side, a bacterial source (*Escherichia coli*), which supplied the volatile gradient. Structural heterogeneity was introduced, by the addition of a monolayer of sterile sand grains, into each of the homogeneous conditions. Nematode trails were observed using time-lapse video, and all trails were digitised using in-house software. The conditions used reflect the situation of a nematode moving towards a root in response to a spreading attractant emanating from the root or rhizosphere bacteria. Typical trails from all conditions are given in Figure 1a-d. General observations show the nematode using sophisticated moving and foraging strategies such as reversals and looping, respectively. More random movement was observed to be operating in homogeneous systems without a bacterial source. Quantification of such behavioural patterns was achieved by both fractal dimension analysis and by analysing the turning angles of the trails using the Kolmogrov-Smirnoff test. The results indicate clearly that non-random behaviour is captured by the methods used. The looping foraging strategy, so clearly seen in Figure 1a, is modulated significantly by the structure of the sand grains. Structure acts not only to straighten out nematode trails, but also to increase significantly reversals. The reasons for these results become clear in the development of the theory and simulation model, and lie in the effect of structure on diffusion of the volatile.

In the model, nematode behaviour is controlled by three factors: random movement, foraging strategies and chemotactic terms. The latter is influenced by the level of attractant diffusion, which has the bacteria

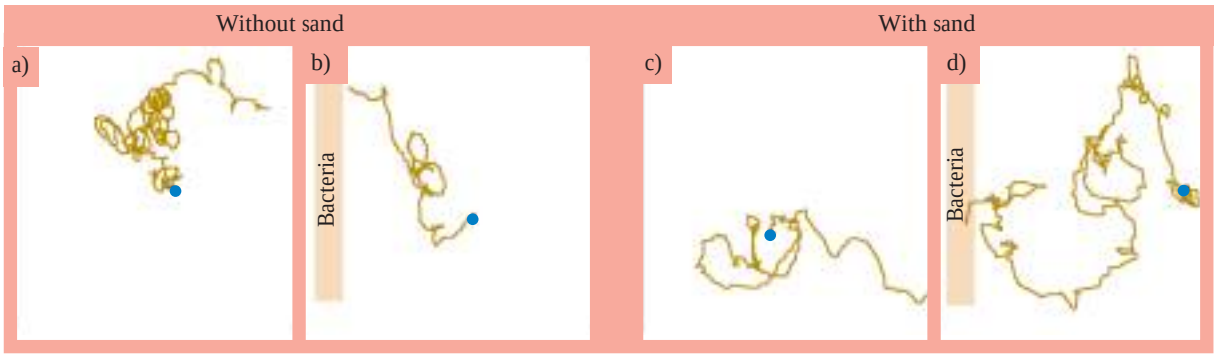

Figure 1 Digitised nematode trails. a) = Homogeneous (nutrient agar with no bacterial source); b) = Homogeneous with approximate location of bacteria shown on the left; c) = Heterogeneous (nutrient agar with a monolayer of sand grains (not shown)); d) = Heterogeneous with approximate location of bacteria shown on the left. (● denotes starting position)

concentration as its source, which in turn is mediated by the level of substrate. A continuous model was developed from a system of differential equations which accounted for the above factors. By using the continuous system as a guide, a discrete model was derived, which approximated the two dimensional (x, y) space of the nematode as a grid of points (mesh size h) and time (t) by discrete increments (magnitude k). The equation governing nematode, N, behaviour is given by,

$$n_{l,m}^{q+1} = n_{l,m}^{q} P_0 + n_{l+1,m}^{q} P_1 + n_{l-1,m}^{q} P_2 + n_{l,m+1}^{q} P_3 + n_{l,m-1}^{q} P_4$$

where subscripts specify the location on the grid and the superscripts the time steps. That is $x = lh$, $y = mh$ and $t = qk$ where l, m, h, k and q are positive parameters. The purpose of this equation is essentially to determine the nematode concentration at position (l,m), time $q+1$ by averaging the concentrations of the four surrounding neighbours at the previous time step q.

The advantage of using a discrete model is the manner in which it splits the equation into the five coefficients P_0 to P_4, which are factors of the nematode concentration at various positions. It is these coefficients that are the driving force behind the model, for they can be thought of as being proportional to the probabilities of the nematode being stationary (P_0) or moving left (P_1), right (P_2), up (P_3) or down (P_4). This is reasonable, as a higher density of nematode trails towards a preferred direction will cause the weight of the coefficient, corresponding to that direction, to be larger and subsequently the probability of movement in that direction will be greater.

The coefficients P_1 to P_4 have the general form,

P_n = Random movement + Chemotaxis + Looping Term,

P_0 has a similar form to the above but without the looping term. The exact forms of P_0 to P_4 involve functions of the attractant concentration near the nematode. Therefore when there is no, or insufficient, attractant the values of P_1 to P_4 are equal with P_0 smaller i.e. there is no bias in any one direction and the nematode is less likely to be stationary. This is in agreement with observation. However, if there is an attractant gradient (strong enough for the nematode to react to), chemotaxis dominates and the coefficients P_0 to P_4 will become biased (larger or smaller) depending on the gradient of the attractant.

To show the effectiveness of the model, simulations were carried out on a 200 x 200 grid, which is a discretisation of a 10 x 10 cm square approximating the

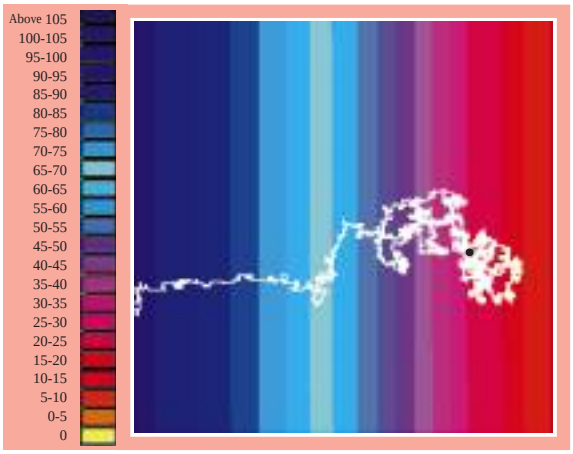

Figure 2 Homogeneous simulation after 60,000 time steps, with attractant (arbitrary scaling) concentration displayed on the left (nematodee trail is given in white and • denotes the starting position.

homogeneous agar plate used in the experiments with a space step of h = 0.5 mm. No flux boundary conditions were imposed on the square grid, restricting the nematodes and the volatile attractant (the only variables which diffuse) to within the grid.

Simulations were carried out with a bacterial source present, both without (homogeneous), and with structure (heterogeneous). Figure 2 shows the nematode trail (in white) reacting to an attractant gradient, in a homogeneous system, after 60,000 time steps. As in the experiments, the nematode was positioned initially on the right-hand side, with a bacteria population on the left. The analogous experimental data is shown in Figure 1b. The nematode trails in both simulation and experiment display clearly the relatively random, looping behaviour which occurs prior to the nematode reacting to the attractant, and subsequent straightening of the trail as the nematode follows a biased walk along the attractant gradient into the bacteria.

Structure was included in the simulation by means of digitised replicas of the sand grains used in the experiment. Every grain was treated as an impregnable region deflecting the attractant and nematodes. Figure 3a-d are snapshots in time of the attractant diffusing through the structure. For clarity the structure is only revealed as the attractant diffuses through it, and can be identified as regions where the concentration is zero.

From a comparison of Figure 2 and Figure 3, the effect of the heterogeneous structure on attractant diffusion and nematode behaviour to the attractant is

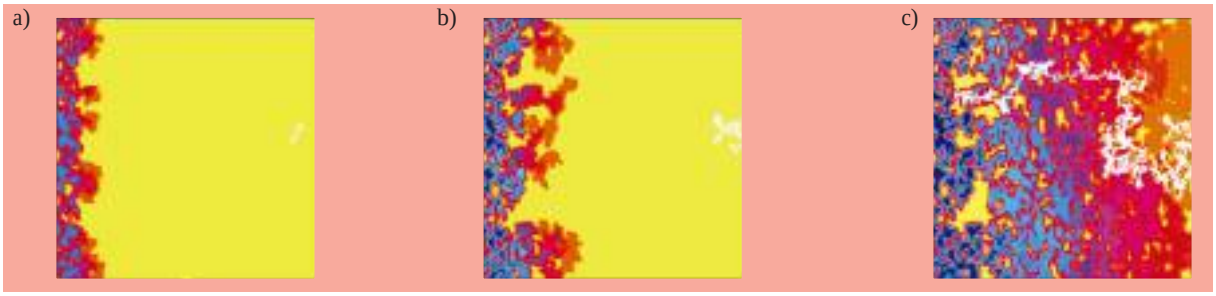

Figure 3 Heterogeneous simulation a) : 1000 time steps; b) : 15,000 time steps; c) : 120,000 time steps (concentrations displayed as in figure 2).

evident. In particular the smooth linear attractant gradient in the homogeneous condition (Fig. 2) has been transformed into a complex mixture of highs and lows when structure is present. The emergence of fingering at the diffusion front is due solely to the structure: the attractant being reflected off the sand grains and into the space between the grains. Within the structure the nematode trail remains localised due to the relatively high local density of structure around the nematode, and the decreased diffusion of the attractant. After 120,000 time steps (Fig. 3c) the nematode trail has moved through the pore network and into the bacterial source.

The trail (Fig. 3b) clearly shows that the foraging behaviour, seen in the homogeneous systems, has been limited due to the constraints imposed by the structure, although it does appear tortuous at times. This trail tortuosity is a reflection of local structure, where the nematode is occasionally trapped in smaller pores. This is evident almost half-way through the structure (Fig. 3c). Where structure is no longer restricting movement towards the bacteria, the nematode trail returns to a directed, straight path. Another feature that could occur is the appearance of small regions of attractant that have a gradient in a different direction to the one emanating from the bacterial source. This would be due to the build up of attractant in enclosed or restricted pore space, and would result in guiding the nematode out of blocked pore pathways, and may also divert the nematode from the general direction of the bacterial source.

There are two central differences between the trails of Figure 2 and Figure 3. Initially the time taken to enter the bacterial population is *c.* twice as long in the case of the heterogeneous set-up. This is in agreement with experimental data. Secondly, the foraging behaviour has been changed in the heterogeneous network. The inclusion of structural heterogeneity modifies significantly the foraging behaviour and the nematode follows a trail which is dictated by the structure, thus taking longer to reach the bacterial source. However, by modifying the foraging strategy, the nematode effectively moves in a biased random manner restricted by structure (i.e. moving much shorter distances than without structure, with a random element aiding the nematode in finding a path through the structure). Therefore, the foraging strategy changes to an 'avoidance' strategy in a structured environment which aids the nematode in escaping structural 'traps'. The persistence of a random element in the nematodes movement, in all of the experimental treatments observed, is clear. This persistence is advantageous to a nematode which is faced with structural (or indeed chemical/biological) traps, whereby the nematode is able to switch from a biased foraging strategy to a semi-biased strategy. This allows a greater degree of freedom for escape, but still permits the nematode to identify the advantageous chemical gradients towards food sources. In such complex heterogeneous structures as soil, such a mix of strategies is vital to the nematode survival and population spread.

There is a large degree of consistency between nematodes behavioural patterns in the experiments and simulations. It would seem that nematodes have other abilities (e.g. memory of direction and/or chemical concentration) which enables them to accommodate the broken nature of the diffusion front. It is clear that the study of such relatively simple experimental and theoretical systems, with the inclusion of appropriate heterogeneities, provide important insights into the behaviour of organisms in structured environments. Such systems may help in our understanding of the probability of disease infection in plants through the action of soil-borne pathogens. This is particularly so since such systems include structural heterogeneity as an important variable, and may be extended to include a variety of other biophysical-chemical variables.

Lectins of the Amaryllidaceae and their potential uses

J.M.S. Forrest, D. Stewart & W.E.G. Müller[1]

Lectins are ubiquitous proteins or glycoproteins which bind sugars and oligosaccharides specifically and reversibly. Pioneering studies with red blood cells revealed the presence of multiple binding sites on the lectins capable of combining with receptors on the surface of different cells and enabling the agglutination or clumping of the cells - hence the alternative name - agglutinins. Lectins have been studied more thoroughly in plants than in animals and a few have gained notoriety as poisons. Kidney beans are toxic if they are not cooked sufficiently to denature the lectin phytohaemagglutinin. More tragically, the Bulgarian journalist, Gyorgy Markov, was poisoned by a minute quantity of *Ricinus communis* agglutinin, one of the most toxic proteins known. Mammalian toxicities of *Narcissus pseudonarcissus* agglutinin (NPA) and *Galanthus nivalis* agglutinin (GNA) remain to be fully tested, but when fed to rats over periods of 10 days, they do not cause hyperplastic changes in the small intestine, and may even reduce the attendant coliform overgrowth which contributes to poisoning by the kidney bean lectin.

NPA and GNA were isolated from members of the Amaryllidaceae family seven years ago, and were the first shown to have exclusive specificity for binding the monosaccharide D-mannose[1]. They do, however, differ in their preference for oligomanno-saccharides, with NPA preferring terminal and internal Man(α-1-6)Man and GNA favouring terminal Man(α-1-3)Man linkages (*Ann. Rep. 1991, 100*). They are proteins of molecular mass 26 kDa and 48 kDa, rich in glycine, asparagine and leucine and resistant to acid, heat and proteolysis. Their concentration in the plant varies seasonally, reaching its highest in the aerial parts, especially the ovaries, at flowering. Contemporaneously, it is lowest in the bulb/corm comprising only 1% of the total protein, but increasing to 15% during the resting period. Lectins in the bulbs of different *Narcissus* cultivars also vary quantitatively (0.7-6.9 mg/g dry weight) and qualitatively. NPA can be extracted from fresh bulbs and readily isolated by affinity and hydrophobicity chromatography.

Other members of the family Amaryllidaceae such as *Leucojum* and *Hippeastrum* spp. also contain lectins, but NPA and GNA have been the most extensively studied. They can be distinguished unequivocally from Concanavalin A and other lectins of the Leguminoseae by the binding specificities and requirements listed in Table 1. The recent elucidation of the structure of GNA confirmed that it is a representative of a unique lectin family, which has a new polypeptide folding motif and novel architecture at the mannose binding site.

These lectins are probably storage proteins with insecticidal and nematicidal activity, as they are present both in young plants, and also at particularly high concentrations in the bulbs/corms. NPA and GNA have been shown to reduce the growth and fecundity of homopterous pests, such as aphids and leafhoppers, when included in artificial diets at a concentration of 0.1%. Proof of their mode of action requires to be provided using lower concentrations of lectin and competing sugars. Lectins may operate on insect gustatory mechanisms to reduce feeding, or, by binding to the midgut epithelium, to block uptake of nutrients. Their insecticidal properties have already been confirmed *in planta* following the insertion and overexpression of the genes in transgenic tobacco[2]. The same potential exists for developing nematode resistance through the expression of high levels of lectin in the roots. Their effects on other plant pathogens have not been reported. These lectins may also play much more subtle roles in cell signalling, but in common with many other lectins, such physiological roles are only beginning to be elucidated.

Lectin	Sugar specificity	Preferred linkage	Ca^{2+}	Agglutination RBC	
				Human	Rabbit
NPA	mannose	α1,6	−	−	+
GNA	mannose	α1,3	−	−	+
Concanavalin A	mannose, glucose	α1,2	+	+	+

Table 1 Differences in binding between lectins of the *Amaryllidaceae* and Concanavalin A.

[1] University of Mainz, Germany

	Cell death		Syncytium formation	
	IC 50% (µg/ml)	Toxicity (µg/ml)	IC 50% (µg/ml)	Toxicity (µg/ml)
NPA	27	>100	7.6	>500
AZT	0.03	>1	0.008	>0.5

Table 2 Effect of NPA and Azidothymidine (AZT) on virus-induced cell death and syncytium formation.

Although NPA and GNA have no known activity against plant viruses, they bind to gp120 and gp125, the envelope glycoproteins of HIV 1 & 2 respectively. It is clear that a number of plant and animal pathogens share the same surface manno-oligosaccharides in accessible arrays. Binding to retroviruses can be characteristically inhibited by mannose and manno-oligosaccharides, confirming their mode of action as lectins rather than non-specific anti-viral agents such as sulphated polysaccharides. This was first demonstrated by W.E.G. Müller and colleagues at the University of Mainz using NPA isolated at SCRI, and formed the basis for the development of a sensitive 'Sandwich ELISA' for quantifying HIV envelope glycoproteins in cell culture fluid[3]. These results have since been confirmed in numerous laboratories. The lectins are now known to bind to other viruses, including Simian Immunodeficiency Virus (SIV), Feline Immunodeficiency Virus (FIV) and Human Cytomegalovirus (HCMV) and so can be used on the solid phase for general trapping. Recognition of individual viruses can be achieved by specific antibodies. Lectin ELISA tests have also been used successfully to quantify viruses in the more demanding milieu of human or feline serum.

NPA and GNA may also have potential as therapeutics because they can prevent HIV from infecting human cells *in vitro*. Target cells of the immune system known as Thymocytes (T cells) are protected when lectin-bound virus particles are inhibited either from contact with the cell, or subsequent membrane fusion, which is essential for entry. Membrane fusion was recently shown to require binding of viral gp120 not only to the cell receptor CD4 but also to fusin, the newly identified co-factor. One possible mode of action of the lectin is to prevent fusion by sterically hindering the attachment of fusin. It may be possible to protect large areas of epithelial mucosal cells normally open to infection by HIV *in vivo* by exploiting the binding properties of the lectins. Interference with the entry of the virus to other types of cell is considered below. In contrast, the drug Azidothymidine (AZT) can only reduce viral multiplication by blocking reverse transcriptase intracellularly. A comparison of the activity and toxicity of NPA and AZT *in vitro* is presented in Table 2 using data provided by a pharmaceutical company. Since HIV is now believed to multiply and mutate rapidly from the time of infection, a combination of therapies acting on different stages of the life cycle may best hinder the appearance of virus strains which have drug resistance or evade immune control[4].

It may be advantageous that NPA and GNA appear to recognise the same pathogens as the collectins, those soluble mannose-specific lectins which coat the surface of the lungs and respiratory tract or circulate in mammalian sera. Although each group of lectins has different requirements for binding, both are specific for terminal mannose structures. Binding of GNA and the collectins is summarised in Table 3 with special reference to HIV and AIDS-related infections. HCMV, *Pneumocystis carinii*, and *Candida albicans* are life-threatening pathogens only in immunodeficient patients. HCMV is carried by the majority of the world's population and enhances multiplication of HIV in sub-sets of T cells. Individuals co-infected with HIV and *Chlamydia trachomatis* (the most common sexually transmitted bacterium) transmit the virus more efficiently.

	HIV	*Chlamydia trachomatis*	HCMV	*Candida albicans*	*Pneumocystis carinii*
GNA	+	+	+	?	?
Collectin	+	+	?	+	+

? = Not known

Table 3 Binding of GNA and collectin to HIV and associated pathogens.

The collectins are the vital first line of defence against bacteria, fungi and a few viruses in pre-immune hosts. Most of the collectins can activate the complement pathway, an antimicrobial enzyme system found in plasma, and induce opsonization (coating of micro-organisms to facilitate phagocytosis and destruction). Coating of HIV however, enhances infection by gaining the virus access to the receptors of many different cells which subsequently take it up. It then escapes destruction because it is protected by other proteins acquired from the host, and a reservoir of infectious virus accumulates with ready access to T cells. It is possible to speculate about the use of NPA and GNA

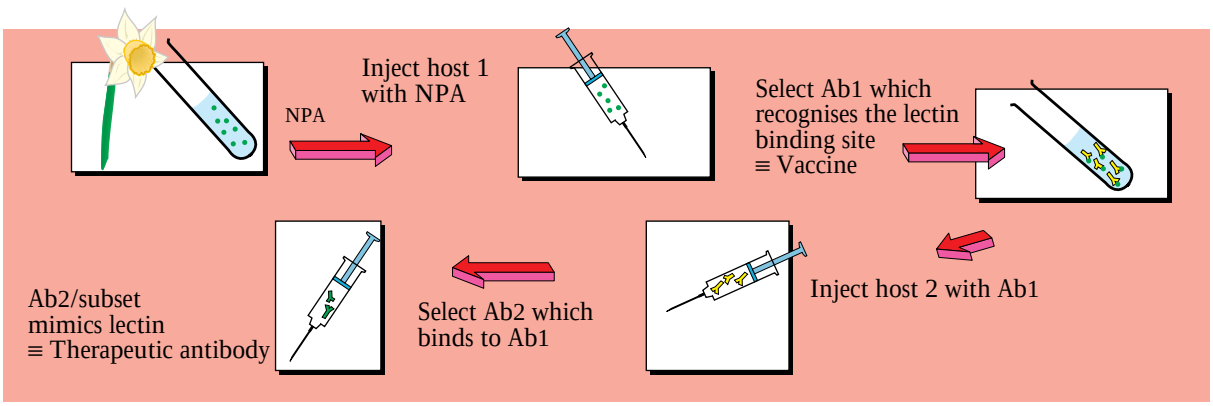

Figure 1 Hypothetical preparation of an idiotype vaccine starting from NPA.

to counteract this mode of infection, by interfering with opsonization via collectin binding and signalling and perhaps by masking the host proteins which afford protection. Anti-lectin antibodies might then increase destruction of infected cells.

Finally, the proposed generation of an idiotype vaccine based on the lectin is illustrated in Figure 1. The rationale of the vaccine is to trigger protective effector mechanisms against the glycosylated ligand for NPA on gp120. It is assumed to be conserved[5]. The vaccine may also give cross protection if the conserved epitope is shared by a number of pathogens. Immunisation with NPA will give rise to anti-lectin antibodies, including Ab1, with a binding site which is an exact replica (internal image) of the ligand on a correctly glycosylated molecule of gp120. Presentation of a soluble protein antigen in an immunostimulating complex (ISCOM) by an appropriate route has been reported as an effective way of maximising a cell-mediated response for the destruction of the virus. Such a course of action may be less risky than using killed virus or whole gp120 which is cytopathic. The process can be continued by immunising with Ab1 to obtain a second antibody, Ab2, with the binding specificity of the lectin. Such an antibody raised in this way should function at the mucosal surfaces and in the blood stream without provoking a neutralising response.

Patents written at and owned by SCRI were partly based on an earlier patent held by W.E.G. Müller on the use of the lectin from the coral *Gerardia savaglia*, as a diagnostic for oncogenic RNA viruses. The patents have now been granted in USA and Europe (1995) as well as in West Africa, and describe more fully the uses of a lectin from the Amaryllidaceae as a diagnostic, therapeutic and means of obtaining a vaccine to retroviruses.

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Natural abundances of stable isotopes reveal trophic patterns in soil food webs

R. Neilson, J. Wishart, D. Hamilton[1], D. Robinson, C.A. Marriott[1], C.M. Scrimgeour, B. Boag & L. L. Handley

Stable isotopes are the naturally occurring forms of chemical elements, such as carbon and nitrogen, which differ only in their atomic weights. Natural abundance levels of stable isotopes can be used as an experimental tool to provide information on complex interactions, without significantly disturbing the system under study (*Ann. Rep. 1991, 59-60*). The trophic structure of terrestrial food webs is inherently complex and difficult to determine. As a result, few terrestrial systems have been studied in detail compared to those

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The units and terminology of stable isotope measurement reflect the minute differences in isotopic composition that are recorded and the fact that those measurements are comparative. The stable isotope ratio of a sample is measured by mass spectrometry. This ratio is then compared with that of a standard, and the result expressed as the parts per thousand (‰) difference from the standard using the 'delta' notation:

δ = 1000 x (isotope ratio of sample – isotope ratio of standard) / (isotope ratio of standard)

The working standards for $\delta^{13}C$ and $\delta^{15}N$ are related to, respectively, an International Standard limestone ($\delta^{13}C$ = 0‰) curated by the International Atomic Energy Agency in Vienna, and air ($\delta^{15}N$ = 0‰). Analytical precision for $\delta^{13}C$ can be as good as 0.01‰ (i.e., a difference of only 1 part in 10^6 in the $^{13/12}C$ ratio), but the analysis of 'real' samples is often less precise than this: $\delta^{13}C$ and $\delta^{15}N$ in plants and invertebrate samples can, typically, be determined to within 1‰; in soil samples, the precision is 1‰ for $\delta^{13}C$, but up to 2‰ for $\delta^{15}N$ if the soil contains low concentrations of total N.

from aquatic systems[1]. A change in the ratios of light to heavy isotopes within a sample indicates the occurrence of a physical or chemical process, which can include ones related to changes in trophic level. In the field, such information typically provides patterns for further investigation rather than unequivocal answers[2].

The stable isotopes $\delta^{13}C$ and $\delta^{15}N$ provide complementary information. $\delta^{13}C$ levels in animals normally reflect the $\delta^{13}C$ of their diets[3]. In contrast, $\delta^{15}N$ in animals are typically more positive than the nitrogen in their diets[4]. $\delta^{13}C$ is better for tracing food sources and $\delta^{15}N$ is more useful for determining the relative positions of animals in a food web.

Grazed	Ungrazed
Mixed grass sward grazed to 4cm by sheep	No grazing
Fertilizer applied comprising: 2 applications of Ammonium nitrate and one application of NPK totalling 150 kg ha^{-1}	No fertilizer

Table 1 Experimental treatments studied.

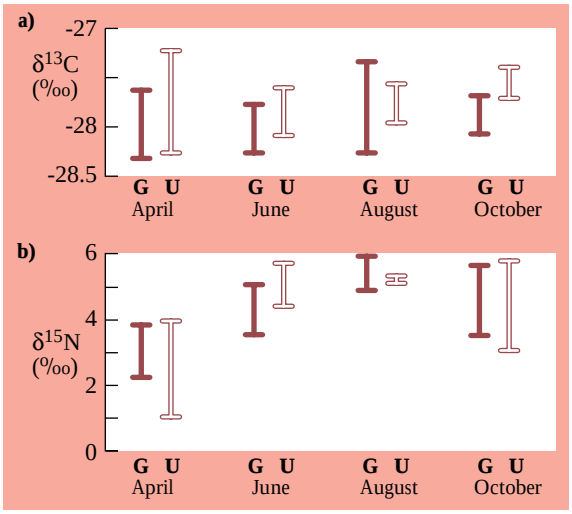

Figure 1 Range of whole soil $\delta^{13}C$ (a) and $\delta^{15}N$ (b) in grazed (G) and ungrazed (U) treatments.

Analyses of slugs and earthworms (soil herbivores) from grazed (by sheep) and ungrazed treatments (Table 1) of an upland pasture typical of central Scotland, showed differences in the natural abundance levels of $\delta^{13}C$ and $\delta^{15}N$ ($p<0.01$ for $\delta^{13}C$; $p<0.06$ for $\delta^{15}N$).

Analyses of whole soil isotope values revealed no differences between treatments (Fig. 1a,b) and cannot be considered to have had a direct influence on the $\delta^{13}C$ and $\delta^{15}N$ values of slugs and earthworms.

Individual plant species analyses showed that they had a wide range in their isotopic composition from both treatments. The $\delta^{13}C$ and $\delta^{15}N$ values separated the plants into three distinct groups: grasses, *Ranunculus repens* (creeping buttercup) and *Trifolium repens* (white clover) (Fig. 2).

As expected, the species composition of the vegetation in the grazed and ungrazed treatments were different.

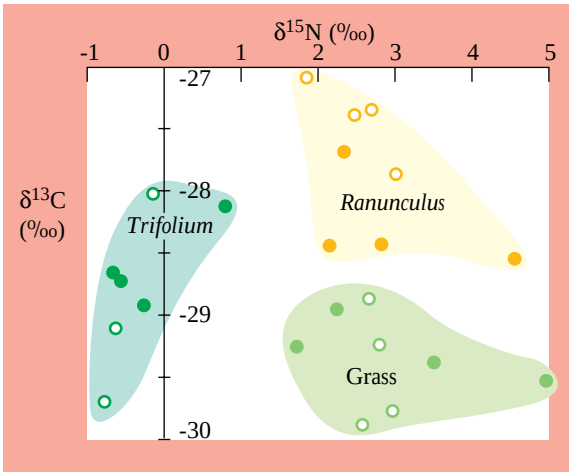

Figure 2 $\delta^{13}C$ v $\delta^{15}N$ of vegetative groupings from grazed (open symbols) and ungrazed (solid symbols) treatments.

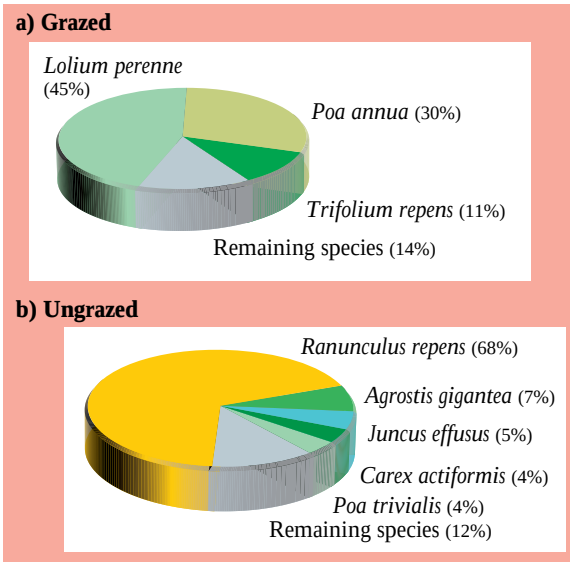

Figure 3 Dominant plant species in a) grazed and b) ungrazed treatment.

Two grass species represented nearly 75% of the vegetative cover in the grazed treatment, whereas *Ranunculus repens* represented nearly 70% of the cover in the ungrazed treatment (Fig. 3a,b). When a weighted isotopic average value was calculated for all of the plants in a plot, adjusted for percentage species cover, i.e. the available food sources for soil herbivores, the difference of $\delta^{13}C$ and $\delta^{15}N$ values reflected the differences in plant composition. Analyses of the slugs and earthworms showed $\delta^{13}C$ and $\delta^{15}N$ values which reflected the differences in vegetation (Fig. 4) . The groups are separated most clearly for $\delta^{13}C$. The difference in $\delta^{13}C$ values between treatments can be attributed to the high percentage cover of *Lolium perenne* (perennial ryegrass) and *Poa annua* (annual meadow grass) in the grazed treatment ($\delta^{13}C$ *c.* -29.50) and *Ranunculus repens* in the ungrazed treatment ($\delta^{13}C$ *c.* -27.50). The absolute $\delta^{13}C$ values of soil herbivores in both treatments were approximately 2‰ more positive than in the above-ground vegetation. Because of the difficulty of separating roots of different plant species in the field, we sampled only above-ground plant parts. The observed 2‰ difference between soil herbivores $\delta^{13}C$ and that of plant shoots could be partially explained if (1) roots were, on average, slightly enriched relative to shoots[5] and (2) the sometimes observed slight enrichment of $\delta^{13}C$ at lower trophic levels[6] were expressed at our sites in the herbivores.

The difference in $\delta^{13}C$ values of slugs and earthworms between treatments would suggest that they were not

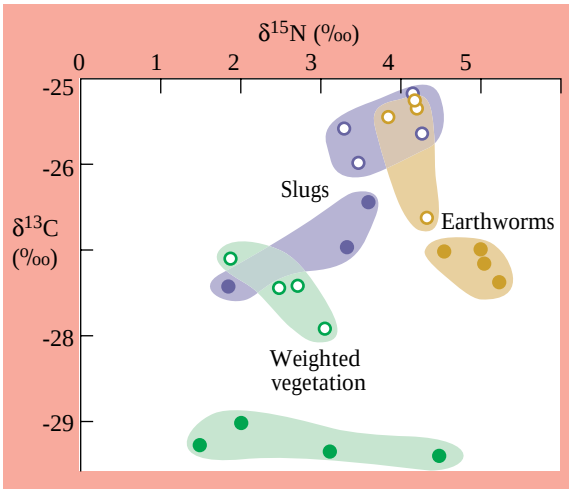

Figure 4 $\delta^{13}C$ v $\delta^{15}N$ of soil herbivores and weighted vegetative groupings - grazed (open symbols) and ungrazed (solid symbols).

feeding exclusively on living plant material, and may, apart from plant carbon, be assimilating carbon derived from another source with a $\delta^{13}C$ value more positive than the surrounding vegetation. A possible source is fungal carbon which has been found to have $\delta^{13}C$ values up to 4‰ more positive than vegetative material[7].

We can report for the first time, using natural abundances of stable isotopes as an experimental tool, that changes in land management are reflected in soil herbivores. By using this technique in other environments, both managed and natural, it should be possible to clarify the patterns found in this study and advance our understanding of the complex interactions between soil, plants and invertebrates. The potential for stable isotope techniques for monitoring land use changes has been established.

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Chemistry

William W. Christie

The progress achieved through the chemical expertise of the Institute is reviewed. It encompasses the investigations of the Chemistry Department, including the Lipid and Magnetic Resonance Units. Some topics are covered directly by the Institute's commissioned remit, and some are assistance for this remit through other disciplines or are supported financially by outside agencies.

Cyclic fatty acids are produced in vegetable oils at the high temperatures pertaining during refining or frying operations. They are believed to have toxic properties. Saturated cyclic fatty acid fractions, formed from oleic acid in normal and high-oleate sunflower oils heated to 275°C, were isolated by silver ion high-performance liquid chromatography. By means of gas chromatography of the methyl esters and gas chromatography-mass spectrometry of the dimethyloxazoline derivatives, eight monocyclic saturated fatty acids comprising four basic structures were identified: cyclopentyl fatty acids with rings between C-5 and C-9 or C-10 and C-14, and cyclohexyl fatty acids with rings between C-4 and C-9, or C-10 and C-15 of the original fatty acid chain (Fig. 1). Each structure was represented by two components corresponding to *cis* and *trans* ring isomers. Cyclopentyl fatty acids were twice as abundant as those with a cyclohexane ring. We believe that free radical intermediates are involved in their formation.

The loss of specific molecular species of triacylglycerols from sunflower, high-oleate sunflower and palm oils has been investigated in commercial frying operations and simulated frying experiments. The non-oxidized triacylglycerols were isolated and molecular species separated by silver ion high-performance liquid chromatography. Linoleate-containing species were lost more rapidly than those containing oleate, as expected. However, all species were liable to oxidation and those containing oleate were lost more rapidly than might have been anticipated. It is suggested that oxidation of linoleate is the probable initiation step, but then the reaction can be propagated readily to all unsaturated species.

Progress in stable isotope analysis has centred on compound specific analyses, using a Europa Scientific ORCHID system. This involves the coupling of a gas chromatograph to an isotope ratio mass spectrometer via a micro-reactor which converts each component of a complex mixture to a suitable gas for isotopic analy-

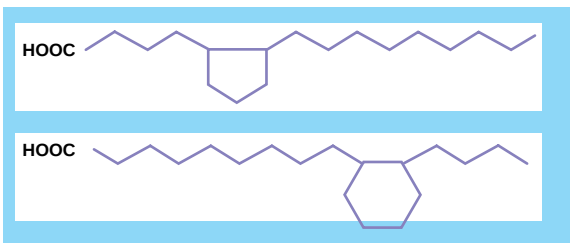

Figure 1 Structures of some of the cyclic fatty acids formed from oleic acid in heated frying oils.

sis. Following installation, the system was tested with a range of lipid compounds including fatty acids and sterols. These are converted to CO_2 for carbon isotope analysis using a micro-oxidation furnace containing copper oxide. This technique can be used in a range of applications, including metabolic tracer studies, food authentication and establishing the biochemical basis of natural isotopic variation.

Subsequent work has established the ability of the system to analyse both hydrogen and oxygen isotopes of water using a micro-reduction/pyrolysis system. The ability to analyse hydrogen relies on a novel analyser design, which allows isotopic analysis of hydrogen in the presence of helium carrier gas. This analyser was developed in collaboration with Europa Scientific Ltd as part of an EU-funded project.

A range of volatiles emanating from *Vicia faba* plants in flower was entrained in the laboratory on Tenax TA porous polymer. A large number of volatiles was detected, including some novel compounds which are probably derived from the metabolic pathway involved in the production of phenolic acids prominent in these plants. Further studies of oil seed rape (*Brassica napus* ssp *oleifera*) volatiles were also carried out using Rylsan II forensic nylon bags, extending existing sampling techniques to encompass growing plants in a field situation.

The leaf surface chemistries of two genotypes of raspberry, which display extremes of resistance and susceptibility to raspberry aphid, have been characterised by use of GC and GC-MS. There were significant differences in epicuticular wax composition, particularly in the steroid and primary alcohol components. Detailed investigations were conducted to determine the relationship between the leaf wax chemistry and aphid resistance or susceptibility. Physiological activity was demonstrated by simultaneous bioassay with raspberry aphid. Preliminary results show that aphids leave a chemical signature on the leaves of their host plant, principally in the form of unusual triglycerides. These were apparently derived from aphid skins shed on moulting, rather than being an induced effect. This has prompted an analysis of the cuticular chemistry of several varieties of raspberry aphid, which will determine if aphid cuticular chemicals can be used to identify the varieties infecting susceptible plants *in situ*.

The development of after-cooking blackening in potato tubers is known to be due to the formation of iron-chlorogenic acid complexes, which on exposure to air result in the formation of blue-gray coloured compounds. Chlorogenic acids can occur in three isomeric forms namely 3, 4 or 5-caffeoylquinic acid. Methods based on both high-performance liquid chromatography (HPLC) and high-performance capillary electrophoresis (HPCE) have been developed to determine their concentration in freeze-dried potato tubers. Application of these methods has revealed that the predominant isomer present in commercial potato cultivars is 5-caffeoylquinic acid, although quantifiable amounts of 4-caffeoylquinic acid may also be detected. Studies have also shown significant increases in the concentration of both isomers in response to light exposure with a proportionally greater increase being found for the 4-caffeoylquinic acid isomer. A comparison of the relative amounts of the two isomers indicated that, in tubers stored under light-free conditions, 4-caffeoylquinic acid accounted for less than 3% of the total chlorogenic acid content as compared with over 30% in tubers from the same cultivar exposed to light for 168 hours.

Studies *in vitro* utilizing synthetic mixtures of all three isomers of chlorogenic acid revealed that neither the colour intensity nor the wavelength maxima of the iron complexes formed were dependent on the relative proportions of the isomers present. This would suggest that, in light-exposed tubers, the observed intensity of after-cooking blackening would be independent of the isomeric ratios and dependent solely on the total chlorogenic acid content.

During the year the automated DNA sequencing instrument was upgraded to allow the use of 48 cm gel plates. This, coupled with the adoption of recent advances in sequencing chemistry and the provision of improved base calling software, has lead to a significant increase in length of read obtained. Sequences of between 650 and 700 bases per run, at 98% accuracy, can be achieved routinely. A reliable method of sequencing PCR products directly was also implemented, resulting in considerable time savings as the need to clone products before sequencing was removed. Using these approaches, many templates from a multitude of plant, insect and viral DNAs were sequenced.

The study of irradiated foods of plant origin has concentrated largely on evaluating LC/MS for use in detection methods, particularly with reference to fruit and proteinaceous foods. These latter are proving to be more difficult than carbohydrate or lipid-rich foodstuffs and we have not so far reached the stage where

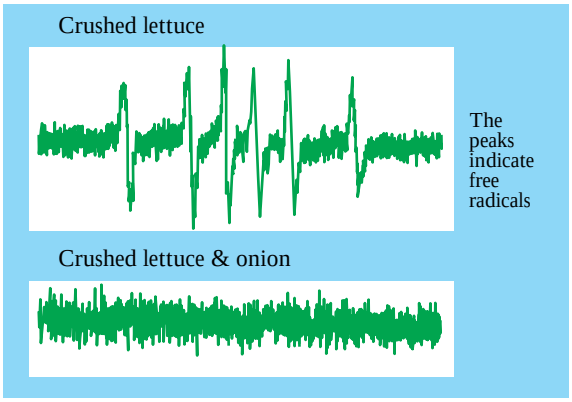

Figure 2 Electron paramagnetic resonance signals of crushed lettuce or lettuce crushed in presence of onion.

unique radiation-induced products can be detected at radiation dosages likely to be used in practical situations. One of the difficulties here seems to be the internal protective processes that exist in complex multicomponent systems, especially those that are biologically viable.

The various EPR signals from the paramagnetic components in potato tissue have been characterised and interpreted in terms of Fe(III), Mn(II) and Cu(II), as well as free radical species. The objective is to study how these vary under different types of stress condition and it has been demonstrated so far that the various important fungal pathogens show differences in their effects on the iron and free radical signals. There is clear evidence that changes in such signals precede the appearance of visible signs of disease in infected tubers. Discrimination of such healthy and diseased tissues can also be seen non-invasively in NMR microimages, which can be used to follow the dynamics of the development of the disease.

Abiotic generation of free radicals has been investigated in normal and recalcitrant seeds in a collaborative project involving both the Royal Botanic Gardens, Kew and the University of Abertay, Dundee. This work has demonstrated that, although free radicals are involved during the desiccation of recalcitrant seeds, the processes are different to those reported in the literature. These studies are leading to a re-evaluation of free radicals in the loss of seed viability.

The study of the generation of free radicals in foodstuffs, particularly biologically viable foods such as fresh fruit and vegetables, that was commenced last year, has been extended and is gaining in importance in understanding the mechanisms of health protection of such foods. For example, free radicals generated from lettuce are quenched by antioxidants in onion (Fig. 2). Some varieties of mushroom generate very large quantities of free radicals on physical damage of tissue, and these have now been trapped by chemical spin traps. In addition to EPR spectroscopy, they have been characterised by combined HPLC and mass spectrometry using an APCI (atmospheric pressure chemical ionisation) interface. This is the first occasion on which such measurements have been possible with free radicals generated in biological systems.

Extensive measurements have been made on grape berries, with special attention being given to the characterisation of seeds. Complete 3-dimensional reconstructions have been made and can be related to features seen by conventional methods. Of particular significance is the embryo, which has enhanced intensity in T_1-weighted images. Clear discrimination of the embryos from surrounding tissues has been observed in other types of seed and is presumably a consequence of free radical generation during metabolic turnover. With barley grains it is possible to monitor the imbibition of water followed by seed germination in 3-D images. Attempts are being made to relate NMR micro-image characteristics to malting quality.

Complete 3-dimensional structures have been generated for pupae of the cabbage white butterfly (*Pieris brassicae*) and the evolution of the internal features has been observed for individual specimens throughout the period of metamorphosis. With its ability to map individually the distributions of water and lipids, combined with the generation of intensity weightings according to the chemical compositions of these fluid media, this promises to be an extremely valuable technique for fundamental studies on such specimens.

Characterisation of metalloenzymes by electron paramagnetic resonance (EPR) spectroscopy

B.A. Goodman

Many vital biological processes, including photosynthesis, respiration, metabolism and nitrogen fixation, require metal ions; these are also utilised by a large number of diverse species in protection against pathogen attack and the activity of potentially toxic chemical agents. The number of recognised essential metals has increased steadily in recent years and currently encompass the entire 1st row transition series, along with molybdenum, tungsten, cadmium and some of the alkali and alkaline earths. These metals usually function as components of proteins, and metalloproteins that are able to perform specific catalytic functions are known collectively as metalloenzymes. Metalloenzymes act on specific types of molecule to perform defined chemical transformations, many of which have no simple comparable analogues in small molecule chemistry.

Biologists traditionally describe enzymes in terms of the reactions they perform. However, in order to achieve a greater understanding of these processes, it is necessary to develop our knowledge of the structure and chemical characteristics of the active sites in these molecules. Such science is vital, not only in the quest to prepare synthetic chemical analogues, but also in modern molecular biological activities, since the genetic engineering of a particular protein framework will be useless to the organism if the specific metal ion, that is necessary for the active site, is unavailable.

A wide range of physical methods has been developed and utilised in the characterisation of metalloproteins. This article concentrates on the use of one of them, EPR spectroscopy, which has specific applications to the characterisation of chemical species with unpaired electrons. The technique is dependent upon the fact that electrons have magnetic moments (i.e. non-zero spin quantum number) and, when in the presence of a magnetic field, adopt states with different energies. Transitions between these states can be induced by the absorption of electromagnetic radiation of the appropriate frequency, which for conventional spectrometers is in the microwave region, according to the relationship (Fig. 1),

$$\Delta E = h\nu = g\mu_B B_0 \qquad (1)$$

where ΔE is the separation between energy levels, ν is the (microwave) frequency (in Hz), B_0 is the magnitude of the magnetic field (in Tesla) at resonance, h is Planck's constant (6.626×10^{-34} J s), μ_B is the Bohr magneton (9.273×10^{-24} J T^{-1}), and g is a constant that is sample dependent. EPR spectroscopy is essentially the electron analogue of nuclear magnetic resonance (NMR), which involves the study of nuclear magnetic moments in the presence of external magnetic fields.

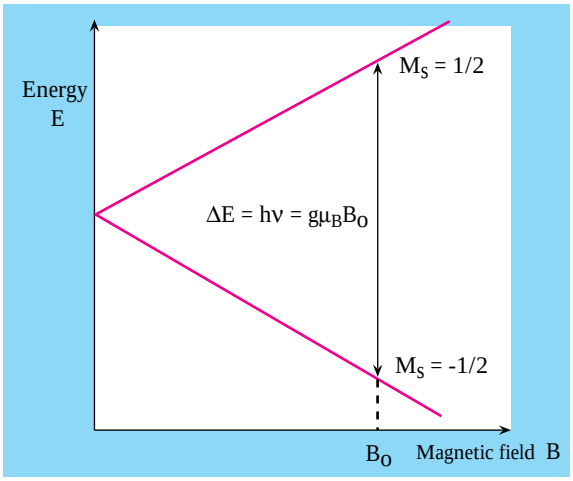

Figure 1 The electron paramagnetic resonance condition.

Examples of copper-containing molecules that are amenable to study by EPR spectroscopy have been presented in a previous Annual Report[1]. The back-

No. of *d*-electrons										
0	1	2	3	4	5	6	7	8	9	10
V(V) Mo(VI) W(VI)	V(IV) Mo(V) W(V)	V(VIII)	Cr(III) Mn(IV) Mo(III)	Mn(III)	Mn(II) Fe(III)	Fe(II)	Fe(I) Co(II)	Co(I) Ni(II)	Ni(I) Cu(II)	Cu(I) Zn(II)

Table 1 Common paramagnetic ions encountered in metalloenzymes.

Metal	Function
Vanadium	Nitrogen fixation, oxidase
Chromium	Not known
Manganese	Photosynthesis, oxidase, structure
Iron	Oxidase, electron transfer, 0_2 transport, nitrogen fixation
Cobalt	Oxidase, alkyl group transfer
Nickel	Hydrolase, hydrogenase
Copper	Oxidase, 0_2 transport, electron transfer
Zinc	Hydrolase, structure
Molybdenum	Oxidase, nitrogen fixation, oxo transfer
Tungsten	Dehydrogenase

Table 2 Some examples of functions of enzymes containing transition metals.

ground and scope of applications of the technique are discussed in more general terms in the present article, which also presents one specific example to illustrate an experimental approach that can be used to optimise the amount of structural information which can be obtained on the environment of the metal centre(s).

Paramagnetic metalloenzymes all involve transition group metals with various numbers of d-electrons; the most commonly encountered ions are summarised in Table 1 and examples of their functions are presented in Table 2. With d-electrons, the magnitude of the g-value is dependent on the orientation of the electronic orbital relative to the direction of the magnetic field, because of coupling between electronic spin and orbital properties. For a molecule with tetragonal or higher symmetry, the g-value varies between $g_{||}$ and $g_{\perp}$, which correspond to alignment of the principal symmetry axis of the orbital parallel or perpendicular, respectively, to the direction of the magnetic field; for lower symmetry molecules, three principal g-values are observed. In a single crystal, the position of the EPR spectrum varies with the orientation of the specimen within the spectrometer, but in polycrystalline, powder, or frozen solution specimens, molecules with all possible orientations are present. The resulting spectrum (which is conventionally displayed as the 1st derivative of the absorption line) consists of features that correspond to the extreme positions in a single crystal measurement (Fig. 2). In fluid solution, the shape of the resulting spectrum is governed by the rate of molecular rotation compared to the timescale of the resonance transition. Thus rapid motion results in a spectrum with an average g-value, whereas slower molecular motion (less than ~10^{-8} sec) gives a polycrystalline-like spectrum; the large molecular weight of metalloenzymes means that the latter situation usually applies.

In molecules containing more than one unpaired electron, the dipolar interaction between electron magnetic moments results in major spectral modifications. Where the electrons are associated with the same metal ion, this interaction produces a relatively large splitting of electronic energy levels in the absence of an external magnetic field (known as a zero field splitting). One consequence of this is an inability usually to observe EPR spectra from ions with an even number of unpaired electrons on conventional spectrometers. Additional spectral features resulting from electron-electron interactions are often observed with ions containing an odd number (>1) of unpaired electrons. In this respect Fe(III) (with either 1 or 5 unpaired electrons depending on the chemical nature of its bonding to ligands) is of particular importance, but the interpretation of EPR spectra of Fe(III) in its high spin state is often fraught with difficulties.

Where unpaired electrons are on different ions, the spectral splittings which result from electron dipolar interactions may allow the determination of the spatial separation of the paramagnetic centres. However, if there is antiferromagnetic coupling between elec-

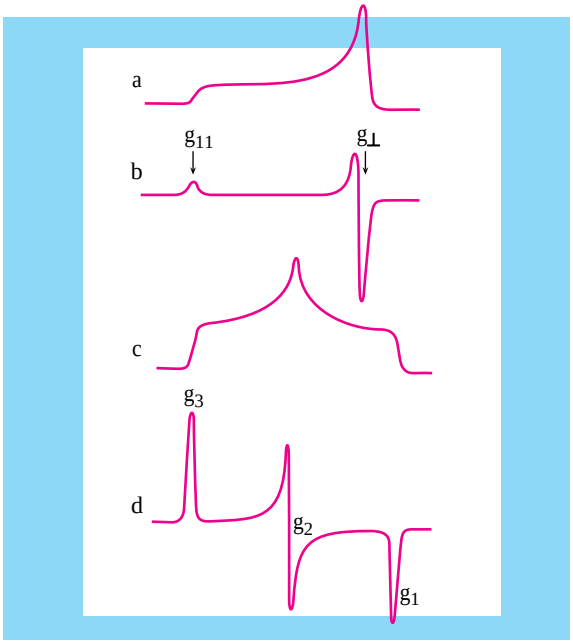

Figure 2 Origin of (a,c) absorption and (b,d) 1st derivative spectral line shapes for a powder with (a,b) axial and (c,d) rhombic symmetry.

