Wealthier & Healthier

Derek Stewart

A combined objective that is key to the development of a vibrant sustainable Scottish economy is the generation of food that creates wealth while being healthier and safer. The Scottish diet has long been held up as the worst in Europe, with Scots regularly topping the tables of degenerative disease such as cardiovascular disease, cancer etc. However, the national diet has improved with a commensurate reduction in disease levels, and through several national and international projects we aim to continue this trend. For example, as part of the EU-funded project, DEVELONUTRI, we are aiming to identity the points throughout the potato, tomato and wheat food chains at which nutritive value is lost.

In addition, we are also trying to enhance the value of our staple foods such as bread by substituting a proportion of the wheat with barley. Barley is one of the few crops that has led to a product with an approved health claim due to the natural component β-glucan which – if present in the diet at ~4% – can lead to a reduction in the risk of cardiovascular disease. This project, BarleyBread, was funded by the EU and involved interaction with SMEs across Europe including the Scottish food ingredients company, Macphie of Glenbervie Ltd, who generated trial breads which were hugely successful in consumer trials. Work on crop nutritional value and human health benefits is also being pursued in soft fruit such as blackcurrant, raspberry and other related crops.

The following articles show that our research into wealthier, healthier and safer food encompasses science from the fundamental through to the applied, and highlights our ability to translate this through to products.

Understanding the genetics of raspberry fruit quality for improved consumer appeal

Julie Graham

Berry sales in the UK are now second only to those of apples, accounting for 19% of all fruit sold. Just under 33% of consumers now purchase raspberries (estimated retail value £125.7 million). Currently one week's raspberry sales are equivalent to what would have been purchased in a whole year six years ago. This success of the raspberry industry is due in part to commercial raspberry production having been revitalised by new horticultural strategies, specifically protected cultivation. Protected cultivation has been pivotal in the success of UK berry cultivation, and experiments at SCRI and in Blairgowrie have demonstrated significant increase in all quality parameters from tunnel grown raspberries. Predictions are for even greater increases given availability of appropriate high quality varieties that meet market requirements across a wider season.

Consumers are willing to pay a premium for quality raspberries and health would benefit from increased consumption. The most important factor for improvement in eating quality is flavour or specifically

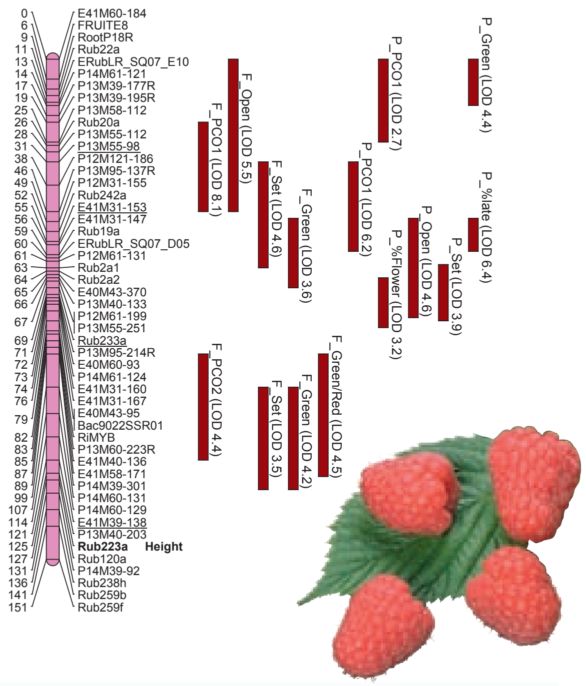


Figure 1 Chromosomal regions controlling fruit development and quality.

taste, made up of sweetness and sourness and the balance of these two attributes and overall intensity of flavour with impacts from flavour volatiles. However, appearance is as important as taste for initial consumer selection, as only berries of high visual quality will be purchased. Berry size and colouration are key to consumer success here. Consumer disappointment, given a high price for raspberries, discourages repeat purchases.

