Sustainability

Philip J. White

The future brings unprecedented challenges to agriculture. The world must produce sufficient nutritious food for its burgeoning population, despite global climate change, the exhaustion of its natural resources, and environmental pressures to reduce pollution, greenhouse gas emissions and chemical inputs (Fig. 1). Great changes in agronomy are required to meet these challenges and scientific research is necessary to inform these changes.

An increasing number of research projects at SCRI address agricultural sustainability, which is defined as the ability of a system to maintain stable levels of food production and quality in the long term, without compromising economic profitability or the environment. To achieve this, SCRI scientists are devising appropriate management strategies and developing novel crop varieties that will allow agriculture to preserve soil fertility, reduce chemical inputs, and optimise the use of water and fertilisers. The articles in this section illustrate aspects of SCRI's research towards agricultural sustainability.

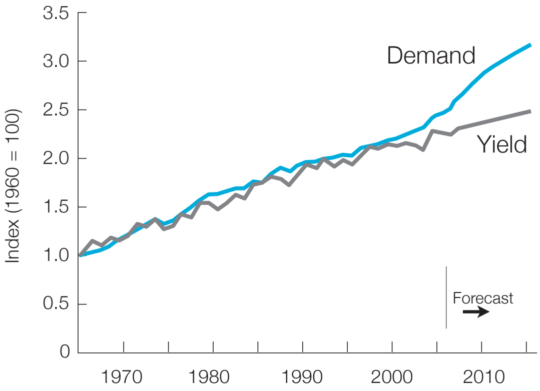

Figure 1 Trends in global demand and yield for food and feed. Data are from "Food, Feed and Fuels: An outlook on the agriculture, livestock and biofuel markets" by Jeffrey Currie, Goldman Sachs International, March 2007.

It is estimated that over one third of potential crop yield is lost to pests and diseases. Historically, these losses were minimised using agrochemicals, but recent legislation has removed from use over half the active substances controlling agricultural pests and diseases. Alternatives to agrochemicals, such as the development of resistant varieties or management practices that reduce the incidence and severity of pest or pathogen attack, are urgently required. John Jones and colleagues describe how basic knowledge

of the molecular interactions between plants and their pathogens can be used to identify genes that can be introduced into crop varieties to confer disease resistance, while Tom Shepherd and Nick Birch describe the use of biochemical signals to lure pests into traps. This research complements other work at SCRI, such as the development of monitoring tools, predictive models and biological control measures, which underpin integrated strategies for management of pests and diseases in sustainable crop production.

Another major challenge for crop production will be restrictions on irrigation water. Ankush Prashar and colleagues describe how physiologists and geneticists at SCRI are identifying traits that improve water use efficiency by the potato crop. Genetic markers for these traits can then be identified and used in breeding improved varieties. This work is one of several projects investigating agronomic and genetic strategies for the efficient use of water and fertilisers by arable crops, whose objective is to maintain stable yields with reduced inputs in the face of global change.

SCRI is putting theory into practice in the Centre for Sustainable Cropping – Balruddery. Here, six fields will become a long term experiment to test whether our knowledge of agronomy, crop physiology, soil and environmental processes, and integrated pest and disease management strategies can be used to develop a sustainable agroecosystem that will optimise inputs, biodiversity and ecosystem processes in addition to crop yield. The experiment will comprise a six year rotation that will be maintained over at least four cycles. The design will incorporate crop traits and agronomic practices that enable the reduction of water and fertiliser inputs, soil and weed management strategies that allow high yields while maintaining soil fertility and arable biodiversity, and environmentally benign and cost effective integrated pest and disease management strategies. Further aspects of the rationale and design of this ambitious experiment are summarised by Cathy Hawes and David W. Hopkins.