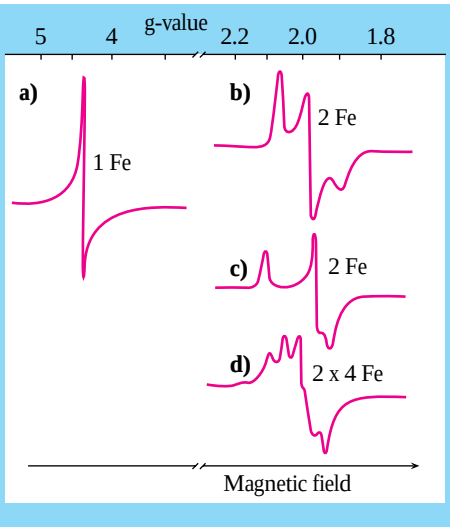

Figure 3 EPR spectra of some iron-sulfur proteins. a) *C. pasteurianum* rubredoxin. b) Spinach ferredoxin. c) *B. stearothermophils* ferredoxin and d) *C. pasteurianum* ferredoxin.

trons, then there may be no net magnetic moment and no resulting EPR spectrum. Alternatively, in atomic clusters containing a number of metal ions, such as for example in iron-sulfur proteins which contain centres of the type Fe_2S_2, Fe_3S_4 or Fe_4S_4, there may be a finite net magnetic moment. EPR spectroscopy is a valuable tool for characterising the chemical states of such proteins (e.g. Fig. 3).

If the metal or coordinating ligand nuclei have magnetic moments (i.e. non-zero nuclear spin), they also adopt different energy states in the presence of a magnetic field; the energy separations of these energy levels (the basis of NMR spectroscopy) are generally much smaller than those corresponding to the electron, with

Isotope	Natural abundance (%)	Spin
^{14}N	99.6	1
^{51}V	99.8	7/2
^{53}Cr	9.5	3/2
^{55}Mn	100	5/2
^{57}Fe	2.2	1/2
^{59}Co	100	7/2
^{61}Ni	1.25	3/2
^{63}Cu	69.1	3/2
^{65}Cu	30.9	3/2
^{95}Mo	15.8	5/2
^{97}Mo	9.6	5/2
^{183}W	14.3	1/2

Table 3 Natural abundance and physical properties of selected nuclei with non-zero spin.

the result that the presence of magnetic nuclei results in relatively minor splittings of the EPR peaks. The characteristics of these nuclear splittings (known as hyperfine structure) are determined by a number of factors with each EPR peak split into 2I+1 components, where I is the nuclear spin, with a separation that is proportional to the nuclear magnetic moment (a constant) and the fractional unpaired electron density in orbitals on that nucleus. The most important nuclei with non-zero spin that are encountered in paramagnetic metalloenzymes are summarised in Table 3.

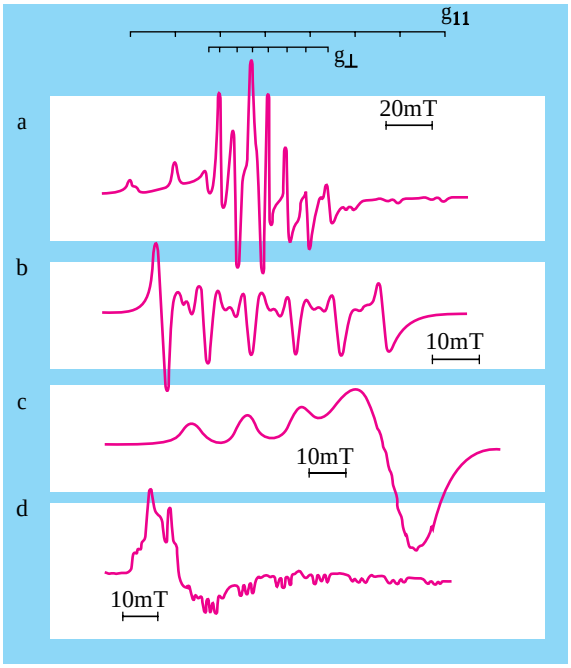

Figure 4 Typical EPR spectra from the major transition metal ions of importance in metalloenzymes, (a) V(IV) in insulin, (b) Mn(II) in soybean agglutinin, (c) Cu(II) in superoxide dismutase powder, (d) Co(II) in the reduced form of vitamin B_{12}.

Examples of typical EPR spectra that are obtained with different types of metal centre are shown in Figure 4. From these, it can be seen that interactions of unpaired electrons with vanadium, manganese, cobalt or copper produce spectra made up of octets, sextets, octets and quartets, respectively. In addition, the spectra of paramagnetic ions of chromium, molybdenum and tungsten each have definitive hyperfine structural patterns flanking the major unsplit peaks from non-magnetic nuclei. Thus, an EPR spectrum provides a fingerprint of the types of nuclei interacting with the unpaired electron, along with information on the distribution of the molecular orbital containing the unpaired electron.

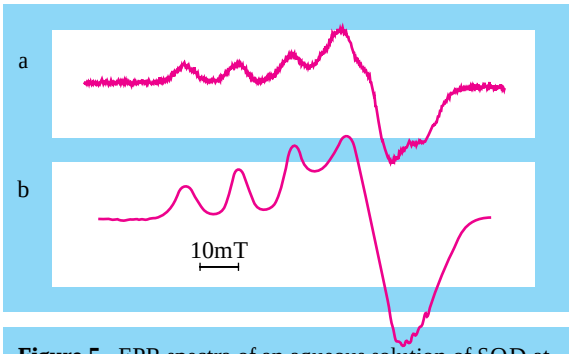

Figure 5 EPR spectra of an aqueous solution of SOD at (a) 294K and (b) 77K.

In addition to identification and characterisation of paramagnetic metal ions, information on the chemical nature of the coordination environment of a metal can sometimes be obtained. The magnitudes of the g-values and hyperfine couplings are determined by the symmetry and bonding characteristics of the metal ion(s), but in some cases the bonding of ligands through nitrogen groups may be identified directly as a result of additional hyperfine structure from the ^{14}N nucleus (Table 3). The additional triplet splitting of the peaks in Fig. 4d arises from one ^{14}N, probably through coordination to the cobalt of the dimethylbenzimidazole, which terminates the long nucleotide side-chain.

Various procedures are available to maximise the information that can be obtained from EPR spectra; some of these are illustrated below in an analysis of the spectra from a superoxide dismutase (SOD), a metalloenzyme involving copper and zinc centres coordinated to histidine centres in the peptide framework.

The spectra from aqueous solutions of the enzyme in fluid and frozen states (Fig. 5) show subtle differences from one another and from that of the polycrystalline powder (Fig. 4c). In fluid solution, there is a splitting of the $g_{\downarrow}$ feature which indicates the existence of distortions from axial symmetry that are considerably greater than those observed in the solid state. This has implications for understanding the mode of action of the enzyme, since models to explain its activity have almost invariably used structures based on X-ray diffraction patterns from single crystals, whereas the EPR results demonstrate that there is significant relaxation of the structure of the organic framework in solution.

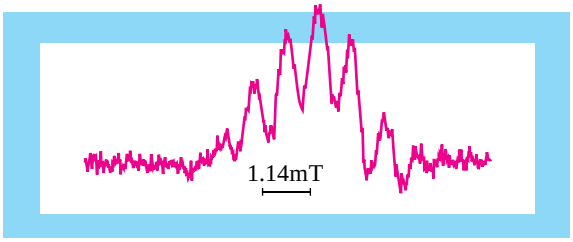

Figure 6 2nd derivative display of the $g_{\perp}$ region of Figure 6(b).

The frozen solution spectrum also shows the presence of minor features superimposed on the $g_{\downarrow}$ resonance; their resolution can be enhanced by higher derivative spectral recording, which generates enhanced amplitude from narrow line components compared to those that are intrinsically broader. This is illustrated in Figure 6, in which the spectrum is consistent with the coordination of the copper to four ^{14}N nuclei (on histidines), which give a nonet pattern with relative intensities 1:4:10:16:19:16:10:4:1.

These examples show briefly the nature of the information that can be obtained from the EPR spectra of metalloenzymes. Spectral analyses are usually confirmed by numerical simulation using software based on the theoretical principles. Thus, EPR spectroscopy is an important technique for identification and characterisation of paramagnetic metal-containing sites in biological molecules. In addition, as the results obtained with SOD demonstrate, structural changes between solid and solution phases can be detected, and suggest that structures derived from X-ray crystallography may not always be sufficient for understanding the fine details of enzyme funtioning.

The EPR spectra of SODs were obtained by Dr. C.G. Palivan whilst on Study Leave from the Department of Atomic and Nuclear Physics, University of Bucharest, Romania.

Reference

1 Goodman B.A., Glidewell, S.M. & Deighton, N. (1993). *SCRI Annual Report 1992*, 66-68.

Plant leaf surface waxes

T. Shepherd

Waxy substances produced by living organisms have been long known, and indeed, beeswax was used in ancient times to make record tablets, models and toys. The word "wax" itself is derived from the Anglo-Saxon "weax", the term applied to beeswax. Today, natural waxes are still industrially important in the manufacture and processing of many materials, ranging from candles, rubber, adhesives, paper and inks, to cosmetics and foods.

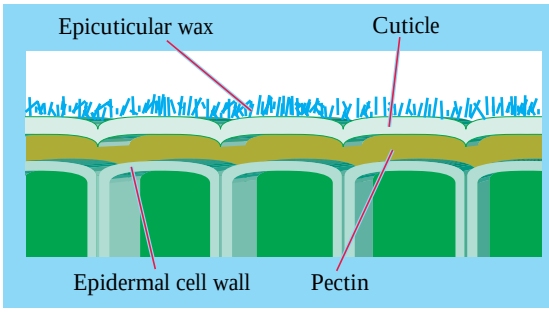

Figure 1 Cross section of plant cuticle.

Function of leaf epicuticular waxes A thin microcrystalline wax layer forms the outer boundary of most higher plant cuticular membranes (Fig. 1), and is the primary interface between the plant and the atmosphere. This wax layer provides most of the resistance to cuticular diffusion of water and solutes, and is also an important route for diffusion of volatiles[1]. These properties help plants to resist drought, allow them to exist in a non-aqueous environment, help to regulate their nutrient status, control foliar penetration of agrochemicals and pollutants, and give protection against disease and pests.

Many crystal forms are seen within the wax using electron microscopy (Fig. 2a), including tubes, rods, ribbon-like structures and dendritic lattices (Fig. 2b). Underlying these is a layer, thought to consist mainly of long alkane molecules lying perpendicularly to the cuticle, with individual layers cross-linked by intercalation of longer molecules, such as esters. Intermixed with these ordered regions, there are amorphous zones where molecules are more mobile, providing the pathway for diffusion of solutes and other molecules (Fig. 3)[1]. The other alkyl- wax constituents, which carry polar substituents, are believed to form the visible and probably compound-specific crystalline structures, although some chemical intermixing is likely.

Leaf waxes have a major role in plant-insect interactions, and this is the primary stimulus for studies of epicuticular wax chemistry at SCRI. Two systems under investigation which involve host/pest interaction are *Brassica* spp/turnip root fly (*Delia floralis*) and raspberry/raspberry aphid. This article will focus on the former. Detailed knowledge of the chemical composition of the host plants' leaf waxes is essential to any investigation of their role in such interactions, and this has been determined in full for the first time.

Chemical analysis of epicuticular waxes Compounds found in brassica wax, following analysis by capillary gas chromatography coupled with mass spectrometry (GC-MS, Fig. 4), are shown in Figure 5. In earlier

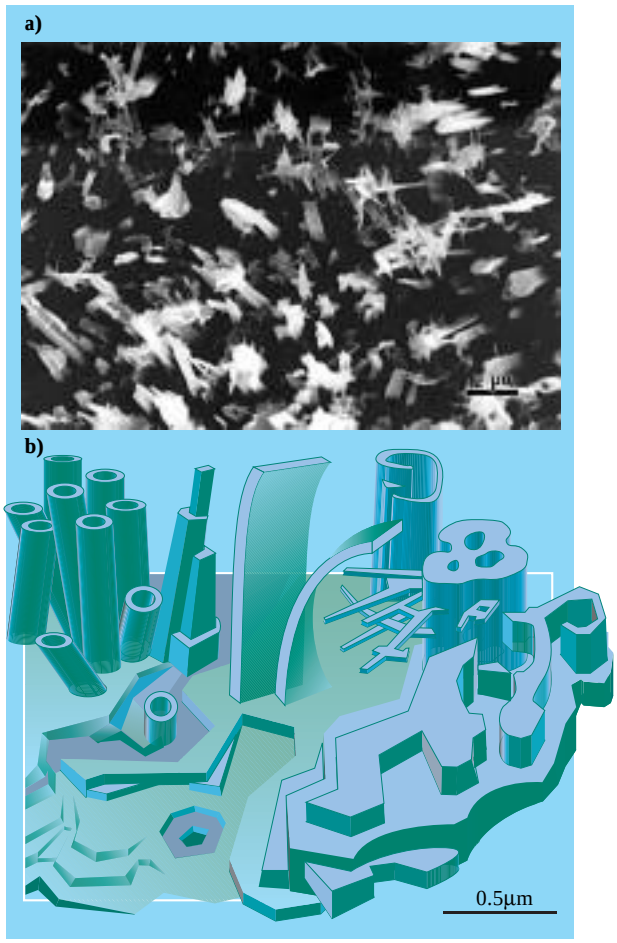

Figure 2 a) Scanning electron micrograph of epicuticular wax from swede genotype Doon Major. Plants were grown indoors at SCRI. b) Schematic representation of crystal morphologies observed in brassica epicuticular wax.

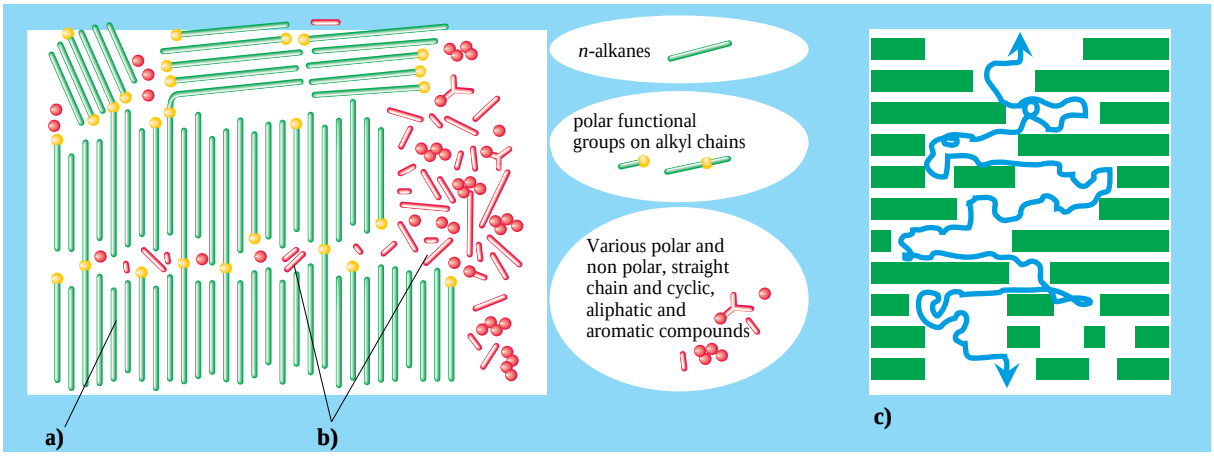

Figure 3 Schematic representation of wax microstructure, showing: a) ordered regions, b) amorphous regions and c) the pathway for solute diffusion.

studies, the structures of intact wax esters could not be determined, because of the difficulty of introducing esters into the mass spectrometer over the temperature ranges required for their separation by GC. Recent developments in GC-MS technology (automated or manual pressure programming) have simplified ester analysis, and we have developed methodology for separation and analysis of intact wax esters from brassicas [2].

Most wax components are readily identified from their characteristic mass spectral fragmentation patterns. Sometimes individual peaks in the GC or total ion (MS) chromatograms contain co-eluting constituents, usually positional isomers of the same compound (e.g. wax esters). These are quantified from the relative proportions of their characteristic fragment ions. Analysis of brassica wax esters is complicated by the presence of branched (*br*-) compounds, with branches in both acid and alcohol moieties, in addition to the normal (*n*-) straight chain compounds found in most plant waxes. The identities of individual acid and alcohol moieties were determined from the molecular ions $[M]^+$ (RCO_2R') in the mass spectra of intact esters, and from fragment ions $[RCO_2H_2]^+$ derived from the acid moieties by McLafferty rearrangement. Additional evidence was provided by the mass spectral and chromatographic characteristics of the methyl esters of the acid components (C_{14}-C_{23}), and trimethylsilyl derivatives of the alcohol components (C_{23}-C_{31}), following transesterification and silylation, respectively. The *br*- acids and alcohols have both the iso- (*i*-) and anteiso (*a*-) structures, which in combination with *n*- acids and alcohols, give nine possible acid:alcohol combinations. Of these, the *a:a*, *a:i*, *i:a*, *a:n*, *n:a*, *n:n* combinations were identified. The *i:n* and *n:i* combinations could not be

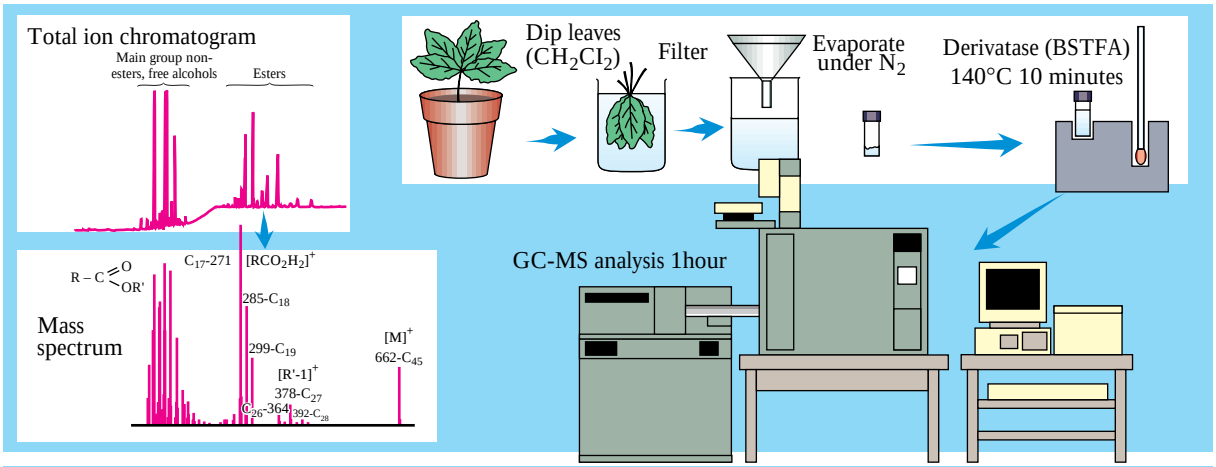

Figure 4 Sample collection, preparation and analysis. During derivatisation with BSTFA, free hydroxyl groups are silylated. The mass spectrum shown is that of a mixture of three coeluting C_{45} esters, and prominent peaks include the molecular ions $[M]^+$ and the McLafferty rearrangement products $[RCO_2H_2]^+$ for each constituent ester.

Main group compounds (74.1-87.6 of wax)

Type C_n	Positional isomers/ homologs	% of main group compounds
n-ketones C_{29}	4	(20.8-30.1)
n-α-hydroxy-ketones C_{27},C_{29}	4	(0.4-1.5)
n-alkanes C_{27}-C_{31}	5	(49.1-56.9)
n-acids C_{28}	1	(8.8-16.1)
n-secondary alcohols C_{29}	2	(0.4-1.3)
n-β-hydroxy-ketones C_{29}	2	(0.1-0.7)
n-aldehydes C_{28}, C_{30}, C_{32}	3	(1.4-11.4)

Free primary alcohols (0.5-2.2% of wax)
Esterified primary alcohols, esterified acids

Type C_n	Positional isomers/ homologs	% of free primary alcohols
n-alcohols (C_{24}), C_{26}, C_{28},C_{30}, (C_{32})	3 + (2)	(33.7-47.7)
i-alcohols (C_{24}),C_{26}, C_{28}, C_{30}	2 + (2)	(8.3-6.9)
a-alcohols (C_{23}, C_{25}), C_{27}, C_{29}, (C_{31}, C_{33})	2 + (4)	(27.1-56.7)
n-acids (C_{16},C_{18}, C_{20}, C_{22})	(4)	
i-acids (C_{16}, C_{18}, C_{20}, C_{22})	(4)	
a-acids (C_{15}, C_{17}, C_{19}, C_{21}, C_{23})	(5)	

Long chain esters (8.6-22.7% of wax)

Type C_n	Positional isomers/ homologs	% of long chain esters
n:n C_{42}, C_{44}, C_{46}	6	(3.7-12.1)
i:n/n:i C_{42},C_{44}, C_{46}, C_{48}	7 + (3)	(1.4-6.2)
a:n C_{41}, C_{43}, C_{45}, C_{47}	11 + (1)	(4.1-8.8)
a:a C_{44}, C_{46}, C_{48}, C_{50}	10 + (2)	(34.1-54.0)
n:a C_{43}, C_{45}, C_{47}, C_{49}	5 + (4)	(18.4-33.9)
i:a C_{43}, C_{45}, C_{47}	5	(0.3-3.8)
a:i C_{43}, C_{45}, C_{47}	7	(2.7-9.5)

Figure 5 Chemical compounds detected and quantified in brassica epicuticular wax. Acids and alcohols shown in parentheses are only found as components of wax esters. Wax esters shown in parentheses were detected but not quantified.

distinguished from each other, and *i:i* esters were not detected (Fig. 5).

Biosynthetic origin of long-chain epicuticular wax components Acyl chains up to C_{16} and C_{18} are formed biosynthetically *de novo* by sequential elongation of a C_2 starter unit (acetate) with C_2 units (malonate). Subsequently, the chain length is extended from C_{16} to C_{32} or higher by a second elongation system, also using malonate. The acyl CoA esters give rise to fatty acids (Fig 6(a)); alkanes (Fig. 6(b)); secondary alcohols, ketones and polyoxygenated compounds (Fig. 6(c)); and to aldehydes and primary alcohols (Fig. 6(d)). Wax esters are synthesised by reaction of alcohols with free or activated acids (Fig 6(e)). Minor components of intermediate carbon number arise similarly, starting from a C_3 precursor (propionate). At least two independent systems seem to function, with the first producing *n*- alkanes, alcohols, ketones and aldehydes, and the second giving *n*-esters and free primary alcohols. A third system may operate for the *br*- compounds in brassica waxes, which originate apparently from the use of *br*-precursors in the synthesis *de novo*; C_4 (from valine) and C_5 (from isoleucine) starter units give rise to *i*- and *a*-compounds respectively.

Our studies with kale and swede show that esterification is non-random, with *n:n* and doubly branched combinations favoured over mixed *n-/br*- combinations. Esterification is also sensitive to acid and alcohol moiety chain length. Combinations with extremes of acid or alcohol length were uncommon, although longer alcohols were more common in some swede esters. This suggests the operation of two different esterification systems, with primary specificities for *n*- and *br*- substrates respectively, but with a degree of cross-over.

Effects of environment on brassica wax composition Variation in light intensity affects wax yield and composition, as shown by comparison of leaf waxes from plants grown indoors (I) and outdoors (O) at SCRI, and outdoors in Switzerland (S). When plants are grown outdoors there is a big increase in wax synthesis, and an increase in formation of acids, decarboxylation products and wax esters, relative to products of the redox pathways. There is also a shift in the chain length distribution for *n*-compounds, with those of shorter chain length more abundant in some locations (O, S), while those of longer chain length were more abundant in another (I). There was evidence that during the formation of compounds oxygenated at mid-chain positions, oxygenation occurs during acyl CoA chain elongation, prior to decarboxylation, and is both site specific and chain length sensitive. Variation in the chain length distribution for *i*- and *a*-ester precursors under the different environmental conditions was more varied than those for *n*-compounds, and this is evidence for separate elongation/reduction/oxidation/reduction systems for synthesis of *br*- and *n*- ester precursors. Plants grown outdoors also produce more *br*- esters and ester precursors in relation to *n*- compounds[3].

These observations suggest that the availability of *br*- and *n*- C_2-C_5 starter units, and the specificity and/or activity of enzymes involved in the pathways shown in Figure 6 for non-ester *n*-compounds and *n*- and *br*-

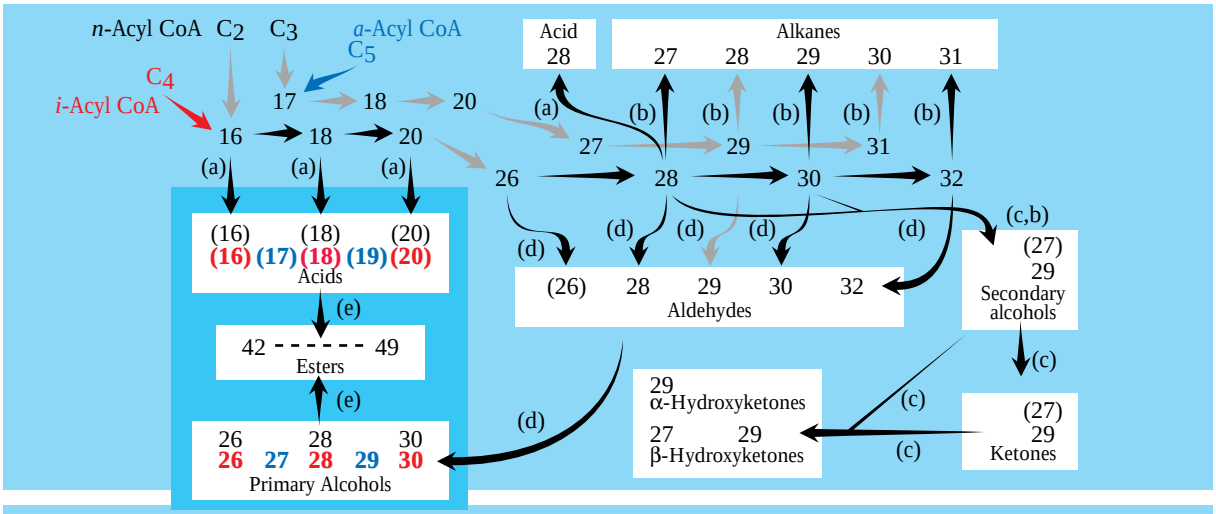

Figure 6 Generalised representation of biosynthesis of long chain epicuticular wax components, formed from acyl CoA chains by: (a) release of acids, (b) decarboxylation, (c) oxygenation/ hydroxylation, (d) reduction, and (e) esterification.

ester precursors, change under the different environmental conditions. Kale waxes also have proportionally more constituents of shorter chain length than swede waxes, again suggesting differences in enzyme specificity.

Role of epicuticular waxes in the brassica/turnip root fly interaction Higher plants use numerous mechanisms to inhibit insect pests. These include detachable wax on long flexible structures (petioles, pedicel, peduncle); sticky or wax coated fistulae, swellings, and structures acting as insect traps (imbricate leaves, folded leaves, pitchers); wax coatings on bracts, sepals and modified leaves; and specific physiochemical interactions with leaf surface waxes.[1]

As a purely physical process, wax blooms reduce surface adhesion by insects. Waxy (glaucous) cultivars with a highly crystalline wax morphology give poor adhesion, probably via detachment of wax crystals, whereas glossy (glabrous) cultivars with amorphous wax structures, give the best adhesion. However, for some plants, loss of glaucousness is associated with resistance to pests. This points to specific chemical interactions at the leaf surface as being a major determinant of resistance/susceptibility.

Several chemical resistance factors have been proposed. These include increased wax coverage, and within the wax, greater abundances of long-chain esters and short to medium chain fatty acids (C_3 - C_{16}), or in glabrous strains, an increase in acid levels with a reduction in levels of alkanes, ketones and other oxygenated compounds. Proposed susceptibility factors include increased levels of alkanes and medium to long chain fatty acids (>C_{16}), and in glaucous strains, an increase in levels of alkanes, ketones etc. with a decrease in acid levels. Stimulatory non-wax compounds may be present, e.g. the glucosinolate sinigrin is an identified stimulant of aphid infestation in cabbage.

Of four genotypes studied at SCRI, a swede, cv Doon Major, is highly susceptible to turnip root fly while the others (swede, GRL; kales, DGC and Fribor) show increasing antixenotic resistance. Behavioural analysis of root fly activity indicates the leaf surface as the major factor determining host selection. A specific polar (non-wax) stimulant has been identified by bioassay of polar (methanol) leaf surface extracts on artificial leaves. However, these extracts are only effective in the presence of hydrocarbons (paraffin wax), and it is likely that epicuticular wax components, in addition to specific polar signal compounds, are involved in host-selection (*Ann. Rep. 1992, 104.*)

Patterns of wax yield were similar to those of antixenotic resistance (Fribor, greatest; Doon Major, least), indicating that wax thickness and/or density may restrict the insects' access to oviposition stimulants. Possible resistance factors are the long chain esters and the C_{28} fatty acid, levels of which were > 50% greater for Fribor than Doon Major. In general, levels of compounds at the shorter end of the chain length ranges found for most wax constituents were also > 50% greater in wax from resistant genotypes.[3]

The activity of specific resistance factors and the interaction of the polar stimulant with the epicuticular wax layer remain to be determined. It is probable that the

wax, or specific wax components, influence the insect's perception of the stimulant, by effecting its diffusion to zones of accessibility and possibly its assumption of an orientiation suitable for detection. Physiochemical analysis and bioassay of the stimulant in the presence of specific wax fractions will provide further information.

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Fungal & bacterial diseases

James M. Duncan

In 1995 the Department of Mycology and Bacteriology changed its name to the Department of Fungal and Bacterial Plant Pathology to reflect its commitment to all aspects of Plant Pathology. Although traditional and molecular Mycology and Bacteriology are very important components of the departmental programme, they do not embrace all aspects of pathology. A few changes in personnel also occurred with the renaming of the Department: work on grey mould of soft fruit was transferred to the Department of Soft Fruit and Perennial Crops, where the gene from raspberry that encodes polygalacturonase inhibitor protein, which is associated with resistance to grey mould, will be genetically manipulated into a number of hosts. The remainder of the programme within the Department was split between two units: Fungal Plant Pathology and Bacterial Plant Pathology.

Whatever plant pathologists call themselves, and however they organise their work, the diseases remain the same. Those that form the basis of the core programme within the Department remain late blight (*Phytophthora infestans*), bacterial blackleg and soft rot of potato (*Erwinia* spp.), phytophthora diseases of horticultural crops, and cereal mildew (*Erysiphe graminis*). The emphasis on *Phytophthora* and *Erwinia* is again reflected in this year's report, with two articles on the former and one on the latter.

Fungal Pathology

Potato growers within the United Kingdom and Western Europe may not always perceive late blight as a serious problem, especially in warm summers such as that of 1995. This is because modern chemical control can be so effective and the myriad of cultural adaptations introduced by earlier generations of pathologists, *e.g.* control of cull piles, have helped them to cope with the disease. Nevertheless, it should not be forgotten that a toll has to be paid to late blight each year. One estimate puts the cost of routine chemical control on ware crops in the UK at *c.* £140 per hectare with up to eight sprays in a season.

Growers in other parts of the world are less sanguine. In the last few years late blight has been very serious in many parts of N. America and it continues to be a problem in the developing world where the potato has become an important crop, prized for its productivity and excellent nutritive value. Peasant farmers in developing countries may not be able to afford chemical control even if they have access to suitable fungicides. Even then, the cost of chemical control worldwide

may well exceed $1,000,000,000 per annum - actual losses in the field and storage are probably several fold greater.

Accordingly, durable resistance is still an important goal for breeders and pathologists. However attempts to select for durable resistance can be confounded by a number of factors, *e.g.* differences and changes in virulence, and aggressiveness among tester isolates used to screen breeding material. Perhaps mixtures of isolates, each of which overcomes all known R-genes should be used, but getting and maintaining such isolates is not a trivial exercise. Other important sources of variability are inoculum concentration and the effect of the environment, both on the infection process and on the resistance of the host itself. As the article by Lowe explains, differences in the environment before and after inoculation affect the expression of host resistance more in some genotypes than in others. Particularly pertinent are the effects of temperature, light intensity and day length, which may go some way to explain differences that can occur when the same genotypes are grown at different latitudes. The net effect of the interaction of all these sources of variability in testing can be considerable uncertainty about the amount and durability of resistance. This problem, which was studied initially in the laboratory at SCRI is now the subject of a coordinated series of worldwide experiments involving close collaboration with the International Potato Centre (CIP) in Lima, Peru; it was given a high priority at a recent planning meeting on an International Late Blight Initiative at CIP.

Variation within *P. infestans* could pose a possible threat to host resistance and to effective fungicide strategies, especially as the fungus, with the escape of the A2 mating type from Mexico, now has the potential to undergo sexual reproduction and form oospores in many parts of the world including the United Kingdom. Oospores are thick-walled spores with the potential to survive in soil for long periods. Thus, apart from being the mechanism by which the fungus generates variation, they might also initiate earlier epidemics by infecting tubers, stems and roots before or at emergence. Such a threat could be particularly serious in Scotland with its important seed potato industry. Seed tubers carrying oospores in or on themselves could transfer the sexual stage of the fungus and populations with mixed mating types from seed- to ware-producing areas. To assess this risk and its possible consequences, in 1995 SOAEFD agreed to fund a joint flexible-funded project between SCRI and the Scottish Agricultural Science Agency (SASA) to study blight populations within Scotland. Through a combination of plant pathology, risk assessment, and population genetics, this project will attempt to gauge the importance of sexual reproduction in Scottish populations of late blight and try to pinpoint 'hot spots' where significant reservoirs of oospores may have been formed. The Scottish programme is closely allied to a MAFF-funded programme in England & Wales, starting in 1996 and involving a team at the University of Wales, Bangor; a part of their work will be looking at blight populations on ware crops established from seed from Scotland and elsewhere.

The summer of 1995 was extremely dry and warm and not very favourable for outbreaks of blight. Nevertheless, with the aid of the Potato Inspectorate of SOAEFD, the Scottish Agricultural College and enthusiastic amateurs, nineteen collections comprising about 150 isolates of the fungus were made. These have been checked for mating type, metalaxyl sensitivity, and in part, for virulence, and molecular studies have now begun. To date, the most interesting results in Scotland have generally come from gardens and allotments where a number of populations containing both mating types have been identified. Monitoring of such sites and others will be continued over the next two growing seasons.

All *Phytophthora* spp. examined to date, including *P. infestans*, produce small protein molecules known as elicitins in culture. Several families of elicitins exist but generally they are very conserved; all those described have 98 amino acid residues. The general conservation of these proteins with some variation among species and the large amounts in which they are produced in culture, have prompted the suggestion that they may play a role in pathogenicity and host range determination. Moreover, they were named elicitins because when applied to tobacco leaves in nanogram quantities, they elicited a rapid and strong hypersensitive reaction but not when applied to other plants. Variation among isolates of *P. nicotianae* in their pathogenicity towards tobacco has been ascribed to variation in the production of elicitins.

In a Franco-British collaboration involving SCRI and the INRA laboratory at Antibes, the gene encoding the elicitin gene of *P. cryptogea* has been genetically manipulated into *P. infestans* using constructs developed at INRA and transformation technology developed at SCRI. A number of transformants of *P. infestans* have been obtained that contain the gene for cryptogein, the elicitin produced by *P. cryptogea*. The presence of the gene has been confirmed by Southern analysis and PCR, and its expression confirmed by reverse transcriptase PCR. The transformants also appear to be producing the elicitin itself, as extracts produce typical necrosis in a bioassay for cryptogein, whereas extracts from the parent isolate do not. The British Council has provided support to continue the collaboration with INRA, Antibes in 1996.

The features of elicitins that have attracted the interest of the molecular biologist also make them attractive targets for molecular diagnostics, an area in which the Department has had a considerable interest for some time (see below). An OECD grant allowed Isabelle Lacourt, a post-doctorate scientist from France, an opportunity to try and develop such a diagnostic for *P. nicotianae*. Using the sequence for the ParA1 gene which encodes parasiticein, the elicitin of *P. nicotianae*, she developed a PCR-based technique by which she could detect this fungus in plants and in water. A full account of her work is given in another paper in this report.

Elicitins are not the only targets for diagnostics. A number of other molecular techniques have been developed in the Department over the past few years for various *Phytophthora* species. These have concentrated on the internally transcribed spacer regions of the ribosomal gene repeat. The programme has been highly successful and to date has produced sensitive, specific and practical tests for seven species, based on a nested PCR technique that should be applicable to any *Phytophthora* species of choice. In 'blind' tests conducted with the collaboration of SASA, the PCR test performed at least as well as the bait tests that are used routinely to test for *P. fragariae* in the strawberry and raspberry certification schemes. A patent for certain aspects of the technology has been applied for. The expertise of the Department in this area has been recognised further by the award of a Potato Marketing Board contract to develop similar diagnostics for powdery scab of potato.

'Molecular diagnostics' is a highly applied aspect of plant pathology but it has proved to be the stimulus for very fundamental studies on speciation and hybridisation within *Phytophthora*. A molecular phylogeny based on the sequences obtained in the diagnostics programme has provided important insights into these fundamental processes, and highlighted the possible importance of hybridisation between species in the generation of new taxa with new host ranges. Some of this work has been part of the SCRI/INRA collaboration. SCRI, using its techniques, has largely confirmed results that various groups of isolates within *P. cryptogea* are the result of hybridisation between other groups. It is hoped that this important work can be continued under a European programme.

Much of the foregoing report has been concerned with molecular studies and perhaps fails to give the impression that field and glasshouse work are still important aspects of plant pathology. This is not so. Well designed and conducted field trials are in some ways the synthesis of all other types of research. It is there that the pathologist learns whether or not his

'beloved' hypotheses stand the test of the 'real' world. The importance of designing trials properly to get the most out of them cannot be over-emphasised. Good design usually means good results and often improved cost-effectiveness. This lesson is brought home in the joint BioSS/FBPP article on the role of generalised additive modelling in improving the accuracy of field trials. Interestingly, this article also demonstrates that FBPP is not completely in thrall to the 'pseudo fungi', a fashionable term incorrectly applied to *Phytophthora* and its close relatives. The main disease discussed is barley mildew!

Bacterial Pathology

Nineteen ninety five saw the retirement of Michel Pérombelon and the end of a distinguished career in bacterial plant pathology, appropriately marked by the award of an MBE in the Birthday Honours List. Although Michel will be missed, Bacteriology still has an important role to play in the Institute's Programme and especially in all aspects of diseases caused by *Erwinia*. All bacteriological studies have now been concentrated in one unit, including some aspects of disease screening previously dealt with by Crop Genetics. This integration of effort should help both breeders and pathologists in the search for resistance to blackleg, which is an important target for the breeders.

The recent series of outbreaks of brown rot in the Netherlands is a reminder that the potato is particularly prone to bacterial diseases. There are four severe diseases found in Europe: common scab caused by *Streptomyces scabies*, brown rot by *Pseudomonas solanacearum*, ring rot by *Clavibacter michiganensis* and soft rot and blackleg caused mainly by *Erwinia carotovora* (subsp. *carotovora (Ecc)* causes soft rot and subsp. *atroseptica (Eca)*, soft rot and blackleg) and *E. chrysanthemi* (soft rot). *Streptomyces scabies* and both sub-species of *Erwinia carotovora* are endemic in the UK; the other three species are not, although two outbreaks of brown rot have been recorded in the south of England. The need to recognise each of these pathogens and to be able to distinguish among them is therefore as important as ever, if or when, the non-indigenous species arrive here. This is especially important for Scotland, a major area for seed production in Europe. In the case of the endemic erwinias, there is also a need to be able to determine amounts on seed tubers, as *Eca* can be found just about anywhere potatoes are grown. All tubers are likely to be contaminated with *Eca*, except those in micropropagation. Above a certain level of contamination (1000 viable bacterial cells per tuber is the generally acknowledged limit) significant levels of blackleg are likely to be found in crops grown from the contaminated seed, if environmental conditions are favourable for development of the disease.

Much of the past work at SCRI focused inevitably on methods of distinguishing among these species, enumerating them, their enzymic activities, pathogenicity and determinants thereof. The importance of this work is recognised throughout the world and SCRI leads a Concerted Action collaboration in Europe to standardise methods for identifying and counting *Erwinia*. Its more recent contribution to this programme on phage typing and differential use of carbon sources, has been a particularly successful part of the programme. The same techniques are also being used to study distribution and movement of the pathogen in the environment.

DNA technology has started to play an important role in such studies, especially new PCR techniques, but other important aspects of variation in the production and variation of pectolytic enzymes still need to be clarified. The principal pectolytic enzyme, pectate lyase (Pel) exists as a series of isoenzymes in each *Erwinia* species. The numbers and amounts of these isoenzymes produced by the bacterium vary with growth conditions *in vitro* and *in vivo* and are clearly affected by the nature of the host, and environmental factors such as temperature. Monoclonal antibodies to a number of these isoenzymes have been developed at SCRI and used to good effect to profile the production of the latter under a variety of conditions by different *Erwinia* species and strains. This work is reported on in article in this Annual Report.

The Unit of Bacterial Plant Pathology has extensive contacts worldwide and is closely involved in EU programmes that are attempting to harmonise methods for enumerating levels of bacteria on tubers. The work on isoenzyme profiling has been an important part of that work in recent years and may also have relevance to resistance breeding. The worldwide involvement of the Unit was recognised in 1995 by the award of a Royal Society grant to help maintain and foster links between the Unit and the group of Dr Alexander Pesnyavich at the University of Byelorussia in Minsk, Belarus.

Pectic enzymes of soft-rot *Erwinia* species: Isoenzyme profile and immunological relationships of pectate lyase and polygalacturonase

L.J. Hyman & I.K. Toth

The genus *Erwinia* comprises fifteen species mostly associated with plants as pathogens or epiphytes. The pathogenic species can be divided into two groups: those that are responsible for necrotic or wilt diseases, typified by *E. amylovora*, the causal agent of fire-blight, and the nine species and subspecies causing soft rots, namely *E. carotovora* subsp. *carotovora* (Ecc), *E. c. atroseptica* (Eca), *E. c. betavasculorum* (Ecb), *E. c. odorifera* (Eco), *E. c. wasabiae* (Ecw), *E. chrysanthemi* (Ech), *E. ananas*, *E. carnegieana*, *E. cypripedii* and *E. rhapontici*. Only Ecc, Eca and Ech have been studied extensively because of the economic importance of the diseases they cause, e.g. blackleg on potatoes.

The soft rot erwinias, with their poor host specificity, lack many of the features associated with pathogenicity in other plant-pathogenic bacteria. All of the studies to date on their molecular phytopathogenicity strongly suggest that, although they might not be the sole determinants of virulence, the secreted pectic enzymes, which all soft rot erwinias produce, are the key pathogenicity determinants. Of the four types of pectic enzymes produced, only pectate lyase (PL) and polygalacturonase (PG) are believed to play an important role in pathogenesis. The former breaks a-1,4 glycosidic bonds and the latter hydrolyses polygalacturonic acid (PGA), a major component of cell-wall pectins which cement together plant cells. These enzymes, particularly PL, are characterized by the formation of several isomeric forms, often with similar molecular weight but different isoelectric point (pI), coded and regulated independently by different genes.

There is some evidence to support the view that the number and certain characteristics of these isoenzymes are implicated in the host range and/or specificity, as well as in the virulence of the bacteria. As a first step towards a better understanding of the relative importance of pectic enzymes in pathogenesis and host specificity within the soft rot *Erwinia* species, it is necessary to determine the isoenzyme profile and relatedness, defined here in terms of immunological relationships, of these two important enzymes.

Isoenzyme profile Isoenzyme profiles were determined by vertical gel isoelectric focusing (IEF)-activity staining of concentrated culture supernatants of bacteria grown in media containing PGA or pectin[1]. In agreement with previous genetic analysis of PL isoenzymes of Ech, four or five isoenzymes were found: one acidic (pI 4.0-5.0), one or two neutral (pI 8.0-9.0) and two basic (pI 10.0-10.5) when the bacteria were grown in broth containing PGA. In contrast, the number of PLs produced by *E. carotovora* was variable, depending on the subspecies and strains tested, ranging between two and four with pI 10.0-10.5. However, most Ecc and Eca strains produced three extracellular PLs with pI values which matched those of the isoenzymes coded by genes cloned in *Escherichia coli*[2]. Of the remaining erwinias, only *E. carnegieana* showed enzyme activity with one basic band (Fig. 1).

In contrast to PL, only one basic (pI >10.0) PG isoenzyme was produced by Ecc, Eca, Eco, Ecw and some strains of Ech, and one neutral by *E. ananas*.

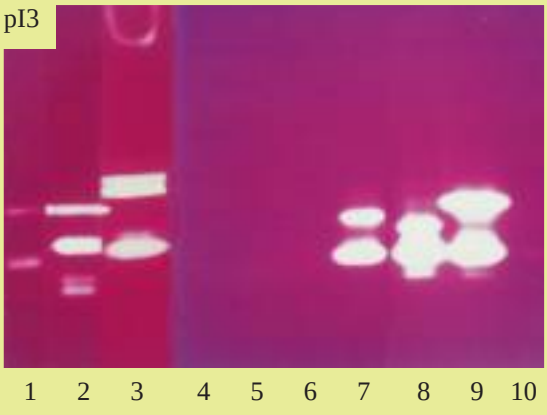

Figure 1 Isoelectric focusing (IEF) (vertical system) with PL-activity staining on culture supernatants of *Erwinia* species grown in PGA-containing medium.
Lane 1, *E. c. atroseptica* strain SCRI 1043; lane 2, *E. c. carotovora* SCRI 193; lane 3, *E. chrysanthemi* AC 4150; lane 4, *E. ananas* SCRI 485; lane 5, *E. cypripedii* SCRI 440; lane 6, *E. rhapontici* SCRI 421; lane 7, *E. c. betavasculorum* SCRI 479; lane 8, *E. c. wasabiae* SCRI 481; lane 9, *E. c. odorifera* SCRI 482; lane 10, *E. carnegieana* SCRI 483.

In addition to the well-established and often genetically defined PL and PG isoenzymes produced in PGA-containing growth media, novel isoforms of these enzymes have been found to be induced by highly methylated pectin[3]. When the ten species and subspecies of soft rot erwinias were grown in media containing PGA and pectin, novel additional PL and PG acidic and neutral isoenzymes with pI of 3.5-4.0 and 8.0 could be detected in the culture supernatant of Ecc and Eca, but the precise number was strain-dependent of the other bacteria, only some Ech strains produced additional iso-enzymes (Fig. 2).

Further evidence confirming the induction of novel isoenzymes by pectin was obtained with an Ech strain (CUCPB 5012) with all its known *pel* and *peh* genes deleted by site-directed mutagenesis (a gift from A.C. Collmer, Cornell University, USA). This retained its pathogenicity, yet failed to produce PL and PG when grown in PGA-containing medium, but produced novel isoenzymes when grown in pectin-containing medium.

Immunological relationships Immunological relationships were assessed by Western blot, following electrophoretic transfer of the isoenzymes from IEF gels, using enzyme-specific polyclonal (PAb) and monoclonal (MAb) antibodies produced against purified PL and PG isoenzymes of Eca strain SCRI 1043[1]. Western blot analysis showed that Eca PL and PG antibodies cross-reacted only with the basic but not with the pectin-induced novel PL or PG isoenzymes of *E. carotovora* subspecies (Fig. 2). This suggests that the latter probably belong to a different class, though with a similar mode of action. PL and PG antibodies reacted with the corresponding enzymes of *E. carotovora* subspecies only. However, the reaction patterns of the PL isoenzymes with three PL MAbs were different within and between the five subspecies (Fig. 2), whereas the three PG MAbs reacted with the single PG isoenzyme of *E. carotovora* subspecies.

None of the four or five PL isoenzymes, nor the PG isoenzyme of Ech cross-reacted with the Eca PL or PG polyclonal or monoclonal antibodies, which is in agreement with the low degree of immunological relationship between *E. carotovora* and Ech[4]. However, since there is an apparent correlation between amino acid sequence and immunological reaction within a series of related proteins, some relationship could have been expected as there is a close DNA homology (70-85% overall amino acid identity) between Ech PLs B, C and Ecc/Eca PLs c, d. PL and PG immunology

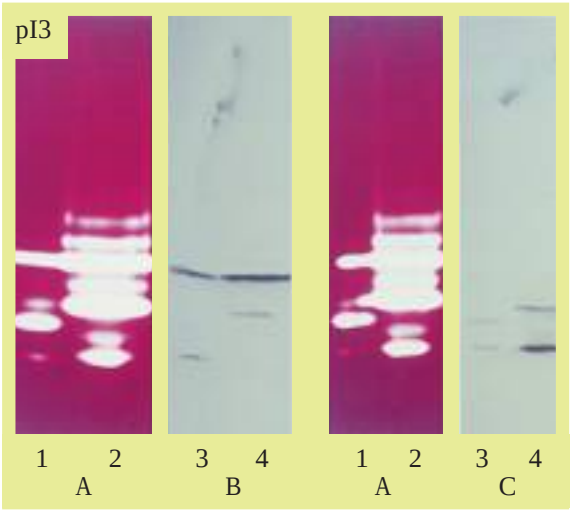

Figure 2 Isoelectric focusing (IEF) (vertical system) with PL-activity staining of culture supernatants of *E. c. atroseptica* (Eca) strain SCRI 1043 and *E. c. carotovora* (Ecc) SCRI 193 grown in pectin-containing medium; and Western blots with monoclonal antibodies (MAb) against PL of Eca SCRI 1043.
A IEF-PL activity stain; Eca SCRI 1043, lane 1; Ecc SCRI 193, lane 2.
B, C Western blots with MAbs B5/158 (B) and B6/39 (C); Eca SCRI 1043, lanes 3; Ecc SCRI 193, lanes 4.

tends to follow more closely the taxonomy of the bacteria than the DNA sequence homology of the relevant genes.

Implications to pathogenicity and host specificity These results tend to support the view that the PL isoenzyme production pattern of the soft rot erwinias can be related to their relative pathogenicity and host specificity. Erwinias which produce multiple PL isoenzymes, usually at high levels, (*E. carotovora* subspecies and Ech) were found to be more pathogenic on a wider host range than the others when they were inoculated in different plants (Fig. 3).

Although the soft rot erwinias are believed to be basically opportunistic pathogens with potentially a wide host range[5], there is nevertheless some degree of host specificity, e.g. Eca to potato, Ecb to beetroot, Eco to chicory and Ecw to Japanese horse-radish. The role of isoenzyme production of PL, and no doubt the other pectic enzymes, in defining host specificity remains to be established. Similarly, the role of the novel isoenzymes produced by Ecc, Eca and Ech needs to be clarified. However, the fact that the culture supernatant containing pectic enzymes of most soft rot erwinias can macerate tissues of host species, regardless of whether they are resistant or not when inoculated with the bacteria, suggests that erwinia host specificity

Figure 3 Chicory leaves inoculated with *Erwinia* spp.
1 Control; 2 *E. c. atroseptica* strain SCRI 1043; 3 *E. c. carotovora* SCRI 193; 4 *E. chrysanthemi* 3937; 5 *E. c. odorifera* SCRI 482; 6 *E. c. betavasculorum* SCRI 479; 7 *E. c. wasabiae* SCRI 481; 8 *E. ananas* SCRI 485; 9 *E. cypripedii* SCRI 440; 10 *E. rhapontici* SCRI 421; 11 *E. carnegieana* SCRI 483.

could also be related to host susceptibility. As pectic enzyme production is globally regulated through a bacterial cell density-dependent system[6], the bacteria must be able to multiply in the host first. Therefore, if host tissue resistance does not allow the invading *Erwinia* population to increase to the critical level, large quantities of pectic enzymes would not be produced and disease would fail to develop. It is clear that the role of isoenzyme production of PL, and probably also other pectic enzymes, including novel isoenzymes, in the expression of disease development and host specificity, needs to be examined for a better understanding of host-pathogen interaction.