Breeding new high quality raspberries which can be scheduled across a wider season is a long, difficult process hampered by several genetic problems. Concern over environmental impact and sustainability of agricultural and horticultural practices is leading to a greater emphasis on pest and disease resistance, as well as the ability of plants to withstand local environmental stresses. The changes in environmental, cultural and agronomic practices within the industry will impact strongly on the nature of the germplasm required for the future, with a greater interest in the conservation of genetic resources and utilisation of diverse locally adapted germplasm. Breeding methods used in raspberry have changed very little over the last 40 years and little novel germplasm has made its way into commercial cultivars. However, with the narrowing genetic base coupled with the increasing demands from consumers, new breeding methods are required to meet demands. The speed and precision of breeding can be improved by the understanding of the genetic control of key traits and then the deployment of molecular tools or markers (linked to good traits) for germplasm assessment and management. The correlation between traits and chromosomes is done by developing linkage maps which represent the plant chromosomes with locations of markers and traits placed along them. Implementation of marker assisted breeding which links easily scorable molecular markers to complex traits which require extensive field evaluations, can yield defined improvements in fruit quality across the cropping season in harmony with developments of disease resistances and production agronomic traits. In the raspberry this is now possible through the development of the SCRI raspberry genetic map that forms the basis for linking phenotype

(plant traits) to genotype (plant genes). A number of chromosomal locations (quantitative trait loci [QTL]) for key quality traits in raspberry have been identified and in some cases the major genes responsible for trait variation have been identified (Fig. 1). These include steps towards understanding the ripening process which is essential to enable the development of high quality varieties fruiting across a wide season. Chromosomal locations responsible for ripening have been identified. A number of key genes including a MADS-box gene and Gene H were associated with the QTL and markers associated with plant height have also been identified. The major anthocyanin pigments in red raspberry have also been mapped to the same chromosome region on linkage groups (LG) 1 and 4 across years and from different environments. The most significant markers were genes including bHLH, and bZIP transcription factors which are thought to regulate the anthocyanin pathway. Colour has also been assessed with progress made in understanding the major genetic and environmental control. Sensory traits and the composition of fruit, both of which influence flavour, have been studied and again preliminary chromosomal regions for control identified. Methods have been developed that will allow us to measure and subsequently understand the genetic regulation of health components for improvements through marker assisted breeding.

Progress in the area of the genetic regulation of fruit quality is well underway, with marker assisted breeding now possible for a number of quality traits which can be transferred into conventional breeding programmes for targeted and timely improvements.

Fruit components and their impact on fundamental disease mechanisms

Gordon J. McDougall

The health benefits associated with a diet rich in fruit and vegetables may be derived from the intake of natural phytochemicals. One theory suggests that polyphenol antioxidants (which are especially high in fruits and berries) protect against oxidative damage

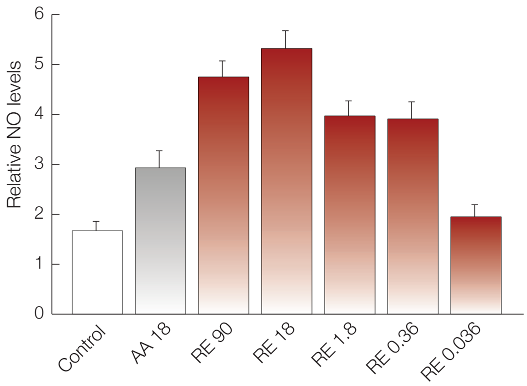


Figure 2 Protection of nitric oxide levels in a rat model by berry extracts. AA = ascorbic acid, RE = raspberry extracts. All values are μg/ml.

involved in disease development. However, many polyphenols (e.g. anthocyanins) are poorly bioavailable and are unlikely to act as antioxidants at the cellular level. Indeed, large portions of berry polyphenols are not taken up into the blood and remain in the gut.