Functional analysis of pathogen effectors: how to go from genomics to durable resistance

Anna O. Avrova, Paul R. J. Birch*, Ingo Hein, John T. Jones & Leighton Pritchard

Pests and diseases cause extensive damage to crops and represent a threat to sustainable production. In Scottish agrosystems pathogens such as late blight (*Phytophthora infestans* – Fig. 2), potato cyst nematode (*Globodera*) and *Pectobacterium* on potato and *Rhynchosporium* on barley cause yield losses and require application of a wide range of pesticides. Although these pathogens use different mechanisms to infect plants, all need to produce a number of protein molecules, known as effectors, that interact with the plant to manipulate host processes to the benefit of the pathogen. Effectors may adjust plant metabolism so that nutrients are transferred to the pathogen, or may suppress host defences. Recognition of these effector molecules by host resistance genes triggers a strong local defence response that leads to resistance. The identification of pathogen effectors is therefore of key importance for understanding how pathogens infect plants and for identifying resistance genes that can be introduced into crop varieties.

The increased accessibility of high throughput sequencing tools means that genome and transcriptome sequencing is now achievable for a wide range of plant pathogens. Researchers at SCRI are part of the consortia that have sequenced or are sequencing the genomes of *P. infestans*, *R. secalis* and *G. pallida*. We have also sequenced the genomes of a range of bacterial plant pathogens including *Pectobacterium atrosepticum* and several species in the genus *Dickeya*. Some of these recently defined *Dickeya* species are emerging as severe potato pathogens in the UK, possibly as a consequence of climate change.

Figure 2 *P. infestans* infected potato leaf.

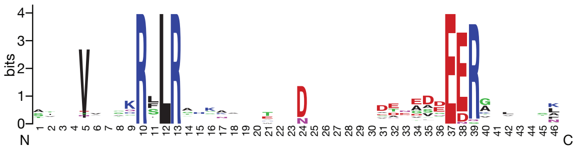

Figure 3 Sequence logo indicating the relative frequency with which particular amino acids occur in the RxLR translocation motifs of *P. infestans* effectors.

Bioinformatics can be used to aid identification of pathogen effectors. Effectors within a pathogen may have conserved sequence motifs that are important for their function. For example, we have found that *P. infestans* effectors contain a conserved RxLR motif (Fig. 3) that allows them to be translocated into the host cell, and this motif can be used to identify novel candidate effectors from a genome sequence. We can also link bioinformatics to experimental evidence, such as expression analysis, to identify genes that are up-regulated as the pathogen infects the plant, or that are expressed in pathogen tissues known to be a source of effector molecules, in order to predict which genes may be effectors.

These approaches have allowed identification of effector gene families from all of the key pathogens that we work with. Bacterial effectors that are secreted through the Type III Secretion System have been identified, and some have been shown to suppress host defences. Over 500 predicted proteins containing the RxLR motif which is required for some oomycete effectors to enter host cells have been identified in *P. infestans*, and a number of these have also been shown to suppress plant defences. Effectors identified from *G. pallida* include a very large family of proteins (SPRYSECs) that may suppress host defences and that have been shown to localise to a range of plant subcellular structures (Fig. 4). Finally, a project in which the transcriptome of germinated conidia of *R. secalis* was studied has led to the identification of a large number of secreted proteins, including candidate effectors. The roles of these effectors in suppressing host defences are now being investigated within the Plant Pathology programme.

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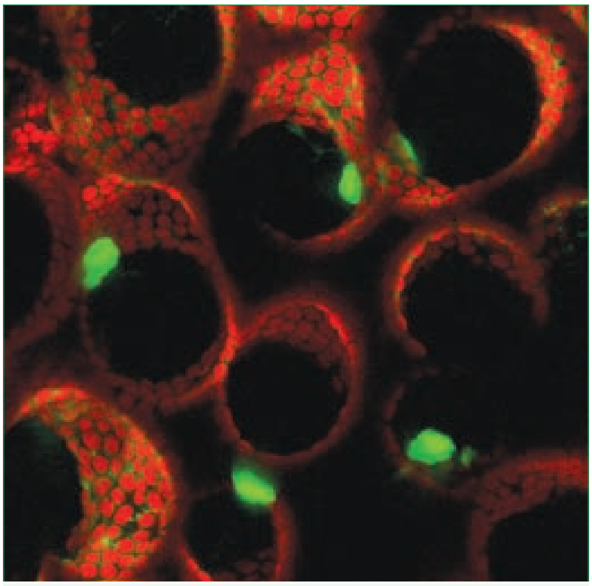


Figure 4 Subcellular localisation of a nematode effector to the nuclei of plant cells.