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Detection systems for *Phytophthora nicotianae* based on elicitin genes

I. Lacourt

Phytophthora is an economically important genus of plant pathogens comprising about sixty species; it is an oomycete and closely related to *Pythium*. All *Phytophthora* species examined to date, and some *Pythium* spp., secrete elicitins, a family of proteins which are so named because very low amounts (ng) of them in purified form elicit a strong and rapid hypersensitive response when applied to tobacco, and protect against further infection with *P. nicotianae*, the agent of the economically important black shank disease[1].

Although all species produce elicitins, some isolates of *P. nicotianae*, which are highly aggressive pathogens of tobacco, generally do not. These isolates are generally restricted to tobacco, whereas isolates of *P. nicotianae* that do produce elicitins are often polyphagous, *i.e.* they can attack a wide range of plant species, *e.g.* tomato. Interestingly, the triggering of defense mechanisms by elicitins has been observed on tobacco only. Elicitins have no other known function, but similarities with other fungal hydrophobins, such as ceratoulmin, toxin of the Dutch Elm disease, have suggested

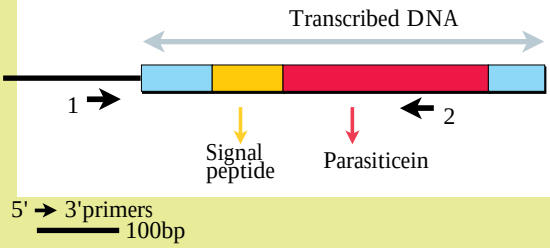


Figure 1 Structure of par A1 : a parasiticein gene.

that elicitins could be involved in determining pathogenicity and host range.

Elicitins are constitutively secreted *in vitro* and are a major product in culture filtrates of *Phytophthora*. Although all *Phytophthora* species produce different elicitins, these proteins have the same size of 98 amino acid residues, (*Pythium* elicitins have 100 amino acids residues), strong sequence homology and are serologically related. High Performance Liquid Chromatography (HPLC) analysis has shown that *Phytophthora* isolates can produce several isoforms of elicitin in the same culture filtrate. However, for each species, one characteristic elicitin predominates in culture and characteristically is named after the species: cryptogein for *P. cryptogea*, cinnamomin for *P. cinnamomi*, parasiticein for *P. parasitica* (also named *P. nicotianae)* etc...

Elicitin genes have been cloned from *P. cryptogea* and *P. nicotianae* and in both cases, clusters of genes have been found with some corresponding to different isoforms already purified from culture filtrates. In the case of *P. nicotianae*, although most black shank isolates do not produce parasiticein, it has been shown that they do have the corresponding gene. As each *Phytophthora* (and *Pythium*) species has its own characteristic elicitin genes, they provide a good target around which specific primers can be designed for the detection of *Phytophthora* species based on PCR (Polymerase Chain Reaction).

Design of elicitin primers. To identify and detect a single species of *Phytophthora*, elicitin-based PCR primers must be specific for a gene characteristic of that species. On the other hand, primers must also be located in regions of the gene that are conserved enough to avoid problems with intraspecific variation, so that all the isolates of the same species will give the same amplification signal. The published sequence[2] of the parasiticein gene ParA1 has been used to design *P. nicotianae*- specific primers (Fig. 1). The ParA1 gene sequence has an open reading frame of 354 base pairs

Isolates	Host Plant	Geographical origin	Production of parasiticein
26	Carnation	France	+
149	Tomato	France	+
329	Tobacco	Greece	-
385	Tobacco	Bulgaria	+
409	Tobacco	Argentina	+
399	Tobacco	USA, Georgia	-
432	Tobacco	USA, North Carolina	+
183	Tobacco	USA, Kentucky	-
301	Tobacco	USA, Kentucky	-
378	Tobacco	Zimbabwe	+
405	Tobacco	Australia	-
308	Tobacco	Cuba	-
IMI 208688	?	?	?

+ Production - Non production ? Unknown

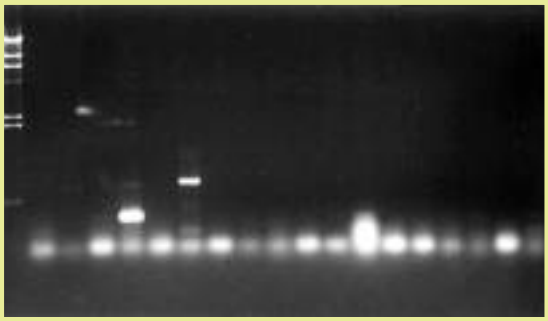
Table 1 Diversity among the *Phytophthora nicotianae* isolates tested with primers 1 and 2.

which encodes for a pre-elicitin, protein of 118 amino-acids. This pre-elicitin contains a 20 amino-acids N- terminal signal peptide which after processing gives the 98 amino-acid parasiticein. Specific amplification of *P. nicotianae* by PCR was achieved by designing one primer which annealed to the coding sequence of the ParA1 gene (primer 2- reverse) and another which annealed upstream of the transcription start (primer 1-forward).

Primers 1 and 2 amplified an intense 378 bp DNA fragment from 13 *P. nicotianae* isolates tested, regardless of geographical origin, original host or whether or not they produced parasiticein (Table 1). In further tests with sixteen other species representing all six groups of *Phytophthora* spp.[3], these primers only amplified DNA from two of four isolates of *P. palmivora.* The DNA product from these two isolates was rather faint and much longer (800 bp) than the *P. nicotianae* product and therefore easily distinguishable from it (Fig. 2).

The small size and strong intensity of the amplified product from *P. nicotianae* isolates and the failure to generate an equivalent product from other *Phytophthora* spp., encouraged further testing of primers 1 and 2 for the detection of the pathogen *in planta.*

Detection of the fungus in the plants Although black shank isolates of *P. nicotianae* are specialised on tobacco, the fungus generally infects a broad host range; it has been isolated from tomato and egg plant, many flowers such as petunia and saintpaulia, and citrus spp. *etc.* To test detection *in planta*, two known



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

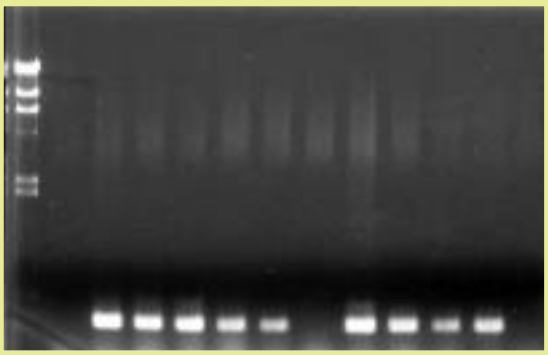
Figure 2 Agarose gel electrophoresis of amplification products obtained with the primers 1 and 2 and DNA of seventeen *Phytophthora* species: Lane 1 - DNA marker size *λ/HindIII*; lanes 2 to 19 respectively - *P. pseudotsugae* (Pse1), *P. cactorum* (Cac 23), *P. idaei* (Ida7), *P. nicotianae* (26), *P. palmivora* (178, Pal 1), *P. capsici* (238), *P. citrophthora* (Ctp 1), *P. syringae* (Syr 2), *P. citricola* (Cit 1), *P. infestans* (I117), *P. fragariae* (FrR 70), *P. megasperma* Meg 6), *P. cinnamomi* (Cin 8), *P. dreschleri* (Dre 1), *P. cryptogea* (Cry 3), *P. cambivora* (Cam 1), *P. erythroseptica* (Ery 1).

hosts, tomato and tobacco were chosen. The former is a common host for several other *Phytophthora* species such as *P. capscici* or *P. cactorum* and rapid identification of the species is required as they vary in their severity of attack on this host and therefore need to be identified for appropriate control measures. Tobacco was chosen because of the importance of black shank disease on this crop.

Two-week old tomato and tobacco were inoculated by placing them in a tube of water also containing a mycelial plug of the fungus. Subsequently, the fungus attacked the collar region of the hosts and the stems, causing characteristic rotting symptoms. Inoculation directly onto the stem of six week-old tomato plants, also resulted in clearly visible and expanding lesions. Using primers 1 and 2, no PCR amplification was observed with healthy uninoculated plants, whereas a band identical to that obtained with pure DNA from *P. nicotianae* was found in infected tomato and tobacco plants (Fig. 3). PCR worked better with DNA extracted from two week-old rather than six week-old tomato tissues. However, signals were generally stronger when, instead of grinding the sample in liquid nitrogen, it was crushed directly in the lysis buffer using a pestle. Improvement of the signal was also achieved when Bovine Serum Albumin (BSA) was added to the PCR mix. The signal was more intense without loss of specificity and the addition of BSA to samples therefore was adopted as standard.

These results show that with PCR and the elicitin-based primers 1 and 2, *P. nicotianae* in a lesion on a plant can be identified quickly without resort to isolation of the pathogen from the host. However, much greater sensitivities have been achieved in other work on detection of *P. fragariae* in raspberry and strawberry plants. Using PCR and nested primers based on sequences from the ribosomal RNA gene repeat, detection is possible on plants not showing any symptoms of disease.

Detection of the zoospores on a membrane by PCR amplification Motile zoospores are the infective agent of *Phytophthora*. They are released from asexual sporangia and are probably the most common propagule found in water; numbers can reach as high as 400 per litre in recirculated irrigation water. They are an important target for detection because they rapidly spread the disease in water, infecting the host after encystment. Their accumulation onto a dipstick plunged into water has been used to detect *P. cinnamomi* serologically[4] and zoospores of *P. nicotianae* have also been detected using ELISA by filtration and trapping on a membrane[5]. A similar approach using filter membranes, but this time with PCR, has been used to detect bacteria[6]. We have used this last approach to detect zoospores of *P. nicotianae* using the elicitin-based primers.



1 2 3 4 5 6 7 8 9 10 11 12 13

Figure 3 Detection of *Phytophthora nicotianae in planta.* Agarose gel electrophoresis of amplification products obtained with primers 1 and 2: lane 1 - DNA marker size *λ/HindIII*; lane 2 negative control with no DNA, lanes 3 and 9 positive control with pure fungal DNA of P. nicotianae isolates 149 and 329; all other lanes with DNA extracted from plants : lanes 4 to 7 - 2 week-old (lanes 4,5) and 6 week-old (lanes 6,7) infected tomato stems: lane 8 - uninoculated 2 week-old tomato stem; lanes 10 to 12 - stem bases of infected 2 week-old tobacco plants; lane 13 - stem base of inoculated 2 week-old tobacco plant.

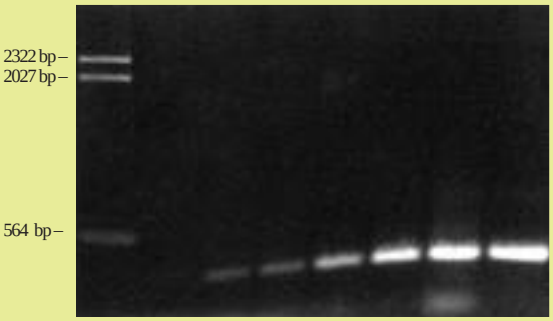


Figure 4 Agarose gel electrophoresis of a second PCR amplification of products obtained with primers 1 and 2 from various numbers of zoospores of isolate 149 of *Phytophthora nicotianae* fixed on a nitro-celulose membrane: Lane 1 - DNA marker size *λ/HindIII*; lanes 2 to 7 respectively - zoospore numbers 0,10^2,10^3,10^4,10^5,10^6; lane 8 - amplification product of a single PCR on pure fungal DNA (isolaote149).

Two successive cycles were required to obtain a signal, even when one million zoospores were fixed to the membrane. However, with a second round of PCR, a signal was observed with just one hundred zoospores, and the intensity of the signal increased with increasing number of zoospores (Fig. 4). When one million zoospores were present, the signal was identical to that obtained when a single PCR was made with pure fungal DNA. A high annealing temperature during the second round of PCR increased the specificity of DNA amplification. As expected, a negative control of water lacking zoospores gave no signal after the two cycles of PCR.

Whereas ELISA-based techniques detected as few as 10 zoospores, a *P. nicotianae*-specific amplification signal was obtained with an estimated 100 zoospores trapped on a membrane, although the efficiency of the trapping has not yet been determined. Our protocol therefore may yet require further refinement. However, these results offer a realistic alternative to the use of ELISA-based tests for the detection of *P. nicotianae* in plants and as zoospores. Given that all species of *Phytophthora* probably have equivalent elicitin gene sequences, PCR-based diagnostic techniques (detection and identification) based on elicitin genes could be developed for all species in the genus.

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Isolation of genes encoding polygalacturonase-inhibiting proteins from raspberry

B. Williamson, V. Ramanathan, C.G. Simpson, G. Thow, P.P.M. Iannetta & R.J. McNicol

Ripe raspberry fruits are highly susceptible to grey mould disease, caused by the fungus *Botrytis cinerea*, and the losses due to the disease after harvest are a major factor limiting the sale of fresh fruits on distant markets and the quality of processed fruits. *B. cinerea* attacks the flowers of raspberry as soon as they open and establishes a quiescent infection in the styles and other floral organs, but the disease does not appear until fruits ripen. Biochemical studies *in vitro* have shown that *B. cinerea* produces endo-polygalacturonases (PGs) which degrade polygalacturonic acid, the 'backbone' of the large pectin molecule in the primary walls and intercellular matrix of fruit tissues (Ann. Rept. 1992, 70-72).

A raspberry polygalacturonase-inhibiting protein (PGIP) purified from immature green fruits inhibits *Botrytis* endo-PG activity; this activity declines sharply with the onset of ripening which corresponds with an increase in susceptibility to infection. PGIPs

Cons	P	-	-	-	-	-	L	-	-	L	-	-	L	-	L	S	-	N	-	L	-	G	-	I
LRR1	E	C	D	P	T	T	H	R	I	N	S	L	T	I	F	T	D	N	N	L	T	G	Q	I
LRR2	P	A	Q	V	G	A	L	P	Y	L	E	T	L	E	L	RK	L	P	H	L	T	G	P	I
LRR3	Q	P	S	I	A	K	L	K	H	L	K	M	L	R	L	S	W	N	G	L	S	G	S	V
LRR4	P	D	F	I	S	Q	L	K	N	L	T	F	L	E	L	N	F	N	K	F	T	G	S	I
LRR5	P	S	S	L	S	Q	L	P	N	L	G	A	L	H	L	D	R	N	Q	L	T	G	Q	I
LRR6	P	S	S	F	G	K	F	V	G	T	VP	A	L	F	L	S	H	N	Q	L	T	G	K	I
LRR7	P	T	S	F		A	N	M	N	F	D	Q	I	D	L	S	R	N	K	L	E	G	D	A
LRR8		S	V	I	F	G	L	N	K	T	T	Q	IV	D	L	S	R	N	M	L	E	F	D	L
LRR9		S	K	V	V	F	S	T	S	L	R	A	V	D	L	N	H	N	S	I	T	G	S	I
LRR10	P	A	Q	L	T	Q	L	D	D	L	V		LF	N	V	S	Y	N	R	L	C	G	K	I

Figure 1 Alignment of leucine rich repeats in raspberry PGIP1. Tandem leucine Rich Repeats (LRR1-10) extend from amino acid 63 to amino acid 302 and are aligned in comparison to the consensus (cons) derived for previously characterised PGIPs.[1]

have been purified from a number of plant families and recently the genes encoding some of them have been published (see Ann. Rept. 1994, 65). One strategy to improve resistance to *Botrytis* infection is to increase the levels of active PGIP in ripening fruit by activating expression of PGIP genes.

Structure of PGIP genes Two PGIP genes have been isolated from a raspberry fruit cDNA library. PGIP1 contains an open reading frame encoding a 331 amino acid protein. The deduced amino acid sequence shows a high degree of similarity with previously isolated PGIPs (Table 1), and contains features characteristic of PGIPs found in several plant families. PGIP1 contains an N-terminus of 22 amino acids which targets the peptide for export through the cell membrane to its final location in the plant cell wall. Four cysteines towards the N-terminus of the mature peptide and four cysteines towards the C-terminus are probably required for correct folding of the extracellular peptide to its final structure as a molecule capable of interacting with fungal endo-PGs. PGIPs are glycosylated and the raspberry PGIP contains four N-glycosylation sites (N-T/S), three of which show a conserved position with previously isolated PGIP genes. Variation in the glycosylation of PGIPs may modulate the wide differences in activity and specificity reported in purified PGIPs from different plants.

	Amino acid % similarity	
	PGIP1	PGIP2
Pear	86.8	76.1
Kiwifruit	77.3	71.5
Tomato	77.2	67.3
Antirrhinum	71.8	62.8
Bean	66.0	56.5
Soybean	61.3	58.2
Raspberry PGIP2*	88.5	-

*Raspberry PGIP2 is compared to the other sequences up to the frame-shift mutation

Table 1 Comparison of raspberry PGIP peptide with published peptide sequence.

Leucine-rich repeat motifs PGIP1 shows a high leucine content (15.7%) and contains 10 loosely conserved leucine-rich repeat (LRRs) motifs (P-----L--L--L-LS-N-L-G-I) (Fig. 1). LRRs have been described in several recently isolated plant resistance genes. The LRRs of PGIPs have a high homology to the *Cf9* and *Cf2* genes of tomato, conferring major gene resistance to the fungal pathogen *Cladosporium fulvum*. LRRs are a key feature in other protein-to-protein interactions, such as the characteristic non-globular structure of the porcine ribonuclease inhibitor and its molecular interaction with RNase A.

PGIP2 may be a pseudogene. Comparison of PGIP1 and PGIP2 shows 88% similarity at the amino acid level (Table 1) and preserves all of the characteristics of PGIPs at the amino acid level. However, a single nucleotide insertion puts the coding sequence out of frame, leading to premature termination 226 amino acids downstream from the translation start.

Southern blotting shows PGIPs in raspberry to be part of a low copy number gene family. Expression analysis by reverse transcriptase-PCR and Northern blotting shows consistent levels of expression in flowers, immature and mature fruit. Since earlier biochemical studies showed that the activity of the enzyme inhibitor extractable from cell walls declined rapidly as fruits ripened (Ann. Rept. 1992, 70-72) the expression analysis suggests that post-translational modification may be occurring.

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Stability of R-gene resistance to late blight in potato leaves

R. Lowe & J. G. Harrison

Late blight of potato (*Solanum tuberosum L.*), caused by infection of leaves, stems and tubers by *Phytophthora infestans* (Montagne) de Bary, is the most serious disease of this crop. Blight is controlled by one or more of several strategies including planting only clean seed tubers, the use of early maturing varieties that are harvested before the disease becomes serious, and the destruction of waste dumps on which the fungus overwinters. Intensive efforts over the past few decades to breed blight resistant cultivars have had only limited success.

Vertical resistance (Van der Plank, 1975) of potatoes to late blight, caused by *Phytophthora infestans*, depends on the presence of specific resistance genes (R-genes) in the host. The pathogen exists as a number of physiological races, each of which can overcome a particular combination of host R-genes. The R-gene complement of a potato genotype is usually determined by inoculating leaves with known physiological races of *P. infestans* and assessing the reaction visually. Assessment of host-pathogen interaction is subjective and there is evidence that expression of resistance can be modified by several factors. For example, changing the environment in which the host is grown prior to inoculation and varying inoculum density, can lead to reactions which appear intermediate between hypersensitivity and rapid colonisation.

R-genes can effectively confer immunity to blight, but major gene resistance has been largely discredited for long term disease control because races of *P. infestans* carrying virulence genes, which can overcome the plant's resistance, soon dominate the pathogen population.

Mean fresh weight (µg) of *P. infestans* (race 3,4) per leaflet as determined by ELISA.

Potato genotype with	Photoperiod 10h	20h
R1	366	296
R2	830	685
R10	71045	6362
R11	35608	9124

Table 1 Switching on/off of R-genes by photoperiod.

Resistance of potatoes to blight is also controlled by minor genes; also known confusingly as non race-specific, horizontal, field or polygenic resistance. This resistance does not confer immunity but slows down blight progress. It is much more durable and currently offers the only long-term solution to blight control. Differences in field resistance have also been attributed to variations in the aerial environment.

The environmental factors that affect the expression of resistance have been the subject of speculation. The work reported here investigates the effects of daylength and inoculum density on disease expression, using cultivars possessing known major genes. Eleven R-genes have been reported and others may also exist. We suggest that the stability of R-gene resistance may decrease as the series is ascended. We hypothesise that genes R1 and R2 are "strong" and that the environment and inoculum density have little or no effect on expression of resistance. A "weak" R-gene such as R10 or R11 may not be fully expressed in some environments and may easily be overcome by high inocula.

Experiments to test this hypothesis were carried out using potato genotypes possessing genes R1, R2, R10 and R11, by challenging detached leaflets of these cultivars with incompatible races of *P. infestans*. The effects of photoperiod (Table 1) and inoculum concentration (Table 2) were investigated. Scoring was carried out visually by noting the characteristics of any lesion formation. In a typical incompatible reaction, no major lesion should develop on the leaflet surface, whereas in the compatible reaction a large, usually

Mean blight score* per leaflet

Potato genotype with	No. of sporangia of *P. infestans* (race 3,4) per inoculum drop 8000	1600	320	64	0
R1	0.6	0.0	0.0	0.0	0.0
R2	1.0	0.0	0.0	0.0	0.0
R10	3.0	1.0	0.2	0.0	0.0
R11	3.0	2.6	0.2	0.0	0.0

* 3=sporulating lesion 2=spreading lesion, but no sporulation
1=limited lesion 0=lesion absent

Table 2 Effect of inoculum density in overcoming R-gene resistance.

Figure 1 Illustration of the compatible and incompatible reactions of detached potato leaflets of the same genotype when challenged with zoospore suspensions of different races of *P. infestans*. The groups on the left have been challenged with a race which does not possess the virulence factor(s) to overcome the R-gene(s) of the host. Those on the right show a typical response to a compatible isolate.

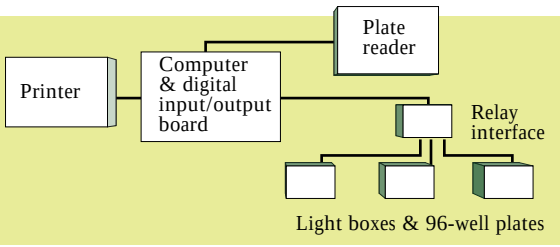


Figure 2 Computer connections.

sporulating, lesion is common. An illustration of these reactions is shown in Figure 1.

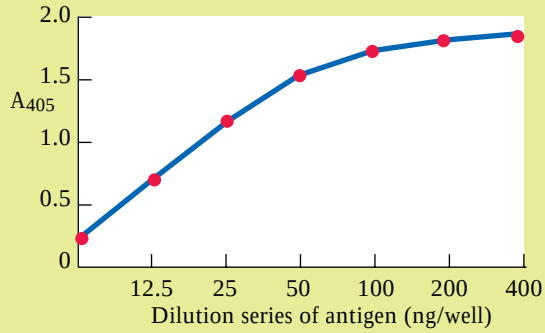


Figure 3 Typical calibration curve from standard series of *P. infestans* on plate.

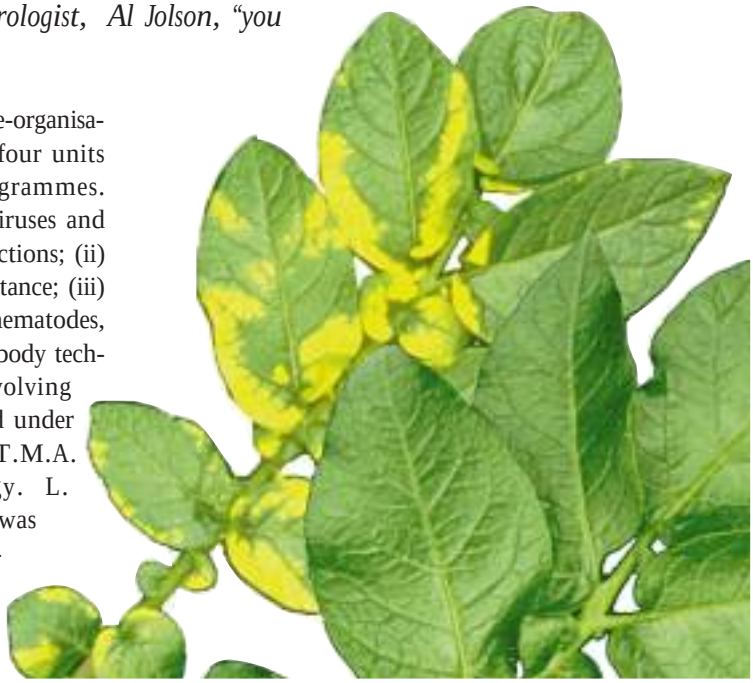
An ELISA technique using a polyclonal antiserum raised against *P. infestans* mycelium was used to quantify colonisation of individual leaflets. Experience in running this type of assay suggested randomisation of sample wells is desirable as inter- and intra-plate variations are significant. A computer programme was developed which improved the speed and reliability of loading the samples into replicated and randomised positions on microtitre plates sitting on 96-place LED light boxes. A block diagram of the computer connections is shown in Figure 2. Dilution series of the antigen were included on each plate to allow the computer to calculate a calibration curve (Figure 3) and print results in de-randomised form.

Results indicated that expression of resistance conferred by genes R1 and R2 is indeed more stable than that conferred by genes R10 and R11. Genotypes with R1 and R2 remained resistant after inoculation with large numbers of spores, but resistance of genotypes with R10 and R11 broke down. Genotypes with R1 and R2 were resistant when plants were grown in 10 or 20 hour photoperiods, but those with R10 and R11 were strongly resistant only in 20 hour photoperiods.

Plant Viruses

Peter F. Palukaitis

Research at SCRI on plant viruses and diseases of viral aetiology continues to lead to significant breakthroughs, not only in our understanding of virus-plant and virus-vector interactions, but also for new sources for resistance, new diagnostic tools, and the use of plant viruses as vectors in wealth-creating biotechnology. With the array of molecular tools that are available to virologists today, we are no longer limited by the technology, but rather by resources available for any particular project. Thus, grants and contracts continue to be essential sources of support for work involving the development of viruses as vectors, risk assessment, development of diagnostic methods, and characterisation of viruses affecting crops in the developing world. Such funds are critical to our ability to make quantum leaps in understanding and controlling viral diseases of plants in a time when cultivation of crops for food, fibre or biomass is under tremendous pressure by expanding populations. After 100 years since the concept of viruses was proposed, we are making rapid gains in understanding viruses and their interactions and are able to exploit that information to improve agriculture and enter new arenas of biotechnology as never before. To quote that non-virologist, Al Jolson, "you ain't seen nothing yet!".



The virus research programmes saw some re-organisation during 1995, with the formation of four units encompassing most of the previous programmes. These are (i) molecular biology of plant viruses and molecular mechanisms of plant-virus interactions; (ii) host gene and viral transgene-mediated resistance; (iii) mechanisms of transmission of viruses by nematodes, aphids and fungi; and (iv) recombinant antibody technology and diagnostics. A fifth unit involving viruses, the biotechnology unit, was formed under the leadership of the Deputy Director, T.M.A. Wilson, the previous Head of Virology. L. Torrance, Head of the diagnostics unit, was Acting Head of Virology in 1995. The electron microscopy laboratory was re-organised as an institutional support facility, and the research programme on viral diseases of soft

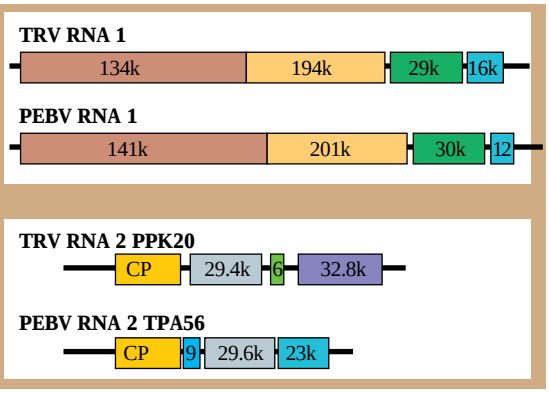


Figure 1 Genome organisation of tobacco rattle virus (TRV) and pea early browning virus (PEBV).
PPK20 and TPA56 are isolates named after the nematode species that will transmit these viruses.

fruit was integrated into the re-organised Soft Fruit and Perennial Crops Department.

Research on tobravirus molecular biology and transmission continued to make interesting and important advances. In the 1994 Annual Report, an article described the mapping work showing that transmission of pea early browning virus (PEBV) isolate TPA56 required the presence of the coat protein (CP) as well as the 23K, the 29.6K, and surprisingly, the 9K gene products of RNA 2 (Fig. 1). Subsequent work has shown that small deletions at or near the C-terminus of the CP of PEBV allowed particles to assemble, but either severely reduced or abolished transmission, indicating that this region of the CP has a role in nematode transmission by *Trichodorus primitivus*. In work done in collaboration with J Bol (University of Leiden, The Netherlands), it was shown that mutants in RNA 2 of tobacco rattle virus (TRV) isolate PPK20, with deletion in either the CP gene or the 29.4K gene, were not transmitted by the vector *Paratrichodorus pachydermus*, while deletions in the TRV 32.8K gene did not interfere with vector transmission of TRV PPK20. Thus, either different genes or combinations of genes are involved in transmission by different nematode species, or various tobraviruses have diverged sufficiently to require different combinations of proteins for nematode transmission. As a first step in unravelling this process, RNA 2 of TRV isolates PAY4 (transmitted by *P. anemones*) and TPO1 (transmitted by *T. primitivus*) have been cloned. TPO1 RNA 2 has been sequenced. Transcripts of isolate PAY4 have been confirmed as transmissible, and the nucleotide sequence of PAY4 RNA 2 will be determined, to allow comparison of the transmissibility with the genome organisations and sequence similarities of the encoded proteins of TP01 and PAY4.

Two genes on RNA1 of tobraviruses have also been the subject of analysis: (1) An ELISA assay for the TRV non-structural 16K protein (Fig. 1) was devised and was used to show that the accumulation of 16K protein was much less in plants infected with a TRV NM-type than plants infected with a TRV M-type. This difference in level of accumulation (and possibly expression) made it impossible to localize the 16K protein in tissue infected by the NM-type isolate using immunogold labelling. (2) Frameshift and deletion mutants in the 12K gene of PEBV RNA 1 (Fig. 1) resulted in large reductions in viral RNA accumulation in *Nicotiana benthamiana* and *N. clevelandii*, but resulted in an increase in viral RNA accumulation in pea. It is not yet known whether replication or movement is being affected here. However, on the basis of work done on an 11-12K protein encoded by cucumber mosaic virus, which affects movement in some hosts but not others (S W Ding and R H Symons, University of Adelaide), the PEBV 12K protein might have a similar host-dependent movement function.

Isolates of TRV from Belgium, Greece and the Netherlands that were transmitted by *T. similis*, were found to be serologically distinct. *T. similis* from Fife transmitted the Belgian and Dutch isolates, but apparently not the Greek one. This contrasts to previous findings on *P. pachydermus*-transmitted isolates, all of which are related closely, serologically. Trichodorid virus-vector nematodes were shown to acquire virus from symptomlessly-infected tubers, which thus were effective sources for virus dispersal and have the potential to introduce virus to new sites at which the vector, in the absence of the virus, is already present.

There have been other significant developments in programmes analyzing the interaction between viruses and vectors. For example, agroinoculation was established as a method to recover infectivity from cloned potato leafroll virus (PLRV) cDNA. Infectious virus was recovered from plants inoculated with *Agrobacterium* containing a full-length cDNA clone of PLRV. This will allow "reverse genetics" to be used to analyze the role of particular sequences in both transmission and infection. The full-length cDNA clone of PLRV was expressed in insect cells from a baculovirus vector. Unexpectedly, virus-like particles were produced, although they were not infectious. However, this provides us with a good model system to produce virus-like particles to study movement through the insect vector. Other aspects of virus-vector interactions related to the epidemiology of PLRV and potato virus Y (PVY), are covered in a separate report in this section.

This past year also saw the first demonstration of acquisition and transmission of potato mop top virus (PMTV) by the plasmodiophorid (fungal) vector *Spongospora subterranea*. PMTV was detected in primary zoospores using a monoclonal antibody-based ELISA assay. The laboratory strain of PMTV was not transmitted by *S. subterranea*. Sequence differences in the laboratory strain and a transmissible field isolate are present in the C-terminal half of the CP-readthrough protein.

Research on virus resistance has made great strides this past year. *N. benthamiana* plants were transformed with the PMTV CP gene and shown to be resistant to some Scandinavian isolates of PMTV, which are a serious problem. Since there is no natural resistance to PMTV in potato, this pathogen-derived resistance offers the potential to control PMTV infection in the field. The potato cvs. Saturna and Pentland Marble (both very susceptible to PMTV) have been transformed with the PMTV CP gene, and transgenic plants have been regenerated. These will be tested for resistance to PMTV.

In collaboration with M K Cheung and D Twell (Leicester University), it was shown that *N. benthamiana* plants transformed with the CP gene of Indian peanut clump virus (IPCV), showed some resistance to the accumulation of manually inoculated IPCV.

The potato cv Barbara was shown to contain one gene that controls extreme resistance to PVY and PVA (the *Ry* gene) and another gene that controls extreme resistance to PVA alone. The latter should be called the *Ra* gene. This gene may prove to be very useful in defining gene-for-gene interactions between potatoes and potyviruses.

Other research describing the prospects for improving resistance to PLRV by targeted breeding is described below.

Work on viral diagnosis, detection and the development of unique diagnostic tools is covered in two reports (below): one on artificial antibodies and their use in immunochemistry and diagnosis, and another on the properties and detection of a virus showing affinities with nepoviruses that is closely associated with blackcurrant reversion disease.

The use of PVX as a vector was described in the 1994 Annual Report. This past year, strategic and tactical improvements have been made in the PVX vector system, greatly increasing its utility and commercial potential. These improvements are described below as the use of a plant virus vector for production of foreign proteins in plants. In addition, the earlier PVX vector system has been used to express cloned single-chain variable fragments (scFv) of antibodies to PVY/PVA *in planta*. The scFv antibodies were functional and could react with virus particles in ELISA. Such systems may be useful as diagnostic tools and also allow the rapid screening of modified scFv genes targeted to different locations within plant cells.

Other highlights of the year include the following:

•Crystals of parsnip yellow fleck virus, which has unusual similarities to animal picornavirus, were produced for x-ray crystallographic analysis in a collaboration with the Dept of Biophysics, University of Oxford;

•The sequence of RNA 2 of IPCV (isolates of the L and T serotypes) was determined in collaboration with R A Naidu (ICRISAT), and comparisons with those of the IPCV H serotype isolate and peanut clump virus (PCV) from West Africa indicated that as much divergence existed among the IPCV serotypes as between IPCV and PCV;

•Nucleotide sequence analysis of the CP genes of 10 isolates of PLRV and 8 isolates of PMTV from the Andean highlands (in collaboration with L F Salazar, CIP, Peru and E N Fernandez, Northcote, PROINPA, Bolivia) revealed only minor differences in sequences compared to European isolates;

•The complete sequence of the genomic RNA of groundnut rosette virus (GRV) was determined, the genome organisation was shown to be the same as in RNA 2 of pea enation mosaic virus (PEMV), and there were notable sequence similarities between the two RNAs and their putative translation products;

•PEMV supported the replication of the GRV satellite RNA in *N. bethamiana* and GRV supported the replication of PEMV satellite RNA in both *N. benthamiana* and groundnut, which is not a host for PEMV;

•Mutagenesis studies of several variants of GRV satellite RNA showed that none of the potential open reading frames were required for satellite replication, while specific domains required for satellite replication, symptom modification, and suppression of the replication of the helper virus were identified.

Prospects for improving resistance to potato leafroll virus by targeted breeding

H. Barker, R. Solomon-Blackburn, J. McNicol & J. Bradshaw

Potato is the world's fourth major food crop and its most widespread and important virus disease is caused by infection with potato leafroll virus (PLRV). Infection with PLRV can cause large losses of yield and crop quality. Losses caused by PLRV infection can be limited by controlling the aphid vectors (of which the most important is *Myzus persicae*) that transmit the virus, and planting virus-free seed tubers. In future, however, aphid control may become more difficult because of the increase in insecticide-resistant aphid populations, long-term changes in climatic conditions leading to changes in vector populations, concern about the environmental effects of large-scale insecticide use, and consumer concern about pesticide residues in food. The production of virus-free seed tubers requires sophisticated facilities and, in some environments, frequent application of insecticides to seed crops. In some countries, such facilities and insecticides may not be available or are too expensive. The most economic and environmentally acceptable way of controlling PLRV is by the use of resistant cultivars.

In potato breeding programmes, resistance to PLRV is usually identified in field trials by growing plants of new lines in close proximity to PLRV-infected plants and determining how many test plants become infected by aphid-borne virus. Efforts to improve PLRV resistance in potatoes have been hindered by a lack of knowledge about the nature and inheritance of resistance, and the difficulties and expense of the screening procedures. Furthermore, resistance is expressed as a quantitative trait and has been thought to be controlled polygenically[1, 2] thus making targeted breeding difficult. For these reasons there are few cultivars with substantial resistance to PLRV.

In recent years, work at SCRI has attempted to understand more about the mechanisms underlying PLRV resistance. Such work has revealed that there are three components which can be found in some clones and cultivars[3]. These components are (i) restriction of virus accumulation, (ii) resistance to infection, and (iii) inhibition of virus movement from foliage to tubers. The first two forms of resistance have been systematically assessed in SCRI clones to determine their value in a breeding programme and whether substantial improvements can be obtained by their deliberate selection and combination.

Resistance to PLRV accumulation The use of enzyme-linked immunosorbent assay (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 accumulation is expressed to varying degrees in a number of SCRI potato clones and some recently selected clones (e.g.

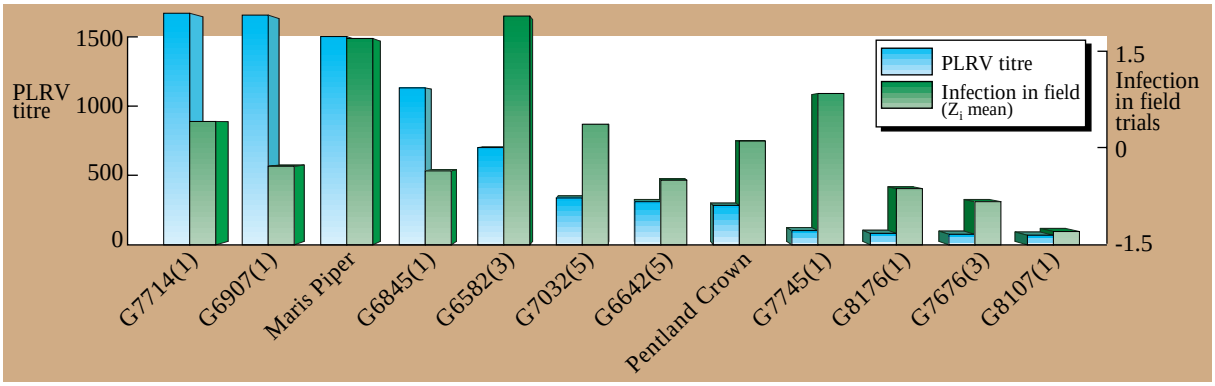


Figure 1 Expression of two types of resistance to PLRV in 12 potato clones (resistance to accumulation and resistance to infection). Mean PLRV titre is expressed as the concentration in ng/g leaf. Estimate of infection is given as a mean of Z-transformed percentages from three field trials. The formula used to transform percentage infection to Z_i is: $Z_i = \frac{X_i - \overline{X}}{s}$

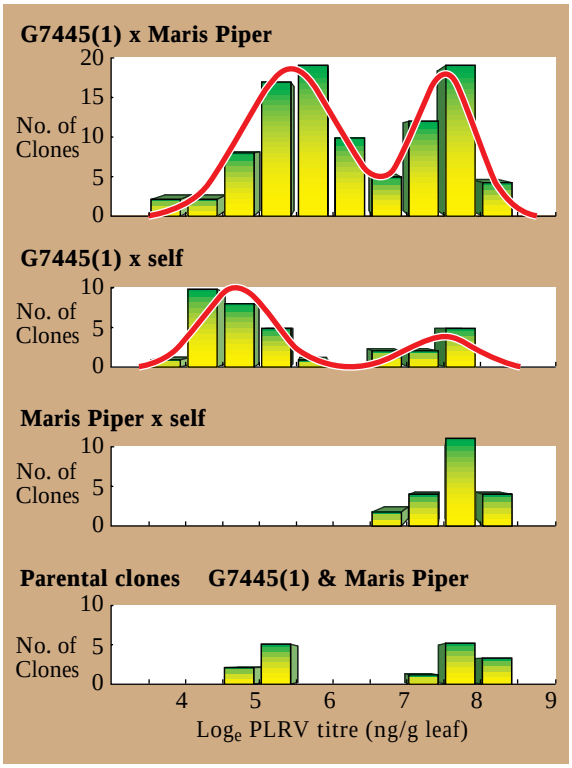


Figure 2 Histograms and fitted mixture densities (curves) of Log$_e$ PLRV titres (ng PLRV/g leaf) of parent clones of G7445(1) and cv. Maris Piper and the genoypes from progenies obtained from crosses using these parents.

G7445(1), G8176(1), G7676(3) and G8107(1)) are considerably more resistant than Pentland Crown (Fig. 1). The occurrence of this character in SCRI clones was largely fortuitous as it had not been deliberately selected in the breeding programme. The resistance is quantitative in nature, but is nevertheless very effective in diminishing aphid-borne spread of PLRV to uninfected plants in a potato crop. A field experiment sought 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. 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. For example there was 6-fold less spread 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.

Inheritance of resistance to PLRV accumulation To examine the genetic control of resistance to PLRV accumulation, reciprocal crosses were made between the susceptible cv. Maris Piper and the resistant clone G7445(1), and the two parents were selfed. Seedling progenies of these families were grown to generate tubers of individual genotypes (clones). Clonally propagated plants were graft inoculated, and their daughter tubers were collected and used to grow plants with secondary infection in which PLRV concentration was estimated. The expression of resistance to PLRV accumulation in a progeny from a cross between Maris Piper and the resistant clone, and also in a progeny from selfing the resistant parent, had a bimodal distribution with genotypes segregating into high and low virus titre groups (Fig. 2). Only the progeny obtained from selfing Maris Piper did not segregate, all genotypes accumulating high concentrations of PLRV. From initial studies[4] it appeared that resistance is conferred by a single dominant gene, but the patterns of segregation seen in the above progenies and those of another resistant parent, fit more closely with the hypothesis that two unlinked dominant complementary genes (both needed for resistance) are involved[5] (Fig. 3). The notation *R1* has been provisionally assigned to these genes until data from confirmatory crosses are obtained.

Resistance to infection For many years the second of the resistance components, the ability to resist infection, has been estimated in SCRI potato germplasm by field exposure trials as described above, and some parent clones with good resistance to infection have been produced. However, a feature of such field trials is that the overall level of infection can vary from year to year. Twelve potato clones were exposed to infection in three field trials in order to assess their resistance to infection[6]. Up to 92% of the plants of some clones e.g. G6582(3) and Maris Piper became infected, although other clones were relatively resistant to infection and clone G8107(1) remained virus-free in all three trials. To facilitate comparison and combination of the data from the different trials, the

	Resistant parent	Susceptible parent
Model 1 - single dominant resistance genes	**Rrrr**	**rrrr**
Model 2 - two complementary dominant genes	**Aaaa**	**aaaa Bbbb**

Figure 3 Two alternative genetic models for the inheritance of resistance to PLRV accumulation. Evidence for model 1 (a single dominant resistance gene) was provided from preliminary studies and Model 2 (two complementary unlinked dominant resistance genes) by data from more extensive crosses.

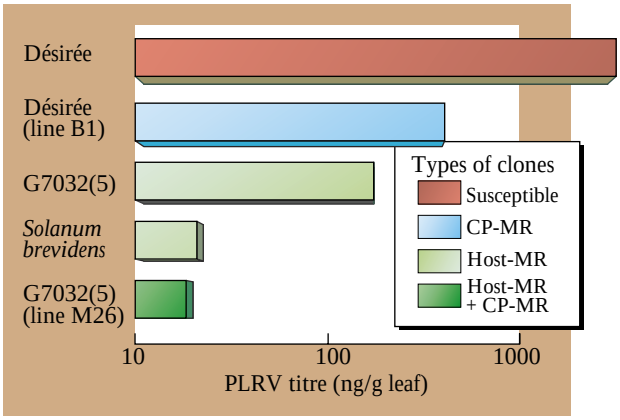


Figure 4 Host gene mediated resistance (Host-MR) and coat protein transgene-mediated resistance (CP-MR) to PLRV accumulation can be combined to provide greatly enhanced virus resistance.

percentage infection was transformed to Z_i using the formula shown in the footnote to Figure 1. In this formula X_i is the percentage of infection in a clone, $\bar{X}$ is the trial mean of the 12 clones and s is the standard deviation of the X_i values. The mean values of Z_i derived from the three field trials, are shown in Figure 1 as an estimate of the susceptibility of the clones to PLRV infection. Clones that were resistant to virus accumulation were not necessarily those which were most resistant to infection in the field, and there was no association between the two types of resistance. Nevertheless, both types of resistance were found in some clones such as G8107(1), in which both resistances are expressed to a greater degree than in Pentland Crown.

Prospects for further improvements in resistance to PLRV Our recent work has shown that the deliberate combination of different types of resistance to PLRV can result in selection of potato clones that should be considerably more resistant than any existing cultivar. The observation that resistance to PLRV accumulation is controlled by major dominant genes will lead to improvements in the breeding programme. For example, it should facilitate a more targeted approach to crossing parents with a view to obtaining resistant progeny. In future, selection of the trait of resistance to PLRV accumulation would be made more efficient by the use of linked molecular markers. We are initiating a programme to obtain suitable cDNA markers by bulked segregant analysis. The use of such markers for the *Rl* gene will greatly facilitate the identification of clones with resistance to PLRV accumulation, without the expense of the laborious screening procedure that is used at present.

Another form of resistance to PLRV has been obtained by genetic manipulation. Plants transformed with sequences that encode the coat protein gene of PLRV, were found to be more resistant to PLRV accumulation than non-transformed control plants[7]. More recently, it has been shown that combining host-coded genes with transgenes (for example in G7032(5)) results in a PLRV concentration that is about 1% of the level in susceptible cvs such as Désirée, and demonstrates that there is an additive effect against the virus (Fig. 4). This concentration of PLRV is similar to that estimated to accumulate in leaves of the highly resistant wild potato species *Solanum brevidens*[8]. This combination of three forms of resistance should provide almost complete protection in the field, even in areas where there is high inoculum pressure.

Our attempts to produce potato clones which have a unique degree of resistance to PLRV should, in future, prove to be of great benefit to both growers and consumers alike.

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Antibody-like proteins from a phage-display library

A. Ziegler, K. Harper, M.A. Mayo & L. Torrance

Antibodies are proteins which bind specifically to particular antigens, often with high affinity. For many years, this type of reaction has been used in plant pathology as a way of identifying viruses because the protein coats of virus particles are immunogenic, and the antibodies they elicit can discriminate between proteins with relatively small differences in amino acid sequence.

Two recent developments in molecular immunology have resulted in the production of antibody-like proteins (ALP) which behave like antibodies in the way in which they bind to specific antigens The methods developed are (1) cloning the DNA which encodes the parts of antibody molecules that are responsible for binding to antigens, which are known as the variable domains of the heavy chain (V_H) and light chain (V_L), and (2) displaying proteins as part of the surface structure of infective bacteriophage particles which carry the gene which encodes the protein being displayed. These developments were described in the Annual Report for 1994.

The process by which genes encoding V_H and V_L regions are cloned and then expressed is summarised briefly in Figure 1. Messenger RNA from hybridoma cells which secrete a single species of monoclonal antibody (MAb) is copied to form cDNA encoding the

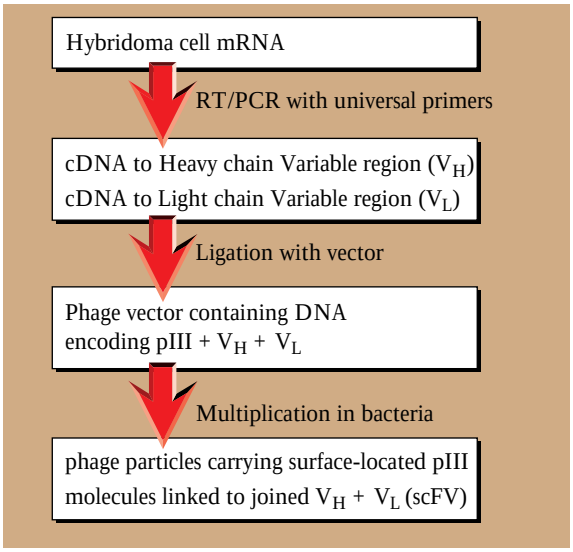

Figure 1 Outline of origin of phage-displayed scFv.

V_H and V_L domains of that antibody. The cDNA fragments are joined, by means of a linker sequence, into one piece of DNA (Fig. 2) which encodes a single chain Fv (scFv) with the binding specificity of the original MAb. Figure 2 shows an example of a scFv molecule. The scFv cDNA is ligated into phage DNA and the recombinant phage particles formed in cells transfected with the vector DNA, carry the scFv on their surfaces as well as the scFv gene in their DNA. When phage is grown in a different, non-suppressor strain of host bacterium, phage particles are not produced and the scFv gene is expressed to produce soluble ALP.

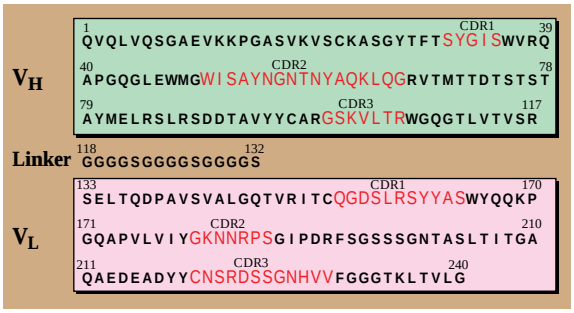

Figure 2 Amino acid sequence of an anti-CMV scFv.