Nevertheless, evidence continues to accrue that berries or polyphenol-rich berry preparations can influence the progression of neurodegenerative diseases, cancers, cardiovascular disease and diabetes *in vivo* even if an overarching theory to explain their mechanism of action has not been formulated. We present evidence that berry polyphenols have bioactivities (often independent of their antioxidant capacity) relevant to cardiovascular health, obesity and glycaemic control.

Nitric oxide is a key signalling molecule which controls vasodilatory responses in blood vessels. Raspberry extracts (at 90 - 0.36 μg/ml) significantly increased nitric oxide levels in a rat model system and were more effective than ascorbic acid, a known cardio-protectant (Fig. 2). Protection of nitric oxide levels, presumably through an antioxidant mechanism by reducing free radical degradation, could influence cardiovascular fitness *in vivo*. Such model studies are backed by studies which highlight the cardiovascular benefits of berry intake.

Slowing the rate of digestion of starch rich foods could influence post meal blood glucose levels and glycaemic control, thereby benefiting type II diabetes. In model systems, polyphenol extracts from berries inhibit α-glucosidase, the key enzyme in glucose release from starch, at doses easily achievable from normal dietary intake (Fig. 3). Inhibition of α-glucosidase is the target for acarbose-type pharmaceuticals (e.g. Glucobay) which are currently prescribed to type II diabetics to reduce starch breakdown after meals. Identifying the active polyphenol ingredients is under further study.

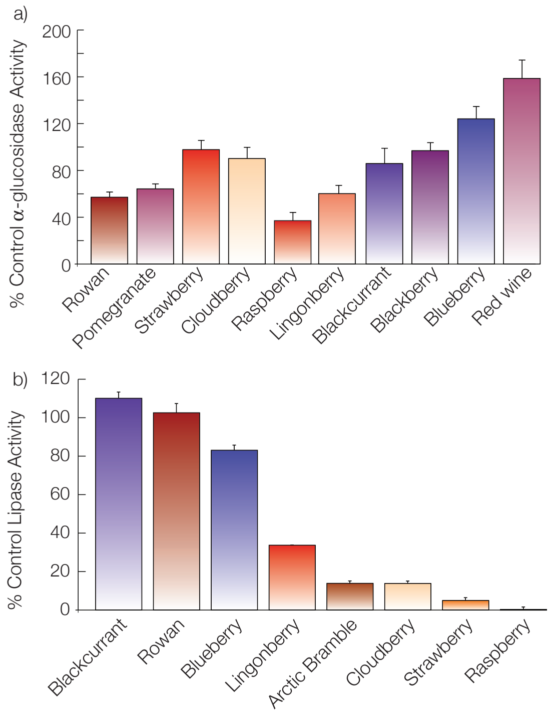


Figure 3 a) Inhibition of α-glucosidase activity by berry extracts. b) Inhibition of lipase by berry extracts. All extracts assayed at 50 μg/ml.

Inhibition of fat digestion could reduce blood lipid levels and calorie intake from fat rich meals and benefit cardiovascular complications and directly influence obesity. In model systems, polyphenol extracts from berries inhibited pancreatic lipase, which is the key enzyme for fat digestion (Fig. 3). Inhibition of this enzyme by drugs, such as orlistat (Xenical also sold as Alli), is a current treatment for reducing obesity. Inhibition of lipase and reduction in blood lipid levels may explain the effects of berries on obesity in animals. The large differences in effectiveness of the berry extracts indicate that particular polyphenol components are more effective.

Derek Stewart and a tasting panel at work.

Selenium biofortification of bread

Philip J. White

Selenium (Se) is an essential mineral element for human nutrition. It is incorporated into several important proteins as the amino acid selenocysteine. Among these proteins are iodothyronine deiodinase, which is responsible for converting the prohormone thyroxine to the active thyroid hormones; sperm-capsule selenoprotein, which is implicated in sperm motility; the antioxidant enzyme glutathione peroxidase; and selenoprotein P, the main Se-compound in plasma. According to the UK Food Standards Agency, "Selenium plays an important role in our immune system's function, in thyroid hormone metabolism and in reproduction. It is also part of the body's antioxidant defence system, preventing damage to cells and tissues".