Figure 5 Recognition of AVR3a EM and KI in CPC accession *S. chacoense.*

Although effectors play a role in suppressing the plant's innate immune system to allow infection, they may nevertheless be detected by surveillance systems in the plant. The constant evolution of pathogen effectors and host resistance proteins to, respectively, evade or gain molecular recognition, provides an intense

arms race that may be exploited to seek durable forms of resistance for future potato breeding efforts. We have adopted a strategy to find effectors that are relatively conserved at the sequence level in pathogen populations; that are expressed in all pathogen isolates; and that provide an essential virulence function. Such effectors provide a point of vulnerability, an Achilles' heel, for the pathogen *if* resistance proteins can be found that detect and respond to them. To find such resistances, we are screening the Commonwealth Potato Collection for wild *Solanum* genotypes that respond to expression of these key effectors. A number of promising resistances have already been found in this way (Fig. 5), and their judicious combination in finished cultivars provides the potential for durable resistance to defeat our major crop diseases, and to reduce the levels of chemicals currently needed to control them.

Fooling the bugs – using plant volatiles as chemo-attractants in integrated pest management schemes

Tom Shepherd & A. Nick E. Birch

In northern and central Europe, raspberry production can be seriously affected by two major pests: raspberry beetle, *Byturus tomentosus* and raspberry cane midge, *Resseliella theobaldi.* The beetles lay eggs on opening buds and flower heads, and the resultant larvae burrow deeply into the berries to feed on the plug. This can result in damage to flowers and fruit with subsequent crop rejection. Splits in young canes caused by damage or natural splitting are the sites of egg laying by adult female raspberry midges. Emerging larvae feed on the pith, causing lesions which provide a means of entry for diseases such as cane blight fungus and midge blight. Currently there is an urgent need to reduce pesticide usage under revisions to EU pesticide policy 91/414/EEC, therefore development of environmentally friendly control measures forming part of an Integrated Pest Management (IPM) system are becoming vital. Two aspects of a successful IPM approach may include monitoring of insect numbers to improve the timing of insecticide treatments and use of control measures to disrupt the normal life cycle. Methods for monitoring and biological control of pests involve development of monitoring and mass trapping systems using natural chemical attractants as lures ('biomimicry' of the host plant). In the case of raspberry beetle, analysis of floral volatiles at SCRI using gas chromatography-mass spectrometry (GC-MS) in conjunction with electrophysiology, resulted in identification of several beetle attractants which now serve as a lure used in a

Figure 6 Bucket trap for monitoring raspberry beetles. The trap mimics the exact colour and scent of a raspberry flower.