In work described in the Annual Report for 1994, scFv were prepared from RNA extracted from hybridoma cells secreting monoclonal antibodies to potato leafroll luteovirus (PLRV). The resulting phage-displayed scFv had some antibody-like binding activity, but soluble scFv produced from the same vector was found to bind only very weakly to virus particles. In this respect, our experience has mirrored that of other groups. In further work, we have isolated virus-specific scFv from clones selected from the MRC human synthetic scFv library, obtained from G. Winter and J. Harrison of the Centre for Protein Engineering, Cambridge.

The naive library The heavy and light chains of antibody molecules each contain 3 complementarity determining regions (CDR). The scFv sequence shown in Figure 2 illustrates these regions. As there is a finite number of amino acids in each of the CDR, and a finite number of possible amino acids at each

scFv dilution	CMV-Fny	TAV	Healthy
1/2	0.43	0.06	0.09
1/5	0.21	0.13	0.08

ELISA plates were coated with extracts of plants infected with Fny strain of cucumber mosaic virus (CMV) or tomato aspermy virus (TAV) or of healthy plants. Values are absorbance (A_{405nm}).

Table 1 PTA-ELISA using soluble anti-CMV scFv.

position in the CDR, there is a finite, though large, number of possible combinations of all possible CDR sequences. The naive library is, in principle, a mixture of phage particles which display these combinations on their surfaces. By selecting for those phage which bind to a particular antigen, it is possible to obtain a clone of phage which encodes a scFv with the desired binding activity.

Currently, we are experimenting with the MRC human synthetic scFv library which contains 50 germline V_H fragments (CDR1 and CDR2) combined with randomized CDR3 sequences joined to a single V_L sequence. In practice, this library approaches the diversity of a theoretical naive library because much of the binding specificity of antibodies is determined by the sequence of CDR3 of the heavy chain. We have used this library as a source of ALPs for use in a variety of lines of work.

Selection by panning The library contains between 10^8 and 10^9 phage types. The desired phage clone is selected by successive rounds of panning. For this procedure, virus particles are adsorbed to plastic tubes. The library is then added and those phage which bind specifically after extensive washing, are eluted and multiplied. After repeating this procedure 3-4 times, individual phage clones are isolated and tested for binding activity.

Cucumber mosaic virus-specific ALP The antigen was a purified preparation of isolate R of cucumber mosaic cucumovirus (CMV). After 4 rounds of panning, 24 individual clones were tested for antigen binding. Nine of the clones produced phage which bound to CMV in plate-trapped-antigen (PTA) ELISA. When the phage were inoculated into cells of non-suppressor host bacteria, soluble scFv was produced which bound specifically to CMV particles in ELISA tests (Table 1). The same preparation also reacted with denatured CMV coat protein in immunoblots (Fig. 3).

Restriction enzyme mapping suggested that the DNA sequences which encoded the V_H parts of the scFv in each of the 9 clones were very similar, and the nucleotide sequences of three of the clones were found to be identical (Fig. 2).

Potato leafroll virus-specific ALP As in the experiments with CMV, when the library was panned using PLRV particles bound to plastic tubes, clones were obtained which produced phage which bound to PLRV particles in ELISA tests. However, the clones produced only small quantities of active soluble antibody. This problem was overcome by subcloning the scFv gene into a different, newly constructed expression vector, pDAP2 (a gift from R. Kerschbaumer and G. Himmler, Institute of Applied Microbiology, Vienna).

Cloning scFv in pDAP2 The vector pDAP2 was constructed so as to allow the expression of a soluble fusion protein which consists of scFv linked to *Escherichia coli* alkaline phosphatase. Such a fusion protein could be used in ELISA tests directly, in contrast to conventional antibodies which normally must be conjugated with a reporter molecule, such as alkaline phosphatase, before use. In pDAP2, the alkaline phosphatase gene and sequence encoding a C-terminal 6-histidine tag to facilitate protein purification, is downstream of a multiple cloning site and a leader

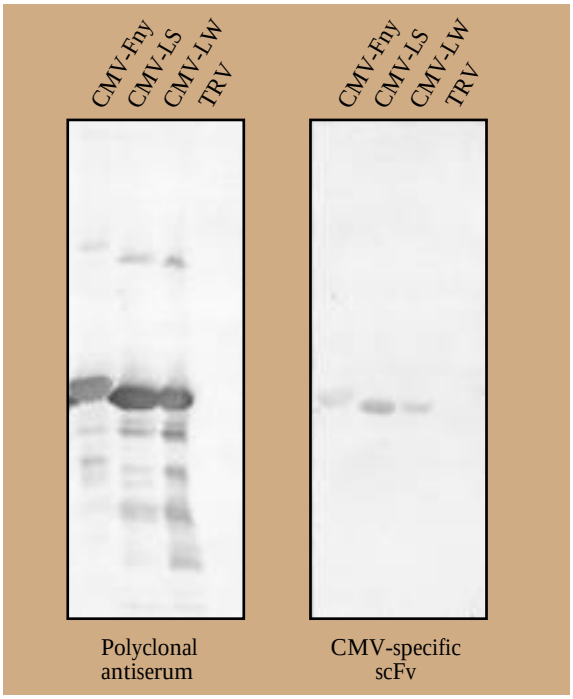

Figure 3 Immunoblots comparing CMV antibodies and scFv.

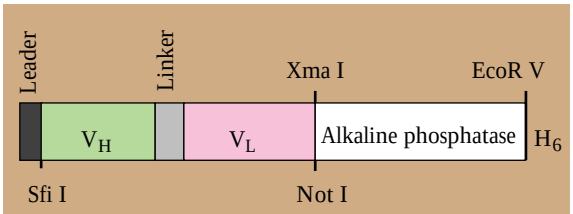

Figure 4 Diagram of the cloning site for scFv cDNA in pDAP2.

sequence (added to direct the protein to the bacterial periplasmic space). The scFv gene is cloned between *Sfi* I and *Not* I restriction sites such that it is in-frame with the alkaline phosphatase gene (Fig. 4).

An scFv expression vector was constructed in pDAP2 by cloning a scFv gene, which had been isolated from a clone selected from the phage display library by panning with PLRV. Expression of this vector resulted in the production of a scFv-alkaline phosphatase fusion protein which was functional in ELISA (Table 2).

scFv dilution	Infected	Healthy
1/10	0.70	0.15
1/100	0.31	0.10

Values are absorbance (A_{405nm}) obtained in ELISA of extracts of potato leafroll-infected *Physalis floridana* using scFv-alkaline phosphatase fusion protein

Table 2 PTA-ELISA using anti-PLRV scFv-AP.

Prospects So far, the anti-virus scFv which have been obtained have reacted less well in serological tests than conventional polyclonal or monoclonal antibodies. However, there are several ways in which the performance of ALPs could be made more comparable to that of conventional antibodies. For example, it is possible to enhance the amount of ALP produced in *E. coli* by modifications to the cultural conditions. Also, it should be possible to improve the binding affinity of the ALP by molecular means such as re-selection from a library in which the CDR3 of the V_L part of the scFv is randomized from that obtained in the primary selection. Current work is concerned with modifications to anti-viral ALPs such as these with the overall aim of developing the ALPs to the point where they are of practical use in virus disease diagnosis.

The naive library should also be a source of ALPs which bind to non-viral antigens. Such reagents could be applicable in a number of diverse fields of research and currently we are exploring this potential. Selection for phage with binding activity to an enzyme preparation purified from plant extracts, has already given very promising results.

Aphid vector population biology and the control of virus diseases

J.A.T. Woodford, M.A. Mayo, H. Barker, R. Harrington[1] & J. Pickup[2]

Because viruses are intra-cellular parasites, their biological success, and the consequent economic impact of the diseases they cause, is critically dependent on an ability to spread from one host to a new one. For the great majority of viruses, this spread from host to host depends on the activities of other organisms known collectively as vectors. Humans can be virus vectors as, for example, during the grafting of grapevines. However, it is almost always plant-feeding invertebrates or fungi which perform this function.

Most vectors are plant-sucking insects (mainly aphids and whiteflies), but other invertebrates, such as beetles, mites and nematodes, and certain soil-infesting fungi can also transmit plant viruses. Several of these virus-vector associations are the subjects of current research work at SCRI. This article describes some aspects of the work aimed at understanding virus biology at the level of the host populations of economically important crops. Other aspects of the vector research programme, including studies of aphid

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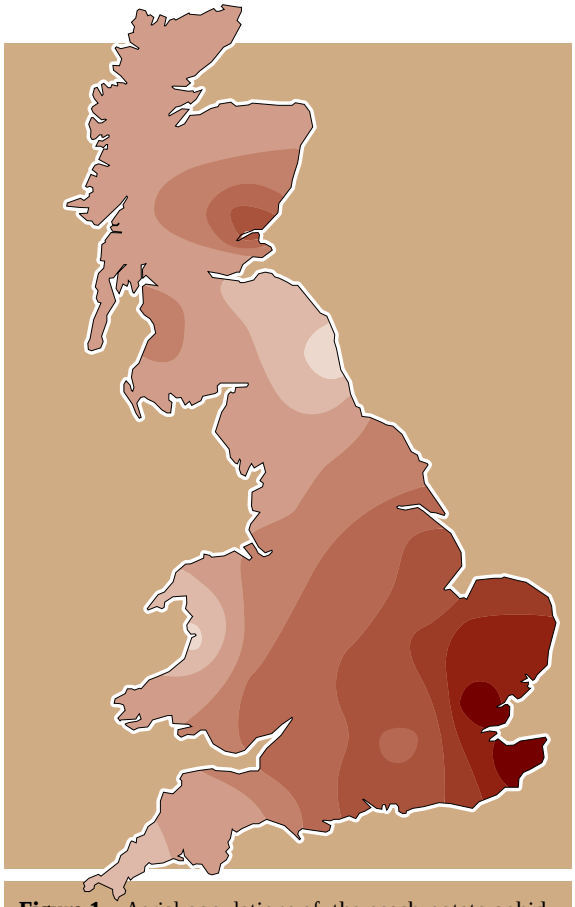

Figure 1 Aerial populations of the peach-potato aphid *(Myzus persicae)* in early summer 1982; note larger numbers (darker shading) in more intensive arable areas.

behaviour and virus transmission mechanisms, have been described in previous Annual Reports.

Slightly more than half of the plant viruses are transmitted by aphids and about 30% of plant virus genera contain aphid-transmitted viruses. Aphids are therefore one of the most economically significant groups of vectors, and over 300 species of aphids have been reported to transmit viruses. Determining what makes one aphid a vector, when another species or even biotype is not, is one of the ultimate goals of current research. Aphids transmit viruses in one of three ways. For some, virus particles are bound to mouth parts when an aphid probes an infected plant and, if released into cells of an uninfected plant in a subsequent feed, can cause infection. An important example of this is transmission of potato virus Y (PVY) by a variety of aphid species. A more subtle mechanism is involved when virus particles are taken up by the aphid gut, circulate in the haemolymph, pass through a salivary gland and then pass into a new plant in the injected saliva. An important example of this is transmission of potato leafroll virus (PLRV). A third, less common mechanism, is when the plant virus also infects the aphid, multiplies in aphid tissues and thereby contaminates the saliva.

In economic terms, damage from virus disease can be related to the size of vector populations, and measuring populations is a key component of disease control. Some 30 aphid species, which are pests of a wide range of arable and horticultural crops, have been estimated to be capable of causing economic losses in excess of £100 million per year to British agriculture as a result of direct feeding damage and the virus diseases which they spread. However, very few species cause widespread damage every year. Most cause sporadic damage; their numbers fluctuate widely in time and space. Methods of forecasting the time and size of crop infestations, which is the first step in determining the risk of virus infections, usually depend on estimating the extent of aphid migrations. Many annual crops are colonised in the spring or early summer by winged aphids flying from overwintering plants. In 1964, Dr Roy Taylor at Rothamsted Experimental Station developed a suction trap to overcome the impracticalities of monitoring aphid populations on many different crops. His design led to the establishment of a network of similar traps which has been used to forecast aphids and virus diseases in several crops, including potatoes, sugar beet

Figure 2 SCRI suction trap.

and cereals, and now extends to most EU countries. The traps collect aerial populations sampled from 2700m^3 air/h at 12.2 m above the ground, a height chosen to give a good estimate of the numbers of small wind-dispersed insects. They provide daily, standardised, quantitative information on many species, which is unbiased by local sources of migrants, and represents aphid activity over wide areas (Fig. 1). Fifteen suction traps are currently operated in the UK. The 'Dundee' suction trap (Fig. 2), located at SCRI, was one of the first to be operated and has provided continuous aphid records since 1967. There are now four traps in the Scottish network (Dundee, Edinburgh, Elgin and Ayr), sited in areas of importance for potato production.

Predicting the incidence of potato leafroll virus (PLRV) in seed potato crops The date when *Myzus persicae*, the main vector of PLRV, is likely to arrive in seed potato crops can be predicted. Significant relationships have been found between the date that the first *M. persicae* is recorded in suction trap samples each year, and temperature during the preceding winter. Variations in winter temperature, which affect the survival of these aphids, can also be used to determine the size of the spring migration. Figure 3 shows the relationship between the mean air temperature at SCRI in January and February, and the date when the first *M. persicae* was recorded in the 'Dundee' suction trap (a), and the total numbers of *M. persicae* trapped until mid-July each year (b). Dates on which *M. persicae* was first recorded in this trap have ranged from 29 April in 1989, following a particularly mild winter, to 30 July after a cold winter in 1977; after mild winters, larger numbers of *M. persicae* are caught. The figures show that data must be accumulated over many years so as to obtain a wide range of temperatures. The pitfalls inherent in attempting to derive these relationships from short-term studies, are exemplified by two periods of four consecutive years which would have given misleading results; between 1973 and 1976, when the range of winter temperatures was small, and between 1992 and 1995, when the relationships were contrary to long-term trends. Analyses of historical trap data now form the basis for early warnings of aphid activity in high risk years. These warnings are produced in conjunction with data on the incidence of potato leafroll in the previous year, and issued in time to decide on the use of insecticide granules when seed crops are planted.

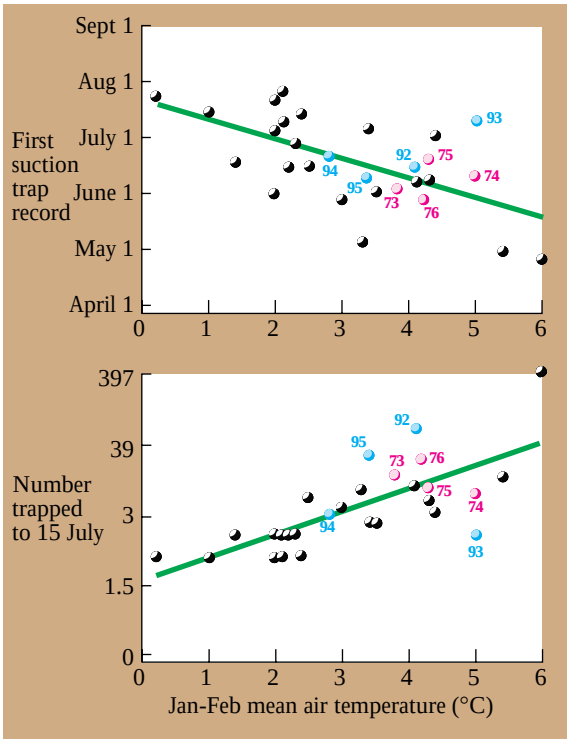

Figure 3 Relationships between mean Jan-Feb air temperatures at SCRI and *M. persicae* in the suction trap (a) first record (1967-95); (b) total numbers caught until mid-July each year.

When we tested relationships between temperature and subsequent spread of PLRV in field experiments, we found that mean temperatures from April to June, when aphids were becoming active in the crop, were more closely correlated with virus spread than were winter temperatures. The total number of *M. persicae* caught in the suction trap during the spring and summer gives a measure of the numbers of potential

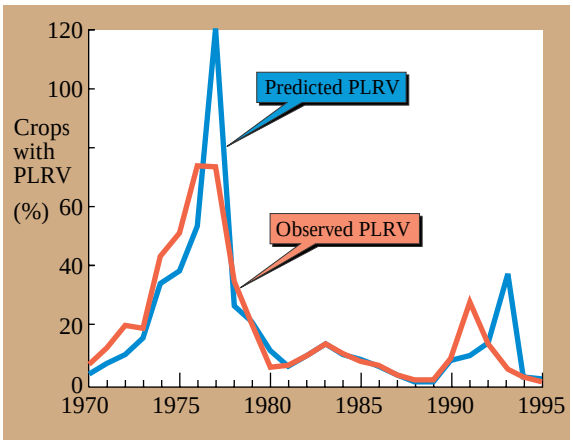

Figure 4 Predicting PLRV incidence in Scottish seed potato crops using a model based on the virus incidence and catch of *M. persicae* in the Dundee suction trap during the previous year.

Figure 5 Swinging net trap to catch live aphids.

colonisers and of the subsequent emigrants from potato and other crops. This information can be used, together with records collated by SOAEFD of classified seed potato crops in which PLRV was present, to forecast the incidence of PLRV in the coming year (Fig. 4).

Assessing the spread of PVY in potato crops Forecasting disease incidence is more difficult when viruses are spread by many different vectors. In contrast with PLRV, which has no alternative host plants in Scotland and few aphid vector species, PVY can infect a few weed species and is transmitted by several aphid species, most of which do not colonise potato crops. In experiments to determine which species are potential vectors of PVY, winged aphids flying over potato fields have been trapped alive in conical nets facing upwind (Fig. 5). When the trapped aphids were taken to the laboratory and allowed to walk across potato leaves infected with PVY, some species never stopped to probe and could not, therefore, acquire the virus. Aphids that did make brief probes sufficient to enable them to transmit the virus to healthy potato plants, differed in their transmission efficiencies

Species	No. tested	Percentage transmitting
Myzus persicae	34	41.2
Brachycaudus helichrysi	54	7.4
Rhopalosiphum padi	430	2.8
Aphis fabae	42	2.4
Metopolophium dirhodum	299	1.3
Sitobion avenae	304	0.7

Table 1 Efficiency in transmitting PVY^N of aphids caught in the swinging net trap.

(Table 1). Figure 6 shows the numbers of potential vectors of PVY that were caught in yellow water pan traps in four crops of cv. Record grown from super elite Scottish seed potato stocks in 1994. Although no virus infections were detected in any of the planted seedstocks, between 36 to 54% of the potatoes harvested from the English crops became infected, compared with 3% of the crop grown in Scotland. Further work is in progress to determine if the 3-fold difference in aphid populations (Fig. 6) could account for such a large difference in virus spread.

Conclusions Relationships between the numbers of aphid vectors and virus disease incidence, as illustrated by the examples in this report, indirectly reflect those aspects of insect behaviour, i.e. movement and probing activity, that are crucial to virus spread. Current developments in monitoring aim to provide more detailed information about aphid vectors including, for example, work at SCRI to develop sensitive DNA tests for individual aphids to identify genotypes adapted to particular crops, such as potatoes. Other refinements include the development of immunoassays to determine the proportion of aphids in suction trap samples that are resistant to insecticides, or that are carrying persistently-transmitted luteoviruses, e.g barley yellow dwarf virus.

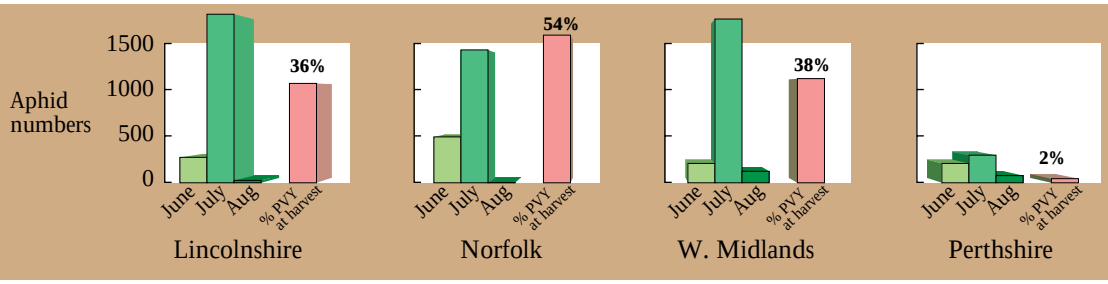

Figure 6 Relation between the numbers of trapped PVY vector aphid species and the spread of PVY^N at four sites in 1994.

Blackcurrant reversion disease - tracking down the causal agent

A.T. Jones, K. Lehto[1], A. Lemmetty[2], S. Latvala[1], P. Susi[3], W. J. McGavin & S. Cox

Of about 14 distinct virus or virus-like diseases that have been recognised in *Ribes*, blackcurrant reversion is unquestionably the most important for blackcurrant crops world-wide; it also affects redcurrant but apparently not gooseberry[1]. The disease is particularly widespread in blackcurrant (*Ribes nigrum* L.) in Eastern and Central Europe and Russia, in some parts of the UK and Scandinavia[1] and, in recent years, it has become a serious problem in New Zealand (A.T. Jones, unpublished information). A form of the disease (R) present in countries of the former Soviet Union, Eastern and Central Europe, and Finland[1] is much more severe in plants than the common European (E) form but, with each of these forms, the progression of the disease in plants and crops is similar. In nature, the causal agent of the disease is transmitted between plants by the blackcurrant gall mite, *Cecidophyopsis ribis*, but not through seed. Experimentally, it can be transmitted between infectible *Ribes* plants by grafting[1].

The disease was first described in the Netherlands in 1904 by Ritzema Bos, but undoubtedly occurred before this, and presently is reported from all countries where blackcurrant is grown commercially, with the exception of the Americas. As its name suggests, the disease reflects the change in plant habit, mostly in the leaf appearance, that is suggestive of 'reversion' to a primitive wild plant type[1]. Compared to leaves of healthy plants, those of reverted plants are narrower, show a decreased number of main veins, have larger but fewer marginal serrations, and have a basal sinus that is less lobed (Fig. 1). These symptoms are usually more pronounced in infections with the R than with the E form of the disease but symptoms vary in intensity and severity between blackcurrant cultivars[4]. Another, more consistent, symptom of the disease, seen only in newly emerging flower buds, is a brighter pigmentation of the buds compared to those of healthy plants, due to a loss of the downy hairs on buds (Fig. 2). On plants affected by the R form of the disease, flower buds in addition develop strong malformations, including the absence of stamens, elongation of the style and an increase in the number of petals, and pigmentation is intensified further (Fig. 3). Affected flower buds in each form of the disease are usually sterile causing a severe loss in fruit productivity. Leaf and flower symptoms in redcurrant are less noticeable than those in blackcurrant. Much less consistent in appearance in plants affected by either form of the disease is the development in spring of foliar chlorotic line patterns and/or ringspots, as the expression of this symptom is dependent on genotype/environment interactions that are not well studied (Fig. 4). The leaf markings often disappear as the leaves age and are usually not evident on growth made during the summer. Such line-pattern symptoms are not diagnos-

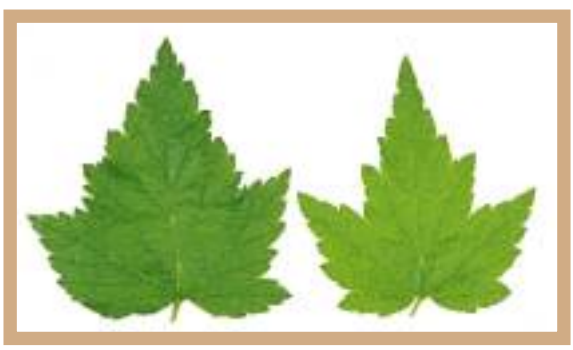

Figure 1 Leaf symptoms of reversion disease in Ben Nevis blackcurrant (right). Healthy leaf (left).

Figure 2 Brighter pigmentation of the flower buds of a blackcurrant plant affected with the R form of reversion disease (left) compared to those of a healthy plant (right).

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Figure 3 Increase in the number of petals, and intensification of pigmentation of flower parts that are characteristic of the R form of reversion. Note the erratic distribution of the symptoms in different nodes.

tic of reversion because similar symptoms can be induced in blackcurrant by infection with some nepoviruses, cucumber mosaic cucumovirus and alfalfa mosaic virus ([1]; A.T. Jones, unpublished information).

Blackcurrant reversion, together with some other mite-transmitted virus-like agents[3], have been an enigma in science for over 50 years because much research has failed to identify the causal agents of these diseases. The seriousness of reversion disease in blackcurrant has initiated many attempts to identify its causal agent. However, earlier claims that it was a mycoplasma-like agent[4] or a potyvirus[5] have not been

Figure 4 Conspicuous chlorotic line-patterns on the leaves of reverted blackcurrant plants.

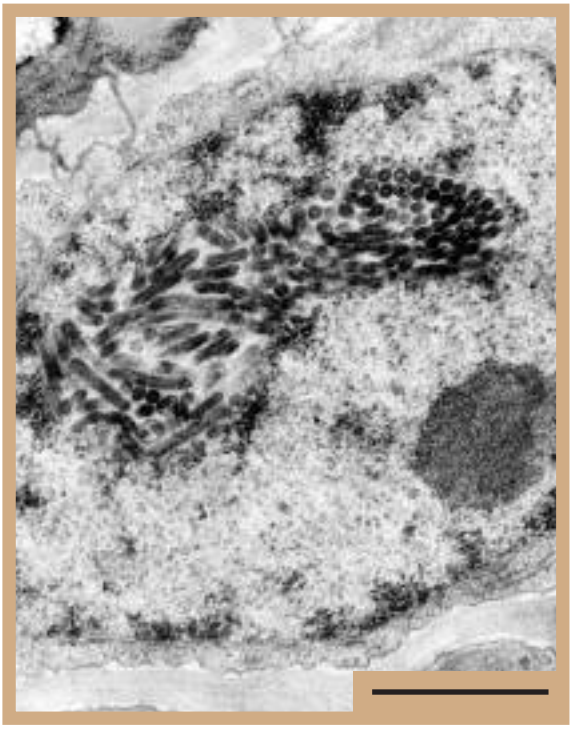

Figure 5 Rhabdovirus-like particles in the nuclei of xylem parenchyma cells of blackcurrant affected with the R form of reversion disease. Bar = 1µm.

substantiated by other workers ([1]; A.T. Jones and I.M. Roberts, unpublished data). Recent work at SCRI identified several dsRNA species in reverted blackcurrant plants that were not present in virus-tested mother stock plants, but further work has shown that some of these dsRNA species are present in some older blackcurrant cultivars that are free from reversion disease. No specific dsRNA species were therefore consistently associated with the disease (S. Cox, A.T. Jones and M.A. Mayo, unpublished data). More recently at SCRI, extensive studies were made to identify the possible morphological structure of the reversion agent and/or changes in cell ultrastructure associated with it, by using electron microscopy to examine ultrathin sections of different organs of reverted blackcurrant plants and of gall mite vectors from such plants. No such structures were observed in mites[6] but, in a very small proportion of parenchyma cells of vascular tissue of a few plants affected with the R form of the disease, rhabdovirus-like particles *c.* 65-80 x 215-485nm were detected (Fig. 5)[7]. Such particles have not been reported previously from *Ribes* and we detected them in only three of seven plants affected with the R form of reversion, but not in any of five plants affected with the E form. It is not clear if the failure to detect these particles in some plants is

Figure 6 Systemic chlorotic flecking in *Chenopodium quinoa* two weeks after mechanical inoculation with BRAV.

due to their absence, or the difficulty of detecting them due to their very low frequency of occurrence in cells. However, as morphologically similar particles were also observed in vascular cells of gooseberry that is reported to be immune to the reversion agent[1], it suggests that these rhabdovirus-like particles are unlikely to represent the causal agent of reversion disease, at least not on their own.

This lack of knowledge of the disease agent has prevented the development of tests for its rapid detection, so that the test for infection in plants today is still dependent on traditional graft-inoculation of test material to sensitive blackcurrant cultivars that was developed over 40 years ago. Furthermore, because the reversion agent is erratically distributed in infected plants (Fig. 3), it is necessary to test material from several branches of the same test plant and to await symptom development for up to 2 years[1]. However, this impasse in identifying the causal agent of reversion disease may have been overcome following work in Finland that isolated, with great difficulty, a sap-transmissible virus from a rooting cutting of a black-currant plant affected with the R form of the disease, and which developed pronounced chlorotic/yellow line patterns in newly produced leaves (Fig. 4). This virus was mechanically transmitted to *Chenopodium quinoa* (Fig. 6) and, from this host, to a range of other herbaceous test plants, that allowed it to be cultured, purified, partially characterized, and an antiserum to it produced. In collaborative studies, the virus was found to have isometric particles *c*. 27 nm in diameter (Fig. 7) that sedimented as two nucleoprotein components. Virus particle preparations contained a major protein species of *M*r 55kD and two major RNA components of *c*. 6700 and 7700 nucleotides, each with poly-A tails. Several of these properties are shared by members of the proposed sub-group 3 of nepoviruses, but the virus was serologically unrelated to 9 possible members of this sub-group or to 5 other nepoviruses, or putative nepoviruses tested. However, the deduced nucleotide sequence of part of the 3' end of one of the viral RNA species contained short regions of homology to the 3' terminal sequences of RNAs 1 and 2 of cherry leaf roll nepovirus, and to the RNA 2 of cowpea mosaic and red clover mottle comoviruses; apart from these short regions, the partial viral sequence is distinct from those reported for other viruses. The virus therefore appears to be newly described.

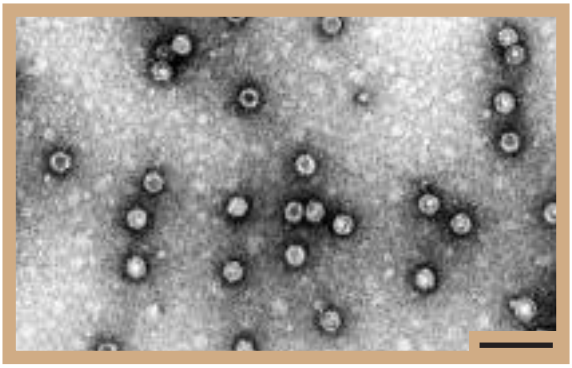

Figure 7 Electron micrograph of a purified preparation of BRAV particles stained in uranyl acetate, pH 3.5. Bar = 100nm.

The isolation and partial characterisation of this virus have provided the materials for its rapid and sensitive detection in plants. Primers were designed from the known partial sequence to amplify a 210 bp region of the cDNA of the virus RNA using an immuno-capture-reverse transcriptase-PCR (IC-RT-PCR) protocol. This technique has given the necessary sensitivity and reliability to assay this virus in a wide range of *Ribes* plants infected with different viruses or virus-like agents, and to draw some conclusions on its possible involvement, or otherwise, in known diseases of *Ribes*. Data from such analyses have shown a very close association of the virus with reversion disease in plants. It was detected by IC-RT-PCR in plants showing symptoms of the E or R forms of the disease (Fig. 8), whether they were of Finnish, Scottish or New Zealand origin and also in gall mites collected from reverted plants. It was also associated with the field spread of reversion into initially healthy blackcurrant plants and was detected in initially healthy blackcurrant plants on which gall mites from reverted plants

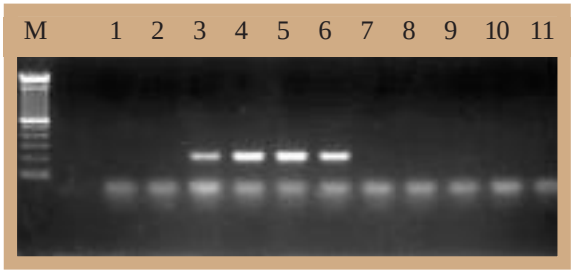

Figure 8 IC-RT-PCR amplification of the 210 bp cDNA fragment of BRAV RNA from nucleic acid extracts from individual blackcurrant bushes. Lane 'M' contains a 100 bp mol. wt ladder (Gibco BRL). Samples are (lanes in parenthesis), healthy plants of the blackcurrant cultivars Ben Alder (1, 7), Ben Lomond (2, 11), Ben Nevis (8), Ben Tirran (9) and Ben Sarek (10), and unnamed cultivars affected with the R (3, 4) and E (5, 6) forms of reversion disease.

had fed experimentally. However, the virus was not detected by IC-RT-PCR in healthy *Ribes* plants, in *Ribes* plants free from reversion but affected by three other distinct virus-like diseases of *Ribes*, or in plants infected with arabis mosaic, strawberry latent ringspot or raspberry ringspot nepoviruses. In assays of individual buds from branches of a plant of Ben Lomond blackcurrant naturally infected in the field with the E form of reversion, the virus was detected in only 50% of the buds, ranging from 28-60%, depending on the branch assayed (Fig. 9). There was no clear pattern of distribution but a large proportion of the cluster of buds near the tips of some branches assayed were infected, suggesting that this region might be the best to sample to detect infection in plants. This erratic distribution of the virus is in keeping with the known erratic distribution of the reversion agent in plants.

Taken together, these data suggest strongly that this virus may be the causal agent of reversion disease and it is tentatively called, blackcurrant reversion associated virus (BRAV). However, unequivocal evidence that BRAV is the causal agent of the disease depends on the ability to fulfil Koch's postulates by infecting healthy blackcurrant plants with the purified virus and reproducing the disease. Currently, technical difficulties prevent this because *Ribes* plants are very difficult to infect with virus by mechanical inoculation, due presumably to the high levels of tannins and polyphenols in leaves. Also, the development of reversion symptoms in inoculated blackcurrant plants may take up to 2 years[1] Despite the absence of this final proof, our finding of a very close association of BRAV with reversion disease and the development of a sensitive

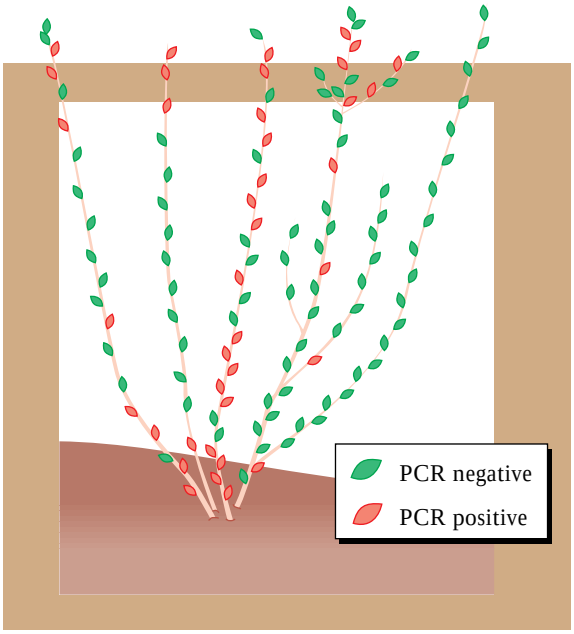

Figure 9 Diagrammatic representation of the occurrence of BRAV in assays on individual buds of branches of a reverted plant of Ben Lomond blackcurrant.

protocol to detect it in *Ribes* tissue, are major steps forward in the possible identification of the causal agent and a rapid means of diagnosing it in plants. They also provide a means to study, in more detail, the relationship of BRAV with its plant host and possible mite vector.

Finally, if BRAV is found to be the causal agent of reversion disease, and if the remaining sequence of its RNAs confirm its status as a nepovirus, then it poses interesting questions regarding the transmission of the majority of nepoviruses that have no known vector. In the light of our work with BRAV, it may be opportune to examine the possibility that mites may also act as vectors for some of these nepoviruses for which the mode of transmission is not known.

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Use of plant viruses for production of foreign proteins in plants

S. Chapman, S. Santa Cruz & T.M.A. Wilson

Foreign gene expression vectors based on plant viruses have been used as virological research tools, and for the production of commercially valuable proteins and peptides. In this article, SCRI research to achieve the latter objective is described. The utility of some of the vectors we have produced for following virus movement and for understanding plant transport processes is described elsewhere in this report (see p. 78) .

Plants are an attractive alternative source of foreign protein, compared to more traditional cell culture and fermentation technologies, because large amounts of a protein can be produced at relatively low cost, without the requirement for a sterile environment. Most interest has centered on producing proteins with medicinal or veterinary applications because of the high prices these command. At present, the two ways of manipulating plants to produce proteins are through transformation of the plant DNA genome, and by using RNA or DNA viral vectors. Transformation of plant genomic DNA permits the stable, heritable production of novel proteins in plants. However, transient production of proteins by viral vectors has several advantages. New vectors can be created and used to infect plants quickly and easily, avoiding the arduous tasks of plant transformation and regeneration. Moreover, because of the efficiency and high yield of virus multiplication, novel proteins produced from modified viral forms can accumulate to high levels, which exceed those achievable by stable transformation. Use of viral vectors can also circumvent some problems encountered with stable transformation, such as plant species that are difficult to regenerate, gene escape in pollen, and variation in levels of expression between different transformed lines.

The strategies adopted to produce viral vectors depend on the mechanisms used by the viruses to express their genes and whether the novel protein is to be produced as a free protein or as a fusion to a viral protein. Fusion of the novel protein to the coat protein offers several commercial advantages. First, the coat protein is generally the most highly expressed viral gene product and hence, as the foreign protein is produced in equal amounts, the latter will also accumulate to extremely high levels. Second, a protein fusion strategy avoids the use of duplicated subgenomic RNA promoter sequences, a strategy that has often been used for the production of free proteins by plant viral vectors, but which allows homologous recombination that results in instability of the foreign gene sequence insert. Therefore, coat protein fusions are genetically more stable. Third, if the coat protein fusion is still capable of virion formation, the special physical and chemical properties of the virus particle can be used to facilitate separation of the foreign protein with its virus "carrier" from the huge array of other plant cellular components. Lastly, one major application identified for small proteins or peptides produced in plants is in vaccine production. The stability and immunogenicity of such peptides should be enhanced by their assembly into a macromolecular carrier molecule such as a "decorated" virus particle.

Previously at SCRI, vectors based on potato virus X (PVX) were used for the production of free proteins in plants[1]. To extend the utility of our PVX-based system for foreign protein production, it was decided to develop a vector in which fusions could be made to the coat protein, while retaining the ability to form virus particles. Earlier studies on tobacco mosaic virus (TMV) which, like PVX, forms elongated particles of helically stacked coat protein subunits, had indicated that direct fusions of only a few amino acids could be made to the coat protein for the modified subunits to still form particles. Larger fusions to the TMV coat protein were made possible by the introduction of a translational read-through sequence between the normal 3' end of the coat protein gene and a sequence encoding a peptide[2]. This strategy resulted in production of a mixed pool of free and fused coat protein subunits. The presence of the free subunits permitted occasional (5%) incorporation of coat protein subunits with foreign carboxy-terminal fusions of up to twenty one amino acids into virions. However ninety five percent of the subunits produced and incorporated into virions did not have the peptide attached to their carboxy-terminus. This strategy cannot be applied to PVX as the carboxy-terminus of PVX coat protein is not on the outer surface of the virus particle. Therefore an alternative strategy was devised to

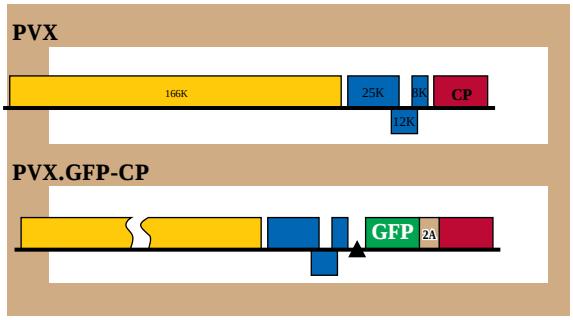

Figure 1 Schematic representation of potato virus X and PVX-based vector that produces a green fluorescent protein-coat protein fusion.

produce a mixed pool of free and fused coat protein. A fusion was made to the amino-terminus of PVX coat protein through a short foot-and-mouth disease virus peptide known as the "2A protease". The 2A peptide mediates a co-translational processing event that results in disruption of peptide bond formation. A sequence encoding sixteen amino acids from the 2A peptide was fused between the 3' end of a gene encoding the green fluorescent protein (GFP) from a jellyfish and the PVX coat protein gene (Fig. 1), in a full-length cDNA clone of PVX from which infectious RNA could be produced. Translation of this open reading frame in plants was expected to result in the production of a GFP-2A fusion protein, the PVX coat protein, and a small amount of unprocessed GFP-2A-coat protein fusion. The GFP was chosen as a reporter gene because its intrinsic green fluorescence can be seen readily under ultraviolet illumination.

Inoculation of plants with RNA produced from the modified PVX cDNA construct resulted in virus infections, as judged by the development of disease symptoms on systemically infected leaves. The first evidence of virus infection was the appearance of green fluorescent lesions or spots when the inoculated plants were viewed under ultraviolet illumination, indicating that the virus produced GFP. Fluorescence was later visible in systemically infected leaves that showed disease symptoms. Development of systemic disease symptoms on plants inoculated with the modified form of PVX, was retarded with respect to wild-type PVX indicating an impairment in some viral process.

The intracellular distribution of fluorescence, observed under the confocal laser scanning microscope, differed from that observed in tissues infected with a PVX vector which produced free GFP (SCRI Annual Report 1994, p135). Uniform fluorescence

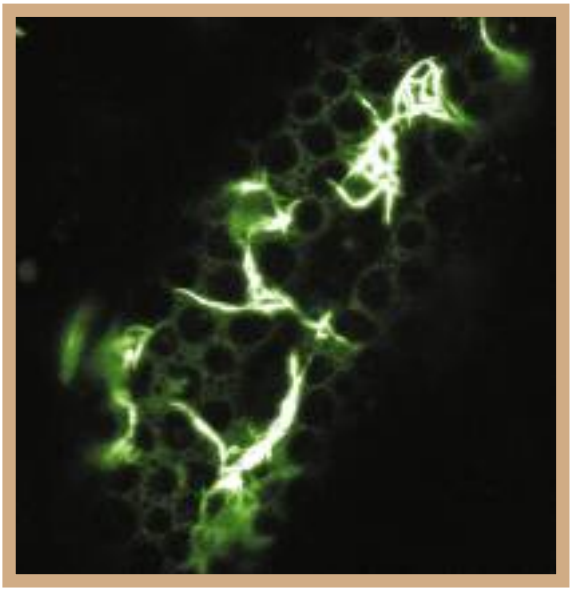

Figure 2 Fluorescent viroplasm in cell infected by the PVX-based vector of figure 1 viewed under a confocal laser scanning microscope.

was observed throughout the cytoplasm of cells infected with the PVX vector which produced free GFP. In contrast, cells infected with the PVX coat protein fusion vector showed that most of the fluorescence was associated with filamentous structures in the cytoplasm that were presumed to be viroplasms, paracrystalline arrays of virus particles (Fig. 2). That the structures were viroplasms, consisting of virus particles with attached GFP, as opposed merely to amorphous aggregates of GFP-containing protein, was confirmed by electron microscopic analysis of immunogold labelled ultrathin leaf sections. Under the electron microscope, the structures were seen to consist of virus particles. When the sections were probed with GFP- or PVX coat protein-specific antisera, most of the immunospecific gold label was associated with these structures. Furthermore, when negatively stained particles from leaf tissue infected with the modified virus were viewed in the electron microscope, they were seen to have a different morphology to the filamentous particles of wild-type PVX. Particles of the modified virus had a diameter twice that of normal PVX particles and globular extensions were visible along the length of the particles, presumably because the surface of the particles was decorated with GFP.

To test whether processing of the fusion protein was occurring at the 2A sequence, and if free and fused coat protein were produced, total protein extracts were prepared from inoculated leaves. Probing of blots of

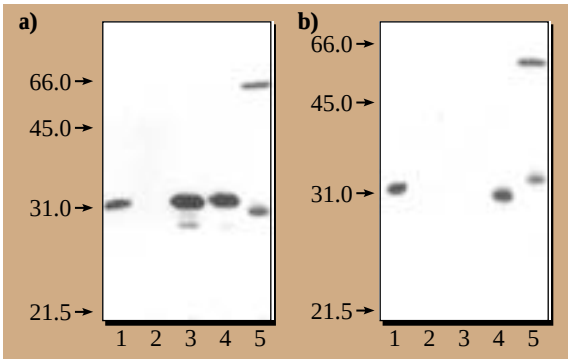

Figure 3 Blots of plant protein extracts probed with antisera specific for PVX coat protein (a) or green fluorescent protein (b). Lane 1, purified PVX coat protein (a) or GFP (b). Lane 2, mock infected plant. Lane 3, plant infected with PVX. Lane 4, plant infected with previously described PVX-based vector that produces free coat protein and free GFP. Lane 5, plant infected with PVX-based vector that produces a GFP-coat protein fusion.

these protein extracts showed that free coat protein and free GFP accumulated in infected tissue (Fig. 3). However the majority of the immunoreactive protein present was in the form of the fusion protein. This result was unexpected, as previous *in vitro* experiments had shown that the sixteen amino acid sequence of 2A used in the modified form of PVX should have given a higher level of processing.

Confirmation that protein processing at the 2A sequence and production of free coat protein was necessary for infectivity of the modified PVX and for virus particle formation, was obtained by targeted mutation of the 2A sequence. Amino acid substitutions, known to disrupt the *in vitro* processing activity of 2A, were introduced into the PVX construct. Inoculation of plants with RNA from the mutated construct did not result in PVX infection. However, when RNA was inoculated onto transgenic plants which expressed low levels of wild-type PVX coat protein, the plants became infected and virus particles were produced. The ability of low levels of transgenically-expressed PVX coat protein to complement the defective virus offers an additional means of biological containment for the modified virus.

The discovery that a protein as large as GFP (27kDa), with a higher molecular weight than PVX coat protein itself (25kDa), can be fused to the surface of PVX virions, represents a very significant advance in plant viral vector technology. Previously, the largest, assembly-competent coat protein-fusion made contained thirty eight amino acids attached to a surface loop of the smaller coat protein subunit of cowpea mosaic virus (CPMV)[3]. Furthermore, whereas only sixty equivalent fusion sites are possible on CPMV particles, the PVX particle contains in excess of twelve hundred coat protein subunits to which fusions could be made. This new "Overcoat Protein" technology should therefore permit the production of valuable therapeutic or industrial proteins, rather than just peptides, fused to readily purified virus particles. The technology should also extend the capacity and range of plant virus vectors for vaccine production. Previously, because only small peptides could be attached to viruses, only short continuous antigenic epitopes could be expressed. Now that larger protein sequences can be expressed, it should be possible to produce discontinuous epitopes in which the antigenic determinant is formed from more than one short peptide region. In addition, it is now feasible to produce multiple epitopes, so that both B- and T-cell epitopes can be produced on the same PVX coat protein subunit to elicit a better immune response.

Current research is aimed at demonstrating that this system can be used to make proteins of high commercial value and to produce more effective vaccines. As a first step to show the general applicability of this approach for protein production, other reporter protein genes with a range of sizes have been fused to the PVX coat protein gene *via* the 2A sequence. Initial steps to develop the PVX-based system for vaccine production have focused on the fusion of small antigenic epitopes to the PVX coat protein. The ability of these chimeras to elicit immune responses and protect animals is being examined by our partners elsewhere. Further work is being carried out to optimize the 2A processing. *In vitro* studies have shown that 2A sequence activity can be modulated by varying the number of amino acids of the 2A sequence used and by the introduction of amino acid substitutions. Increasing the level of processing may permit particle assembly with fusions to larger proteins, while its reduction may be of use in maximizing the yield of smaller proteins. The prospects for commercialization of this system has so far attracted funding from the BBSRC under the "Wealth Creating Products of Plants Initiative" and from Axis Genetics Ltd a private company involved in vaccine development.

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Zoology

David L. Trudgill

Studies on crop pests often involve other scientific disciplines and, because we are a multi-disciplinary institute, we are particularly well placed to be involved in such interactions. Work on nematode transmitted viruses has been one area where our interactions with our virological colleagues have been strengthened, especially with basic studies on the virus genome in relation to the specificity of transmission. We are a lead centre for studies on potato cyst nematodes where we have the advantage of being associated with a programme breeding for resistance. Soft fruit breeding is another important area of interaction, particularly involving the entomologists. Both areas provide opportunities for developing and assessing the utility of both natural and transgenic resistance. Most nematodes and insects are not crop pests but are beneficial and nematodes are proving to be valuable indicators of soil conditions in interactions with colleagues studying vegetation dynamics and soil processes.

Aphids are important and highly successful crop pests because of their ability to colonise and rapidly reproduce on available host plants over wide areas. Nematodes are similarly remarkable plant pathogens, which induce profound changes in their hosts. Consequently, we have a strong programme of basic research which includes molecular studies in relation to aphids and nematodes and to their virulence characteristics. Other studies include work on nematode surfaces and secretions in relation to the changes they induce in resistant and susceptible hosts, and new studies on nematode developmental biology and the development of transgenic resistance.

We have made considerable progress in the development of molecular techniques to distinguish aphid genotypes. One application, reported in the Annual Report for 1994 (pp 153-155), has been to demonstrate that biotypes of the large raspberry aphid, *Amphorophora idaei*, which differ in their ability to overcome resistance genes in their raspbery host plants, each consist of several distinct genotypes. We recently found that variations in the size of the intergenic spacer regions (IGS) between ribosomal genes can also be used to distinguish genotypes of the peach-potato aphid, *Myzus persicae*. The large field infestations in 1995 enabled us to examine *M. persicae* from several potato and brassica crops to seek answers to the population structure of this parthenogenetic aphid species. DNA digests of some genotypes identified among samples from a potato crop showed identical banding patterns to those of aphids collected from oilseed rape 1 km distant and upwind of the potato crop during the main migration period. This is the first direct evidence for the colonisation of potato crops by short-range flights of aphids from local sources.

Our previous studies on the population structure of the large raspberry aphid, revealed by molecular analysis of aphid heterogeneity, led to the conclusion that new virulent genotypes would soon be found on resistant raspberries. In surveys of UK raspberry plantations made in 1995 in collaboration with ADAS, we detected a new resistance-breaking biotype, capable of colonising raspberry varieties containing the A_{10} resistance gene. This new biotype now poses a threat to aphid control based on currently available sources of genetic resistance and raspberry germplasm requires screening for new aphid resistance genes.

In ongoing collaboration with scientists at Wödenswil, Switzerland; Uppsala, Sweden; and RBG Kew, further progress has been made in elucidating the chemical ecology of host recognition behaviour by root flies. By combining behavioural and electrophysiological studies on both *Delia* species, we have now identified primary and secondary oviposition stimuli on the leaf surface on Brassicas. Adult flies landing on resistant plants consistently spend more time on the leaf searching for these oviposition stimuli, before rejecting the plant. This supports our hypothesis that resistance to root flies can be due to or the lack of a suitable positive chemical stimulus on the leaf surface. Bioassays indicate that some resistant Brassica plants also contain oviposition deterrents, so a balance between positive and negative signals on the leaf surface is likely to determine host recognition

The impact of aphids on potato crops was particularly evident during the hot, dry summer of 1995, with substantial spread of the aphid-transmitted potato virus Y (PVY) and yield losses in some crops. In collaboration with virologists and potato processors we studied crops grown from virus-free seed and found that PVY became endemic in one season (over 90% infection of tubers at harvest) in some areas of the UK. Work is progressing to identify the virus sources and aphids responsible for these high rates of spread. In addition, in a non-irrigated field experiment at SCRI in 1995, large populations of potato aphids (*Macrosiphum euphorbiae*) on cv. Desiree induced severe symptoms of top-roll (false leafroll) and decreased yields by up to 60%.