The UK reference nutrient intakes (RNI = the dietary intake that meets the needs of 97.5% of the population) for adult males and females are 60 and 75 μg Se d^{-1}, respectively. However, the average dietary Se intakes in the UK have declined from over 60 μg Se d^{-1} in 1985 to between 48 and 58 μg Se d^{-1} today (Fig. 4). In Scotland, recent dietary surveys suggest that average dietary Se intakes may be lower than this. This downward trend is thought to be a consequence of replacing North American milling wheat, which is grown on high-Se soils and has a high Se concentration, with wheat grown on soils containing little Se in the UK. It has been suggested, therefore, that UK dietary Se intakes could be improved by increasing Se concentrations in homegrown milling wheat by applying Se-fertilisers to the crop. This practice is referred to as 'agronomic biofortification' and has been adopted

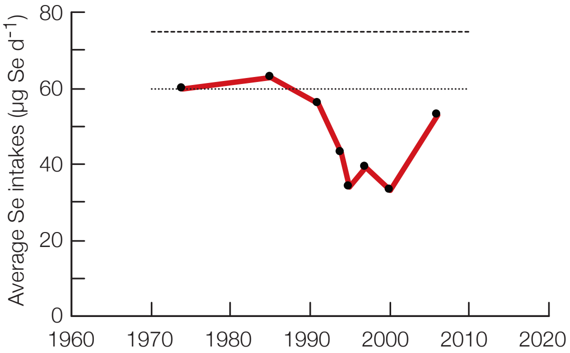


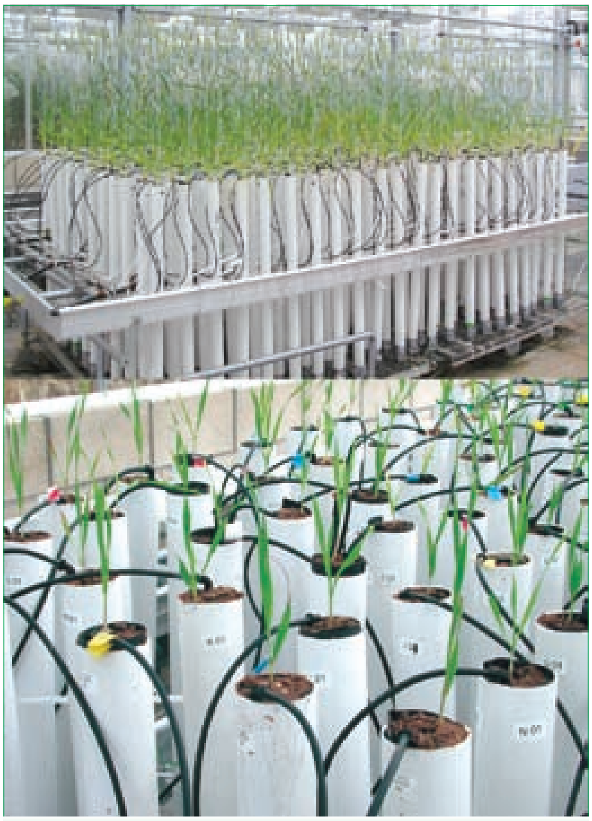
Figure 4 Average selenium intakes (µg Se d^{-1}) estimated from UK Total Diet Studies undertaken between 1974 and 2006. Horizontal lines indicate the UK reference nutrient intakes (RNI) for adult males (75 µg Se d^{-1}) and females (60 µg Se d^{-1}). Data sourced from the UK Food Standards Agency.

successfully in countries such as Finland. This year, the BAGELS consortium, which included Philip White from SCRI's Environment Plant Interactions Programme, have reported that the application of Se-fertilisers to UK wheat crops increases grain Se concentrations, and that Se concentrations in bread baked from this grain can be increased without affecting other quality attributes important for breadmaking.