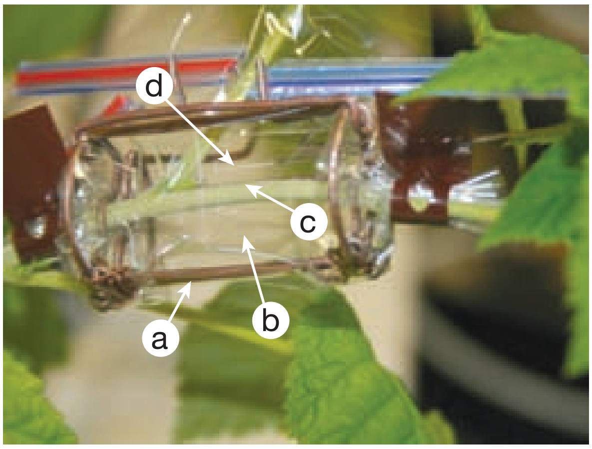
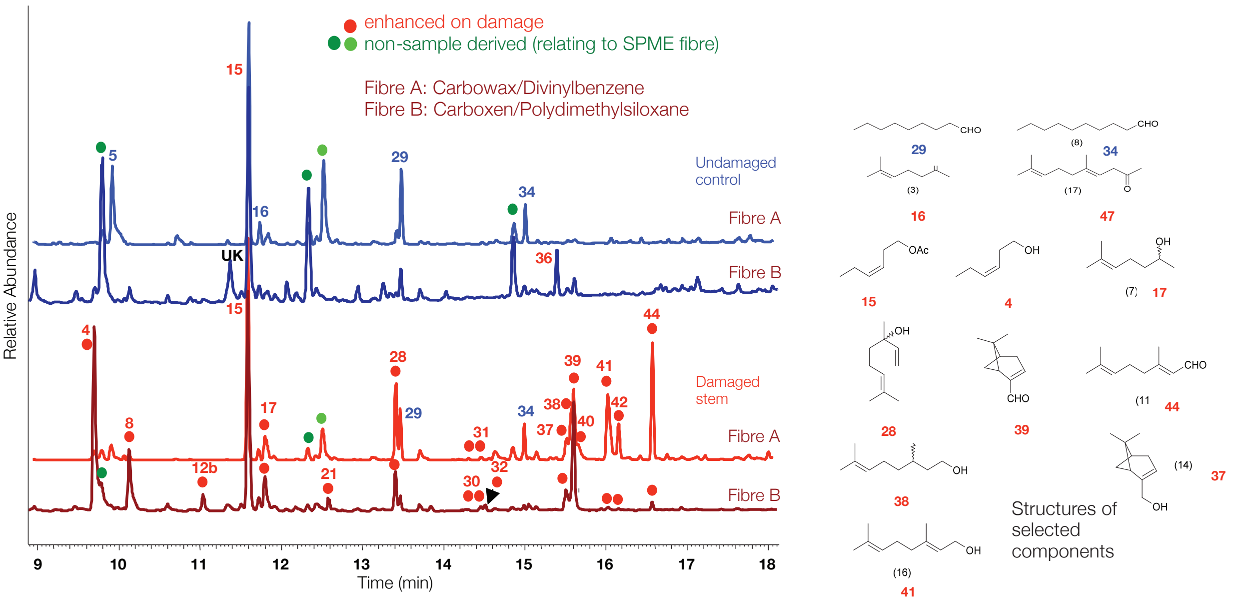


Figure 7 Enclosure for sampling volatile chemicals released from raspberry stems.
Wire frames (a) supporting an inert plastic film (b) were positioned around the cane at sites having a 2–3 cm manually created split (c). SPME fibres (d) inserted into the enclosure adjacent to the split trap released volatiles which were then desorbed in the GC injector and analysed by GC-MS.



4: *cis*-3-hexen-1-ol; 5: camphene; 8: 2-heptanol; 12b: unknown terpene; 15: *cis*-3-hexen-1-ol acetate; 16: 6-methyl-5-hepten-2-one; 17: 6-methyl-5-hepten-2-ol; 21: 5-ethyl-2(5H)-furanone or 5-methyl-4-hexen-3-one; 28: linalool; 29: *n*-nonanal; 30: citronellal; 31: *trans*-pinocarveol or *trans*-verbenol; 32: unknown; 34: *n*-decanal; 36: methylsalicylate; 37: myrtenol; 38: β-citronellol; 39: myrtenal; 40: nerol; 41: geraniol; 42: neral; 44: geranial

Figure 8 Volatile metabolites released from stems of raspberry cv Malling Promise analysed using SPME and GC-MS.

device (Fig. 6) marketed by AgriSense Ltd for precision monitoring of this key pest, so 'hot spots' can be treated as required.