SCRI entomologists have collaborated with fruit advisors (ADAS, SAC) to produce technical notes to assist soft fruit growers in identifying pests and their damage symptoms. Data on raspberry beetle flight, monitored with white sticky traps, has provided valuable information on direction of movement into and through raspberry plantations over the growing sea-

son. The traps have shown that adult beetles are active for longer periods than had been considered previously. A "flowering bridge" over the growing season could explain how autumn-fruiting varieties become infested. Further work to optimise the siting of white sticky traps to monitor raspberry beetle and raspberry moth is now progressing with the collaboration of several commercial growers in Scotland. The aim is to develop forecasts of damage and optimum dates for controls, based on threshold numbers of insects in the traps.

External funding continues to be of increasing importance and both scientific advances and changing farming patterns create new problems and opportunities. These include the increasing use of nematicides to protect seedling carrots and parsnips from feeding by ectoparasitic nematodes which is a major cause of "fanging". Together with our soil physicists, we have received a SOAEFD grant over 3 years to investigate the processes involved in the movement of the chemicals which attract nematodes to roots, the movement of the nematodes in response to the attractants, and the movement of nematicides applied to protect the crop. Manipulation of the soil to decrease nematode movement and damage is the ultimate goal.

Our molecular studies on nematode heterogeneity have led to our involvement in an EC funded project to develop rapid and sensitive diagnostics for detecting *Meloidogyne chitwoodi*. This is a newly discovered pest threatening potato production, especially in the Netherlands and neighbouring countries. Previous studies on the use of lectins (proteins that bind to sugars e.g as used in blood typing) to control nematodes, for which we have a patent, have resulted in our sharing in a SOAEFD funded initiative to examine their effectiveness in transgenic plants and their non-target effects. Work on novel, natural nematicides, which led to the patenting of DMDP, continued with external funding and further funding is being discussed.

We continue to assess the resistance to potato cyst nematodes of the progeny of our own, and two external potato breeding programmes, screening several thousand plants each year. Our continuing EC funded projects on heterogeneity in relation to the introductions of, and virulence differences in potato cyst nematodes (*Globodera* spp.) and on the integrated control of root-knot nematodes (*Meloidogyne* spp.), using the bacterial parasite *Pasteuria penetrans*, are both about to enter their final year. Both projects are particularly successful and more than achieving their objectives. Considerable variation in *G. pallida* has been identified, with a Scottish population probably representing a distinct, highly virulent introduction. A survey of the virulence characteristics of *G. pallida*, funded by the Potato Marketing Board, emphasised the wide range in virulence of populations in the UK in relation to resistance derived from *Solanum vernei*, and confirmed, as suggested by nematologists several years ago, that resistance from *S. tuberosum* ssp. *andigena* CPC 2802 is equally effective and would be easier to exploit in a breeding programme. The project on *P. penetrans* has demonstrated that the parasite is much more widespread than previously recognised and has shown that it is possible to produce soils highly suppressive to *Meloidogyne* spp. by repeated cropping with susceptible crops. Progress has also been made in understanding the specificity of the relationship between populations of *P. penetrans* and of *Meloidogyne* and of the role of soil factors in the binding of spores to the surface of nematode juveniles.

An EC funded Concerted Action project of which we are part and which involves many research centres throughout Europe is also nearing completion. This project has been exploring the changes in plant gene expression associated with the development of the multinucleate cells induced at the feeding sites of cyst, root-knot and certain virus vector nematodes. These studies, in which our role was to investigate the virus vector nematodes, identified many changes in gene expression at nematode feeding sites and led to the isolation of various "nematode inducible" promoters. These are now being used at several centres to manipulate the expression of genes, such as those coding for lectins to develop transgenic resistance to nematodes.

The profound changes to plant cells at nematode feeding sites are thought to be induced by the injection of secretions from certain nematode gland cells and we have been seeking ways of isolating and characterising these secretions. Various stimuli, including certain plant hormones, have been identified which induce secretion *in vivo*. Analysis by GC-MS of these secretions indicates a range of novel compounds but their characterisation is likely to be long and difficult because of the problems of obtaining sufficient material for detailed analysis. Antibodies have been prepared to some of these secretions and used to screen a *Globodera pallida* expression library in order to isolate and characterise the genes coding for secreted proteins. A gene has been isolated and partly characterised using this approach and evidence has been obtained that it produces two products of different size.

Root-knot nematodes are the most important nematode pests world-wide and comprise two main groups.

The species in the larger group reproduce sexually, have long life cycles and are host specific. Those in the smaller group reproduce asexually, have short life cycles and wide host ranges and it is on these we have concentrated. The molecular information indicates that these root-knot nematodes recently have been widely spread around the world and that they have brought with them the capacity to parasitise much of the natural vegetation (which would not have had the opportunity to develop specific resistance) and many crop plants. In experiments with four populations of *M. incognita*, the most important species in the asexual group, all except one of 19 British wild plants were susceptible, confirming the hypothesis that these nematodes have the potential to parasitise the majority of the flowering plants from around the World. Our discovery during the EC project on *P. penetrans* that *M. mayaguensis*, a species even more virulent than *M. incognita*, is widespread in west Africa and that it also occurs in the West Indies, is a cause for alarm.

The effect of growing potato cultivars with resistance to the yellow potato cyst nematode (PCN, *G. rostochiensis)* on the increased incidence of the white species *(G. pallida*) was predicted and is now a reality for many farmers. The white species (*G. pallida*), for which we lack strong resistance, is now reaching epidemic proportions; recent data indicates that more than 50% of the ware land in England is infested with *G. pallida* with 30% justifying nematicide treatment to prevent damage. Infestations of *G. pallida* are also increasing in Scotland, posing a creeping threat to seed land and giving added urgency to the development of resistant cultivars. We have put considerable effort, supported by the Potato Marketing Board, into modelling the population dynamics and control of *G. pallida* and have shown that we are indeed involved in a slow epidemic in which it takes c. 20 years for *G. pallida* to replace *G. rostochiensis*. Partially resistant cultivars will not prevent populations of *G. pallida* from increasing and we have been emphasising that it is a nematode which is much more difficult to control. Integration of control measures is essential to prevent damaging populations from developing and it is much more difficult to decrease large, damaging populations than it is to prevent small ones from increasing.

Molecular biology of the control of potato cyst nematode development

J.T. Jones

Background Novel methods for controlling the white species of potato cyst nematode (*Globodera pallida*, PCN), particularly the development of plants with artificially engineered resistance, are a priority because of the environmental toxicity of many nematicides and the lack of natural major gene resistance against some pathotypes of this nematode. Currently, approximately 25,000 ha of potatoes in the UK are treated each year with nematicides and only two cultivars, each of which has only partial resistance, are available to help control *G. pallida*. The development of plants with transgenic resistance has been a major area of interest and, as part of these studies, changes induced by the nematode at its feeding site have been investigated.

After the juvenile nematodes invade the root they settle to feed and the root cells around their heads become modified to form an enlarged, multinucleate feeding site termed a syncitium. Syncitium formation is thought to be induced by nematode secretions injected into the cells at the feeding site. Recent studies have shown that establishment of the syncitium by the nematode involves massive changes in the expression patterns of a range of plant genes. The promoters controlling the expression of some of these host genes have been isolated and are now being used in transgenic plants as tools to deliver anti-nematode proteins, including lectins and protease inhibitors, directly to the feeding nematode.

Despite this progress, there is a need for a greater range of anti-nematode gene products to be used in transgenic plants. Studies on nematode processes, which might form the basis of such novel control strategies, have not progressed as rapidly as those on the changes in plant processes involved in feeding site formation. Details of the mechanisms by which plant

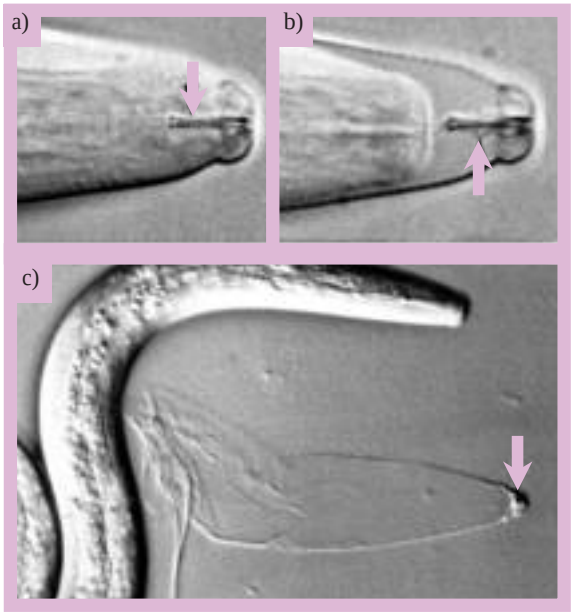

Figure 1 Moulting in a plant parasitic nematode *(Aphelenchoides hamatus)*. a) Nematode before the onset of moulting. b) Nematode retracts from the cuticle of the head end. c) Succesful moulting! The nematode escapes from the remains of the old cuticle (arrow). Photographs by Dr R.N. Perry, IACR Rothamsted.

parasitic nematodes induce feeding sites in their hosts remain unknown and despite the wealth of information available on the free living nematode *Caenorhabditis elegans*, surprisingly little is known about many important nematode developmental processes. Only when a thorough understanding of the function of a parasite is gained will it be possible to accurately target attempts to disrupt its life cycle. We are currently examining the molecular biology of PCN at several stages of its life cycle including hatching, feeding site induction and the control of moulting and development. This article describes our studies on the control of moulting in PCN.

Nematode moulting Once the nematode has established a syncitium in the roots of its host plant it goes through three moults before reaching the sexually mature adult stage. The moulting process in nematodes is superficially similar to that in insects, in that an old cuticle is broken down, partly reabsorbed, shed and a new one is laid down (Fig. 1). In contrast to the situation in insects however, nematodes continue to grow between moults, suggesting new cuticle is continually synthesised. Although the mechanisms involved in the control of insect moulting have been intensively studied, almost nothing is known about the mechanisms which control nematode moulting. We are currently investigating nematode moulting and cuticle growth and formation, with a view to identifying means of disrupting these vitally important parts of the nematode life cycle using two approaches. In one approach we are seeking to isolate genes likely to be important in the nematode moulting process and to examine the control of their expression. In the second approach we are investigating whether mechanisms similar to those which control moulting in insects operate in nematodes.

Control of expression of genes important in moulting - collagens The most obvious feature of moulting is the removal of an existing cuticle and its replacement with a new one. About 80% of the cuticular protein is made up of various collagens, the combination of collagens varying between nematode stages to allow flexibility in the nature of the nematode surface. Isolation of genes coding for various collagens and subsequent analysis of the regulation of their expression is one way of identifying control mechanisms important in moulting and development. Using an antibody to screen a mixed stage *G. pallida* expression library, we have isolated and sequenced a full length collagen gene from *G. pallida* (Fig. 2). Northern blots suggest this gene is expressed specifically in adult, gravid females as compared to second stage juveniles and virgin females (Fig. 3). Given the conserved nature of collagen genes it will now be possible to use this gene to isolate further collagens from *G. pallida* for comparative purposes. Comparison of the expression patterns of the various collagens will

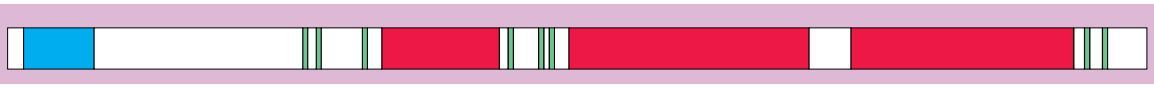

Figure 2 Diagram showing important sequence features of collagen gene cloned from *G. pallida*. At the N-terminal end a stretch of hydrophobic amino acid residues is present, thought to function as a signal sequence for extracellular transport (blue). Three Gly-X-Y repeat regions (where Gly is glycine and X and Y are frequently proline or hyroxyproline), which are common to all collagens and are thought to give the molecule its triple helical structure, are located in the central part of the molecule (red). Several highly conserved cyteine residues (positions shown in green) are also observed: the number and position of these can be used to classify each collagen into a particular sub-group of genes and are thought to be important in interactions between different collagen molecules.

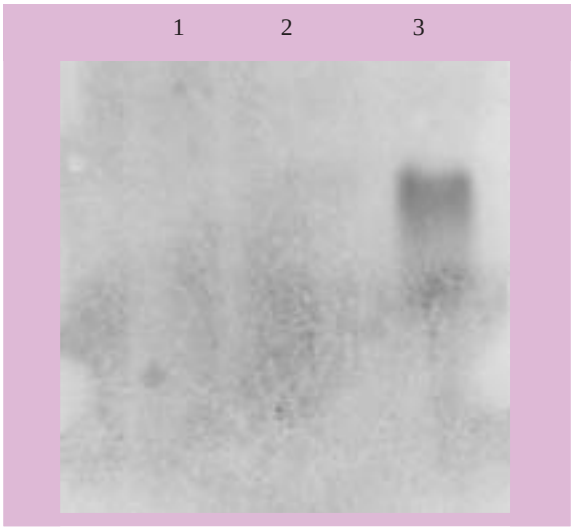

Figure 3 Northern blot using cloned collagen gene as a probe. The collagen gene is expressed specifically in gravid adult females (lane 3) - no signal is detected in mRNA from second stage juveniles (lane 1) or virgin females (lane 2).

increase our knowledge of the functional role of these molecules. Comparison of the sequences of the regulatory regions and of expression patterns of the genes isolated will also give insight into the mechanisms used by the nematode to control its moulting and development. Enzymes which attack nematode collagens (collagenases) may be used as anti-nematode genes in transgenic plants. Knowledge of when various collagens are expressed in the nematode and the ability to test any collagenases against specifically chosen, expressed cloned collagens will facilitate this approach.

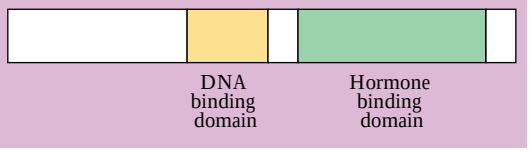

Figure 4 Structure of a steroid receptor molecule. A highly conserved DNA binding domain and a less well conserved ligand binding domain are present in all known steroid receptors.

Do insects and nematodes use similar mechanisms to control moulting and development ? - steroid receptors In insects, moulting and development are controlled largely by steroid hormones, the most important of which are ecdysone and juvenile hormone. Ecdysone stimulates the physiological and behavioural processes responsible for moulting, while juvenile hormones control the timing of the decision to moult from juvenile to sexually mature adult. Given the apparent similarities between insect and nematode moulting it has long been tempting for nematologists to suggest that similar control mechanisms operate in insects and nematodes. Ecdysone has been identified in animal parasitic nematodes, and exogenously applied ecdysone has been shown to affect some developmental processes in a range of animal parasitic nematodes. However, conclusive evidence that nematode moulting is controlled by steroid hormones in the same way as insect moulting is lacking.

In insects, ecdysone exerts its effects through its interaction with the ecdysone receptor (EcR). The EcR is a member of a large superfamily of ligand responsive

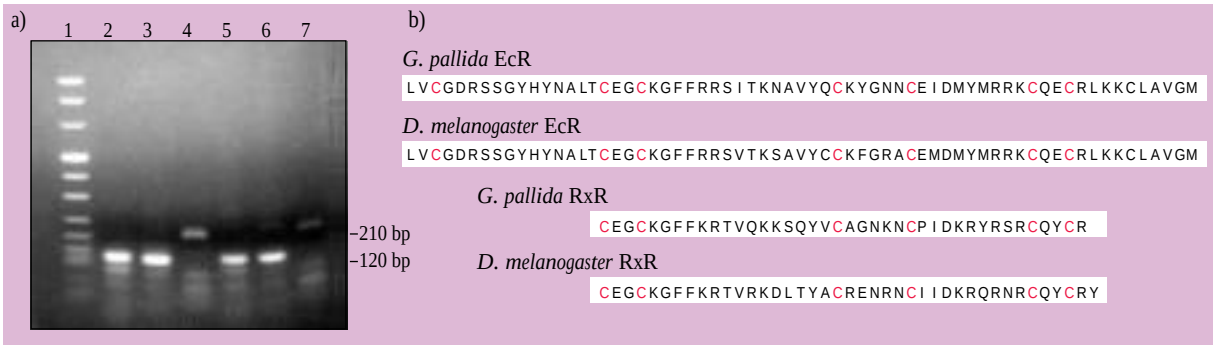

Figure 5 Steroid receptor fragments amplified from cDNA of the potato cyst nematode *Globodera pallida*. a) PCR products obtained from a reaction using primers designed to recognise the DNA binding domain of various steroid receptors. Fragments of the sizes expected - approximately 120bp for RXR homologs and 210bp for EcR homologs - were obtained and were cloned and sequenced.(Lane 1: low molecular weight marker, Lanes 2-4: cDNA from second stage juvenile mRNA, Lanes 5-7: cDNA from adult female mRNA). b) Deduced amino acid sequences of two steroid receptor fragments amplified from *G. pallida* cDNA and published sequence of the *Drosophila melanogaster* EcR and RXR DNA binding domains. The fragments obtained from PCN show extensive homology to the DNA binding domains of the *D. melanogaster* molecules. Several conserved cysteine residues (shown in red) thought to be involved in zinc finger formation, which allows the protein to bind to DNA, are present in the appropriate positions.

transcription factors: the nuclear hormone receptors. These receptors are characterised by a set of conserved structural features including a highly conserved DNA binding domain, a fairly well conserved hormone binding domain and a large hypervariable region (Fig. 4). These receptors directly affect gene expression, binding as homo- or heterodimeric complexes. In the intensively studied insect *Drosophila melanogaster*, the EcR affects gene expression as a heterodimer with another steroid receptor; the gene product of the *ultraspiracle* locus which has been identified as a homolog of the vertebrate retinoid X receptor (RXR). The genes coding for the EcR and RXR have been cloned and sequenced from a number of insects; we have used primers designed against the region coding for the conserved DNA binding domain of these molecules to search for genes coding for homologues of these molecules in PCN. We have successfully amplified and cloned a number of gene fragments from PCN whose sequence suggests they code for molecules similar to insect RXRs (Fig. 5). These fragments are now being used as probes to identify other members of this superfamily of receptors in nematodes. Future studies on the timing and localisation of their expression will be used to determine their role in the control of nematode moulting. Other work to examine the nature of the genes whose expression is controlled by these genes will also be made possible by this approach. Although identification of these molecules does not prove that insect and nematode moulting are controlled by the same mechanisms, it does suggest that similar molecules are available to the nematode. The functional role of these molecules remains to be assessed.

Responses to flower volatiles by the raspberry beetle, *Byturus tomentosus* and field evaluation of white traps for monitoring flight

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Introduction The raspberry beetle, *Byturus tomentosus*, is a major pest of cultivated and wild *Rubus* species (raspberries and blackberries, and hybrid berries) in Europe. A closely related species, *B. unicolor*, (raspberry fruitworm) causes similar damage in N. America. *B. tomentosus* larvae normally inflict more damage than adults, by tunnelling into the developing fruit, allowing entry of fungal pathogens (e.g. *Botrytis*) or directly contaminating harvested fruit. Adult beetles can also damage unopened buds, unfolding leaves of first year canes, and opened flowers during feeding. Adults usually start to emerge from the soil in late April to mid-May, and in warm weather migrate to flowers of other Rosaceous plants, including hawthorn. They are then attracted back to early flowering raspberry cultivars where they feed, mate and females lay eggs, mainly on the stamens of raspberry flowers. It is thought that raspberry beetles follow long-distance volatile cues (e.g. flower odours) and also use visual cues (e.g. flower colour) to find suitable host plants. Larvae emerge from eggs after a few days and start to feed on the basal drupelets, before tunnelling into the fruit's central plug.

Limitations of current pest control method At present insecticides are routinely applied to the ripening fruit ("green/pink" stage) close to harvest, targeted against eggs and recently emerged larvae, before feeding larvae have caused fruit damage. The most commonly used products are short-persistence organophosphorus insecticides such as fenitrothion. Although there is no evidence that maximum residue limits (MRLs) have been exceeded for these insecticides on raspberries, there is growing public concern about applying organophosphates, particularly close to fruit harvest. The threat of future removal of these insecticides from approved product lists means that it is highly desirable for the soft fruit industry to develop pest control methods which reduce the use of pesticides.

New, environmentally-sensitive approaches at SCRI As part of a general strategy at SCRI to develop more environmentally-benign control methods for soft fruit, we are investigating the fundamental biology and behaviour of raspberry beetles and other important soft fruit pests. Our main emphasis is on the role

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Figure 1 White sticky traps are hung at 0.6m and 1.2m from the ground in raspberry plantations. The design of the traps (two interlocking sticky surfaces at right angles) provides information on the direction and timing of raspberry beetle flights.

of host plant volatiles in attraction to and recognition of host plants by insects, linked to targeted breeding for pest resistance. Sources of resistance to raspberry beetle in wild *Rubus* species, including *R. coreanus*, *R. crataegifolius*, *R. occidentalis* and *R. phoenicolaisus*, have been reported in the past and have been used in breeding programmes, but little is known about the resistance mechanisms involved. Our approach should enable us to develop selectable markers (chemical and/or molecular) for pest resistance traits, and also to identify plant-derived attractants, repellents and deterrents which complement host plant resistance in sustainable Integrated Crop Management programmes.

White sticky traps to monitor raspberry beetle activity in plantations Ongoing field trials and scanning electron microscope studies indicate that visual and contact stimuli are important behavioural cues for raspberry beetles. In applied studies white, non-U.V. reflective traps, developed in Switzerland, have been tested in raspberry plantations in eastern Scotland (Fig. 1). The aim is to determine when raspberry beetles are active in plantations, so that insecticides can be more carefully timed and spraying thresholds developed. Results show that adult beetles tend to fly at low levels in Scotland, with considerably more adults caught at 0.6m above ground level than at 1.2m. The traps at SCRI also showed that many beetles were flying in a south-westerly direction (i.e. against the prevailing wind) when caught. Further trials at nine commercial plantations in Scotland have shown that numbers and times of peak beetle activity are very variable, so localised monitoring should be advantageous for timing insecticide sprays more effectively.

Screening tests on beetle-resistant *Rubus* species *R. phoenicolasius* (Japanese wineberry; Fig. 2) was selected as a known beetle-resistant, wild *Rubus* species for behavioural and chemical studies. In previous sleeve inoculation tests under field conditions, raspberry beetles laid very few eggs on flowers of *R. phoenicolasius* and the fruit were virtually free from

Figure 2 The Japanese wineberry (*Rubus phoenicolasius*) is resistant to raspberry beetles. Odours emitted from the flowers and glandular hairs on sepals repel beetles when they are searching for egg laying sites.

larval infestation, indicating antixenosis (resulting in non-preference during egg laying) as an important component of resistance. In confirmatory olfactometer tests, giving female beetles the choice of flower volatiles from resistant *R. phoenicolasius* and the susceptible red raspberry cv Glen Prosen, beetles showed a strong preference for red raspberry flower volatiles. *R. phoenicolasius* is covered in glandular hairs which emit a distinctive aromatic odour. These volatiles appear to repel or confuse raspberry beetles during the early stages of host recognition involving flower volatile cues. In more recent "sleeve" inoculation tests at SCRI, resistance was also noted in a purple raspberry breeding line, indicating that the source of resistance may be from a *R. occidentalis* (black raspberry) parent. The *R. occidentalis* cv. Munger has previously been reported to be highly resistant to raspberry beetle. The mechanism of this resistance in purple raspberry selections to raspberry beetle is currently being investigated.

Testing for volatile attractant and repellent odours from host and non-host flowers A linear track olfactometer is used to quantify behavioural responses of male and female raspberry beetles to flower odours and to identified volatile components[1,2] . These tests show that flower volatiles from raspberry or from other Rosaceous hosts are highly attractive in a choice test against a control (moist air with no flower volatiles). In a similar choice test between volatiles from a non-host (oilseed rape flower) and the moist air control, no clear preference was observed. Further choice tests between flower volatiles from the host (raspberry) and a non-host (oilseed rape) again showed a clear preference for the raspberry flower volatiles. Interestingly, raspberry beetles that emerged early in the 1991 season, before raspberries had flowered in the field, showed a distinct preference for hawthorn flower volatiles (an early season "temporary host" for adult aggregation and feeding) over raspberry volatiles (normal host for mating, oviposition and larval feeding). However, this preference for hawthorn flower volatiles was not seen in later tests over three subsequent years. These and other studies suggest that the phenological stage of the host (raspberry) flower alters the chemical composition of volatiles released, and hence the relative attractiveness of raspberry flowers to raspberry beetles during host finding.

Flower volatile chemistry Flower volatiles from four raspberry cultivars and from wild hawthorn were trapped on the porous polymers Haysep Q and Tenax TA. These volatile flower odours were analysed on an automated thermal desorption (ATD)-gas chromatography (GC)-mass spectrometry (MS) system at SCRI and by GC-MS analyses of ether extracts in a collaborative study with IACR, Rothamsted. The major classes of compounds (> 100 detected in each entrainment) included aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols and esters, monoterpenes and sesquiterpenes and a number of unusual nitrogen compounds. The volatile profiles of the four raspberry cultivars at full flowering were complex but exhibited only minor differences between them. Volatiles of hawthorn flowers, although similar to raspberry, contained elevated levels of several compounds not found in raspberry cultivars. The volatile profile of the resistant wild species *R. phoenicolasius* contained elevated levels of terpenoid and other compounds, in addition to the typical volatile profile of red raspberry The chemical identities of possible repellent compounds in this and other wild *Rubus* species are currently being investigated at SCRI using ATD-GC (Automated Thermal desorption-Gas Chromatography) linked to an Electroantennogram (EAG) system. This records electrical signals from insects' antennae, generated by exposure to stimulatory volatiles, separated on the GC.

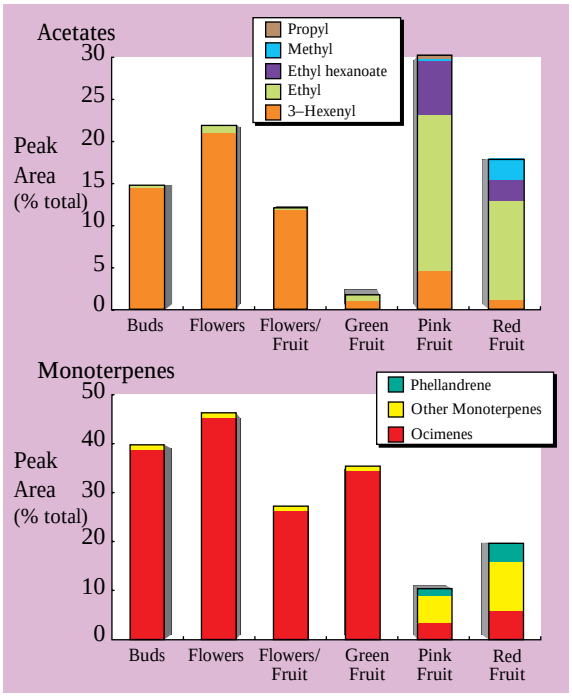

Figure 3 Several groups of plant volatiles are emitted from raspberries change during flower and fruit development (examples shown are acetates and monoterpenes). Raspberry beetles respond differently to these changing chemical signals when selecting suitable sites for egg laying.

Field observations from a joint AFRC (now BBSRC) Link programme with St Andrews University and confirmatory olfactometer tests at SCRI showed that the female's preference for floral oviposition sites is strongly influenced by the phenological stage of the raspberry flowers[3]. In a second series of entrainment experiments, we investigated changes in volatile profiles during flower development of raspberry cv. Glen Prosen. ATD-GC-MS analyses of volatiles from green buds, flowers, green fruit, pink fruit and ripe red fruit showed major changes in the composition of the odour profile. As the flowers matured, levels of "green leaf" volatiles declined whilst several monoterpenes increased (Fig. 3). During fruit ripening several additional compounds appeared, which are highly characteristic of raspberries, followed by the production of higher levels of several types of acetates[4]. Further experiments are now in progress to identify the key, behaviourally-active compounds which convey information on the development state of the flower and fruit to raspberry beetles.

EAG studies to target chemical identification of flower volatiles perceived by raspberry beetle Our studies have revealed the complexity and dynamic nature of volatile profiles emitted from host and non-host flowers. In most GC-MS analyses >100 compounds are detected, many of which are minor components more characteristic of the host than some of the major components (e.g. more ubiquitous "green leaf" volatiles). A further complexity in obtaining "biologically relevant" chemical data for plant-insect studies is that each volatile extraction or entrainment method and GC-MS system used, will produce different sets of chemical data, both in terms of relative proportions of components detected and in the classes of compounds trapped. Because of this we have adopted a strategy of using EAG to identify compounds which the insect's antennae can detect. This then provides a "short-list" of candidate volatiles which must be characterised in terms of their behavioural effects (e.g. attraction, repulsion) over a range of naturally-occurring concentrations.

In collaborative studies at SCRI and IACR Rothamsted, components of raspberry and hawthorn flower extracts separated by GC, were passed over a raspberry beetle antenna. Initially, electrophysiological recordings were made from the whole antenna (EAG; Fig. 4) and later from individual olfactory receptors (single cell recordings) of raspberry beetles at IACR. Several volatile GC peaks which showed electrophysiological activity were identified by GC-MS[5] and are currently undergoing further EAG and behaviour bioassays. Recent results using olfactometers, a wind tunnel and EAG dose-response studies, indicate that both qualitative (number of chemical components in the odour blend) and quantitative (ratios of volatile components) characteristics of the flower profile are important to elicit a normal behavioural response (equivalent to raspberry flowers) by raspberry beetles. Raspberry beetles also appear to obtain extra information on the phenological stage and physiological condition of the developing fruit from characteristic volatile profiles emitted from buds, opened flowers, green and fully ripened fruits. This olfactory informa-

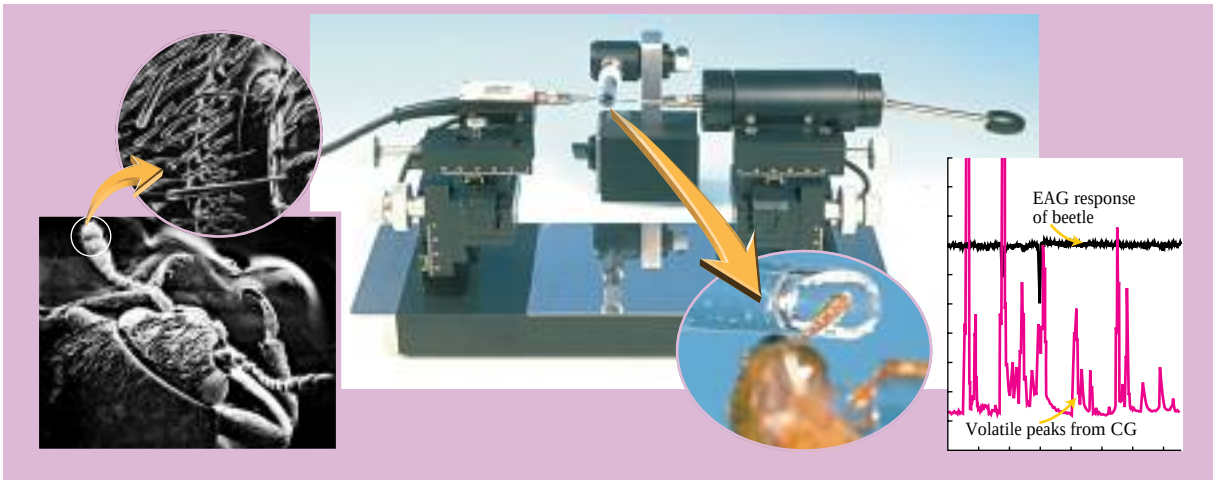

Figure 4 Raspberry beetle's antennae are covered by olfactory receptors which respond to plant volatiles. Electrical responses to volatiles can be recorded from the antenna using an EAG recording system. A typical EAG is shown; the change in DC voltage is measured in millivolts and is proportional to the odour intensity and number of antennal receptors stimulated by the volatile chemical.

tion modifies beetle behaviour in host selection for feeding and egg laying sites.

Conclusions These approaches have led to the successful identification of a number of candidate flower volatiles for evaluation as attractants or repellents. Although this list of EAG-active compounds is not yet complete, it has enabled us to show that both qualitative (the number of chemical components) and quantitative (ratios of components in the odour blend) characteristics appear to be important in order to elicit a strong behavioural response. Similar observations, indicating the importance of the "optimal blend" of volatile components for attracting insects to baited traps, have been reported for other insects. This information is now being used to design improved traps for monitoring raspberry beetle activity in the field and so reduce unnecessary pesticide usage. We will also be able to target plant breeding for resistance to raspberry beetle and other important pests and diseases. These control strategies are currently being combined into an Integrated Crop Management system, which it is hoped will be to the ultimate benefit of soft fruit growers and consumers.

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Effects of plant natural compounds on nematodes

W.M. Robertson, A.N.E. Birch & I.E. Geoghegan

Many existing synthetic nematicides are broad-spectrum compounds and have residues which are highly toxic and persistent. They can contaminate ground water thereby posing an additional threat. The search for environmentally-benign methods for controlling plant parasitic nematodes has led to the exploration of several strategies for replacing commercially available nematicides. As many plant species have evolved pathways for producing defence compounds against a wide variety of pathogens, one possible alternative to synthetic nematicides is to identify and use plant-derived compounds which are effective against nematodes. Although these compounds may also have a broad spectrum of toxicity they are biodegradable and will not persist in the soil for long periods or leave toxic break-down products.

Plant defence compounds can act in subtle ways. Some deter attack by acting as repellents, whereas others inhibit hatching of nematode eggs rather than directly killing nematodes. Such activity would not be detected by the standard test that is used, where invasive stage nematodes are immersed in a series of aqueous solutions of a test compound to determine the lethal dose. Furthermore, compounds that have been found to be toxic in immersion tests have been found subsequently to have no effect when applied to soil.

Members of the *Leguminosae* appear to have particularly well developed chemical defence strategies and we have worked with compounds from several species. Jack bean (*Canavalia ensiformis*) contains at least four

compounds that have nematicidal effects on potato cyst (PCN, *Globodera spp.*) and root-knot nematodes (RKN, *Meloidogyne spp.*). In the seed, concanavalin A (con A) and canavanine occur with the greatest abundance. Con A lectin is a sugar-binding protein and its nematicidal activity is associated with its ability to bind to mannose. Canavanine (L-2-amino-4-4 guanidino-oxybutyric acid) is a non-protein, amino acid analogue of arginine which results in non-functional proteins when it substitutes for arginine. Similarly, the tropical legume, *Lonchocarpus* contains a polyhydroxy alkaloid, 2R, 5R-dihydroxymethyl-(3R,4R)-dihydroxypyrrolidine (DMDP) which is an analogue of fructose, in its seeds and leaves. It acts as a glucosidase inhibitor and is an insect anti-feedant.

A range of bioassays has been developed at SCRI to detect the following range of anti-nematode or nematode-tolerant activities (Fig. 1) Examples of the effects of canavanine and DMDP illustrate some of these anti-nematode activities.

Anti-hatching activity Canavanine mixed with the washings from potato roots which are routinely used to hatch the second stage juveniles (J2) of *G. rostochiensis* from cysts significantly (P<0.001) inhibited PCN hatch *in vitro*. At 100 µg ml^{-1}, numbers of live, hatched J2 were reduced by 65% and the number which died after hatching was increased 3-fold. At 200 µg ml^{-1}, the number of live J2 was reduced by 80% and the number which died after hatching was similarly increased 3-fold, compared with the control (Fig. 2).

Anti-feedant activity The anti-feedant activity of DMDP was estimated by the decrease in root galling caused by J2 of RKN in a pot test.

When applied as a soil drench to tomato seedlings grown in sand, root galling damage by *Meloidogyne javanica* was significantly (P<0.001) reduced (Fig. 3). At 1 and 10 µg ml^{-1} DMDP, galling was reduced by

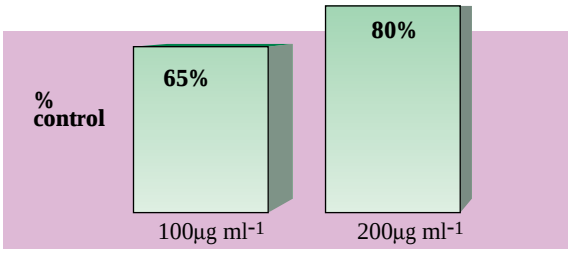

Figure 2 Percentage reduction in numbers of hatched *Globodera rostochiensis* juveniles after treatment with 100 and 200µg ml^{-1} canavanine.

up to 71%. At 100 µg ml^{-1} DMDP, control was marginally less (57%) than for 1 and 10 µg ml^{-1}, indicating that low concentrations were at least as effective as higher concentrations of DMDP. When sprayed on the leaves of tomato seedlings, systemic activity of DMDP was demonstrated with aq. solutions at 1, 10 and 150 µg ml^{-1}. The soil surface of each pot was covered during spraying to prevent contamination by run-off. All treatments significantly (P<0.001) reduced root galling by *M. javanica*, with maximum control (61%) at 1 µg ml^{-1} foliar spray concentration (Fig. 3). Control at the highest concentration was again less effective (40%) than at the lower concentrations.

Tomato plants grown from seeds soaked in aq. solutions of DMDP for 24 h prior to planting were significantly (P<0.001) less galled when inoculated with *M. incognita* at the 2-3 true leaf stage than plants grown from water only soaked seeks.

After seed soaks in 10 and 100 µg ml^{-1} solutions of DMDP galling was decreased by 53-33% whereas at 1 µg ml^{-1} there was only a 25% reduction.

Inhibition of female development In tests with PCN, plants treated with canavanine at 12.5 µg ml^{-1} showed no significant decrease in number of cysts/plant, compared with water controls. However,

1.	**Anti-hatching**	- numbers of juveniles hatched from cysts immersed in test solution.
2.	**Repellent**	- petri dish test with nematodes on agar.
3.	**Nematotoxin (immobilise/kill)**	- counts of nematodes in solutions.
4.	**Anti-feedant**	- counts of nematode galls from plants treated by drench, foliar or seed soak treatment.
5.	**Inhibition of female development**	- counts of cyst formed on host plant roots.
6.	**Anti-virus vectoring**	- virus-vector nematodes treated during acquisition and transmission phases of bioassay.
7.	**Plant growth stimulant**	- roots and foliage of test plants scored for growth promoting or phytotoxic effects.
8.	**Activities complementary to host plant resistance**	- test chemicals applied to partially resistant host plants and assessed for additive or synergistic effects on nematode development.

Figure 1 Bioassays developed at SCRI to detect anti-nematode or nematode-tolerant activities.

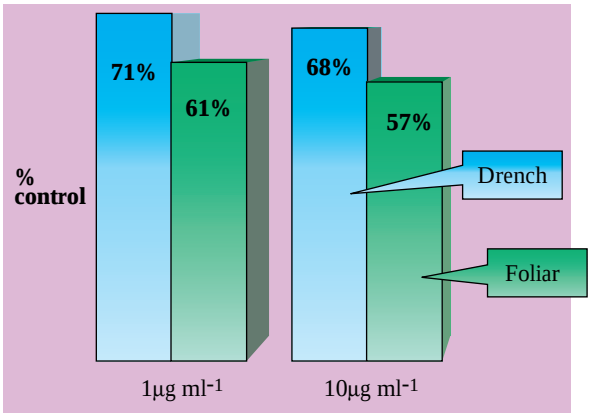

Figure 3 Rates of control of root knot nematode *(Meloidogyne javanica)* using soil drench and foliar applications of DMDP.

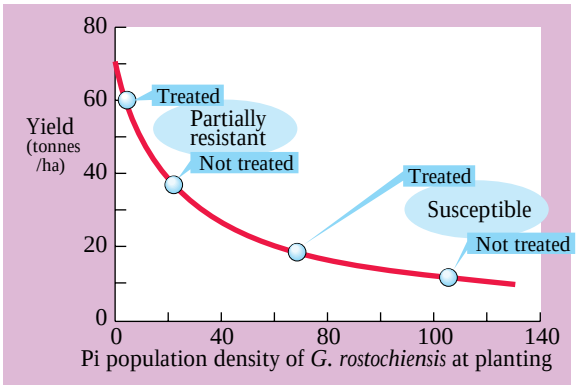

Figure 4 Effect of nematode *(G. rostochiensis)* population on yield from susceptible (Désirée) and partially resistant (Heather) potato cultivars following treatment with 100µg ml^{-1} DMDP.

the mean number of eggs/cyst was reduced by 67%. There was no significant difference in the reduction (43%) of the number of cysts/plant at 25 and 100 µg ml^{-1}. However, there were significantly fewer (50%) eggs/cyst in the 100 µg ml^{-1} compared with the 25 µg ml^{-1} treatment.

Activities complementary to host plant resistance DMDP applied as a soil drench was found to complement the partial resistance of potato cv. Heather to PCN (*G. rostochiensis*) using a cyst inoculum (Fig. 4). When applied at 100 µg ml^{-1} to the susceptible cv. Désirée the mean number of cysts/plant was not reduced but the mean number of eggs/cyst was reduced by 38% ($p<0.05$). When DMDP was applied to partially resistant cv. Heather at 100 µg ml^{-1}, mean number of cysts/plant was decreased by 65%, eggs/cyst by 36% ($p<0.05$) and the resultant eggs/g soil by 81%, compared with the Heather treated with water control. The overall effect of combining partial resistance with DMDP was to reduce the numbers of eggs/g soil by 96%, compared with the Désirée control.

Conclusions We have selected examples of positive activities for plant-derived compounds using both *in vitro* and *in vivo* tests. However, in reality we and other workers have found many plant compounds do not show consistent activities in these two types of screening tests. For example, the fructose analogue, DMDP, was found to have low activity *in vitro* but has a range of interesting activities, including systemic activity, when tested in an environment involving the nematode-plant interaction. This could be because such compounds interfere with host location, recognition, feeding and reproductive activities which are normally only triggered in the nematode by specific host plant compounds (e.g. root diffusates, root surface and cellular components). The unusual lack of dose-related control found in our nematode bioassays by several sugar analogues such as DMDP, suggests that they may trigger or interact with nematode or plant receptors, even at low concentrations.

Our initial research has already identified and led to the patenting of anti-nematode compounds with interesting systemic activity which can be applied at low doses using foliar sprays and seed coating technology. Many more such compounds await discovery but the ecological approach to screening we have outlined is needed to identify their activity.

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A view of genetic diversity in potato cyst nematode in Britain and beyond

V.C. Blok, B.E. Harrower & M.S. Phillips

The yellow and white species of potato cyst nematode (PCN), *Globodera rostochiensis* and *G. pallida*, are persistent and damaging pests of potato which are thought to have been introduced into Europe in the mid-19th century. From the initial site or sites of introduction, they have been dispersed throughout Europe (Fig. 1) to become a widespread and major problem to potato growers. Control of PCN relies on rotations, nematicides (which are toxic and expensive) and resistant cultivars. A number of sources of resistance genes have been found and incorporated into breeding programmes and some potato cultivars. These different sources of resistance, however, have lead to the recognition of a number of groups of populations of PCN, or pathotypes, which show different levels of virulence depending on the source of resistance[1]. Kort *et al.*, 1977 recognised five pathotypes of *G. rostochiensis* (Ro1-5) and three of *G. pallida* (Pa1-3). While pathotyping can be useful, it has its limitations because some of the differentials contain single major gene resistance which seperate pathotypes on qualitative bases, whilst others show quantitative differences based on polygenic resistance.

In Britain, populations of the yellow species of PCN *G. rostochiensis* (Ro1) have been managed through the use of cultivars with the H1 resistance gene (e.g. Pentland Javelin and Maris Piper). There are a few cultivars with quantitative resistance to *G. pallida*, but no source of major gene resistance. Following repeated growing of cultivars with H1 resistance, *G. pallida* is now the dominant species in most of England. This is leading to increasing concern regarding the future management of this species as it becomes increasingly predominant.

Virulence differences in *G. pallida* An assessment of the range of virulence of British populations of *G. pallida* and populations from other geographic regions (Table 1) has been made in response to quantitative resistance from *Solanum vernei* and *Solanum tuberosum* spp. *andigena* CPC 2802 (Fig. 2). Some populations appear to be totally virulent as they show similar levels of reproduction on the partially resistant potatos as on a susceptible cultivar. Others are much less virulent and the resistant cultivars give a significant level of control. Between the extremes are popu-

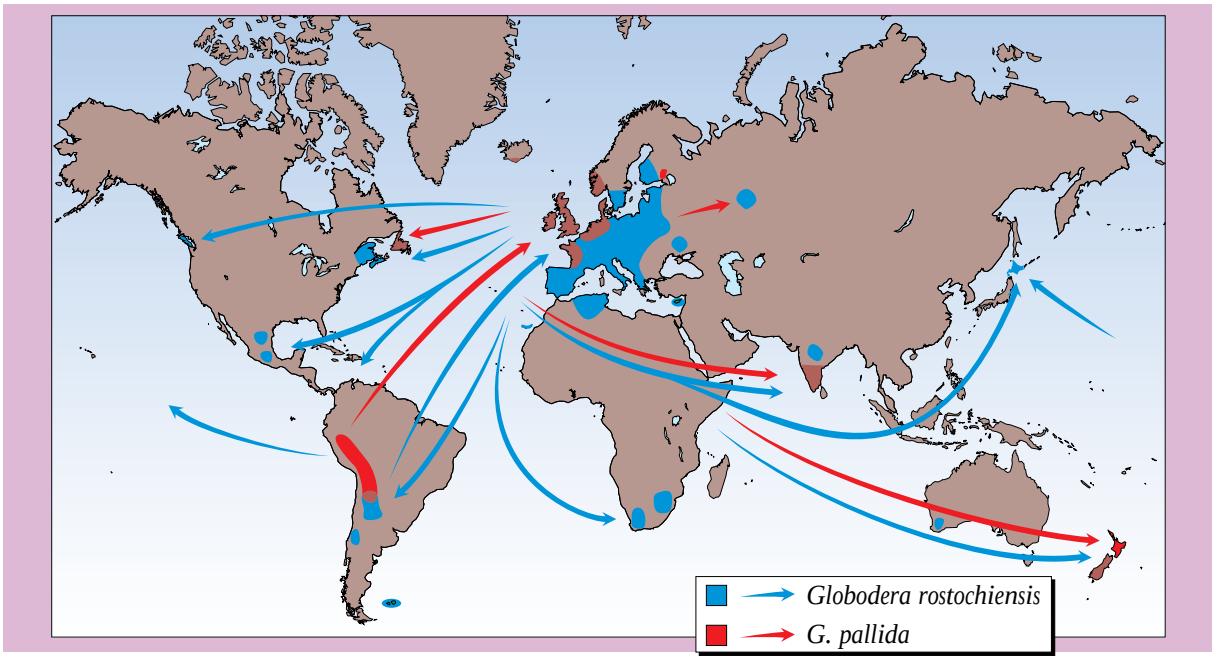

Figure 1 World distribution of potato cyst nematode.

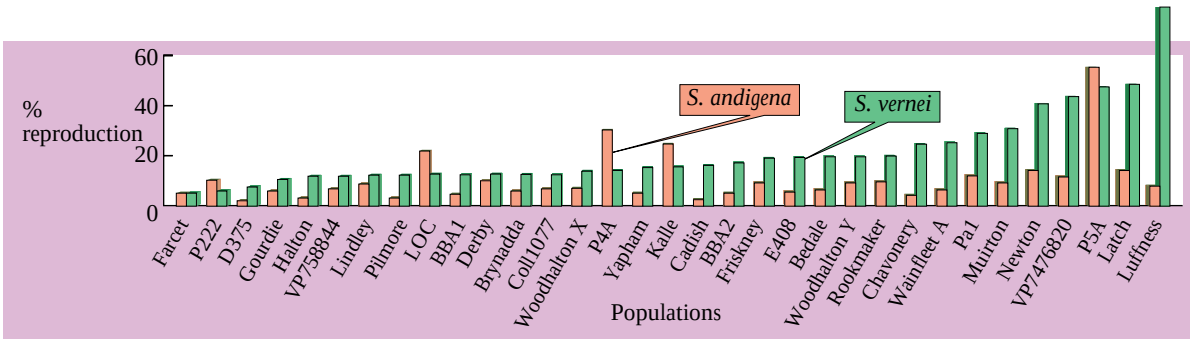

Fig 2 Relative reproduction of 30 populations of *G. pallida*.

lations with intermediate virulence. When different sources of resistance are used, the relative ranking of these populations according to their virulence changes, indicating that there are different genetic interactions underlying these biological responses. The resistance from CPC 2802, whilst not giving resistance to *G. rostochiensis*, gives better control to nearly all the *G. pallida* populations including the most virulent ones.

Population	Species	Pathotype	Source
BBA1	*G. pallida*	Pa2	Germany
BBA2	"	Pa3	Germany
Kalle	"	Pa2	Germany
P2-22	"	Pa2	Netherlands
D 375	"	Pa2	Netherlands
Rookmaker	"	Pa3	Netherlands
Coll. 1077	"	Pa3	Netherlands
VP 75-884-4	"	Pa3	Netherlands
VP 74-768-20	"	Pa3	Netherlands
E 408	"	Pa3	Netherlands
Gourdie	"	Pa3	Scotland
Latch	"	Pa3	Scotland
Muirton	"	Pa3	Scotland
Chavonery	"	Pa3	Switzerland
Farcet	"	Pa3	England
Friskney	"	Pa2	England
LOC	"	Pa2	England
Newton	"	Pa3	England
Wainfleet	"	Pa3	England
Yapham	"	Pa3	England
Woodhalton X	"	Pa3	England
Woodhalton Y	"	Pa3	England
Bryn Adda	"	Pa3	Wales
P4A	"	P4A	South America
P5A	"	P5A	South America
Luffness	"	Pa3	Scotland
Pa1	"	Pa1	Scotland
Bedale	"	Pa3	England
Halton	"	Pa2	England
Lindley	"	Pa3	England
Pilmore	"	Pa3	Scotland
Derby	"	Pa3	England
Mierenbos	*G. rostochiensis*	Ro1	Netherlands
Ro1	"	Ro1	Scotland

Table 1 Populations of potato cyst nematode, their species classification and country of origin.