BAGELS (Biofortification through Agronomy and Genotypes to Elevate Levels of Selenium; bagels.ukcrop.net) was a 'farm to fork' project sponsored by Defra through the Sustainable Arable LINK Programme. The partners spanned the entire food chain and included researchers, manufacturers and retailers. Partners included SCRI, University of Nottingham, University of East Anglia, Rothamsted Research, Institute of Food Research, Nickerson-Advanta, Velcourt, Carrs Fertiliser, Yara UK and Marks & Spencer. The main aim of the BAGELS project was to determine if the selenium levels of UK-grown wheat could be increased safely through the use of selenium-containing fertilisers, without causing harm to the environment. Field trials were conducted at the University of Nottingham, Rothamsted Research and Velcourt sites over two years on two widely-grown cultivars of Grade I bread-making wheat, Hereward and Solstice. These field trails demonstrated that the application of about 10 g Se ha^{-1} at the right time could increase grain selenium concentrations to levels similar to that of imported North

Harvesting a BAGELS field trial at the University of Nottingham. Photograph courtesy of Dr Martin Broadley.

American grain. The fate of the selenium applied in fertilisers was monitored in both the soil and the crop. Analysis of soils indicated that there was no residual build up of selenium in the soil that could pose an environmental risk. Analysis of the crop revealed that it acquired between 20 and 35% of the selenium applied in the fertiliser. About half of the selenium acquired by the crop remained in the straw, while half was recovered in the grain.



Glasshouse survey of genotypic variation in Se acquisition, Se redistribution within the plant, and grain Se concentration among wheat genotypes.

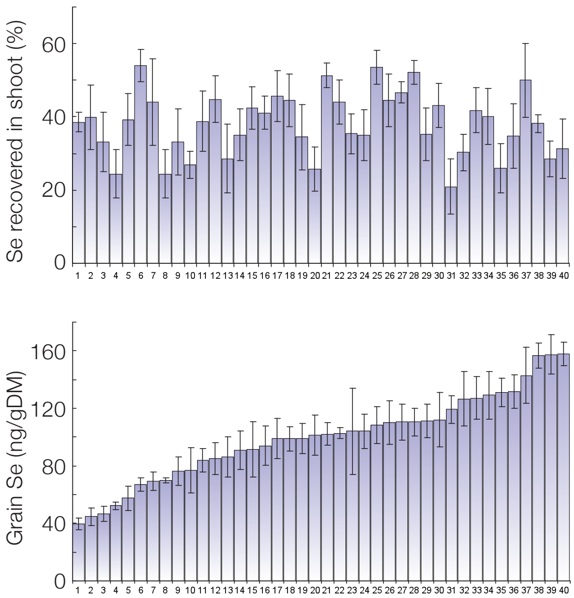


Figure 5 Genotypic variation in Se accumulation in above ground tissues, expressed as a percentage of the Se-fertiliser applied, and grain Se concentration among 40 cultivars of winter wheat grown in the glasshouse at SCRI in 2007/2008. (1) Dover, (2) Batis, (3) Opus, (4) Ochre, (5) Brompton, (6) Riband, (7) Isengrain, (8) Monopol, (9) Petrus, (10) Hereward, (11) Alchemy, (12) Lynx, (13) Mascot, (14) Malacca, (15) Zebedee, (16) Einstein, (17) Solstice, (18) Gatsby, (19) Deben, (20) Gladiator, (21) Rialto, (22) Hyperion, (23) Caphorn, (24) Cordiale, (25) Avalon, (26) Enorm, (27) A50-03, (28) PBIS, (29) Claire, (30) Scorpion 25, (31) Soissons, (32) Sokrates, (33) Gulliver, (34) Maris Widgeon, (35) Arche, (36) Robigus, (37) Flanders, (38) Cappelle-Deprez, (39) Glasgow, (40) ELS.