For monitoring and control of raspberry midge two approaches are being taken. Traps developed and tested by colleagues at the Natural Resources Institute (NRI) and East Malling Research (EMR) use the female sex pheromone for local monitoring of males and, potentially, also for mating disruption. However, effective control requires identification of an attractant to lure emerging females in early spring (first generation) and during the second generation, coincident with fruit harvest and the main period of fungal colonisation. We have developed a novel sampling technique using solid phase microextraction (SPME) fibres to trap cane volatiles close to a split (Fig. 7).

A suite of volatiles has been identified, using several different raspberry varieties, which show a consistent pattern of enhancement following damage (Fig. 8). These compounds consist mainly of a family of structurally related terpenes, many of which are known to have behavioural effects on insects and plants (for example they attract natural enemies) and/or are produced in response to insect herbivory.

The physiological activity of the entrained volatiles is being investigated with collaborators at NRI by means of a GC-electroantennography (EAG) system which identifies the metabolites that elicit a response from the antenna of female midges. Compounds showing enhanced production when canes are damaged, and those shown to elicit a response from the midge are being used to test and develop lures for biological control and monitoring using laboratory and field based behavioural bioassays developed by SCRI, EMR and NRI. This work is funded by a five-year HortLINK project (SF 74): 'Integrated pest and disease management for high quality raspberry production'.

Water use efficiency in potato

Ankush Prashar, Timothy S. George, Gavin Ramsay, Paul D. Hallett, Hamlyn G. Jones*, Peter Hedley, Jim W. McNicol, M. Finlay B. Dale, Philip J. White & Glenn J. Bryan

Water is one of the key resources challenging the sustainability of modern agriculture. In developing countries, potato production is increasing because of its ability to provide nutritious food in a short season. However, the potato crop requires profuse irrigation. In the UK, potato production currently uses about half of

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Figure 9 Phenotypic screening of extreme $\delta^{13}C$ genotypes under controlled conditions.

all irrigation water. Further climate change is inevitable and, as this proceeds, summers are predicted to become drier and water for irrigation scarcer.

Yields of commercial potato varieties are often restricted by water availability. Their root systems are generally sparse and shallow, and they close their stomata, preventing photosynthetic carbon assimilation, while water is still available in the soil. To enable breeding of drought tolerant varieties, we are developing phenotypic screens that will allow us to explore the genetic basis of key traits for water use efficiency (WUE).

From field trials of a genetic mapping population performed in collaboration with Dr Andrew Thompson of Warwick-HRI, we initially identified ten genotypes with contrasting transpiration efficiencies based on leaf $\delta^{13}C$ values. These genotypes were then cultivated under controlled glasshouse conditions in a soil typical of arable fields in Scotland (Fig. 9). After emergence, plants were grown for 30 days in soils watered to field capacity (30% volumetric content, -5 kPa water potential) before being divided into three groups irrigated to 30%, 20% (-300kPa, slight stress) and 12% (-1500kPa, wilting point) volumetric content. As WUE is a complex phenomenon, we evaluated a number of associated physiological and morphological traits. Tissue samples were also collected at different time points to determine differentially expressed genes at these moisture levels.

Response to water stress not only includes closing stomata but also reducing the density of stomata during leaf development. Preliminary data from our experiments shows that transpiration-efficient genotypes, as indicated by low leaf $\delta^{13}C$ values, have consistently lower stomatal conductance at 12% volumetric soil moisture than transpiration-inefficient genotypes (Fig. 10). Thus transpiration-inefficient genotypes transpire more water at lower soil water content.