Molecular studies To examine the genetic basis for this variation in virulence, molecular techniques were used to assess the genetic relationships of a number of populations of *G. pallida*. The populations were mainly from the British Isles and continental Europe. Two populations from South America designated pathotypes P4A and P5A, were included for comparison with those already established in Europe and which in terms of virulence are equivalent to the Pa2 and Pa3 populations as defined by Kort *et al.*, 1977. Two populations of *G. rostochiensis* were also included. A PCR based technique, random amplified polymorphic DNA (RAPD), was used to amplify small randomly sampled regions of the nematode DNA. The PCR products were seperated by electrophoresis and the resulting patterns used to investigate the groupings of populations. Comparison of the numbers of shared DNA products was used to produce the unrooted phylogenetic tree shown in Figure 3. This clearly illustrates the wide divergence between the two PCN species. Within *G. pallida*, the two populations from S. America are distinguishable from each other and all the populations from Europe. Indeed this wide divergence could even have implications for the taxonomic relationships within *G. pallida*. The genetic distinctness of these two South American populations from the European populations is not reflected in their biological grouping as they fitted readily into the European pathotype scheme. Amongst the European populations, the PA1 and Luffness populations from Scotland are also distinct from each other and from all of the other European populations. These populations also have distinct virulence characteristics; Pa1 is avirulent to resistance from *S. multidissectum* and has pale yellow females. The Luffness population is extremely virulent on the partially resistant cv Morag (Fig. 2). Among the remaining populations there are indications of groupings by country of origin; particularly the Dutch and a few of the other continental European populations which group together (Fig. 3). The molecular rela-

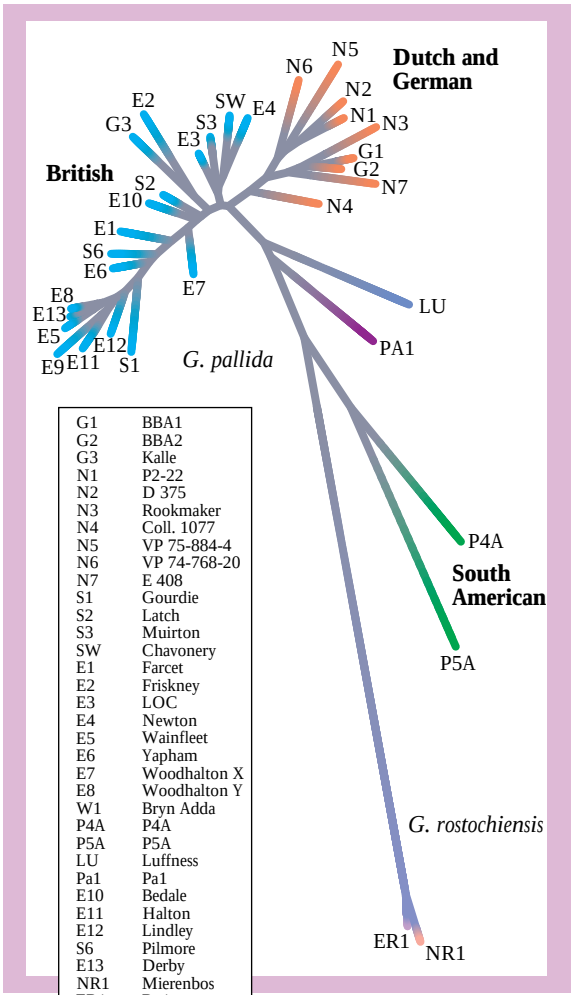

Figure 3 Unrooted phylogenic tree showing relationships between populations of PCN used in RAPD study.

tionships between these remaining populations did not correspond to their virulence classification by the pathotype scheme and the statistical support for these subgroups within the main European group was poor.

Selection for virulence When introducing new sources of resistance to any pathogen or pest, it is always a concern that their use will lead to the selection of virulent populations, pathotypes or races. This is no less of a concern with PCN, and consequently in another study, populations were examined following selection for a number of generations on clones from the breeding program with resistance derived from *S. vernei* or *S. tuberosum* subsp. *andigena* CPC 2802. The changes in virulence in response to selection are shown in Figure 4. Selection on the *S. vernei* derived clones resulted in increased virulence which to a lesser degree was expressed on other *S. vernei* derived clones. In contrast, selection on the CPC 2802 clones also

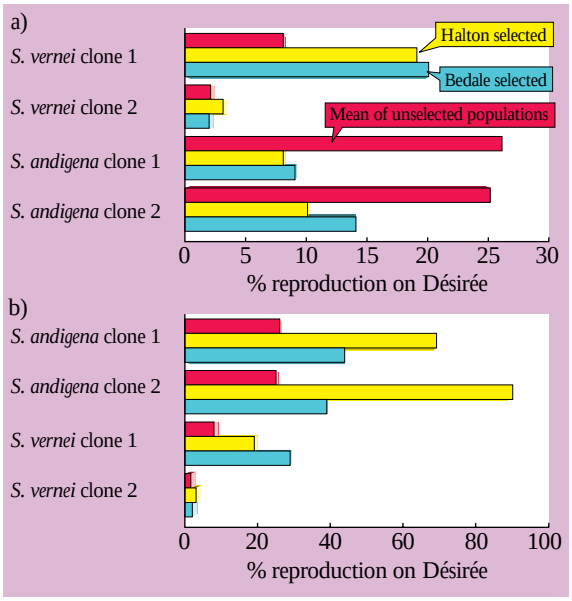

Figure 4 Selection for virulence on a) *S. vernei* clone 1 and b) *S. andigena* clone 1.

resulted in increased virulence but was also expressed on other clones with this source of resistance. The genetic relationships of the unselected and selected populations based on a study using RAPDs (Fig. 5) shows that the selection for virulence has also affected the general genetic constitution of the subpopulations. The differences between these subpopulations can be as great as those between naturally occurring field

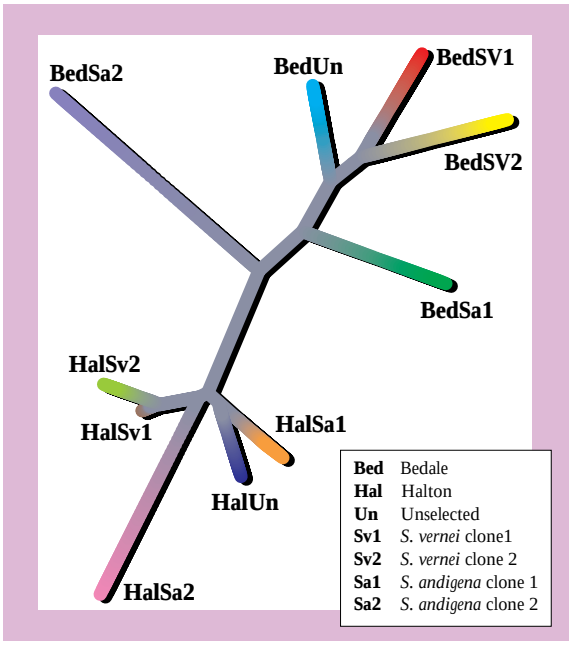

Figure 5 Unrooted phylogenetic tree showing relationships between unselected and selected populations.

populations especially following selection with clones derived from CPC 2802. A possible explanation is that resistance from CPC 2802 selected for a more discrete subset of the total gene pool than did selection with *S. vernei*. This could be either because fewer genes are involved in resistance from CPC 2802 and/or because these clones are more resistant than those derived from *S. vernei*, and thus exert a stronger selection pressure.

Conclusions Our molecular studies indicate that there are probably at least three different introductions of *G. pallida* into Europe (Pa1, Luffness and the rest) and that a single population can give rise to a broad range of variation, both in turns of general genetic variation and in virulence. The next stage in these studies will be to relate biological information to molecular data in order to identify DNA markers which are associated with virulence characteristics. A better understanding of the genetic basis for virulence differences between populations will be of value for the development of resistant cultivars and their utilisation in the field. It will also permit an assessment of the durability of resistance in relation to the various virulence mechanisms operating in *G. pallida*.

Reference

1 Kort, J., Ross, H., Rumpenhorst, H.J., & Stone, A.R. (1977). *Nematologica* 23, *333-339.*

Molecular and adaptive variation in aphid guts

P. Irving & B. Fenton

Aphids are an important pest of most commercial crops, causing direct feeding damage and the transmission of harmful plant viruses. In recent years, with the incidence of warmer summers and milder winters, more aphids have survived from one season to the next, increasing their pest status. Within the aphid group, the control of polyphagous aphids, i.e. those able to feed on several host plants, has proved very difficult. Spraying against polyphagous aphids in one crop results in only temporary control, as aphids recolonise from populations living on unsprayed alternative hosts.

It has been shown that moving aphids between suitable host plants reduced their fecundity, but, after a time, the aphids were able to recover their reproductive potential. The work indicated that there was a period of adaptation, and in the case of feeding aphids this would require some form of internal adaptation to the new host plant. The most likely location for any adaptive response would be the aphid gut, which may react to environmental change with the production of new proteins, probably enzymes.

Electrophoretic studies carried out at SCRI have found significant differences between the gut protein profiles of the raspberry feeding *Amphorophora idaei*, and the polyphagous *Myzus persicae*. However, when a comparison was made between both types of aphid

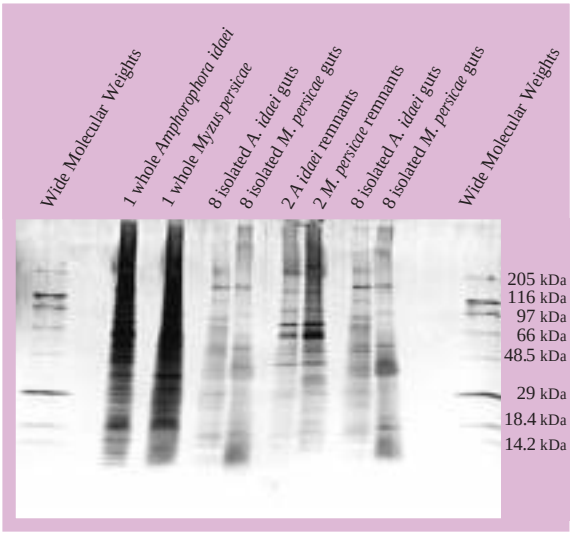

Figure 1 SDS-PAGE of aphid proteins. While there are distinct differences between the protein profiles obtained from the isolated guts of the two aphid species, there are conversely great similarities between the profiles of the two aphid remnants.

remnants, (no guts or embryos), they were found to have similar protein profiles (Fig. 1). While this is not an exhaustive comparison of protein composition between the two aphids, the information clearly indicates that the majority of variation in expressed genes between the two species is contained within the gut.

As a target for insecticides, the insect gut is of further interest as it is often the first barrier which must be penetrated before a compound can act. The gut epithelial cells are separated from the ingested material by a peritrophic membrane, or PM, in many insect species. The PM, a chitinous structure secreted from the gut epithelium, is thought to have protective and energy conserving functions. The PM has also been shown to interact with viruses and ingested parasites in some insects .

Older literature stated that there are no PMs or PM-like structures in aphids, whereas more recent work, on EM sections, suggested the presence of a perimicrovillar membrane with functions similar to a PM. Such a structure in the aphid gut could reduce or even prevent an aphicide from crossing into the haemocoel and acting on its intended target, and might also act as a recognition site for the transmission of plant viruses. Acquisition of plant virus by the beetle *Diabrotica undecimpunctata howardi* only happens through the PM lined areas of the gut, suggesting a specific interaction between virus and beetle PM[1]. The presence of a PM or PM-like structure in the aphid gut could explain why one aphid species was more successful at transmission of a particular virus than another species feeding on the same plant. For example, while many aphids are found feeding on potato, *Myzus persicae* is recognized currently as the most significant vectors of PLRV.

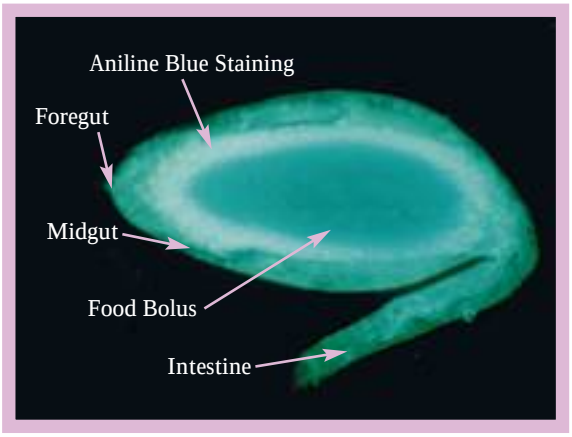

Figure 2 Isolated gut from *Myzus persicae* stained with aniline blue and viewed under UV. The distinct layer surrounding the food bolus may be indicative if a peritrophic-like membrane.

In recent work at SCRI, a distinct and stainable layer within the gut of *Myzus persicae* was found using the chitin specific stain analine blue (Fig.2). This distinct layer in the aphid midgut may be a perimicrovillar membrane[2]. It remains to be seen if this layer can be further characterised in sections of isolated aphid guts, ending the current controversy of whether aphids have PMs or not.

References.
[1]Wang, R.Y., Gergerich, R.C., & Kirn, K.S. (1994) *Phytopathology* 84(2), 147-153.
[2]Terra, W.R. (1990) *Annual Review of Entomology* 35, 181-200

Biomathematics and Statistics Scotland

Rob Kempton

Originally established in 1987 as the Scottish Agricultural Statistics Service, Biomathematics and Statistics Scotland now has an international reputation in the field of mathematics and statistics applied to the biological sciences. BioSS contributes research, consultancy and training to agricultural and biological research institutes in Scotland and collaborates in research programmes with leading UK and European universities and laboratories. In accord with the aims of the Government White Paper 'Realising our Potential', increasing emphasis is being placed on interchange of mathematical and statistical ideas, skills and know-how, with industry and other potential users. SCRI administers BioSS and is one of its major clients.

BioSS activities were reviewed during the year, in advance of our four-yearly Visiting Group in January 1996. The first BioSS Corporate Plan was produced, outlining our strategy for the next three years.

Research is focused on five themes:

- in image analysis, particular attention is being given to image warping, digital microscopy and imaging problems in the soil environment;
- in environmental modelling, the emphasis is on developing techniques to model and monitor spatio-temporal change in the natural environment;
- mathematical modelling is directed towards veterinary and plant epidemiology, and related spatio-temporal and population dynamic models, as well as decision making processes;
- in genetics, work will concentrate on developing methods for linkage mapping and phylogenetic analysis to fully exploit information from molecular marker and molecular sequence data;
- finally, new methods of statistical design and analysis continue to be required to improve the efficiency and effectiveness of scientific research programmes.

There is collaboration with SCRI scientists on all five themes. This collaboration resulted in 21 jointly-authored papers, appearing in refereed journals in 1995, or in press.

New projects started during the year included involvement with MLURI, SAC and SCRI in a six-year study of phenotypic and genotypic bases of population dynamics in heterogeneous species-rich grassland species. Another project, described in the following article, is to model spatial trends and local competition effects in field trials.

The BioSS training programme continues to develop, and 50 SCRI staff and students attended 96 days of on-site training in basic statistics and sequence analysis. We are exploring opportunities in computer-based learning and multi-media applications, and pilot modules are being produced which integrate audio-visual presentations and interactive computer packages. This will allow training to be made available on demand, adapted to individual needs and marketed more widely.

Improving the accuracy of field trials by modelling spatial trends with generalised additive models

C.A. Hackett & A.C. Newton

Background There is continual pressure to improve cost-effectiveness and efficiency of agricultural experiments, and this requires the development of new statistical techniques which build on recent advances in theory and computing power.

In agricultural field experiments, unevenness of soil fertility or disease levels can seriously affect the quality of data from field experiments, sometimes totally obscuring differences between treatments or cultivars. To reduce this problem, the site is usually divided up into areas, or *blocks*, where the environment is as uniform as possible. The blocks are then divided further into plots and a treatment is allocated at random to each plot. If it is known that the site has a major environmental trend in a particular direction, for example if the field slopes, then blocking should take this into account. If a large number of treatments are to be compared, then it may be impossible to have uniform blocks of sufficient size, and incomplete block designs, where each block contains only a fraction of the treatments, may be used.

An alternative approach to field experiments is to adjust for any environmental trends using spatial models. One of the first spatial methods, used in the early 1900s, involved a grid of check plots, a standard treatment placed systematically throughout the experiment. The yields from nearby check plots were used to calculate a fertility index for each treatment plot. In the 1930's this method was refined by eliminating check plots and calculating a fertility index from yields of the neighbouring plots. In the last twenty years there has been a fresh interest in spatial models and sophisticated models have been developed to eliminate spatial trends in different ways.

Another approach is to avoid a formal model and simply *smooth* the data. Such a smoothing approach has been used in the area of medical survival analysis, to relate the health of patients to different factors such as age or blood pressure, without assuming that the relationship has a particular form[1]. This method may be used in field experiments to express plot yield, or disease level, as a smooth function of the position of the plot in the field. Models with a smooth term are referred to as *additive models*.

Additive models A conventional analysis of variance of a field experiment, with block effects estimated for all plots in a block, gives a coarse description of any underlying trends. Trends within a block will not be detected. Additive models provide a method to examine underlying trends as a smooth function of position in the field. An additive model is a flexible generalisation of the familiar regression model.

A regression model for a variable Y, such as yield, relates Y to the sum of several *explanatory* variables, such as cultivar, fertiliser treatment, plant height or plot position. For two explanatory variables, cultivar and position, the regression model has the form:

$$Y_{ij} = \alpha_i + f(X_{ij}) + \varepsilon_{ij}$$

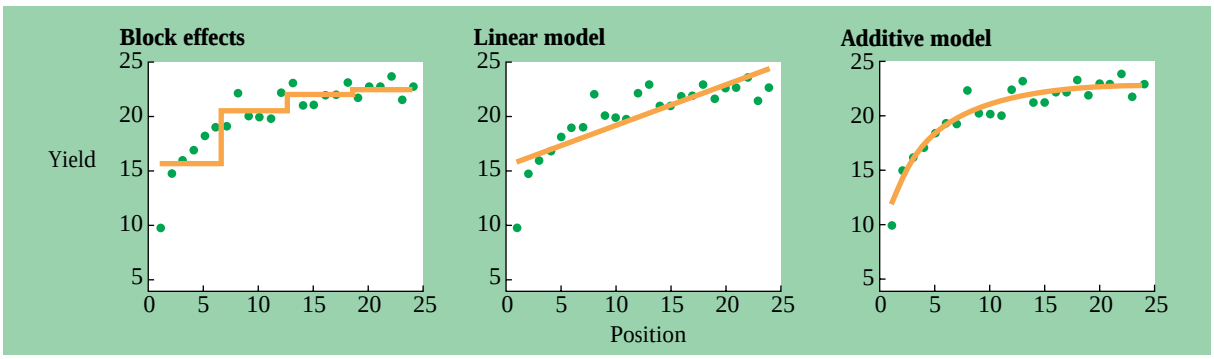

Figure 1 Simulated data showing an increase in yield with plot number. The relationship between the yield and position of the plot in the field may be expressed as a series of means corresponding to different blocks, or as a straight line, or by smoothing the data to give an additive model.

To fit the semi-parametric model $Y_{ij} = \alpha_i + lo(X_{ij}) + \varepsilon_{ij}$
[Y_{ij} = yield, X_{ij} = position of the jth plot receiving treatment i]

- Estimate means for each treatment, $\{\alpha_i\}$, and residuals $r_{ij} = Y_{ij} - \alpha_i$
- Smooth r_{ij} against X_{ij} to give the first estimate of lo (X_{ij})
- Adjust the response Y_{ij} by subtracting the smooth lo (X_{ij})
- Repeat from step 1 using the adjusted response until the estimates of $\{\alpha_i\}$ and lo (X_{ij}) converge

Figure 2 Procedure for fitting an additive model.

where Y_{ij} is the yield and X_{ij} is the position of the jth replicate of cultivar i. The term α_i represents the effect of cultivar i. The error ε_{ij} is assumed to have zero mean and constant variance. The function $f(X_{ij})$ has a given form such as, for example, βX_{ij} (linear), γX^2_{ij} (quadratic) or $\delta \log_e(X_{ij})$. An *additive model* replaces the function f by an unspecified smooth function $s(X_{ij})$, estimated by smoothing the data. Figure 1 represents the relationship between position and yield using block effects, a linear model and an additive model for some simulated data.

There are several different ways of smoothing the data, but all are derived from the idea of a weighted mean of the yields at several neighbouring plots. For each value of X, say x_0, and some fixed value k, the k nearest plots to x_0 are identified and assigned a weight according to their distance from x_0. Plots close to x_0 have a weight close to 1, while the most distant of the k neighbours has a weight close to 0. The value of the smooth is equal to the weighted mean of these k neighbours. The smoothness of the resulting curve depends on the value chosen for k - a small value of k will give a rough curve which follows the data points closely, while a large value of k will give a smoother curve. Some types of smooth, in particular the locally-weighted running line smoother, lo(X), which is used here, may be generalised to estimate a smooth surface for a two-dimensional field plan. Figure 2 shows the procedure for fitting an additive model.

Example Here we will compare the analysis of a barley disease trial using a conventional analysis of variance and an additive model. The experiment was designed to compare yields on plots of single barley cultivars (monocultures) and mixture plots of two or three barley cultivars. Thirteen pure cultivars, six different mixtures of two cultivars and one mixture of three cultivars were grown. The area consisted of 18 beds, separated into three blocks of six beds. Each bed within a block received a different combination of a mildew control treatment and a nitrogen fertiliser treatment, as shown in Figure 3. The beds were divided into 20 plots, and the 20 cultivars or cultivar mixtures were allocated at random to these. The plot yields were recorded at 9% moisture content.

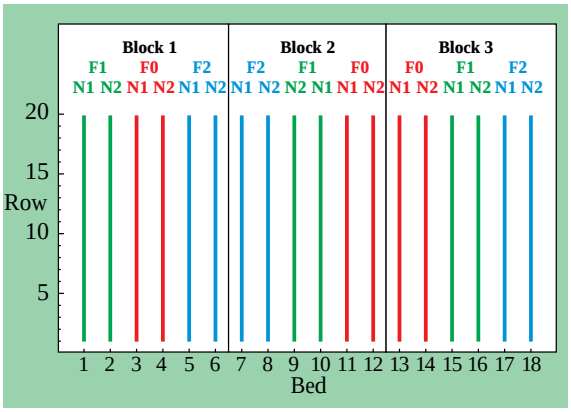

Figure 3 Layout of the field experiment. There were three mildew control treatments (F0 = a control, F1 = a fungicide, F2 = a resistance elicitor) and two nitrogen treatments (N1 = nitrogen at 40kg/ha and phosphate and potassium at 20 kg/ha, N2 = nitrogen at 120kg/ha and phosphate and potassium at 60 kg/ha). The beds were separated along their length into 20 plots, and the 20 cultivars or cultivar mixtures were allocated at random to these.

Analysis of variance of the yields (Table 1) indicated that nitrogen level and cultivar had significant effects on the yield ($p < 0.001$) and that an interaction between cultivar and fungicide treatment might be present ($p = 0.039$). However, when the residuals were plotted against the row number (Fig. 4) they showed a strong downwards trend, implying a lower yield than expected from the high numbered rows.

The residual plot suggests a decreasing trend in the yields with row number. However there could also be

	df	m.s.	pr(F)
Block stratum			
Blocks	2	0.108	
Fungicide	2	0.237	0.096
Residual	4	0.053	
Main plot stratum			
Nitrogen	1	26.347	<0.001
Fungicide.Nitrogen	2	0.055	0.122
Residual	6	0.018	
Bed stratum			
Cultivar	19	0.226	<0.001
Fungicide.Cultivar	38	0.046	0.039
Nitrogen.Cultivar	19	0.031	0.457
Fungicide.Nitrogen.Cultivar	38	0.038	0.174
Residual	228	0.030	

Table 1 Analysis of variance of the yields.
m.s. = mean square

a trend across the beds, giving a choice of several different additive models. Initially one-dimensional trends across the rows and the beds of the experimental area were investigated. Each trend could be excluded, included as a linear term or included as a smooth function with a range of different degrees of smoothing. Initially the smooth was based on a span of k=n/2 nearest neighbours, where n is the total number of observations. This was compared to smooths with spans n/3, n/4 or n/6.

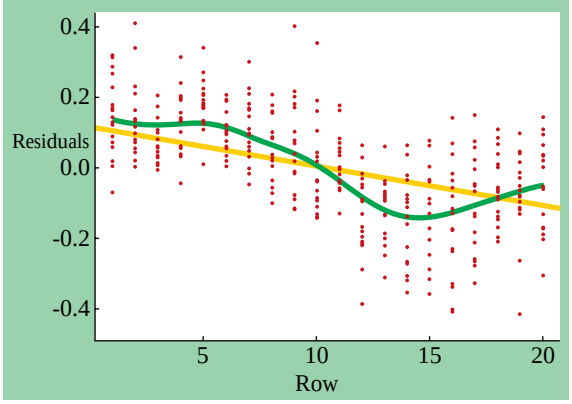

Figure 4 Relationship between the residuals from an analysis of variance and the row number of the plot. This indicates a decrease in yield up each bed of the experiment. The yellow line shows a linear relationship and the green line shows a smooth curve.

A stepwise procedure was used to select the best spatial model, by minimising the Akaike information criterion, AIC. This procedure began with a model with all treatment and interaction effects, but no spatial effects. The first variable selected was a linear function of the bed number or a linear function of the row number - whichever caused the greatest reduction in the AIC. The second variable was selected to reduce the AIC further. This second variable could be a smooth function of bed number or row number if the linear function had been selected earlier. The size of k could be changed to give different degrees of smoothness. At any stage a variable selected to be in the model could be removed if this decreased the AIC. The process continued until the AIC could not be decreased further. If functions of both bed number and row number were selected, the stepwise selection was repeated, including among the set of potential explanatory variables a two-dimensional smooth surface, with various degrees of smoothing.

Table 2 gives the residual mean deviance and the AIC for different spatial models. There were significant

	Residual d.f.	Residual mean deviance	AIC
None	240	0.0312	10.85
Bed	239	0.0301	10.57
s(row,k=n/3)	234	0.0200	8.21
Bed + s(row,k=n/3)	233	0.0188	7.93
s(bed,row,k=n/3)	229	0.0144	6.96

Table 2 The residual mean deviance and Akaike information criterion (AIC) for different spatial models. All models included the three treatment factors and all possible interaction terms.

trends in both directions. Across the beds a linear trend had the lowest AIC, while across the rows a smooth term lo(row) with a span k = n/3 was best. The two-dimensional smooth lo(bed,row) with span k = n/3 had a lower AIC than the sum of the two one-dimensional trends. Figure 5 compares the two-dimensional smooth surface with the sum of the two one-dimensional trends.

All of the main treatment effects were significant. However, the cultivar.fungicide interaction, which

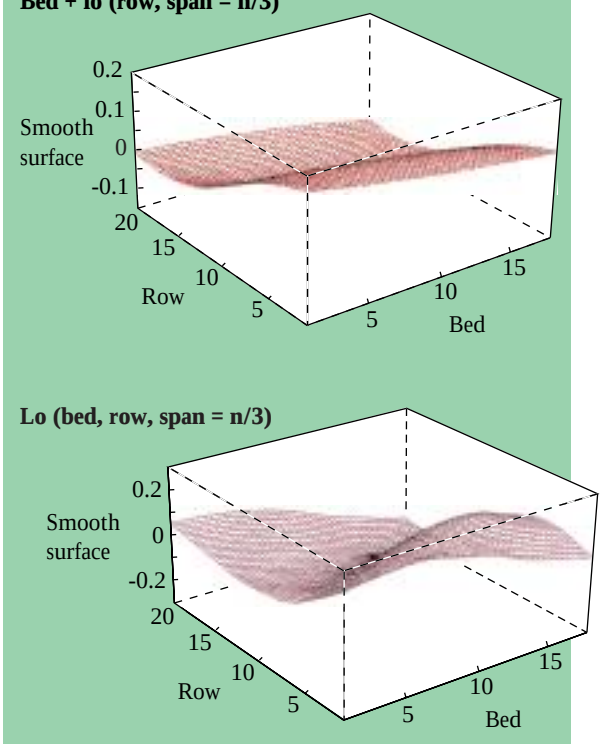

Figure 5 Representation of the trend in fertility as the sum of two, one-dimensional trends (top) or as a two-dimensional surface (below). The two-dimensional surface gives a better representation of the trend, and a lower residual variation.

had a significance level of 0.039 by analysis of variance, was no longer significant. As there were at least 18 replicates of each factor level, the plot adjustments average out and the estimated treatment effects from the generalised additive model are very similar to those from analysis of variance. Cultivar 14 was, however, ranked lower by the generalised additive model. An examination of the field plan showed that cultivar 14 occurred at the high-yielding end for more than half the beds, so when there was an adjustment for trend, the yield of cultivar 14 was reduced. The average standard error has been reduced from 0.041 to 0.028 for cultivar comparisons, from 0.021 to 0.009 for fungicide and from 0.010 to 0.006 for nitrogen by the additive model, so all comparisons have become more precise.

Conclusions Additive models provide a flexible framework for estimating underlying trends in field experiments. The effects of experimental treatments are estimated by additive models with greater precision than when underlying trends are ignored. These models do not assume a fixed form for the trend but estimate it from the data in a form which can easily be displayed graphically. Additive models are not confined to trends in one direction, but may be used to represent two-dimensional surfaces as trends.

Most methods for including spatial trends in field experiments adjust the observations from each plot according to the observations on surrounding plots. This differs from the situation where there is thought to be an interference effect of one cultivar on another. We are currently investigating whether interference effects may be separated from larger-scale spatial trends in field trials by means of additive models.

Reference

1 Hastie T.J. & Tibshirani R.J. 1990. *Generalized Additive Models*. Chapman and Hall: London 335 pp.

Research services

Analytical Facilities

W.W. Christie

Laboratory Accreditation

An essential requirement for further successful marketing of SCRI scientific expertise and facilities on a competitive basis will be the operation of an approved Quality Assurance (QA) System covering all aspects of our activities. This may be important also for the award of research funding from central government and other external sources. In other scientific organisations, the introduction of quality systems has led to an improvement in the overall efficiency of activities at all levels. With this in mind, a programme to implement a QA system within SCRI is underway under the auspices of the Chemistry Department, in which the system is being developed. Dr T. Shepherd is quality assurance officer for the Institute. Stable Isotopes analysis and the MRS Lipid Analysis Unit are the first areas to seek accreditation, but the system is being extended to other areas within chemistry in a phased process, before it is applied to the new Media Preparation Facility and other selected activities within SCRI. With the support of Scottish Enterprise Tayside (SET) and MRS, consultants have been appointed to monitor and advise on the introduction of QA. Within the Chemistry Department, we are working towards application for blanket EN ISO 9000 (BS 5750) registration for the full range of our activities and EN 45000 (NAMAS) registration for selected areas. Such internationally recognised standards are also suitable for coverage of most other activities within SCRI.

Stable Isotope Facilities

Stable isotopes are now basic tools for the study of plant physiology, crop genetics, ecology and food webs. Valuable information comes both from studying natural variation in stable isotope composition and from following the fate of added isotopic tracers. SCRI is equipped with a comprehensive range of modern instrumentation for stable isotope analysis. With these, we can tackle most of the biologically important low atomic number elements - ^{13}C, ^{15}N, ^{18}O and ^{34}S in a wide range of solid, liquid and gas samples. All the instrumentation is based on continuous-flow isotope-ratio-mass-spectrometers that are fully automated and operated through computer data systems. Automation allows a high through-put of samples, essential for many biological experiments where large data sets are required. For solid samples, the Europa Scientific Tracermass and 20-20 mass spectrometers are interfaced to Roboprep CN and ANCA-*NT* SL combustion sample converters. A Roboprep G+ gas purification unit is used for gas analysis. Plant samples of one to five milligrams are

used, containing 25 to 100µg of the element of interest. Where possible, analytical protocols are devised to minimise sample preparation and fully exploit the automation.

SCRI also has expertise and resources for sample preparation from a wide range of sample types. These include plant sample drying and grinding, freeze drying and weighing facilities. Research support is aimed at developing new methods to assist the Institute's commissioned programme.

Services to Molecular Biologists

DNA synthesis

The DNA synthesis facility is based on an Applied Biosystems model 394 DNA/RNA synthesiser. The department also has a single column model 391 synthesiser which provides cover during servicing and repairs. The 394 instrument allows the simultaneous synthesis of four oligonucleotides and is equipped with automated amidite dissolution, on line trityl monitoring and automatic cleavage facilities. Side chain deprotection and recovery of the DNA remain manual operations. Both instruments synthesise DNA by a step-wise solid phase method which allows over 100 nucleotides to be coupled in a single nucleic acid molecule. Oligonucleotides containing between 20 and 30 residues are normally required and cycle times are such that eight oligonucleotides can be made in a day.

The HP Mass spectrometer

Custom peptide synthesis

Peptide synthesis is carried out using an Applied Biosystems model 431A peptide synthesiser, an instrument which automates the coupling of amino acids to form a peptide of any desired sequence. Peptides of up to 35 amino acids can be constructed easily, but with suitable care and alterations to the chemistry, 50 to 60 residue peptides are possible. The cycle times for amino acid couplings are variable, with a minimum of two hours, and so the construction of an average peptide takes several days. Peptides can be made in a variety of formats; as single chain products fully deprotected and either bound to a resin bead or not; or as branched structures containing four copies of the desired peptide in a single, soluble, molecule. An Applied Biosystems 151a HPLC system is available for the analysis and purification of peptides.

DNA sequencing

Automated DNA sequencing is performed on an Applied Biosystems model 373 Stretch Sequencer, with data collection and version 2.1.1 analysis software on an Apple Macintosh Quadra 650. The sequencing reactions can be performed using a Perkin Elmer 2400 thermal cycler in collaboration with the CMG department. By using fluorescent dyes and laser scanning technology, this system can generate large amounts of sequence data rapidly. Up to 35 clones can be loaded on a single gel and, when using 48 cm plates, an accurate sequence of 650-700 bases called in each, resulting in 23 kilobases of sequence generated in 24 hours. Genescan software, from Perkin Elmer, has recently been acquired to enable the instrument to perform DNA fragment analysis tasks.

Mass Spectrometry

The Institute is particularly well equipped in the field of mass spectrometry (MS), with three state-of-the-art instruments devoted to structural analysis of organic compounds. Housed in a new purpose-built laboratory suite, all systems have integrated computer control, library search capabilities and distributed data processing facilities. The core instrument is a new Hewlett Packard 5989B MS ENGINE research-grade quadrupole instrument. The mass spectrometer has electron impact and chemical (positive/negative) ionisation modes with a mass range of 2000 a.m.u., together with an autosampler and distributed processing software which will permit off-line data processing and reduce operating costs. In addition it has a particle beam LC/MS interface which will complement existing LC/MS instrumental techniques. This instrument can provide mass and structural data on a wide range of organic compounds.

In addition, a bench top instrument is dedicated to the analysis of organic volatiles. This consists of a Perkin Elmer automated thermal desorption system (ATD) linked to a VG TRIO-1000 quadrupole gas chromatography (GC)-MS and permits detailed characterization of the profiles of organic volatiles generated by biological systems.

A Finnigan SSQ 710C dedicated liquid chromatography-MS instrument, with atmospheric pressure chem-

ical ionization (APCI) and electrospray ionization (ESI) interfaces, completes the facility. This has an ability to analyse samples whose high molecular weight, lack of volatility or polarity, precludes analysis on the other instruments. APCI and ESI are soft ionization techniques and generally only produce molecular ions, e.g. $[M-H]^+$ or MH^+, but the multicharge ionization mechanism of electrospray can extend the basic 2000 mass range of the instrument by a factor of about 20 giving a mass range of greater than 40,000 amu. This permits accurate mass determination of peptides, proteins and nucleic acids to within 0.1% compared to the 5.0% error usually expected from SDS-PAGE determination.

Mass spectrometric analysis at SCRI covers a broad spectrum of chemical investigations generated by the research programme of the Institute. A wide range of plant metabolites has been analysed, both in the native form and as derivatives, including sterols, monoterpenes, sesquiterpenes, pentacyclic triterpenes, dimeric forms of phenolic acids, glucosinolates, long-chain wax esters, peptides, essential oils, carbohydrates, polychlorinated biphenyls and fatty acids. The facilities are operated by experienced and expert staff, ready to tackle and solve most structural problems. They are actively seeking full laboratory accreditation status and working practices are commensurate with recognised standards.

Scientific Liaison and Information Services

W.H. Macfarlane Smith

The retiral of Dr D.A. Perry - Head of SLIS (see also p.20), together with other retirals and staff changes, provided an opportunity to re-appraise the function and structure of the Department. In August, Dr W.H. Macfarlane Smith was appointed as head of an expanded department, incorporating the Data Processing Unit (since re-named Information Technology Services), and the Health and Safety organisation for SCRI, in addition to Visual Aids, the Library and Reception.

Information Technology Services continue to have a critical role in support of the scientific activities of the Institute. It is essential that Information Technology moves with the times in terms of both hardware and software. As part of this process, five further buildings have been connected to SCRINet, the Local Area Network (LAN) for centrally mounted software services, and the Internet, giving a total of fifteen buildings now linked. Other buildings have had their network link upgraded and the number of access points increased. The main Novell server was upgraded to an Elonex P 590/1, with 32 Mb of memory and 4 Mb of disk space to meet the demands from a higher number of users. Older IBM computer Model 30's, used for specific scientific purposes, have been replaced and other computers purchased, to provide additional services to all Institute users e.g. data bases in CD-ROM format. A substantial effort has gone into developing the Institute's contribution to the World Wide Web. The requirement to produce high quality transparencies has been serviced by the installation of a Mitsubishi colour printer.

The need to widen public knowledge of SCRI's activities and to support the many commercial initiatives has placed a heavy load on the Visual Aids group. Their activities range from general and scientific photography, through the production of videos, to the preparation of displays for exhibitions and conferences.

Photographic and graphics requests have increased by 6% and 27% respectively, from the previous year. The demand for high quality brochures and other publicity materials has required the purchase of additional equipment.

Poster production using a large format colour printer

This includes updates to the Apple Macintosh computers and a Novajet III colour printer. In addition, a cold laminator, a mixer and other accessories for video production, a new black and white film processing unit, and camera upgrades have been obtained.

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

Databases in CD-ROM format are available to give references to the periodical literature from 1972 to date in the fields of biology, agriculture, horticulture, pest control, soils and biotechnology. The most recent years are available over the Institute network. Access to the more general scientific database, BIDS ISI, has increased. The Library staff give advice and help on using BIDS ISI as well as access to university library catalogues and other services available on JANET and the Internet. The availability of these databases over the network has proved very useful, by allowing researchers to gain information without leaving their desks and laboratories.

The use of these databases resulted in a 16% increase in requests for material not held in the Library. These requests have been satisfied from other Libraries or the British Library Document Supply Centre. The increase in the price of scientific literature has been well in excess of inflation and consequently only 145 new books were purchased.

The fact that the subject coverage of the bookstock is appropriate and essential to the work of the Institute is borne out by a 10% increase in the borrowing of such material. A transitional stage is in progress with a number of journals available in both electronic and hard copy form.

Such dual provisions are likely to continue until matters of Copyright are resolved. While all information will be available ultimately in electronic form, the interim stage will impose an additional workload.

Large numbers of commercial contacts, scientists and students now wish to visit SCRI to learn about its work. The number of such visits has increased by 35% over the previous year, with visitors from the former COMECON grouping, including Georgia, Poland, Russia and the Ukraine especially well represented. This rise has been more than matched by a 50% increase in the number of articles concerning the work of the Institute, placed with newspapers, magazines and journals.

Farewell visit of Lord Provost Norman McGowan prior to local authority re-organisation.

The Department also represents the Institute at various public- and private-sector meetings and exhibitions ranging from the local Horticultural Show through to Scotgrow, the PMB Potato Planting Demonstration and the Edinburgh International Science Festival. A major contribution is also made to the organisation of meetings and conferences such as Crop Protection in Northern Britain.

Estate, Glasshouse & Field Research Department

G. Wood

Following the retirement of W.I.A. Jack, an internal review of the Department was undertaken by Professor T.M.A. Wilson, G.R. Mackay and R.J. McNicol. The findings of this review were reflected in a change of name to Estate, Glasshouse and Field Research, coupled with an outline draft which modified the *modus operandi* of the new Department for the future. The essential features included the adoption of a more pro-active rôle in field and glasshouse research work (in cooperation with colleagues in other Departments), a drive to upgrade (a) the breadth and depth of the staff skill-base, and (b) facilities, techniques and equipment, whilst actively seeking ways and means of attracting additional external funding. Subsequently, Dr G. Wood, the Acting Head of Department, was appointed Head with effect from 1 September 1995.

A programme for concreting of floors, installation of roller benching and auto-irrigation systems in glasshouses continued and further gains in exploitable glasshouse space were realised. One of the four large potato seedling houses was modified to provide facilities for GMO containment. A structural maintenance programme was implemented and this will continue on a scheduled basis to ensure that all glasshouse structures are kept up to the required high standard.

The regimen governing the screening, testing, holding, maintenance, propagation and despatch of virus-tested nuclear stock material of *Rubus*, *Fragaria*, *Narcissus*, and *Ribes* was completely overhauled and upgraded. Higher than ever standards of health, trueness-to-type and traceability have to be achieved and maintained. Novel techniques, including tissue culture, PCR-probing and DNA-profiling, have to be introduced. Necessary modifications to facilities associated with these stock holdings and procedures were completed in January 1996.

New field demonstration plots were established for cane fruit, strawberry, winter oilseed rape and winter barley cultivars (spring oilseed rape, spring barley, potato and blackcurrant plots will be completed in 1996). Field sites with soil infestations for work on potato cyst nematode and raspberry root rot, were also established. On SCRI land, long-term and detailed soil analysis records clearly indicate that soil sulphur levels have declined dramatically over the last five years. An investigation into the effects of different crops and fertiliser applications on S levels was initiated.

Several novel developments in the practicalities of field trialling were successfully tested during the year. These included ridge planting of raspberries, raised bed planting of strawberries, the use of a new cereal harvester allowing pre-programmed plot recording and weighing on-the-move, and the use of a machine plot planter and machine plot harvester in potato trials.

Income generation is another aspect of the broadened remit of the Department that has received new attention. Contract trialling at competitive rates has been implemented. A cooperative venture between MRS, BioSS and SCRI has established a Soft Fruit Trialling Unit. This Unit is designed to undertake chemical, pest and disease work contracted by others. Chemical efficacy trials were successfully completed for Pan Britannica Industries Limited on raspberry beetle and cane midge at several sites, and variety trials were undertaken for Sharpes International Seeds Limited on peas. Chemical efficacy and residue analysis trials in oilseed rape and carrots were completed at a number of sites throughout the UK for Inveresk Research International.

The Department, on behalf of the Institute, achieved the Scottish Quality Cereals accredited standard. SQC ensures that members operate production systems encompassing high standards of crop management. The SQC Board made the award after only one year of independent assessments. The assessors covered all aspects of use of fertilisers, use of pesticides, production and harvesting, storage and staff assessment. The latter included assessment of machinery skills, knowledge, record keeping, decision making and management skills. The SQC award is a recognition of the high standards achieved by the staff and the production methods in use both in cereal trials and rotation crops. This is the first in a series of accreditation of quality assurance standards to be sought by the Department.

A system of quality control reporting was also implemented internally. Clients are asked to complete a

report form on each job/aspect of work undertaken for them by the Department. Again, this is to ensure that quality assured standards are attained and maintained throughout all the areas of responsibility of Estate, Glasshouse and Field Research work.

Maintaining high standards is dependent on continually monitoring, reviewing and upgrading the quality, range and depth of the skill-base of personnel. A staff development and training programme, covering many aspects of agricultural/horticultural crop, plant and pest biology, supervisory and management skills, was drawn up. This will be implemented over the next three years in conjunction with the Industry Training Organisation, ATB-Landbase, via its Training Provider, the Carse of Gowrie Training Group, and, in a number of more specialist areas, via internally arranged training courses. This is a programme which also supports the principles underpinning Investors in People.

The year ended with a major landscaping exercise. After more than 20 years, the main entrance driveway to the Institute was redesigned and replanted in consultation with experts from Glendoick Gardens. This task lasted for two months and involved the lifting and planting of several thousand plants. Also, more than 50 tonnes of Discovery Compost was used as a soil improver and mulch. This material is a peat-free recycled compost produced by the City of Dundee District Council's Cleansing Department by natural process from pure botanical waste. We wait to see the driveway beds blossom and develop again over the next 20 years despite their baptism of ice through a very severe winter!

Driveway and new plants from Glendoik Gardens get a boost from Discovery Compost.

Engineering and Maintenance Department

S. Petrie

The Engineering and Maintenance Department offers a technical design and maintenance service throughout the Institute. Preservation of Institute assets is of paramount importance and careful, skilled inspections are frequently carried out. Corrective maintenance work takes place to ensure the expected performance and life of equipment, vehicle, plant or building is achieved. The Department is divided into sections that specialise in a variety of engineering disciplines such as electrical, electronic, refrigeration, heating and mechanical engineering. It provides an engineering design and maintenance service to cover scientific and ancillary equipment, and building services, including heating, ventilation and air conditioning. There is also a farm workshop section providing maintenance facilities for a substantial fleet of tractors and agricultural machinery. The Department pro-

Boilerhouse maintenance

vides 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 rapidly changing and wide ranging scientific aims of the Institute ensure that laboratory alterations will always be a part of the Engineering Department's work. With this in mind, services to laboratories must be as flexible and adaptable as possible. Over the last few years systems have been introduced which allow the Department to respond quickly and efficiently when changes are necessary, thus reducing laboratory down times to a minimum. Scientists can now confidently bring new and diverse projects to the Institute knowing that a team is on hand to ensure the facilities will meet whatever requirement they may have.

During 1995 the work of the Engineering and Maintenance Department was affected by the economic restraints placed on Institutes such as SCRI. This resulted in a number of initiatives coming to the fore in an effort to reduce our costs without any detriment to the level of service which the Department provides to the staff.

One consequence of this was that 18 of the pool of 22 Institute road vehicles were sold and one associated post in the Department was lost. An alternative system of hiring as and when needed was put in place, which has been extremely beneficial not only on a financial basis, but also in coping with seasonal demands for particular types of vehicles. Newer vehicles are now available to staff, and the inherent problem of finding the resources to replace vehicles at irregular intervals is no longer necessary. This allows more streamlined and predictable budgeting. Canteen services were also 'contracted-out', which resulted in a major refurbishment to the kitchen and counter area.

In previous years capital works programs provided the means for the Institute to refurbish and modernise its older facilities. In 1995 no such funding was available, and in an effort to continue the improvements made in recent years, the Buildings, Equipment and Maintenance Committee was formed under the chairmanship of Dr J M Duncan. The Committee focuses a small amount of funding, set aside from core grant, for refurbishments and upgrades to the areas of science where such investment would provide most benefit to the Institute as a whole. Works carried out via funding from this Committee include the establishment of a central media facility; extensions to the confocal microscope area; the formation of an isolated ultra-violet transilluminator facility within Crop Genetics; installation of air-conditioning equipment within a mass spectroscopy laboratory; and alterations to a glasshouse cubicle to allow accommodation of virus-tested mother stocks.

To continue the improvements to glasshouse facilities, which have been carried out through capital works in the past, a small team from within the Engineering and Maintenance Department was formed to refurbish individual glasshouse compartments. This prevents such areas from falling into disrepair, or in some cases below licencing authority levels. To date, work within the Virology, Nematology and Propagation Glasshouses has been carried out.

The Department is also responsible for negotiating utility contracts with electricity, gas, water and telephone companies, and economies have been gained in these areas through reducing tariffs and lowering consumption where possible.

A number of external service contracts have also been discontinued, or the cover provided reduced. In-house maintenance cover has been extended to counter-balance such measures and to minimise any reduction in the service provided to staff.

The Department also has responsibility for maintaining and extending the cabling associated with the Institutes Local Area Network, and key staff have been trained to install and test cables to Category 5 standards. Over the past year a number of areas in outlying buildings have been incorporated into the Network, all to a Category 5 level.

In addition to this, there is an on-going replacement program to upgrade the original thin Ethernet cabling which still serves the majority of Institute buildings. Retaining the ability to carry out such work by our own staff has two main benefits to the Institute in that it reduces costs, and results in a broader in-house knowledge of the Network infrastructure.

Scottish Society for Crop Research

D.L. Hood

The Scottish Society for Crop Research is a registered Friendly Society formed in 1981 by the amalgamation of the Scottish Society for Research in Plant Breeding and the Scottish Horticultural Research Association. It provides a link between SCRI and farmers, processors and other interested bodies by organising meetings for the exchange of information between members and staff of the Institute. It sponsors occasional publications and provides financial assistance to staff for travel and other activities. It is open to membership by any interested person or corporate body on application to the Secretary and it is controlled by a Chairman and Committee of Management. Several crop-orientated sub-committees maintain contact with members on specialised topics relevant to their interests. Membership of the Society was 326 on 31 December 1995.

The AGM of the Society was held on 24 April when Mr A.B.N. Gill, Deputy President of the National Farmers' Union addressed the members, his chosen topic being, "British Agriculture - Perspectives for 2000 and Beyond".

Members of the Society discuss the latest thornless blackberry with Mr R.J. McNicol (r.), Head of Soft Fruit and Perennial Crops Department.

The Committee of Management met on two occasions (24 April and 6 November).

Travel Grants were awarded to:-

Miss H. E. Stewart, Crop Genetics Department to Dublin.

Dr W. H. Macfarlane Smith, Scientific Liaison and Information Services Department, to Cambridge.

Dr R. Brennan, Soft Fruit and Perennial Crops Department, to California.

Dr R. Waugh, Cell and Molecular Genetics Department, to California.

A Cereal Walk was held on Monday 10 July when those attending were addressed by Mr H. Phillips, of Scottish Agronomy and members of Institute staff.

A Soft Fruit Walk was held on Thursday 27 July when updates were given on various aspects of current research.

Crop Sub-Committees were active for Soft Fruit, Potatoes, and Cereals, holding several meetings throughout the year, bringing forward topics for discussion and research.

The Society continues to fund Spring and Winter Barley Trials with the results illustrating the suitability of some varieties for the Scottish environment.

Officers of the Society

Trustees:

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Vice-Chairman: Dr D. A. S. Cranstoun, Corehouse, Lanark ML11 9TN

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Registered Office:

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Mylnefield Research Services Ltd

N.W. Kerby

Since 1989, Mylnefield Research Services (MRS) Ltd has continued to grow both financially and structurally (Fig. 1). Incorporated primarily to exploit commercially the resources and expertise of SCRI, MRS Ltd now undertakes its own near-market research and development. Particular emphasis is placed on product process development, together with the selection and release of new and improved plant cultivars. MRS Ltd promotes the contribution of science and technology to wealth creation and the quality of life.

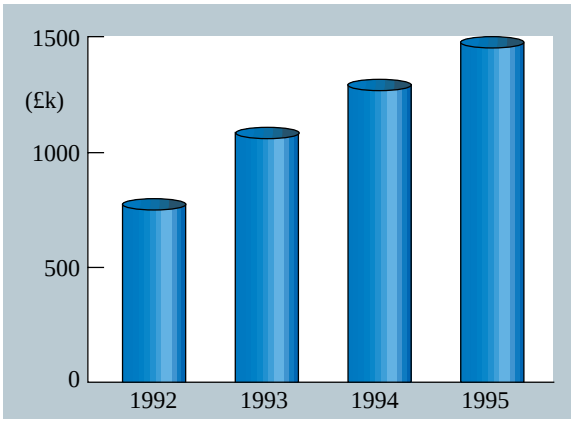

Figure 1 MRS Ltd income.