At present, Se-fertiliser is a non-renewable resource, and it has been estimated that if all of the world's wheat was fertilised at 10 g Se ha^{-1} then commercially viable Se reserves could be exhausted in less than 80 years. We must, therefore, use Se-fertilisers wisely. The efficient use of soil-applied Se-fertiliser by the wheat crop is determined by two physiological processes: (i) the acquisition of Se by the root system and (ii) the translocation of Se to the shoot and its accumulation in grain. At SCRI, we have surveyed genotypic variation in Se acquisition, Se redistribution within the plant, and grain Se concentration among 10 spring wheat and 40 winter wheat cultivars grown in a controlled glasshouse environment. Individual plants were grown in tubular plumbing pipes filled with a gravel:grit:sand mixture (40:40:20 by volume) fertigated with a complete mineral nutrient solution. A single application of sodium selenate was made during early vegetative growth, delivering 10 g Se ha^{-1}. Significant differences were observed between wheat cultivars in their acquisition of selenium, selenium distribution within the plant and grain Se concentration (Fig. 5). There was a strong negative relationship between grain Se concentrations and grain yield. Since previous studies have shown that Se accumulation in grain is generally linearly related to the rate of Se-fertiliser application, one strategy to achieve target Se-concentrations in wheat grain would be to match Se-fertiliser applications to expected grain yields.

The biofortification of bread with selenium.

To determine the fate of selenium during processing, the concentrations and chemical forms of selenium in grain, flour fractions and bread products were analysed at the Institute of Food Research and University of East Anglia. The dominant organic form of selenium in wheat grain, flour fractions and bread products was found to be selenomethionine, which is readily bioavailable to humans. It was also observed that over 90% of the selenium present in the grain was retained in wholemeal bread. The application of 10 g Se ha^{-1} to the wheat crop produced loaves of white and wholemeal bread containing 6.4 and 7.1 µg Se per slice, respectively. Loaves baked from Se-biofortified UK wheat, in which one slice delivered approximately 10% of the RDI, were produced by Marks & Spencer for the Cereals 2008 Event.

In summary, the work of the BAGELS consortium has demonstrated that homegrown wheat can be biofortified with selenium through the agronomic application of Se-fertilisers, and that bread baked from Se-biofortified grain has the potential to increase dietary Se intakes in the UK.

Enterobacteria survival on plants: implications for food safety

Nicola Holden

Food-borne bacteria that belong to the Enterobacteria family are able to persist in a wide range of environments and colonise hosts from every kingdom. Among these are the zoonotic pathogens that are passed from animals to humans, including toxigenic *Escherichia coli* and *Salmonella enterica*. Although traditionally associated with their animal hosts, an increasing number of food-borne outbreaks of these pathogens have occurred as a result of contaminated fresh produce, for example, ready-to-eat bagged salads. Contamination can occur at several stages during production and processing. However, we and others have shown that the bacteria actively interact with plants and can colonise them as alternative hosts. Our research at SCRI aims to understand the mechanisms behind bacterial colonisation of plants with the long term goal of reduction of food-borne bacteria from fresh produce.

At the present time, the number of outbreaks from fresh produce is relatively small. However, there has been a steady rise in outbreaks over the past two decades or so, while the incidence from farm animal products is declining. Several of the reasons for the increase have been recognised and appropriate measures adopted to counteract their effect. Nevertheless, many fundamental questions remain to be answered on the interactions between bacteria and host plants and on the mechanisms of transmission from animal to crop.