In partnership, MRS Ltd and SCRI have built commercialisation into research programmes. Marketing the unique range of skills and services at SCRI requires the identification of both potential opportunities and suitable partners who are committed to converting science and technology into marketable products and processes.

During 1995, MRS Ltd played a key role in financially supporting scientific research at the Institute. As in previous years, MRS Ltd gratefully acknowledges the valuable and essential contributions made by SCRI staff.

Financial

31 March 1995 saw the close of our fourth financial year. Turnover was in excess of £1.48 million, representing a 14% increase on the previous financial year. Turnover increased for the fourth successive year (Fig. 1). A summary of income is shown in Figure 2 and is dominated by contract research income. Gross profit was 35% of turnover in 1995, compared with 28% in 1994.

The financial contribution to SCRI, in addition to intercompany purchases for services provided by the Institute, included a gift aid of £72,000, management fee of £105,000, provision for the Mylnefield Research Fellowship (see below) and a payment of £127,000 towards the costs of the unique Nuclear Magnetic Resonance (NMR) imaging facility.

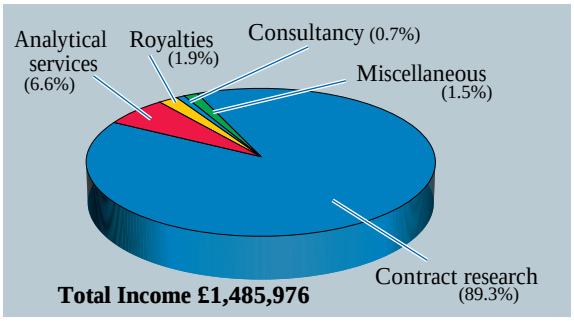

Figure 2 Analysis of MRS Ltd income 1994-95.

External Contracts

During 1995, competitively awarded external contracts in excess of £2.6 million were signed, thereby demonstrating the high level of activity and achievement of SCRI scientific staff. Sponsors included UK Government Departments (excluding SOAEFD), Research Councils, Charitable Trusts, European Union, Levy Boards and the private sector. Notably, significant contracts with the Biotechnology and

Company Aim

The primary objective of MRS Ltd is to exploit commercially the scientific expertise and resources of SCRI while protecting its charitable status and intellectual property.

MRS Ltd acts as the gateway to a variety of skills unique within the UK biological, agricultural and horticultural research service, ranging from fundamental studies on genetics, molecular biology and physiology, through agronomy and pathology to glasshouse and field trials. MRS Ltd is in a position to offer a wide variety of services which typically utilise the unique range of modern facilities and resources resident at SCRI.

Biological Sciences Research Council (BBSRC) were awarded following competitive tendering.

Applications under the following BBSRC schemes were successful: Wealth-Creating Products of Plants Initiative (£259,692); Seed Corn Initiative in Biological Chemistry (£38,200), and Plant and Animal Genome Analysis (£241,600). We were also a partner on a Realising Our Potential Awards (ROPA) scheme with Durham University, as SCRI is currently unable to apply directly to this Scheme. MRS Ltd looks forward to Government research and development funding being open to competitive tender by any competent body, in keeping with the 1993 White paper on Science and Technology (*Realising Our Potential - A Strategy for Science, Engineering and Technology*, Cm 2250).

We also welcomed a new strawberry breeding initiative, which is being funded by Pernod Ricard, one of Europe's biggest growers and processors of strawberries.

Licence Agreements Concluded During 1995

ADGEN DIAGNOSTIC SYSTEMS for the distribution and marketing of antibodies to plant pathogens.

AUSTRALIAN *RUBUS* GROWERS' ASSOCIATION for the marketing of raspberry and blackberry varieties in Australia.

Mylnefield Research Fellowship

Dr Frederick Gildow of the Department of Plant Pathology, Pennsylvania State University, was awarded the first Mylnefield Research Fellowship in February 1995. The Fellowship was created to support research by visiting scientists in innovative areas of agricultural research. Dr Gildow is one of the leading authorities on transmission of plant viruses by insects. Dr Gildow joined Dr Mike Mayo and colleagues for a period of 10 months to research molecular determinants of aphid transmission of potato leafroll luteoviruses. *In vitro* cell systems were utilised to study the effect of capsid protein mutations on virion recognition and transport by aphid tissues.

Intellectual Property

The 1993 Science White paper emphasised the importance of wealth creation and technology transfer. Intellectual property (IP) and "know-how" are at the heart of successful technology transfer. MRS Ltd places particular importance on identifying, managing and exploiting IP. SCRI has embraced the need for wealth creation, and significant numbers of the staff are actively seeking protection for their innovative research (see below). During 1995, six first-filing of inventions, and one full Patent Cooperation Treaty (PCT) application were made. MRS Ltd and SCRI inventors, and a brief description of each application are detailed below:

•D.E.L. COOKE, J.M. DUNCAN, A. DOLAN A method for detection and identification of fungi in culture, plant material, soil, propagation media or water, using oligonucleotide primers to amplify or hybridise to fungal nucleic acid. The invention has particular application for members of the order Peronosporales (*Phytophthora*, *Pythium* and *Peronospora*) which are some of the most important and damaging fungal pathogens of plants.

•W.H. MACFARLANE SMITH A method which reliably demonstrates an allergic or irritant response in humans resulting from exposure to substances emitted by plants, especially oilseed rape (*Brassica napus* ssp *oleifera*).

•K.J. OPARKA AND D.A.M. PRIOR This invention relates to a design for a novel injector-head for a micro-injection system. The system introduces foreign material into single living cells or cell complexes with minimal damage. The invention will have an impact on the techniques of iontophoretic and pressure micro-injections of charged or non-charged material into plant or animal cells. Uniquely, the apparatus can monitor turgor and the membrane potential of the target cells.

•J. PONTES, R.J. MCNICOL, N.W. KERBY A method for treating organic tissue, particularly soft fruit, which helps preserve the structure following freezing and thawing.

•S.P. SANTA CRUZ, S.N. CHAPMAN, T.M.A. WILSON, K.J. OPARKA A method of producing proteins and polypeptides in plants using chimeric plant virus particles. Such proteins and polypeptides "overcoat" the virus coat proteins, facilitating their purification.

•M.A. TAYLOR, H.V. DAVIES A sequence of a potato cDNA clone that encodes an α-glucosidase has been filed. The gene may be used in plant biotechnology and plant genetic engineering to produce novel starches, and to manipulate starch-sugar conversions in transgenic plants by changing the cellular activity of α-glucosidase.

Product and Service Development

SMART Fruit

MRS Ltd, through its SMART Stage II award, successfully developed a technique which dramatically improves the quality of frozen soft fruit using a treat-

ment involving natural gels and gelling agents. Consequences of the process are retention of structure, reduced thawing time and low weight loss following thawing.

We believe that our treated frozen soft fruit satisfy a demand in a variety of different products:
•confectionary
•gateaux
•ice cream
•yogurt
•conserves

As the freeze-thawed fruit will more closely resemble fresh produce, we feel confident that the demand for frozen soft fruit, and products in which they are found, will increase.

Micro-injection System

A micro-injection system developed by K.J. Oparka and D.A.M. Prior was tested in a number of laboratories for a variety of applications including micro-injection of plant and animal cells. Our micro-injection system has several unique advantages over those of competitors in a number of ways: pressure can be monitored during micro-injection thus avoiding "over-pressurising" cells and their eventual destruction; injections can be facilitated using electrical injections with the same injection probe; measurement of turgor and membrane potential of cells can be achieved. A prototype was built by Gaeltec Ltd of Skye, and we are currently investigating marketing of units that will be manufactured under licence.

The Living Plant Cell

The Living Plant Cell, produced by K.J. Oparka, is a unique teaching video containing dynamic images of living cells and their organelles, designed to accompany basic plant-cell biology courses. State-of-the-art video microscopy was used to reveal the structure of cells in a highly visual way that students would not necessarily see by reference to standard texts and diagrams, e.g. the movement of functional mitochondria and endoplasmic reticulum are shown within single living plant cells.

The Living Plant Cell has been extensively marketed by MRS Ltd to UK schools and universities and has proved to be very popular. It was favourably reviewed in educational journals and it is hoped that excerpts will be broadcast on educational television.

The Lipid Analysis Unit

MRS Ltd set up a Lipid Analysis Unit based at SCRI in October 1995 through an amalgamation of the Lipid Units of SCRI and the University of St Andrews. The Lipid Analysis Unit provides analyses ranging from single samples to small research projects, a comprehensive range of lipid expertise, and an extensive range of analytical facilities including:
•gas chromatography
•high-performance liquid chromatography
•mass spectrometry

We are extremely grateful to Professor F.D. Gunstone, formerly of the University of St Andrews and now an MRS Ltd Consultant, and W.W. Christie, G. Dobson, and C. Scrimgeour of SCRI, for their advice, expertise and continuing commitment to making a success of this unit.

Employees

We welcomed the following MRS Ltd scientific staff who were appointed during 1995 on various external contracts: A. Adams, P. Davie, P. Dobson, R. Forrest, P. Iannetta, C. Jones, J. Lyon, S. Mitchell, F. McMahon, C. Reid, L. Surplus.

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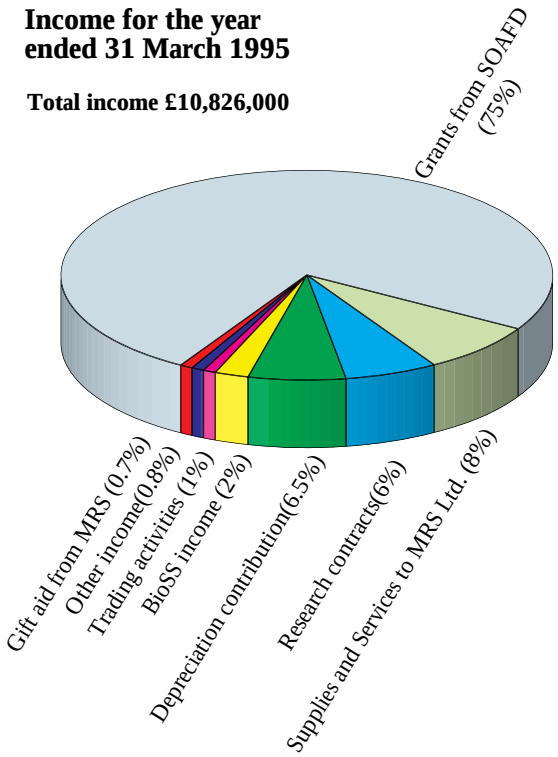
Summary of the Accounts

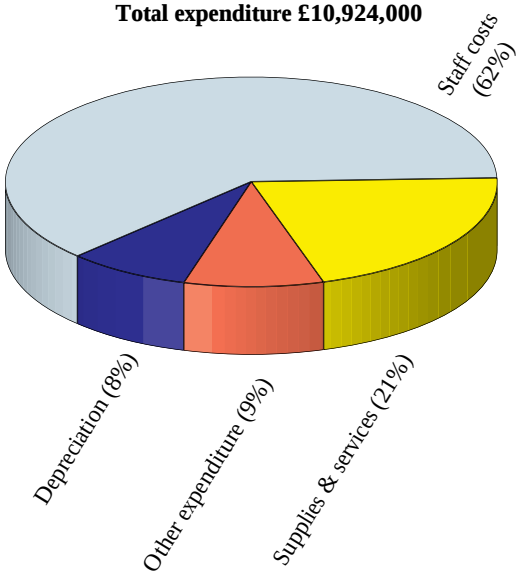
Income for the year ended 31 March 1995

Total income £10,826,000



Expenditure for the year ended 31 March 1995

Total expenditure £10,924,000



Balance sheet at 31 March 1994

Total value £12,561,000

Assets

Fixed assets	94 %
Stocks	1 %
Debtors	5 %

Liabilities

Capital reserve	89 %
Income & expenditure account	2 %
Current liabilities	9 %

The Governing Body

Back row, l to r: A.M. Jacobsen; J.E. Godfrey; T.P.M. Thomson; A.N. MacCallum; J.B. Forrest; A. Logan; G. Rennie
Front row, l to r: J.D. Hayes; Heather M. Dick; J.L. Millar; J.R. Hillman (Director)
(Absent: J.A. Inverarity; D.L. Lee; T.A. Mansfield; J.A. Raven; A.R. Slabas)

Chairman: J.L. Millar, C.B.E., C.A., joined William Low & Co in 1958, took a place on the board six years later, and was Chief Executive from 1980, and Chairman from 1990-94. He was appointed Chairman of Invergordon Distillers Ltd in 1990, Chairman of the Scottish Food Strategy Group in 1993, a non-executive Director of Clyde Port Authority in 1994, and Chairman of Vision Group plc and of W.E.W. Group plc in 1995. He joined the Governing Body of SCRI in 1989, and has been Chairman since 1990. He was awarded the C.B.E. in 1987.

Professor Heather M. Dick, M.D., F.R.C.P.Glas., F.R.C.Path., C.Biol., F.I.Biol., F.R.S.E., studied at Queen's College, Dundee (St Andrews University). She was Professor of Medical Microbiology, University of Dundee, from 1984 to 1996, and Visiting Professor (Immunology) at the University of Strathclyde from 1981. She was Lecturer in the Department of Bacteriology at the University of Glasgow from 1964-71, and Consultant in Clinical Immunology at Glasgow Royal Infirmary from 1971-84. She joined the Governing Body of SCRI in 1992.

J.B. Forrest, F.R.Ag.S., farms at Whitmire, Duns, specialising in malting barley, milling wheat and oats, and pedigree cattle and sheep. He was Vice-Chairman of the National Seed Development Organisation (NSDO) from 1982 until its privatization, and Vice President of the Scottish National Farmers Union (NFU) in 1981. He has been Chairman of British Cereal Exports since 1994, and is a local Director of the NFU Mutual Insurance, and Farms Advisor to the University of Newcastle. He was a Nuffield Scholar in 1980. He joined the Governing Body of SCRI in 1983.

J.E. Godfrey, B.Sc., A.R.Ag.S, gained his degree in agriculture from the University of Reading, and is Director of family companies farming 3400 ha and 3500 sows in Humberside and Lincolnshire. A former Chairman and Vice-Chairman of the Potato Marketing Board, he is Chairman, Member or Adviser to numerous agricultural Committees, including the Bishop Burton Agricultural College farm; the Centre of Agricultural Strategy, University of Reading; The Royal Agricultural Society of England; and the House of Lords Rural Economy Group. He joined the Governing Body of SCRI in 1992.

Professor J.D. Hayes, B.Sc., M.S., Ph.D., C.Biol., F.I.Biol., has a wide experience of the scientific investigation of crop plants. He was in charge of the breeding and research of arable crops at the Welsh Plant Breeding Station. He gained experience of scientific administration while acting as Scientific Adviser in Plant Breeding and Genetics to the Secretary of the Agricultural Research Council, before his appointment as Professor of Agriculture at the University of Wales at Aberystwyth. He has served on Visiting Groups, a range of Scientific Committees, and Boards, including the NSDO, and the United Kingdom Seeds Executive, and published papers on several different aspects of crop improvement. He joined the Governing Body of SCRI in 1986.

J.A. Inverarity, O.B.E., C.A., F.R.Ag.S., F.R.S.A., farms 500 ha to the west of Dundee, and has held senior positions within numerous Committees, including President, Scottish NFU 1970-71; Governor East of Scotland College of Agriculture 1974-90; Director of Scottish Agricultural Securities Corporation plc since 1983, and Chairman since 1987; Director United Oilseeds Producers Ltd since 1985, and Chairman since 1987; Chairman of SAC since 1990; and President of the Scottish Association of Agriculture since 1990. He joined the Governing Body of SCRI in 1984, and was Chairman from 1989-90. He was awarded the O.B.E. in 1989.

A.M. Jacobsen, B.Sc. Agric., farms at Inverbervie, and has a keen interest in brassicas, especially swedes. As a specialist cereal seed producer, he also has a particular interest in cereal breeding. He was Chairman of the Scottish Society for Crop Research from 1993 to 1995, and is on the Aberdeen and Kincardine Area Executive for the NFU. He joined the Governing Body of SCRI in 1992.

Professor D.L. Lee, B.Sc., Ph.D., C.Biol., F.I.Biol., F.Z.S., F.R.S.A., studied Zoology at King's College (Newcastle), University of Durham, and was awarded his PhD by the University of Cambridge for research on parasitic nematodes. He was awarded the Scientific Medal by the Zoological Society of London in 1971 for research on nematodes. He is currently Professor of Agricultural Zoology at the University of Leeds, where he has recently completed a period as Pro-Vice-Chancellor. He is an Independent Member of the UK Advisory Committtee on Pesticides; a Member of the Grants & Education Sub-Committee, Yorkshire Agricultural Society; and is a Director of University of Leeds Farms Ltd, and Mylnefield Research Services Ltd. He was a member of the Governing Bodies of SHRI and SCRI from 1974-82, and was reappointed in 1986. He is a former Governor of the National Vegetable Research Station (now HRI Wellesbourne).

A. Logan, S.D.A., N.D.A., F.I.Hort., studied at the Edinburgh School of Agriculture, and farms in Cupar, Fife. A former Chairman of the Soft Fruit and Field Vegetable Committee of the Scottish NFU; Director of the Scottish Nuclear Stock Association; and Member of the Horticultural Development Council, he is Chairman of Dorward Gray Ltd, and has been Chairman, Director, or Member of numerous agricultural committees, including East of Scotland Growers; the Scottish Agricultural Development Council; the Home Grown Cereals Authority R&D Committee; the Scottish Agricultural Research and Development Advisory Council; and the Scottish Agricultural Organisation Society. He was a Governor of the National Vegetable Research Station from 1977-87. He joined the Governing Body of SCRI in 1986.

A.N. MacCallum, B.Sc., is Group Chief Executive of Don & Low (Holdings) Ltd, Forfar, Industrial Textile Manufacturers, a position he has held since 1986. He graduated from Glasgow University with a degree in Chemistry. Prior to his appointment at Don & Low, he held management positions with Baxters of Fochabers; Devro Ltd of Glasgow; Guard Bridge Paper Company in St Andrews; and Unilever. He was Chairman of CBI Scotland from 1991-93. He currently holds directorships with a number of companies, including the newly formed North Water Authority, and is Chairman of Montrose Harbour Board. He joined the Governing Body of SCRI in 1995.

Professor T.A. Mansfield, Ph.D., C.Biol., F.I.Biol., F.R.S., studied Botany at Nottingham, and was awarded his PhD for research in plant physiology by the University of Reading. He joined the University of Lancaster in 1965, and has held the posts of Head of Department of Biological Sciences and Faculty Dean, and is currently Provost of Science and Engineering. He manages a large research group covering aspects of environmental plant physiology. He was a member of the Council of the AFRC for 4 years, and is now Chairman of the programme *Biological Adaption to Global Environment Change* (BBSRC). He has also served on senior committees for NERC, and has been a member of the Council of the Royal Society. He joined the Governing Body of SCRI in 1989.

Professor J.A. Raven, M.A., Ph.D., Ph.D.h.c., F.R.S.E., F.R.S., was awarded his Masters and Doctorate by the University of Cambridge, and also holds an Honorary Doctorate from the University of Umeå. He is John Boyd Baxter Professor of Biology at the University of Dundee, where he and his colleagues work on resource acquisition, manipulation and protection in photosynthetic organisms, and the impact of past, present and future environments of these processes. He holds senior positions within several organisations, including President of the British Phycological Society, and Vice-President and President-Elect of the Society for Experimental Biology, and is a member of the Council of the Marine Biological Association of the United Kingdom; the Scientific Advisory Committee of the Royal Botanic Garden, Edinburgh; the Advisory Committee on Sites of Special Scientific Interest, Scottish Natural Heritage; and the UVB Measurements and Impacts Review Group, Department of the Environment. He joined the Governing Body of SCRI in 1989.

G. Rennie, O.N.D. Agric., studied at the North of Scotland College of Agriculture. He was awarded a Nuffield Scholarship to visit Australia and New Zealand in 1992, and has published a report "Farming without subsidies" (*44th Agricultural Management Course*, Wye College, 1994). He farms 280 ha at St Monans, Fife, and is agronomist for a further 1,600 ha in Fife. He was Chairman of the Berwickshire Arable Study Group from 1984-89, and is currently a member of the Management Committee of the Scottish Society for Crop Research (SSCR), and Chairman of the SSCR Cereals Sub-Committee. He held the World Record in 1981for the highest recorded wheat yield (13.99 t/ha) and the World Record in 1989 for the highest recorded barley yield (12.2 t/ha). He joined the Governing Body of SCRI in 1992.

Professor A.R. Slabas, B.Sc., D.Phil., is Director of Research, Department of Biological Sciences, University of Durham, where he currently leads a group of 20 involved in various aspects of plant lipid metabolism. He has extensive collaborations with industry, including Monsanto, Unilever, and Nickerson Biocem. He is a member of the UK Technology Foresight Programme Panel Committee on Health and Life Sciences; the Agricultural Systems Directorate Management Committee; the Eukaryotic Cell Link Management Committee; and the BBSRC Innovative Manufacturing Committee. He joined the Governing Body of SCRI in 1995.

T.P.M. Thomson, M.A., trained as a physicist and mathematician. He is Director of Thomas Thomson (Blairgowrie) Ltd, soft fruit growers of 40 ha of raspberries, strawberries and other soft fruit for fresh retail marketing and processing. He is Chairman of the SSCR and the Scottish Nuclear Stock Association Ltd, and was formerly Vice-Chairman of the Soft Fruit Panel of the Horticultural Development Council. He is Director of Scottish Soft Fruit Growers Ltd, Kentish Garden Ltd and NSA Plants Ltd. He joined the Governing Body of SCRI in 1992.

Staff list

as at 31 December 1995

Director	Professor J.R. Hillman, B.Sc., Ph.D., DSc., F.L.S., C.Biol., F.I.Biol., F.R.S.E.[1,2,3]	Band 1
Deputy Director	Professor T.M.A. Wilson, B.Sc., Ph.D., C.Biol., M.I. Biol.[2]	Band 2
Secretary & Financial Manager	R.J. Killick, B.Sc., M.B.A., M.A., Ph.D., C.Biol., M.I.Biol.	Band 4
Assistant to Director	T.J.W. Alphey, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 4

Cell & Molecular Genetics Department (CMG)

Head : W. Powell, B.Sc., M.Sc., Ph.D., D.Sc.[4,5]	Band 3
J.W.S. Brown, B.Sc., Ph.D.[6]	Band 4
R. Ellis, B.Sc., Ph.D.[6]	Band 4
B.P. Forster, B.Sc., Ph.D.[6]	Band 4
W.T.B. Thomas, B.Sc., Ph.D.	Band 4
R. Waugh, B.Sc., Ph.D.[6]	Band 4
A. Kumar, B.Sc., Ph.D.	Band 5
G.C. Machray, B.Sc., Ph.D.	Band 5
J.S. Swanston, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 5
C.G. Simpson, B.Sc.	Band 6
A. Young	Band 6
E. Baird, H.N.C., B.Sc.	Band 7
Gillian Clark, H.N.C.	Band 7
Jackie Lyon	Band 7
G.R. Young, H.N.C.	Band 7
Nicky Bonar, H.N.C.	Band 8
A. Booth, H.N.C.	Band 8
Diane Davidson	Band 8
R. Keith	Band 8
Jennifer Watters, H.N.D.	Band 8 (P/T)
M. Macaulay, H.N.C., B.Sc.	Band 8
A. Wilson	Band 8
Alice Bertie	Band 10
J.D. Fuller	Band 10
Patricia E. Lawrence	Band 10
Joyce I. Young	Band 10

Cellular & Environmental Physiology Department (CEP)

Head : H.V. Davies, B.Sc., Ph.D.[5]	Band 3
K.J. Oparka, B.Sc., Ph.D.[5]	Band 3 (IMP)
B. Boag, B.Sc., Ph.D.	Band 4
J.W. Crawford, B.Sc., Ph.D.[7]	Band 4 (Prom. Apr)
J.M.S. Forrest, B.Sc., Ph.D.	Band 4
B.S. Griffiths, B.Sc., Ph.D.	Band 4
Linda L. Handley, B.A., B.Ed., M.Sc., Ph.D.	Band 4
D.K.L. MacKerron, B.Sc., Ph.D.	Band 4
B. Marshall, B.Sc., A.R.C.S., Ph.D.[7]	Band 4
I.M. Morrison, B.Sc., Ph.D.[6]	Band 4
K. Ritz, B.Sc., Ph.D.	Band 4 (Prom. Apr)
D. Robinson, B.Sc., Ph.D.[6]	Band 4
G.R. Squire, B.A., Ph.D.	Band 4
A.G. Bengough, B.Sc., Ph.D.	Band 5
R.A. Jefferies, B.Sc., Ph.D.	Band 5
G.J. McDougall, B.Sc., Ph.D.	Band 5
M. Taylor, B.Sc., Ph.D.	Band 5
R. Viola, B.Sc., Ph.D.	Band 5
R.E. Wheatley, B.Sc., Ph.D.	Band 5
I. Young, B.Sc., Ph.D.	Band 5
G. Goleniewski, B.Sc., Ph.D.	Band 6
D.C. Gordon, H.N.C.	Band 6
J. Liu, B.Sc., M.Sc., Ph.D.	Band 6
Heather A. Ross, H.N.C., Ph.D., C.Biol., M.I. Biol.	Band 6
D. Stewart, B.Sc., Ph.D.	Band 6
Kathryn M. Wright, M.A., Ph.D.	Band 6
Sandra Caul, H.N.C.	Band 7
R. Neilson, H.N.C., M.Sc.	Band 7
D.A.M. Prior, H.N.C.	Band 7
Susan Verrall, H.N.C.	Band 7
Gladys Wright, H.N.C.	Band 7
D. Crabb	Band 8
G. Dunlop, O.N.C.	Band 8
Margaret Garland	Band 8
Lesley George	Band 8
Diane McRae	Band 8
Julie A. Duncan	Band10 (P/T)
A.T. Hall, B.Sc.	Band10 (Appt. Nov) (P/T)

Crop Genetics Department (CG)

Head : G.R. Mackay, B.Sc., M.Sc., C.Biol., F.I.Biol.[4,5]	Band 3
J.E. Bradshaw, M.A., M.Sc., Ph.D.[6]	Band 4
M.F.B. Dale, B.Sc., Ph.D.[6]	Band 4
I. Chapman, B.Sc.	Band 5
M.J. De,Maine, B.Sc., M.Phil.	Band 5
S. Millam, B.Sc., Ph.D.	Band 5
G. Ramsay, B.Sc., Ph.D.	Band 5
Ruth M. Solomon-Blackburn, B.A., M.Sc.	Band 6
K. Harding, B.Sc., Ph.D.	Band 6
Alison K. Lees, B.Sc., Ph.D.	Band 6 (Appt. Oct)
Helen E. Stewart, C.Biol., M.I.Biol.	Band 6
Jill Middlefell-Williams, H.N.C.	Band 7
G.E.L. Swan	Band 7
D. Todd, B.Sc.	Band 7
R.N. Wilson, N.C.H.	Band 7
Eva Bennett	Band 8
Norma Dow	Band 8
Jane McNicoll, H.N.C., B.Sc.	Band 8
M.P.L. Campbell	Band 8
Sharon Dubbels	Band 9 (Appt. Nov)
Marjorie Grant, H.N.D.	Band 9 (Appt.Jul)
R.J. Milligan, H.N.D.	Band 9
A. Margaret McInroy	Band10
Moira Myles	Band10
Gail Simpson	Band10
Iain J. Young, B.Sc.	Band10 (Appt. Oct)

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

Chemistry Department (Chem)

Head : W.W. Christie, B.Sc., Ph.D., D.Sc., C.Chem., F.R.S.C.	Band 3
B.A. Goodman, B.Sc., Ph.D., C.Chem., F.R.S.C.[6]	Band 4
D.W. Griffiths, M.A., Ph.D, C. Chem., M.R.S.C.	Band 5
G.W. Robertson, B.Sc., C.Chem., M.R.S.C.	Band 5
C.M. Scrimgeour, B.Sc., Ph.D.[6]	Band 5
H. Bain, H.N.C., L.R.S.C.	Band 6
Winifred M. Stein, H.N.C., B.Sc.	Band 6
K. Taylor, H.N.C., B.Sc.	Band 7
Fiona Falconer, H.N.C.	Band 8
Jean Wilkie	Band 10
Quality Assurance Officer : T. Shepherd, B.Sc., Ph.D.	Band 6

Fungal and Bacterial Plant Pathology Department (FBPP)

Head : J.M. Duncan, B.Sc., Ph.D.[5]	Band 3
G.D. Lyon, B.Sc., M.Sc., Ph.D., D.I.C.[6]	Band 4
A.C. Newton, B.Sc., Ph.D.[6]	Band 4
P. Birch, B.Sc., Ph.D.	Band 6 (Appt. Aug)
D. Cooke, B. Sc., Ph.D.	Band 6 (Appt.Feb)
Lizbeth J. Hyman, B.A., M.Sc.	Band 6
R. Lowe	Band 6
G. Thow, B.Sc., Ph.D.	Band 6 (Appt. Mar)
I. Toth, B.Sc., Ph.D.	Band 6 (Appt. Feb)
Jacqueline Heilbronn, H.N.C.	Band 7
D.J. Johnston, B.Sc., PhD.	Band 7 (On sabbatical)
Naomi A. Williams, H.N.C.	Band 7
D.C. Guy, H.N.D.	Band 8
Evelyn Warden	Band 10

Nematology Department (Nem)

Head : D.L. Trudgill, B.Sc., Ph.D., C.Biol., F.I.Biol.[5]	Band 3
M.S. Phillips, B.Sc.	Band 4
W.M. Robertson, N.H.C., F.L.S.	Band 4
Vivian Blok, B.Sc., M.Sc., Ph.D.	Band 6
J.T. Jones, B.Sc., Ph.D.	Band 6
B. Harrower, H.N.D., B.Sc.	Band 7
Ailsa Smith, B.Sc.	Band 7
Anne M. Holt	Band 8 (P/T)
A. Paterson	Band 10 (P/T)

Soft Fruit & Perennial Crops Department (SFPC)

Head : R.J. McNicol, B.Sc.[5]	Band 3
A.T. Jones, B.Sc., Ph.D.[5]	Band 3 (IMP)
B. Williamson, B.Sc., M.Sc., Ph.D., D.Sc.[6]	Band 4
R.M. Brennan, B.Sc., Ph.D.	Band 5
A.N.E. Birch, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 5
S.C. Gordon, H.N.C.	Band 5
B. Fenton, B.Sc., Ph.D.	Band 6
Julie Graham, B.Sc., Ph.D.	Band 6
Wendy J. McGavin, B.Sc.	Band 7
Gaynor Malloch, D.C.R., B.Sc.	Band 7
Sandra L. Gordon, H.N.C.	Band 8
Kay Greig, Dip. H.E.	Band 8
Linzi M. Ross	Band 10
Departmental Administrator : Maureen Murray	Band 8

Virology Department (Vir)

Acting Head : Lesley Torrance, B.Sc., Ph.D.[6]	Band 4
M.A. Mayo, B.Sc., Ph.D., C.Biol., M.I.Biol.[5]	Band 3 (IMP)
H. Barker, B.Sc., Ph.D.	Band 4
D.J.F. Brown, B.A., Ph.D., C.Biol., M.I. Biol., F.R.S.N.R.A.S.	Band 4
I.M. Roberts, H.N.C., Dip.R.M.S.	Band 4
D.J. Robinson, M.A., Ph.D.[6]	Band 4
J.A.T. Woodford, M.A., Ph.D.[6]	Band 4
G.H. Duncan, H.N.C.	Band 5
S.A. MacFarlane, B.Sc., D.Phil.	Band 5 (Prom. Apr)
B. Reavy, B.Sc., D.Phil.	Band 5
S. Santa Cruz, B.Sc., Ph.D.	Band 6 (Appt. May)
Maud M. Swanson, B.Sc., Ph.D.	Band 6
G.H. Cowan, H.N.D.	Band 7
Sheila M.S. Dawson, H.C.	Band 7
Kara D. Webster, H.N.C.	Band 7
Fiona Carr	Band 8 (P/T)
Gillian L. Fraser	Band 8
Sheena S. Lamond	Band 8
Wendy Ridley	Band 8

Scientific Liaison & Information Services Department (SLIS)

Head : W.H. Macfarlane Smith, B.Sc., Ph.D., C.Biol., M.I.Biol.	Band 4
R.J. Clark, B.A., M.B.C.S.	Band 5
T. G. Geoghegan, A.B.I.P.P., A.M.P.A.	Band 5
R. Kidger, B.Sc.	Band 5
T.D. Heilbronn, B.Sc., M.Sc.	Band 6
I.R. Pitkethly, H.N.D.	Band 6
P. Smith, B.Sc.	Band 6
Sarah E. Stephens, B.Sc., M.A., A.L.A.	Band 6
I. Black, H.N.C.	Band 7
S. Clark, H.N.C.	Band 7
S.F. Malecki, A.B.I.P.P.	Band 7
Ursula M. McKean, M.A., Dip. Lib.	Band 7
G. Menzies	Band 7
Barbara V. Gunn	Band 10
Janette Keith	Band 11 (P/T)
Safety Coordinator : Kathryn M. Wright, M.A., Ph.D.	Band 6

Administration Department (Admin)

Secretary & Financial Manager : R.J. Killick, B.Sc., M.B.A., M.A., Ph.D., C.Biol., M.I.Biol.	Band 4
Financial Controller : R.R. Boath, C.A.	Band 4 (Appt. Oct)
Accountant : S.L. Howie, C.A.	Band 5
Assistant Secretary : D.L. Hood, B.Admin., Dip. Ed., L.T.I., A.I.I.M.	Band 6
Personnel Officer : I. Paxton, H.N.C., M.Sc., M.I.P.D.	Band 6
European Liaison Officer : Joan Duffin, B.Sc., P.G.C.E., M.B.A., Dipl. Mngt.	Band 6 (Appt. Sep)
Freida F. Soutar	Band 6
Loraine Galloway	Band 7
Catherine Skelly	Band 7
Anne Pack	Band 8 (Appt. Sep)
Elizabeth L. Stewart	Band 8
Margaret Barnes	Band 9
Dianne L. Beharrie, Dip. Ed.	Band 9 (P/T)
Maureen E. Campbell	Band 9
Rhona G. Davidson	Band 9
Pam Duncan	Band 9
Kristy L. Grant, B.A.	Band 9 (Tr. Nov)
Wendy A. Patterson, H.N.D.	Band 9
Sarah-Jane Simms, H.N.D.	Band 9
Joyce Davidson	Band 10
Sheena Forsyth	Band 10
Elizabeth J. Fyffe	Band 10

Engineering & Maintenance Department (EM)

Institute Engineer : S. Petrie, B.Sc.	Band 5
D. Gray, H.N.C.	Band 6
A. Low	Band 7
K. Low	Band 7
I.C. McNaughton, H.N.C.	Band 8
G.C. Roberts	Band 8
I.M. Scrimgeour	Band 8
R. White	Band 8
J. Anderson	Band 9
D. Byrne	Band 9
W. J. Downes	Band 9
K. Henry	Band 9
E. Lawrence	Band 9
R.D. McLean	Band 9
C.G. Milne	Band 9
R. Pugh	Band 9
J. Flight	Band 10
N. McInroy	Band 10
D.L.K. Robertson	Band 10
J. Rowe	Band 11
J. Oldershaw	Band 11
C. Conejo	Band 11

Estate, Glasshouse & Field Research Department (EGFR)

Head : G. Wood, B.Sc., Ph.D., F.E.T.C.	Band 4 (Appt. Sep)
P.A. Gill, H.N.D.	Band 6
J.R.K. Bennett	Band 7
W.D.J. Jack, B.Sc.	Band 7
B.D. Robertson, N.E.B.S.M., H.N.C., Dip. Mgt., M.B.A.	Band 7
D.S. Petrie	Band 7
A. Grant	Band 8
A.W. Mills	Band 8
R. Ogg	Band 8
D.G. Pugh	Band 8
J.T. Bennett	Band 9
C.R. Dalrymple	Band 9
L.A. McNicoll	Band 9
J. Mason	Band 10
J.K. Wilde	Band 10
G. Dow	Band 10
B. Fleming	Band 10
I. Fleming	Band 10
A.C. Fuller	Band 10
G.S. Lacey	Band 10
C. McCreadie	Band 10
T.A. Mason, N.E.B.S.M.	Band 10
R. Murray	Band 10
Gillian Pugh	Band 10
M.J. Soutar	Band 10
Angela M. Thain	Band 10 (P/T)
Departmental Administrator: Lorna Doig	Band 9(P/T)

Biomathematics and Statistics Scotland (BioSS)

King's Buildings, University of Edinburgh	
Director : R.A. Kempton, M.A., B.Phil. [9]	Band 3
G.J. Gibson, B.Sc., Ph.D.	Band 4
C.A. Glasbey, M.A., Dip. Math. Stats., Ph.D.[9]	Band 4
E.A. Hunter, B.Sc., M.Phil.[9]	Band 4
Janet M. Dickson, B.Sc.	Band 5
D. Hitchcock, B.A., Ph.D.	Band 6
G.W. Horgan, B.A., M.Sc.	Band 5
M. Talbot, F.I.S., M.Phil. [9]	Band 5
F.G. Wright, B.Sc., M.Sc., Ph.D.	Band 5
A.D. Mann, B.Sc.	Band 6
I.M. Nevison, M.A.	Band 6
Muriel A.M. Kirkwood, D.A.	Band 8
Diane Glancy	Band 10 (P/T)
Karyn Linton	Band 9 (P/T)
Amy G. Stewart	Band 10 (P/T)
Secretary : Elizabeth M. Heyburn, M.A.	Band 7
Ayr Unit	
D.A. McNulty, B.Sc., Ph.D.	Band 6
Aberdeen Unit, RRI	
Head : M.F. Franklin, B.Sc., M.Sc., Ph.D. [8]	Band 4
D.J. Hirst, B.Sc., Ph.D.	Band 5
C. J. Harbron, B.Sc.	Band 6
Karen A. Robertson, B.Sc.	Band 7
Aberdeen Unit, MLURI	
Head : D.A. Elston, B.A., M.Sc.	Band 4 (Prom. Sept)
Elizabeth I. Duff, B.Sc.	Band 6 (Prom. Apr)
Dundee Unit	
Head : J.W. McNicol, B.Sc., M.Sc.	Band 4
Christine Hackett, B.A., Dip. Math. Stats., Ph.D.	Band 5 (Prom. Apr)
S.D. Chasalow, B.A., M.A., Ph.D.	Band 6
T. Connolly, B.Sc., Ph.D.	Band 6

Short Term Contracts

SOAEFD Flexible Funding

BioSS

Elizabeth J. Austin, M.A., D.Phil.	Band 6
Maria L.Durban-Reguera, B.Sc., Dip. Maths. Stats.	Band 7 (Appt. Oct)
Verena M. Trenkel, Dipl. Biol., M.Sc.	Band 6
S.A.R. Williams, B.Sc.	Band 7

Cellular and Environmental Physiology

C. Clegg, B.Sc., Ph.D.	Band 6
D. Burn, M.Sc.	Band 7
A.M. Cooper, H.N.D.	Band 7 (Appt. Aug)
N. Ebblewhite, B.Sc.	Band 7
J. Wishart, B.A.	Band 7
Alexandra Holmes, H.N.D., P.G.Dip. Biotech.	Band 10
K. McLean	Band 10

Cell and Molecular Genetics

F. Commerford, B.Sc.	Band 6
A. Ibrahim, B.Sc.	Band 6
J. Provan, B.Sc.	Band 6
C. McQuade	Band 10

Chemistry

N. Deighton, B.Sc., Ph.D.	Band 6
Sheila Glidewell, M.A., M.Sc., Ph.D.	Band 6

Crop Genetics

Elise Flipse, Ir., Ph.D.	Band 6 (Appt. Oct)
M. McEwan, B.Sc., Ph.D.	Band 6 (Appt. Feb)
Sharon McVey, B.Sc., M.S.c	Band 7 (Appt. Apr)
S.A. Tiller, B.Sc.	Band 7 (Appt. Apr)

Fungal and Bacterial Plant Pathology

Francis Gourlay, B.Sc.	Band 7 (Appt. Jul)

Soft Fruit & Perennial Crops

Phil Irving, B.Sc., P.G.Dip.	Band 7 (Appt. Apr)

Nematology

Irene E. Geoghegan	Band 7 (Appt. Dec)

Virology

S. Chapman, B.A., Ph.D.	Band 6
D.A.C. Jones, B.Sc., Ph.D.	Band 6
A. Ziegler, B.Sc., Ph.D.	Band 6
S. Main, B.Sc.	Band 8 (Appt. Apr)

BBSRC (PAGA)

Cell and Molecular Genetics

M. Macaulay, H.N.C., B.Sc.	Band 7 (Appt. Nov)

BBSRC (ROPA)

Chemistry

Samantha Gill, B.Sc.	Band 7

Department of the Environment

Crop Genetics

Yvonne M. Charters, B.Sc.	Band 6
A. Robertson	Band 7

Virology

Livia Dyckhoff, B.Sc.	Band 7

CEC

Cellular and Environmental Physiology

Susan Jarvis, B.Sc., Ph.D.	Band 6
M.R. MacLeod, B.Sc., Ph.D.	Band 6
Sigrun Holdhus, Cand. mag.	Band 7
Paula M. Hebden, B.Sc.	Band 8

Chemistry

G. Dobson, B.Sc., Ph.D.	Band 6
I.S. Begley, B.Sc., Ph.D.	Band 7

Cell and Molecular Genetics

Angela Collins, B.Sc.	Band 6 (Appt. Nov)
Joanne Russell, B.Sc., Ph.D.	Band 6 (P/T)
H. Dewar, B.Sc.	Band 7 (Appt. Nov)

Nematology

M. Armstrong, B.Sc., M.Sc.	Band 7
Jean Harkins	Band 10 (Appt. Jul) (P/T)
A. Stevenson, B.Sc.	Band 10 (Appt. Jul) (P/T)

Virology

K. Harper, B.Sc., Ph.D.	Band 6
A.M. Mueller, B.Sc.	Band 6
Sybil M. Macintosh, B.Sc.	Band 7

DTI

Crop Genetics

D. Matthews, B.Sc., Ph.D.	Band 6

Gene Shears

Cell and Molecular Genetics

D.J. Leader, B.Sc.	Band 6
Jennifer Watters, H.N.D.	Band 8 (P/T)

Leverhulme Trust

Cellular and Environmental Physiology/ Virology

Petra C. Boevink, B.Sc., Ph.D.	Band 6 (Appt. Dec)

MAFF

Cellular and Environmental Physiology

Sheena J. Rodger O.N.C.	Band 8

Soft Fruit & Perennial Crops

Emily Cobb, H.N.C.	Band 10

McCains PLC

Crop Genetics

Venetia Mahoney	Band10 (Appt. Oct)

NERC

Cellular and Environmental Physiology

H. Connolly, B.Sc., M.Sc.	Band 7 (Appt. Nov)

ODA

Crop Genetics

Beverly Ingram, B.Sc., M.Sc.	Band 6
Michele S. Leslie	Band 8
Jane Roberts, H.N.C.	Band 9 (Appt. Apr)

Virology

M. Taliansky, Ph.D., D.Sc.	Band 6
Bridget Jones, B.Sc.	Band 10

SOAEFD/HDC

Fungal and Bacterial Plant Pathology

Isabelle Lacourt, B.Sc., Ph.D.	Band 6 (Appt. Feb)

PMB

Cellular and Environmental Physiology

G.J. Lewis, B.Sc., M.Sc.	Band 6
M. Young, H.N.D.	Band 7
M. Whyte	Band 10 (P/T)

Scotia

Chemistry

R.A. Unwin, B.Sc.	

SmithKline Beecham R&D Fund

SFPC/CEP

Linda Sommerville	Band 7
Mary Woodhead, B.Sc., Ph.D.	Band 7

St Aidans Project

Cellular and Environmental Physiology

B. McGill	Band 11 (P/T)

Estate Glasshouse and Field Research

J. Abernethy	Band 11 (P/T)
M. Torrie	Band 11 (P/T)

UNDP-CIP

Cell and Molecular Genetics

Rhonda Meyer, Ph.D.	Band 6

Miscellaneous funding

Soft Fruit Genetics

P. Lanham, B.Sc., Ph.D.	Band 6

Resignations

Name	Dept.	Band	Month
Amanda Adams	EGFR	10	August
S.A. Brocklebank	CEP	10	August
M. E. Campbell	Admin	9	December
S.A. Clulow	CG	6	September
H. Findlay	Admin	4	July
Michelle Fleming	CG	8	September
Beverly Ingram	CG	6	April
Gayathree Jayasinghe	BioSS	6	March
J.W. Kay	BioSS	4	August
J.S. Miller	Vir	6	December
G.D. Ruxton	BioSS	6	December
A.M. Sword	BioSS	6	February
Carol Taylor	CG	10	June
Aileen Timmons	CG	6	November
W.T.G. van de Ven	SFPC	6	June
N. Wilson	CG	10	October
M.J. Wilkinson	CG	6	September
G. Wood	EGFR	5	August

Staff Retirements

Name	Dept.	Band	Month
M. Barnes	Admin	9	March
M.C.M. Pérombelon	FBPP	4	May
D.A. Perry	SLIS	4	April
M. Purves	Admin	10	November
T. Purves	E&M	11	December
R.L. Wastie	CG	4	June

Redundancies, Voluntary and Flexible Retirements

Name	Dept.	Band	Month
Ann Grant	Vir	8	February
J.G. Harrison	FBPP	4	May
C. Anne Jolly	Vir	7	April
Sandra Millar	CEP	7	March

Mylnefield Research Services Ltd

Managing Director : N.W. Kerby, B.Sc., Ph.D., C.Biol., F.I.Biol.
Administrative Executive Officer : Anne Cameron, H.N.C.
Marketing Executive Officer : Kate Bridgens, B.A., M.Sc.
Personal Secretary : Linda Butler

Amanda Adams, (Appt. Sep)
Carole Bachelier, B.Sc. (Appt. Apr)
J. van den Berg, M.Sc., Ph.D.
P. Davie, O.N.C. (Appt. Jun)
Patricia Dobson, (Appt. Jan)
Alison Dolan, H.N.C.
Jane E. Fairlie, O.N.C.
R. Forrest, B.Sc., Ph.D. (Appt Jan)
D.N. Harris, B.Sc., M.Sc.
R.E. Harrison, B.Sc., M.Sc., Ph.D.
P. Hedley, B.Sc., Ph.D.
P.P.M. Iannetta, B.Sc., Ph.D. (Appt. Mar)
C. Jones, B.Sc. (Appt. Sep)
Janie Lyon, B.Sc. (Appt. Jan)
Fiona McMahon, B.Sc. (Appt. Jan)
Susan Mitchell, B.Sc. (Appt. Nov)
Vasantha Ramanathan, B.Sc., M.Phil., Dip.Biotech., Ph.D.
Claire Reid, B.Sc. (Appt. Nov)
Lynn Surplus, B.Sc., Ph.D. (Appt. Apr)

Honorary Research Professors

Professor P. Broda, M.A., M.Sc., Ph.D., D.Sc., Hon.D.Sc.
Professor F. Gunstone, B.Sc., Ph.D., D.Sc., F.R.S.C., F.T.S.E., C.Chem.
Professor B.D. Harrison, C.B.E., B.Sc., Ph.D., D.Ag.For., F.R.S., C.Biol., F.I.Biol.
Professor N. L. Innes, O.B.E., B.Sc., Ph.D., D.Sc., C.Biol., F.I. Biol., F.R.S.E., F.I. Hort.
Professor P.H. Nye, M.A., B.Sc., F.R.S.
Professor B. Sleeman, B.Sc., Ph.D., D.Sc., C.Math., F.I.M.A., F.R.S.E.
Professor Janet Sprent, B.Sc., D.Sc., Ph.D., A.R.C.S., F.R.S.E.
Professor Sir W. Stewart, B.Sc., Ph.D., D.Sc., C.Biol., F.I.Biol., F.R.S., F.R.S.E.
Professor C.E. Taylor, C.B.E., B.Sc., Ph.D., F.R.S.E., C.Biol., F.I.Biol.

Honorary Research Fellows

R.A. Brown, B.Sc., M.Sc., Ph.D.
Professor H. Griffiths, B.Sc., Ph.D.
J.G. Harrison, B.Sc., Ph.D.
R.J. Jarvis, M.A., D.Phil.
H.M. Lawson, B.Sc., M.Agr.Sc., Dip.Agric., F.I.Hort.
A.F. Murant, B.Sc., A.R.C.S., Ph.D., D.I.C., C.Biol., F.I.Biol., F.R.S.E.
M.C.M. Pérombelon, M.B.E., B.Sc., M.Sc., Ph.D.
D.A. Perry, B.Sc., Ph.D.
P.D. Smith, B.Sc., Ph.D., C.Math., F.I.M.A.

Postgraduate Students

Name	Dept.	Subject
I. Abdalla	BioSS	Automatic detection of tissue boundaries in ultrasound scans of pedigree sheep.
J. Angel-Diaz	Vir	Molecular approaches to the control of raspberry bushy dwarf virus.
Miray Arli	Vir	Studies on potato mop-top virus multiplication.
M. Armstrong	Nem	Molecular heterogeneity in potato cyst nematodes.
G. Asmar	CMG	Characterisation of plant cDNAs encoding putative RNA helicases.
Suzanne Baker	FBPP	The effect of biotic and abiotic stress on the molecular processes underlying *ml-o* resistance in barley.
S.N.B. Barr	CG	Somatic hybridisation of tetraploid and wild potato.
Annette Baty	CEP	Control of cell wall biosynthesis during differentiation of fibre cells.
O. Brendel	CEP	^{13}C Discrimination and genetic diversity of Scots pine.
D. Burn	CEP	Geneflow in agricultural systems.
K. Cheung	CMG	Genetic transformation in Groundnut.
K. Clacher	SFPC	Production of year-round *Rubus* crops in the UK.
D.F. Cox	FBPP	A spatial analysis of disease spread processes.
Sarah Cox	SFPC	Molecular approach to the study of virus-like diseases of *Ribes.*
Clare Croser	CEP	Aspects of root growth through compacted soil.
Pauline Douglas	FBPP	Control of plant defense responses by reversible protein phosphorylation.
Lisa Duncan	Nem	Study of the surface molecules of plant parasitic nematodes.
Maria Durban-Reguera	BioSS	Modelling spatial trends and local competition effects in field trials, using generalised additive models.
M. Ehwaeti	Nem	Root-knot nematodes, biology & control.
S.J. Ferris	BioSS	The investigation and control of carryover effects in observer perception and recording.
J. Forster	CEP/CMG	Genetic manipulation of nitrate reductase activity in potato.
Liliana Franco-Lara	Vir	Development of transgenic resistance to potato leafroll virus in *Solanum phureja.*
J.I. Hamilton	CMG	Molecular characterisation of RNA binding proteins in pre-mRNA splicing.
Patricia M. Harbour	CG	Biological control of potato storage diseases.
S. Hendy	Vir	Development of scFv antibodies for transgenic resistance to potato viruses.
Audra Hunter	CEP	Biochemical markers for maturity in potato.
C. Jones	CEP	Molecular basis of ripening in *Rubus.*
A. Mackie	CEP	Volatile organic compounds as signals between micro-organisms in the rhizosphere.
Anne Maddison	CMG	Molecular dissection of invertase gene expression in potato.
Pauline McConway	FBPP/CMG	Molecular biology of potato resistance to erwinias derived from *Solanum brevidens.*
D. McCormack	SFPC	Aspects of the visual and chemical ecology of *Meligethes aeneus* and *Ceutorhynchus assimilis.*
Grainne H. McGuire	BioSS	The statistical modelling of the genetic structure of bacterial populations.
D. Milbourne	CMG	Molecular marker-assisted targeted breeding for potato cyst nematode and late blight.
Sarah Miller	FBPP	Assessment of the potential to control potato diseases by resistance elicitors.
Adele Mooney	Vir	Replication of pea early browning virus.
A. Munir	Nem	Management of potato cyst nematodes in Pakistan.
Shi Nongnong	Vir	Population genetics of barley yellow mosaic virus-resistance breaking isolates.
F. Nabugoomu	BioSS	REML estimation in a series of varietal trials.
Martha Namfua	Nem	Management of nematodes associated with coffee.
H. Pakniyat	CMG	Genetic control of salt tolerance in barley.
Alexandra Popovich	SFPC	Development of a rapid screening system for gene function.
Sara Preston	CEP	The role of microorganisms in the genesis and stabilization of soil structure.
C. Regalado	CEP	Spatio-temporal dynamics of microorganisms in a heterogeneous environment.
G. Randhawa	CMG	Manipulation of potato genes.
W.Q. Ribeiro	CMG	Genetic variation in *Phaseolus vulgaris.*
A. Richardson	CEP	Coniferyl alcohol oxidases in lignifying tissues of higher plants.
Alison Roberts	CEP	Plasmodesmata and virus transport.
L. Robertson	Nem	Nematode secretions involved in plant pathogenesis.
J. Shaw	BioSS	Techniques for discrimination of seed types using imaging measurements.
Louise Shepherd	CEP	Production of novel starches in potato.
F.N. Wachira	CMG	Molecular variation in tea.
Kathryn Watt	SFPC	Identification of genes switched on in response to wounding in strawberry.
Gemma White	CMG	Population genetics of Mahogany.
A. Wilson*	CG	Gene position in a synthetic *Brassica napus.*

* Permanent member of staff

Service on External Committees or Organisations

Name	Position	Committee or Organisation
T.J.W. Alphey	Secretariat	CHABOS & SMAC
H. Barker	Committee Member	Association of Applied Biologists (Virus Group)
A.G. Bengough	Committee Member	Scottish Soils Discussion Group
R.M. Brennan	Adviser	SmithKline Beecham Blackcurrant R&D Committee
D.J.F. Brown	Co-Chairman	Russian Society of Nematology International Meeting
	Member	Society of Nematology *Ad Hoc* Committee, International Federation of Nematology Societies
	Member	European Plant Protection Organization *Ad Hoc* Committee, Xiphinema Americanum group nematodes
J.W.S. Brown	Member	BBSRC - Genes and Developmental Biology Committee
J.W. Crawford	Member	Industrial Liaison Committee, University of Abertay, Dundee
	Member	Management Group, Centre for Non-Linear Systems in Biology
H.V. Davies	Member	Kluwer Press International Advisory Board
J.M. Duncan	Committee Member	'*Phytophthora* Committee' of International Society for Plant Pathology
R.P. Ellis	Member	BSPB Cereal Crop Group
	Member	SAC Recommended List Consultative Committee - BSPB Representative
B.P. Forster	Co-ordinator	International Committee on Barley Chromosome Genetic Mapping: Chromosome 4
G.J. Gibson	Member	SOAEFD Helminth Modelling Management Group
T.D. Heilbronn	Publicity Officer	Association for Crop Protection in Northern Britain
J.R. Hillman	Chairman	Agriculture, Horticulture & Forestry Sector Panel, UK Technology Foresight Programme
	Chairman	SCRI/SASA/SAC Liaison Group
	Chairman	Tayside Biocentre Group
	Deputy Chairman	Board of Directors, Mylnefield Research Services Ltd
	Member	Board of Directors, CHABOS
	Member	SOAEFD Joint Consultative Committee for Management Board
	Member	ECRE Board of Management
	Member	SNSA Adviser to Committee
	Member	Senate, University of Dundee
	Member	University of Strathclyde Sub-Board for the Degree of B.Sc. in Horticulture
	Member	SSPDC Management Committee
	Member	Tayside Economic Forum
	Adviser	International Foundation for Science, Stockholm
	Adviser	University of Leeds
	Examiner	University of London
D.L. Hood	Secretary & Treasurer	Scottish Society for Crop Research
G.W. Horgan	Committee Member	Royal Statistical Society, Local Group (Edinburgh)
E.A. Hunter	Coordinator	EU Concerted Action - AAIR 2322
A.T. Jones	Director of Studies	British Council International Seminar on 'Modern approaches to the study of plant viruses', 12-24 March 1995
R.A. Kempton	President	British Region, International Biometric Society
	Chairman	International Award Fund Committee, International Biometric Society
R.J. Killick	Member	SMAC
	Member	BBSRC Pay Advisory Group
	Member	Continuing Professional Development Advisory Group, University of Abertay, Dundee
	Company Secretary	Mylnefield Research Services Ltd
W.H. Macfarlane Smith	Member	BBSRC Joint Committee on Health & Safety
	Member	BSPB Oilseed & Industrial Crops Group
	Member	AFRS Safety Officers Group
	Member	SABRI Safety Officers Group
	Member	NPTC Plant Variety Development Panel
G.R. Mackay	Chairman	EUCARPIA, Potato Section
	Member	Biological Sciences Advisory Committee, Coventry University
D.K.L. MacKerron	Chairman	Potato Network, in Focus 3 of GCTE of IGBP
	Secretary	Physiology Section, EAPR
	Secretary	Potato Crop Sub-Committee, SSCR
B. Marshall	Member	NERC, Terrestrial Sciences, Higher Education Grants and Training Awards Committee
	Deputy Head	Management Group, Centre for Non-Linear Systems in Biology
M.A. Mayo	Chair	Plant virus sub-committee, International Committee for Taxonomy of Viruses
	Member	International Commission on Bionomenclature
U.M. McKean	Member	Scottish Agricultural Librarians' Group
D.A. McNulty	Member	United Kingdom Chemometrics Discussion Group

Name	Position	Committee or Organisation
R.J. McNicol	Member	HDC Soft Fruit Trialling Sub-Committee
	Member	Soft Fruit Sub-Committee, SSCR
	Adviser	SNSA Committee
	Adviser	SSFG Ltd Board
	Adviser	Soft Fruit Committee of Horticulture Research International
S. Millam	UK Representative	COST 822
I.M. Morrison	Member	Agriculture & Environment Committee, Society of Chemical Industry
	Member	Energy and Industrial Cropping Group, National Farmers Union of Scotland
A.C. Newton	Member	Local Arrangements Committee, VIIth International Congress of Plant Pathology, Edinburgh
	Committe Member	United Kingdom Cereal Pathogen Virulence Survey
	Membership Secretary	British Society for Plant Pathology
	Web Server Manager	British Society for Plant Pathology
	UK Representantive	EU COST Action 817
K. Oparka	Member	International Organising C'tee IIIrd International Workshop on Plasmodesmata, Israel, 1996
	Member	International Organising C'tee Vth International Conference on Phloem Transport, Canterbury, 1995
	Member	International Organising C'tee Plant-Membrane Transport Conference, Cambridge, 1997
	Member	SEB Plant Biology Committee
W. Powell	External Examiner	B.Sc. Genetics; M.Sc. Cell and Molecular Genetics; B.Sc. Crop Science (UCW Aberystwyth)
G. Ramsay	SCRI Representative	UK Plant Genetic Resources Group
K. Ritz	Co-ordinator	BBSRC/SOAEFD Soil:Plant:Microbe Interactions Initiative
	Member	Management Group, Centre for Non-Linear Systems in Biology
I.M. Roberts	Safety Officer	Royal Microscopical Society
	Chairman	BBSRC EM Advisory Group
D. Robinson	Member	AAB Plant Physiology Committee
D.J. Robinson	Member	Advisory Committee on Releases to the Environment (DoE)
G.R. Squire	Chairman	CHABOS Working Group on Environmental Pollution and Bioremediation
	Member	CHABOS Working Group on Vegetation Dynamics
	Member	CHABOS Working Group on Soil Conservation
	Project Co-ordinator	SOAEFD Co-ordinated Programme in Vegetation Dynamics
	Member	Management Group, Centre for Non-Linear Systems in Biology
S.E. Stephens	Member	Tayside Chief Librarians' Group
	Member	Information Services Group - Scottish Library Association
	Member	Scottish Agricultural Librarians' Group
M.Talbot	Chairman	Statistics Group of UK Plant Varieties and Seeds Committee
	Member	Statistics Committee International Seed Testing Association
	Member	Technical Working Party on Computing of the International Union for the Protection of Plant Varieties
	Member	Management Board of European Network for Information Technology in Agriculture
W.T.B. Thomas	Convenor	Plant Breeding Group, AAB
Lesley Torrance	Committee Member	UK Cost 823
B. Williamson	Secretary	VIIth International Congress of Plant Pathology 1998, Finance Sub-Committee
	Treasurer	Association for Crop Protection in Northern Britain
	Member	Scientific Advisory Committee of XIth International *Botrytis* Symposium 1996, Wageningen
	Member	Department of Agriculture, Aberdeen University Advisory Committee
T.M.A. Wilson	Member	Program/Advisory Committee Xth International Congress of Plant Virology, Jerusalem
	Member	Advisory Committee IVth Positive-Strand RNA Virus Meeting, Utrecht
	Member	Programme Committee VIIth International Congress of Plant Pathology, Edinburgh
	Member	Church of Scotland, Society Religion and Technology Project "Ethics of Genetic Engineering of Non-Human Life"
J.A.T. Woodford	Regional Hon. Sec.	Royal Entomological Society
F.G. Wright	Member	BBSRC Protein Engineering Liaison Group
	Member	SEQNET/CCPII User Documentation Group
	Member	BBSRC Computational Molecular Biology Review Panel
I.M. Young	Committee Member	British Society of Soil Science
	Co-opted Member	British Standards Committee (Soil Quality)

Short Term Workers and Visitors

Name	Country of origin	Dept.	Month/yr of arrival	Length of stay
W. Ahmad	India	CEP	Sep 95	1 month
I. Ahmad	Malaysia	SFPC	Nov 94	9 months
J. Badge	UK	Vir	Apr 95	2 weeks
A. Banck	Sweden	CEP	Feb 95	1 month
P. Bassett	UK	BioSS	Sep 95	1 year
J.F. Bol	The Netherlands	Nem	Jul 95	3 weeks
O. Brendel	Germany	CEP	May 95	5 months
Ivana Burdova	Czech Republic	CG	Aug 95	1 week
M.K. Cheung	UK	Vir	May 95	2 months
M.K. Cheung	UK	Vir	Sep 95	2 months
M. Clokie	UK	Vir	Oct 95	6 months
N. Cryer	UK	Vir	Oct 95	6 months
D.B. Dangora	Nigeria	Vir	Dec 94	9 months
O. David	France	BioSS	Oct 95	2 weeks
Gilles Delecourt	France	CG	Feb 95	4 months
M. Diaz-Ravina	Sweden	CEP	Aug 95	1 month
R. Falloon	New Zealand	FBPP	May 95	3 months
M. Folling	Denmark	CMG	Jan 95	6 months
Suzanne Furby	Australia	BioSS	Oct 95	1 week
Rose Gergerich	USA	Nem	Feb 95	6 months
F.E. Gildow	USA	Vir	Feb 95	10 months
H. Heesterbeek	The Netherlands	BioSS	Mar 95	3 days
Lucia Iasi	Italy	SFPC	Jan 95	8 months
I. Lacourt	France	FBPP	Aug 95	3 months
B. Maanen	The Netherlands	CEP	Jun 95	4 months
Haico Marsman	The Netherlands	CG	Sep 95	5 months
S. McKeown	UK	Nem	Jan 95	3 months
Beatriz Millan-Mendoza	Venezuala	SFPC	Apr 95	6 months
S.T. Minnis	UK	Nem	May 95	4 months
M.G.J. Moens	Belgium	Nem	Mar 95	1 week
J-L. Molina-Cano	Spain	CMG	Dec 95	1 week
S. Moore	UK	CEP	Jul 95	2 months
Anna-Maria Mueller	Austria	Vir	Jul 95	1 year
R.A. Naidu	India	Vir	Sep 95	1 month
C. Neiser	Germany	Vir	Dec 95	2 weeks
T. Olesen	Denmark	CEP	Feb 95	6 months
Marina Paoli	Italy	CG	Nov 95	6 months
Sudam Patil	India	CG	Sep 95	1 month
S. Paul	India	CMG	Aug 95	1 year
Maria Peak	Hungary	SFPC	Jan 95	2 months
D. Phillippeau	France	BioSS	Nov 95	3 days
J. Plazinski	Australia	CEP	Nov 95	2 months
T. Ploeg	The Netherlands	Nem	Jan 95	4 months
M. Radivojevic	Yugoslavia	Nem	Jul 95	1 week
D. Ramirez	USA	BioSS	Nov 95	3 days
J.B. Relloso	Spain	Vir	Aug 95	2 weeks
L. Robert	France	CEP	May 95	4 months
A.H. Schulman	Finland	CMG	Mar 95	1 week
Corinne Schmitt	France	Vir	Oct 95	1 year
L. Shepherd	UK	CMG	Oct 94	1 year
Nasrin Smailzadeh	Iran	SFPC	Apr 95	6 months
M. Smith	UK	CEP	Aug 95	1 year
Bronya Spencer	UK	SFPC	Jul 95	1 year
R. Stirling	UK	Nem	Oct 95	3 months
S.A. Subbotin	Russia	Nem	Nov 95	3 weeks
M. Tilak	India	CEP	Feb 95	1 year
K. Toyota	Japan	CEP	Jan 95	9 months
A. Wallace	UK	CEP	Jul 95	2 months
P. Wightman	UK	Nem	Jul 95	6 months
S. Writh	Germany	CEP	May 95	2 weeks

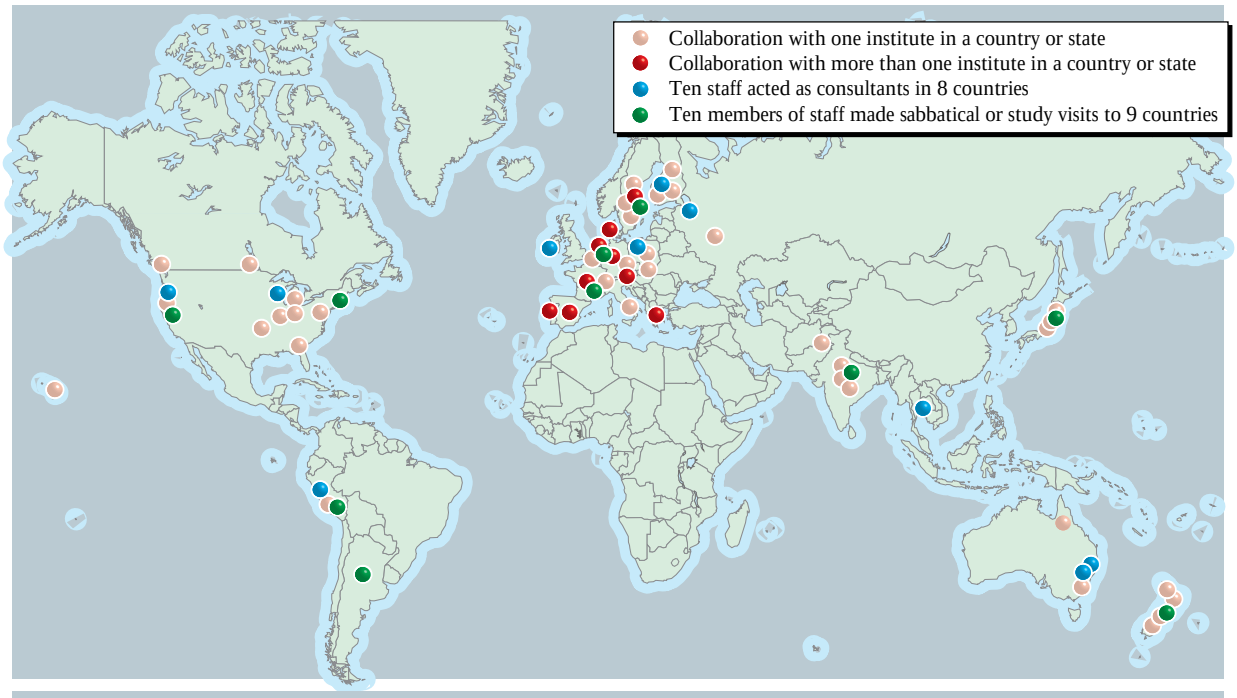
Editorial Duties

Name	Position	Journal Title
H. Barker	Editorial Board	*Annals of Applied Biology*
A.G. Bengough	Editor (joint)	*British Society of Soil Science Newsletter*
B. Boag	Editorial Board	*Annals of Applied Biology*
	Editorial Board	*Nematologia Mediterranea*
R.M. Brennan	Associate Editor	*Journal of Horticultural Science*
D.J.F. Brown	Honorary Chief Editor	*Russian Journal of Nematology*
	Editorial Board	*Nematologia Mediterranea*
W.W. Christie	Editorial Board	*Chemistry and Physics of Lipids*
	Editorial Board	*Lipid Technology*
	Managing Editor	The Oily Press Ltd
J.M. Duncan	Associate Editor	*Mycological Research*
M.F. Franklin	Editorial Board	*Journal of Agricultural Science*
	Editorial Board	*British Journal of Nutrition*
C.A. Glasbey	Associate Editor	*Biometrics*
T.D. Heilbronn	Editor	*SSCR Newsletter*
J.R. Hillman	Publication Committee	*Journal of Horticultural Science*
	Editorial Board	*Agricultural Systems*
	Editorial Board	*Journal of Agricultural Science*
R.A. Kempton	Editorial Board	*Heredity*
	Editorial Board	*Journal of Agricultural Science*
	Editorial Board	*Journal of Agricultural, Biological & Environmental Statistics*
D.K.L. MacKerron	Assistant Editor	*Journal of Horticultural Science*
	Editorial Board	*Euphytica*
M.A. Mayo	Member of Editorial Board	*Virology*
	Member of Editorial Board	*Journal of General Virology*
J.W. McNicol	Editorial Board	*Annals of Applied Biology*
I.M. Morrison	Management Committee & Editorial Board	*Journal of the Science of Food and Agriculture*
K. Oparka	Editor	*Plant Physiology*
	International Advisory Board	*Journal of Experimental Botany*
M.S. Phillips	Associate Editor	*Journal of Nematology*
D. Robinson	Editorial Advisory Board	*New Phytologist*
D.J. Robinson	Editorial Board	*Journal of Virological Methods*
G.R. Squire	Advisory Board	*New Phytologist*
	Editorial Board	*Experimental Agriculture, CUP*
	Advisory Board	*Crop Physiology Abstracts, CABI*
D.L. Trudgill	Editorial Board	*Nematology*
	Editorial Board	*Fundamental and Applied Nematology*
	Advisory Board	*European Journal of Plant Pathology*
	Associate Editor	*Journal of Nematology*
R.L. Wastie	Editorial Board	*Annals of Applied Biology*
	Editor	*Potato Research*
R. Waugh	Editor	*Molecular Biotechnology*
B. Williamson	Associate Editor	*Annals of Applied Biology*
T.M.A. Wilson	Senior Editor	*Journal of General Virology*
	Associate Editor	*Molecular Plant-Microbe Interactions*
I.M. Young	Editor (joint)	*British Society of Soil Science Newsletter*

Awards and Distinctions

Name	Dept.	Degree/Award/Distinction/Appointment
M.C.M. Pérombelon	Fellow	MBE, for Services to Agriculture
W.W. Christie	Chem	Herbert J. Dutton Award, American Oil Chemists' Society
A.G. Roberts	CEP	Katherine Esau Award
D.L. Trudgill	Nem	President-Elect of European Society of Nematologists
D.J.F. Brown	Vir	Fellow of the Russian Society of Nematology of the Russian Academy of Sciences
W. Powell	CMG	Honorary Professor, Oregon State University
L.L. Handley	CEP	Honorary Professor of Biology, Florida International University
J. Duncan	FBPP	Honorary Senior Lecturer, University of Dundee
A.T. Jones	SFPC	Honorary Senior Lecturer, University of Dundee
R.J. McNicol	SFPC	Honorary Senior Lecturer, University of Dundee
M.A. Taylor	CEP	Honorary Lecturer, Institute of Biochemistry and Molecular Biology
T.M.A. Wilson	Admin	C.Biol., M.I.Biol.
W. Powell	CMG	D.Sc., University of Birmingham
B. Williamson	SFPC	D.Sc., University of Aberdeen
M. Arif	Vir	Ph.D., University of Edinburgh
C. Clegg	CEP	Ph.D., University of Exeter
I. Dawson	CMG	Ph.D., University of Dundee
C. Orozco-Castillo	CMG	Ph.D., University of Edinburgh
M.R. Woodhead	CEP/SFPC	Ph.D., University of Dundee
P.E. Hedley	CMG	Ph.D., University of Dundee
R.J. Killick	Admin	M.A., University of Leicester
I. Paxton	Admin	M.Sc., University of Abertay, Dundee
W.M. Stein	Chem	B.Sc., Open University
K. Taylor	Chem	B.Sc., Open University

International Collaboration and Consultancies



Research is executed within an international framework that encourages information transfer. The extent of SCRI's international commitment during 1995 is reflected in the collaborative research that was undertaken with 85 institutions in 25 countries.

SCRI Research Programme
1995-1996

SOAEFD funded research programme showing: SCRI project number; SOAEFD project number; Title (prefixed ROA for ROAMEd core-funded projects; FF for Flexible Fund projects; LINK for SOAEFD-LINK projects); Scientific Project Leader. In addition to this list there are c. 200 research projects undertaken on behalf of various bodies, including other governmental bodies, commerce and levy boards.

47	SCR/017/91	ROA Maintenance, improvement and evaluation of the Commonwealth Potato Collection	Wilkinson M J
281	SCR/053/91	ROA Investigation of the genetic control of characters determining crop performance in barley	Ellis R P
282	SCR/054/91	ROA Development of improved methods of generating and evaluating variation in barley for a range of important characters	Thomas W T B
283	SCR/055/91	ROA Investigation of the genetical determination of biochemical components that relate to cereal quality with the aim of improving selection procedures in breeding programmes	Swanston J S
284	SCR/056/91	ROA Anther and isolated microspore culture in cereals and legumes	Ramsay G
285	SCR/058/91	ROA Anti-nutritional factors in faba beans	Ramsay G
286	SCR/057/91	ROA Tissue culture and transformation in legumes	Ramsay G
287	SCR/059/91	ROA Biochemical markers in faba beans	Ramsay G
296	SCR/027/91	ROA Produce and maintain virus-tested stocks, assess resistance and index British and imported *Ribes* and *Rubus* for virus infections	Jones A T
305	SCR/034/91	ROA Genetic studies within the family Brassicaceae, as model systems for the study of cytotaxonomy, polymorphism and gene introgression	Millam S
312	SCR/070/91	ROA Determine the thermal-time relationships for developmental processes in representative plant parasitic nematodes	Trudgill D L
326	SCR/050/91	ROA Physical and physiological constraints on the growth and activity of plant root systems	Robinson D
332	SCR/045/91	ROA Interactions between environment and microbial transformations in root zone soils	Wheatley R E
340	SCR/004/91	FF Computation of safe isolation distances for field-grown genetically modified crops	Mackay G R
344	SCR/002/91	FF Investigate techniques for identification of foodstuffs of plant origin subjected to ionizing radiation, determination of received radiation dosages and elucidation of the role of free-radicals in senescence related processes	Goodman B A
357	SCR/357/92	ROA To determine the regulation of cell to cell transport and carbohydrate flux in sugar and starch-storing tissues	Davies H V
358	SCR/358/92	ROA Molecular mechanisms involved in tuberisation in potato	Taylor M
361	SCR/361/92	ROA Genetic control of pathogenicity and host specificity at the molecular level in the fungal pathogens *Phytophthora* and *Rhynchosporium*	Duncan J M

362	SCR/362/92	ROA Physiology and biochemistry underlying resistance of potato to late blight (*Phytophthora infestans*) and bacterial soft rots (*Erwinia*), barley to mildew (*Erisyphe graminis*), and soft fruit to grey mould (*Botrytis cinerea*)	Lyon G D
367	SCR/367/92	ROA Post-transcriptional processes in plant gene expression	Brown J W S
381	SCR/381/92	ROA Application of non linear mathematics and fractal geometry to topics on spatio-temporal dynamics in heterogeneous media: diffusion and microbial dynamics in structured soil, morphogenisis and epidemiology	Crawford J W
382	SCR/382/92	FF Investigation into oil seed rape as a possible cause of human allergy and the chemical, palynological and mycological factors which may be involved	Macfarlane Smith W H
386	SCR/386/92	ROA Biochemical and molecular variation in *Myzus persicae* and associated aphid vectors of potato leafroll virus and the potato virus Y complex	Woodford J A T
387	SCR/387/92	ROA Aphid vectors of potato virus Y complex in Scotland in relation to environmental change	Woodford J A T
388	SCR/388/92	FF Development and evaluation of a quantitative theory of soil structure and its relation to transport processes	Young I
389	SCR/389/92	FF Development of non-linear mathematical theory of plant disease epidemiology using as model systems, scald and powdery mildew in barley, blight in potato and redcore in strawberry	Newton A C
393	SCR/393/92	FF Combined NMR and mathematical study of major metabolic pathways in higher plant cells	Viola R
394	SCR/394/93	LINK A molecular approach for the detection and diagnosis of the agent of reversion disease and of other virus-like agents of blackcurrant [SmithKline Beecham/SOAEFD]	Jones A T
395	SCR/395/93	LINK Detection of *Phytophthora* diseases in horticultural planting stocks by the Polymerase Chain Reaction (PCR) [HDC/SOAFD]	Duncan J M
397	SCR/397/93	ROA Novel methodology for the determination of lipid structure and its application to plant biochemistry and food lipids	Christie W W
398	SCR/398/93	ROA Chemical strategies for the study of natural defence compounds present in plant species, including faba beans, potatoes, brassicas and soft fruit crops	Griffiths D W
399	SCR/399/93	ROA *In situ* identification and localisation of plant cell wall components, particularly from fibre cells, by the production of cell wall specific antibodies	Forrest J M S
400	SCR/400/93	ROA Chemical and immunological methods for the isolation and characterisation of proteins, xyloglucons and other cell wall components from endoderm tissue, using barley as a model species	Forrest J M S
401	SCR/401/93	ROA Molecular ecology of arthropod pests, with particular reference to speciation and host adaptation of aphids in the genus *Amphorophora*, mites of *Ribes*, beetles attacking raspberry and Dipteran pests of brassicas	Birch A N E
402	SCR/402/93	ROA Properties, variation, detection and control of the agents of virus and virus-like diseases of *Rubus*, *Ribes* and *Fragaria*	Jones A T
403	SCR/403/93	ROA Molecular basis for variation and genome organisation of nepoviruses	Jones A T

404	SCR/404/93	ROA Functions and modes of action of the gene products of luteoviruses, especially potato leafroll virus, with particular reference to virus transmission by aphids	Mayo M A
405	SCR/405/93	ROA Structure and function of the genomes of tobraviruses (specifically tobacco rattle and pea early browning viruses), with particular reference to virus variation, transmission and pathogenicity	Robinson D J
406	SCR/406/93	ROA Fungus-transmitted viral pathogens of potato, cereal and peanut: fundamental model studies and comparative analyses of their genomes, gene expression, transmission by fungi and molecular cytopathology	Wilson T M A
409	SCR/409/93	ROA Establish methods for cloning antibody-coding sequences to produce recombinant antibodies from bacterial cultures	Torrance L
410	SCR/410/93	ROA Host gene-mediated and transgenic resistance: a study of inheritance, expression and molecular mechanisms to improve crop protection against four important potato viruses	Barker H
412	SCR/412/93	ROA Transformation of *Rubus*, *Ribes*, *Fragaria* and *Vaccinium* and evaluation of the biological value of the resultant transgenic plants	McNicol R J
413	SCR/413/93	FF Development of improved diagnostic tests for potato virus Y in a post-harvest tuber testing scheme	Barker H
414	SCR/414/93	FF Carbon partitioning: role of rhizosphere carbon-flow in regulating soil microbial diversity and activity	Griffiths B S
415	SCR/415/93	FF Antibody gene repertoire cloning to produce a diverse array of specific antibodies	Torrance L
416	SCR/416/93	FF Food web analysis of below-ground components of grassland ecosystems using natural abundances of stable isotopes	Handley L L
418	SCR/418/94	ROA Free radicals, antioxidants and metalloenzymes: their identification and behaviour in plants and plant derived foods	Goodman B A
419	SCR/419/94	ROA Non-invasive approaches to the study of structure, composition and developmental processes in plants and plant parasites using magnetic resonance technologies	Goodman B A
420	SCR/420/94	ROA Production, isolation and characterisation of plant fibres for industrial applications	Morrison I M
421	SCR/421/94	ROA Biosynthetic control of fibre constituents during development and differentiation of fibre cells and genetic modification of these processes	Morrison I M
422	SCR/422/94	ROA Processing of plant fibres by novel and environmentally acceptable methods	Morrison I M
423	SCR/423/94	ROA Physiological and developmental regulation of plasmodesmata	Oparka K J
424	SCR/424/94	ROA Relating soil structure to biological function	Young I M
425	SCR/425/94	ROA Influence of the host on gene expression of plant parasitic nematodes	Jones J T
426	SCR/426/94	ROA Fundamental studies on longidorid and trichodorid nematode vectors in relation to the aetiology of nepo- and tobraviruses which are transmitted to a range of arable and fruit crops	Brown D J F
427	SCR/427/94	ROA Characterisation of nematode cuticular surfaces of *Globodera*, *Heterodera* and *Meloidogyne* involved in pathogenesis	Robertson W M

428	SCR/428/94	ROA Investigate inheritance of low temperature sugar stability and develop effective selection strategies to produce superior potato germplasm for processing	Mackay G R
429	SCR/429/94	ROA Genetic architecture of diploid potatoes and production of enhanced germplasm	Bradshaw J E
430	SCR/430/94	ROA Production, maintenance, distribution and associated management of facilities to produce disease-free tubers of genetic stocks of potato clones	Chapman I M
431	SCR/431/94	ROA Devise and operate methods for detecting and quantifying genetic resistance to pathogens of the potato causing late blight, early blight, blackleg, stemcanker, skinspot, dry rot, silver scurf, gangrene, common scab & powdery scab	Wastie R L
432	SCR/432/94	ROA Integrated approaches for rapid and efficient gene transfer and characterisation in potato	Millam S
433	SCR/433/94	ROA Development of Polymerase Chain Reaction (PCR)-based sequence tagged site markers for potato and barley	Waugh R
434	SCR/434/94	ROA Dissection of regulatory mechanisms governing invertase gene expression in potato	Machray G C
435	SCR/435/94	ROA To clone the *Hero* gene of tomato which confers resistance to potato cyst nematode by transposon tagging	Kumar A
436	SCR/436/94	ROA Molecular approach to study the functions of polyamines in plant cell proliferation and morphogenesis	Kumar A
438	SCR/438/94	ROA Role in pathogenesis of extracellular enzymes of *Phytophthora*, *Botrytis* and *Erwinia*	Williamson B
440	SCR/440/94	FF Investigation of *in vitro* splicing systems for analysing splicing in plants and characterisation of snRNP and spliceosomal complexes	Brown J
441	SCR/441/94	FF Studies of phloem transport using an artificial 'aphid'	Oparka K
443	SCR/443/95	FF Research into nutritional aspects of genetically manipulated potatoes, *Solanum tuberosum*	Mackay G R
444	SCR/444/95	ROA Low temperature stress in *Ribes*, *Rubus* and other woody genera	McNicol R J
445	SCR/445/95	ROA The collection and evaluation of genetic resources of *Rubus*, *Ribes* and *Fragaria*	McNicol R J
446	SCR/446/95	ROA Molecular study of genetic variation in plant parasitic nematodes in relation to virulence and plant resistance especially in relation to potato cyst nematodes (PCN) and root knot nematodes	Phillips M S
447	SCR/447/95	ROA Inheritance of resistance to potato virus diseases and production of resistance enhanced potato germplasm	Solomon-Blackburn R M
448	SCR/448/95	ROA Host recognition chemistry on plant surfaces as a basis for developing chemical and molecular markers for pest resistance	Birch A N E
449	SCR/449/95	ROA Advanced information techniques for the study and management of vegetation systems	MacKerron D K L
450	SCR/450/95	ROA Variation and stability of traits governing plant development and resource capture in relation to environment and plant competition	Marshall B
451	SCR/451/95	ROA Genetic and environmental analysis of epidemics of *Erysiphe*	Newton A C

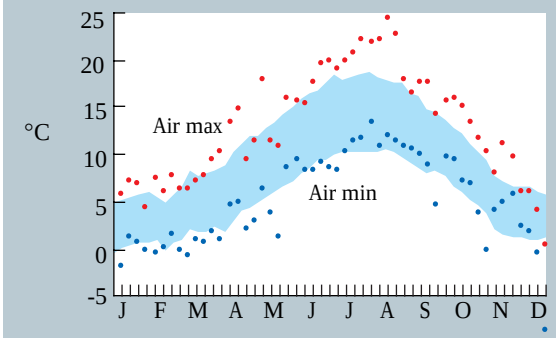
		graminis on barley and oats, *Phytophthora fragariae* on strawberries and raspberries, and *Erwinia* spp. on potatoes	
452	SCR/452/95	ROA Genetic architecture of tetraploid potatoes and production of enhanced germplasm	Bradshaw J E
454	SCR/454/95	ROA Structure of soil microbial and faunal communities, their interaction with vegetation and the relationship to soil processes and health	Griffiths B S
455	SCR/455/95	ROA DARE Dynamics and connectivity in discontinuous plant populations, using wild raspberry and feral oilseed rape as model systems	Crawford J W
456	SCR/456/95	ROA Genetics and ecophysiology of abiotic stress tolerance in *Hordeum vulgare* (barley) and *Arabidopsis thaliana*	Forster B P
457	SCR/457/95	ROA Development and evaluation of novel methodology involving modern chromatography and mass spectroscopy for stable isotopes and antinutritional, quality and other biologically active compounds	Christie W W
458	SCR/458/95	FF Determining the origin and genetic structure of late blight outbreaks on Scottish seed and ware potatoes and assessing the hazard of sexual reproduction by *Phytophthora* to the seed industries of Scotland	Duncan J M
459	SCR/459/95	FF Development of tests to distinguish potato cultivars and their transgenic variants	Machray G
803	SCR/803/94	FF Fundamental studies to develop plant virus-like particles expressed in *Escherichia coli* as vaccine or therapeutic agents	Wilson T M A
805	SCR/805/94	FF Control of certain invertebrate pests of agricultural importance using gut membrane proteins as targets for antibodies	Fenton B
808	SCR/808/94	FF Development of molecular biological and physiological techniques in studies of the interaction between microbes, nutrient cycling and vegetation	Ritz K
815	SCR/815/94	FF Prediction of starch processing potential in relation to cereal and potato production under Scottish conditions	Morrison I M
816	SCR/816/95	FF Vegetation dynamics in heterogeneous species-rich vegetation	Squire G
818	SCR/818/95	FF Genetic engineering of crop plants for resistance to insect and nematode pests: effect of transgene expression on animal nutrition and the environment	Robertson W M

Meteorological Records

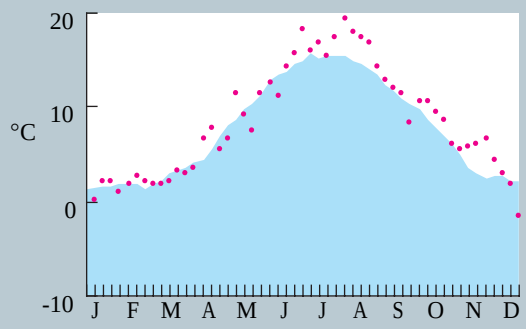
D.K.L. MacKerron

Detailed meteorological records are kept regularly at SCRI. The graphs shown are for weekly values for 1995 and the long term average for 1961-1990( ).

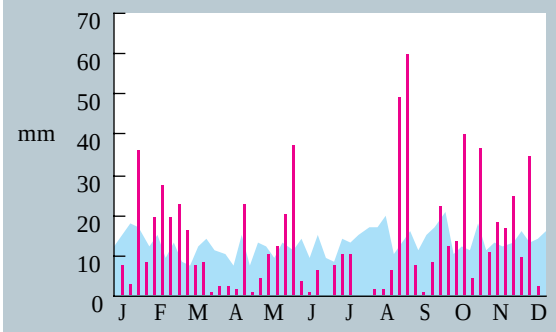
Air temperature



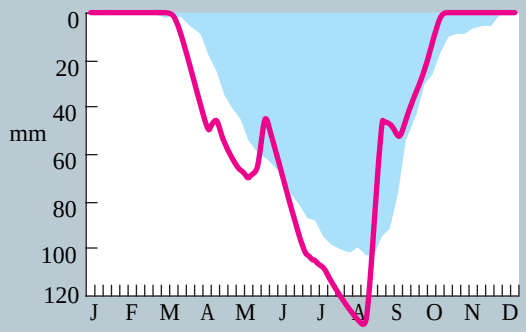
10cm soil temperature



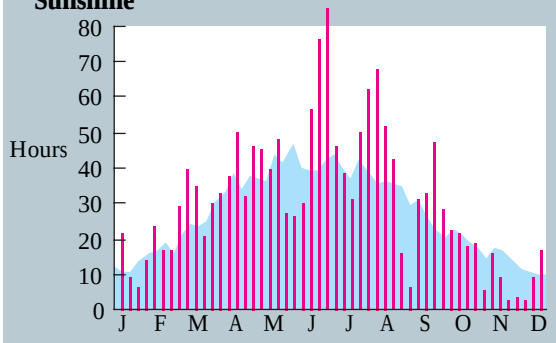
Rainfall



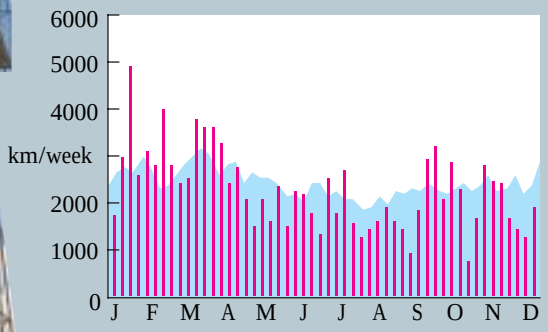
Soil moisture deficit



Sunshine



Run of wind



Cumulative Index 1990 - 1995

In addition to the list below, in every SCRI Annual Report during this period, there are reports of Mylnefield Research Services Ltd; the Research Services; a General Report including accounts, staff lists, publications, research project lists; Overviews by each Head of Department; and a Report by the Director.

Plant genetics

Molecular biology

Cellular and environmental physiology

Chemistry

Fungal and bacterial diseases

Plant viruses

Zoology

Biomathematics and Statistics Scotland

Other Articles

Institutes supported by the Biotechnology and Biological Sciences Research Council

BBSRC Office	Polaris House, North Star Avenue, Swindon, Wilts SN2 1UH	01793-413200
BBSRC Bioscience IT Services	West Common, Harpenden, Herts AL5 2JE	01582-762271
Babraham Institute	Babraham Hall, Babraham, Cambridge CB2 4AT	01223-832312
Laboratory of Molecular Signalling	Babraham Institute, P.O. Box 158, Cambridge CB2 3ES	01223-336600
Institute for Animal Health	Compton, Near Newbury, Berkshire RG20 7NN	01635-578411
Compton Laboratory	Compton, Near Newbury, Berkshire RG20 7NN	01635-578411
Pirbright Laboratory	Ash Road, Pirbright, Woking, Surrey GU24 0NF	01483-232441
BBSRC & MRC Neuropathogenesis Unit	Ogston Building, West Mains Road, Edinburgh EH9 3JF	0131-667-5204
Institute of Arable Crops Research	Harpenden, Herts AL5 2JQ	01582-763133
IACR - Long Ashton Research Station	Long Ashton, Bristol BS18 9AF	01275-392181
IACR - Rothamsted	Harpenden, Herts AL5 2JQ	01582-763133
IACR - Broom's Barn	Highham, Bury St. Edmunds, Suffolk IP28 6NP	01284-810363
Institute of Food Research	Earley Gate, Whiteknights Rd, Reading RG6 6BZ	01189-357055
Norwich Laboratory	Norwich Research Park, Colney, Norwich NR4 7UA	01603-255000
Reading Laboratory	Earley Gate, Whiteknights Rd, Reading RG6 6BZ	01189-357000
Institute of Grassland and Environmental Research	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	01970-828255
Aberystwyth Research Centre	Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB	01970-828255
North Wyke Research Station	Okehampton, Devon EX20 2SB	01837-82558
Bronydd Mawr Research Station	Trecastle, Brecon, Powys LD3 8RD	01874-636480
Trawsgoed Research Farm	Trawsgoed, Aberystwyth, Dyfed SY23 4LL	01974-261615
John Innes Centre	Norwich Research Park, Colney, Norwich NR4 7UH	01603-452571
Roslin Institute	Roslin, Midlothian EH25 9PS	0131-527-4200
Silsoe Research Institute	Wrest Park, Silsoe, Bedford MK45 4HS	01525-860000
Horticultural Research International	Wellesbourne, Warwick CV35 9EF	01789-470382
HRI, East Malling	West Malling, Maidstone, Kent ME19 6BJ	01732-843833
HRI, Wellesbourne	Wellesbourne, Warwick CV35 9EF	01789-470382

Scottish Agricultural and Biological Research Institutes

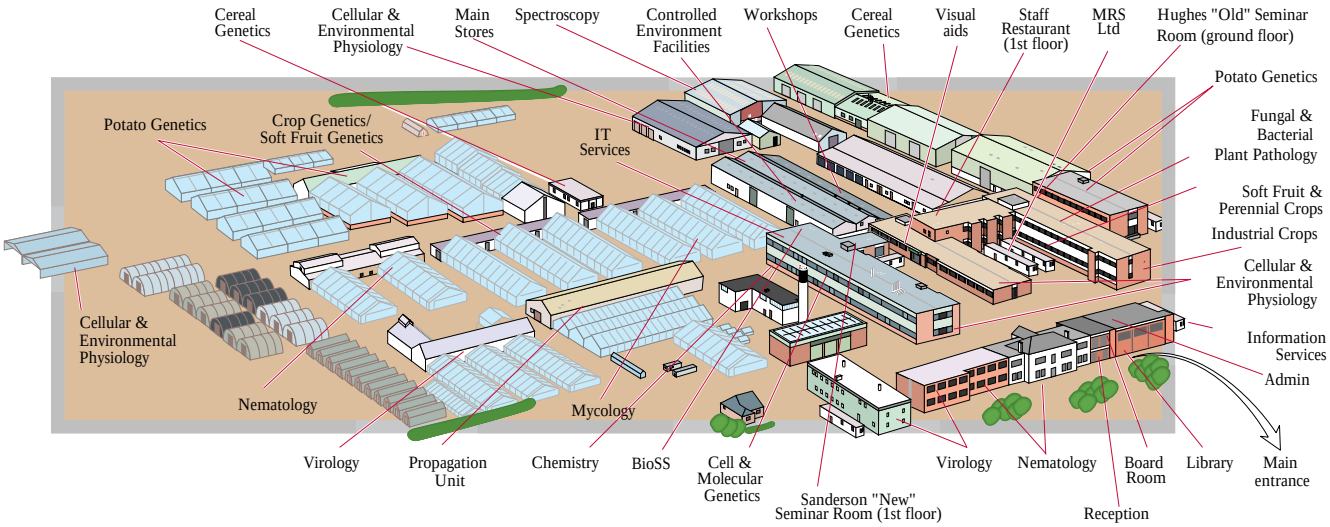
Hannah Research Institute	Ayr, Scotland KA6 5HL	01292-476013
Macaulay Land Use Research Institute	Craigiebuckler, Aberdeen AB9 2QJ	01224-318611
Moredun Research Institute	408 Gilmerton Road, Edinburgh EH17 7JH	0131-664-3262
Rowett Research Institute	Greenburn Road, Bucksburn, Aberdeen AB21 9SB	01224-712751
Scottish Crop Research Institute	Invergowrie, Dundee DD2 5DA	01382-562731
Biomathematics and Statistics Scotland (Administered by SCRI)	University of Edinburgh, James Clerk Maxwell Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JZ	0131-650-4900

List of Abbreviations

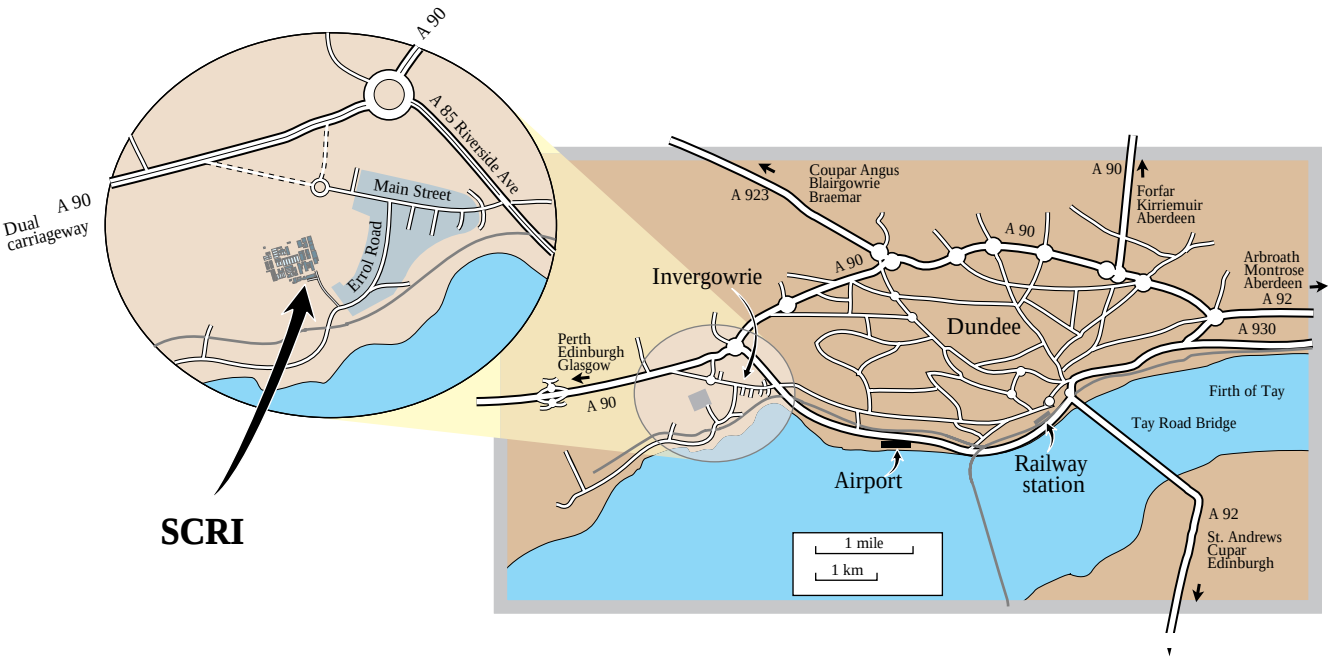
AAB	Association of Applied Biologists
ADAS	Agricultural Development and Advisory Service
BBSRC	Biotechnology & Biological Sciences Research Council
BCPC	British Crop Protection Council
BioSS	Biomathematics and Statistics Scotland
BSPB	British Society of Plant Breeders
BTG	British Technology Group
CAPS	Cleaved Amplified Polymorphic Sequence
CEC	Commission of the European Communities
CIP	International Potato Centre - Peru
COST	European Co-operation in the field of Scientific and Technical Research
EAPR	European Association for Potato Research
ECRE	Edinburgh Centre for Rural Economy
ECSA	European Chips and Snacks Association
EHF	Experimental Husbandry Farm
ELISA	Enzyme linked immunosorbent assay
EPPO	European Plant Protection Organisation
ESTs	Expressed Sequence Tagged Sites
FF	Flexible Funding (SOAEFD)
FLAIR	Food-Linked Agro-Industrial Research
GIUS	Glasshouse Investigational Unit for Scotland
H-GCA	Home-Grown Cereals Authority
HDC	Horticultural Development Council
HPLC	High Performance Liquid Chromatography
HRI	Hannah Research Institute
IACR	Institute of Arable Crops Research
ICTV	International Committee for the Taxonomy of Viruses
IOBC	International Organisation for Biological Control
ISHS	International Society for Horticultural Science
ISPP	International Society for Plant Pathology
IVEM	Institute of Virology and Environmental Microbiology
MAFF	Ministry of Agriculture Fisheries and Food
MLURI	Macaulay Land Use Research Institute
MRI	Moredun Research Institute
NERC	National Environmental Research Council
NFT	National Fruit Trials
NFU	National Farmers Union
NIR	Near Infra-Red
NMR	Nuclear Magnetic Resonance
NPTC	National Proficiency Test Council
ODA	Overseas Development Administration
ORSTOM	Organisation for research in science and technology overseas
PCR	Polymerase Chain Reaction
PMB	Potato Marketing Board
PVRO	Plant Variety Rights Office
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RRI	Rowett Research Institute
SABRI	Scottish Agricultural and Biological Research Institutes
SAC	Scottish Agricultural College
SARI	Scottish Agricultural Research Institutes
SASA	Scottish Agricultural Science Agency
SCRI	Scottish Crop Research Institute
SEB	Society for Experimental Biology
SET	Scottish Enterprise Tayside
SNSA	Scottish Nuclear Stocks Association
SOAEFD	Scottish Office Agriculture, Environment and Fisheries Department
SSCR	Scottish Society for Crop Research
SSFG	Scottish Soft Fruit Growers Ltd
SSPDC	Scottish Seed Potato Development Council
STS	Sequence Tagged Sites
TRIO	Tayside Regional Industrial Office
UNDP	United Nations Development Programme
WHO	World Health Organisation

The Scottish Crop Research Institute

Site plan



Access to Scottish Crop Research Institute



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

It is located at Invergowrie 6km west of the centre of Dundee. Access is via the A85, Main Street and Errol Road.

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

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