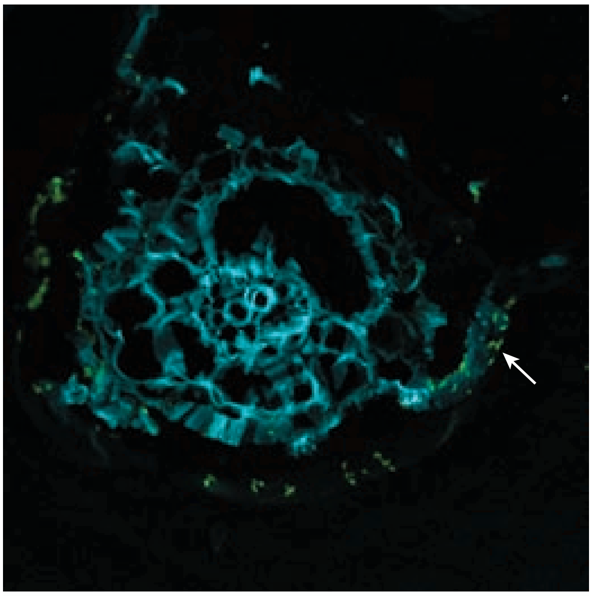


Figure 6 Microscopic image of a transverse section of a lettuce root that has been incubated with *Escherichia coli* O157:H7. The bacteria (in green) are located on the external tissue of the root (in blue) and can be clearly seen on a protruding root hair (arrowed).

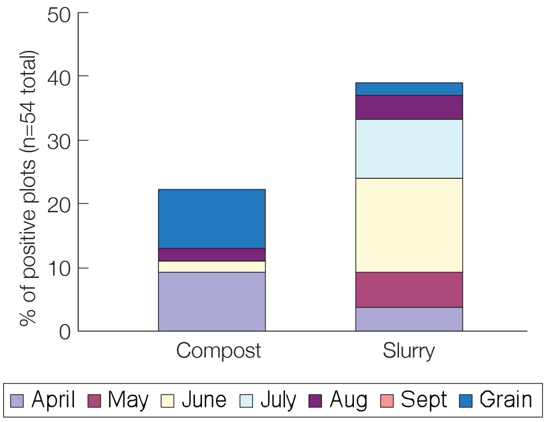


Figure 7 Presence of *Escherichia coli* isolated from the soil and barely grain of a barley trial site. The soil was amended with either municipal compost or animal slurry prior to sowing. Soil samples were collected on a monthly basis, barley grain post-harvest, and were analysed for the presence of *E. coli*-like bacteria. The number of plots, either treated with compost or slurry, that contained bacteria are expressed as a percentage of the total number of plots (n = 54).

Research at SCRI covers both areas. Firstly, in the processes that underpin bacterial colonisation of plants: how the bacteria initially interact with plants (Fig. 6); how colonisation becomes established; how the plants respond to the bacteria. It appears that specific interactions occur between the bacterium and plant host during the initial stages of colonisation, in a similar fashion to colonisation of mammalian cells. Work is continuing to elucidate the nature of the interaction, which appears to involve multiple factors. Another aspect of colonisation is internalisation of the bacteria from the external tissue of the plant to the internal tissue. Again, many fundamental questions remain to be answered about this phenomenon, such as the extent of internalisation and the cues that drive it. This has important implications for food safety where current practices only decontaminate the external parts of fresh produce.

The second area is the transmission of zoonotic bacteria from animal hosts to crop plants. We have investigated the effect of adding farm waste to cereal plots and found that it is possible to detect the bacteria throughout the growing season and at harvest time (Fig. 7). This reinforces the finding that these bacteria are perfectly able to adapt to conditions quite different from their animal hosts and shows the potential for long-term persistence in the soil. Collaborative work between Plant Pathology (Nicola Holden) and Environment Plant Interactions enables investigation of the bacterial populations on plants, which in turn, will aid in modelling the spread of the main food-borne bacterial pathogens in agriculture. This is particularly important in the context of climate change, which in addition to a direct influence on temperature and precipitation, has far reaching consequences for farming practices.