Another approach we are taking to improve the drought tolerance of potatoes is to screen genotypes for root length, specifically that associated with stolons. A

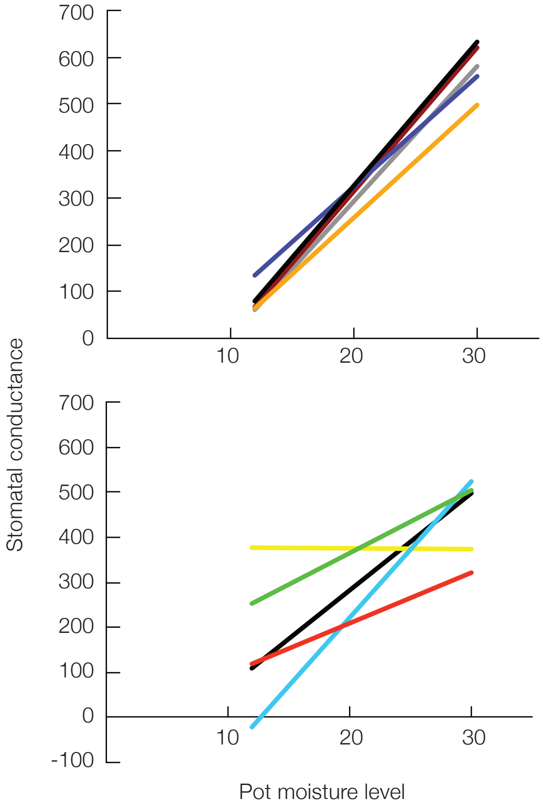


Figure 10 Regression of stomatal conductance against pot moisture levels for the five selected clones with low and high $\delta^{13}C$ values from a prior field experiment.

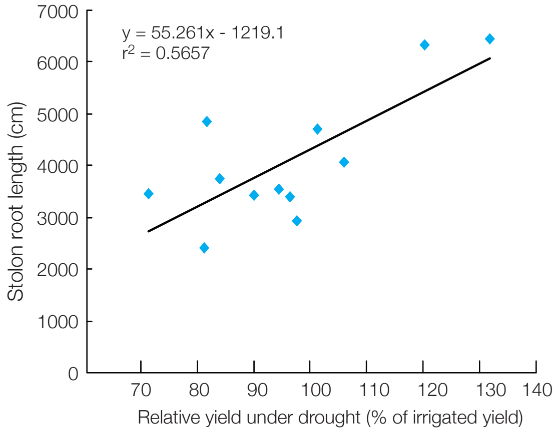


Figure 11 Relationship between stolon root length and relative yield under drought conditions in the field experiment.

number of field trials have demonstrated that there is significant variation (~5-fold) in stolon root length between genotypes of potatoes. During last year's field season we demonstrated that stolon root length

Figure 12 a) Potatoes grown under polytunnels to impose droughted treatments in 2009. b) Measurement of soil water content down the profile to establish the impact of the drought and irrigation treatment of soil water availability.

was strongly correlated with the ability of the potato plants to yield under droughted conditions (Fig. 11). This was achieved by growing potato cultivars with extreme rooting phenotypes under polytunnels with and without irrigation which developed strongly contrasting water availabilities in the field (Fig. 12). It is therefore apparent that as well as transpiration efficiency, rooting characteristics are likely to be critical for drought tolerance in potatoes.

These phenotypic screens in combination with microarray analysis will help us understand the basis of WUE and enable us to correlate WUE with gene expression in appropriate tissues. Identification and mapping of candidate genes that underlie WUE will ultimately enhance breeding for water efficient varieties in multiple environments.

SCRI's Balruddery Farm

A new platform for sustainability research at SCRI

Cathy Hawes & David W. Hopkins

The long-term viability of farming in Scotland depends on the sustainable management of our agricultural habitats. Intensification of arable systems to maximise crop yields in the short term has raised serious concerns about the functioning of arable systems and food security in the long term. The challenge now is to identify appropriate sustainable management practices and associated crop varieties that will allow farmers to achieve a balance between the potentially conflicting goals of maximising crop production, conserving arable biodiversity and maintaining ecosystem functions.

To do this, SCRI is establishing a new experimental research platform at Balruddery Farm near Dundee, for long-term studies on arable sustainability. The Centre for Sustainable Cropping – Balruddery is the first of its scale in the UK and will provide a test-bed for new management practices and 'sustainable' crop varieties developed at SCRI. The six fields that make up SCRI's Centre for Sustainable Cropping cover approximately

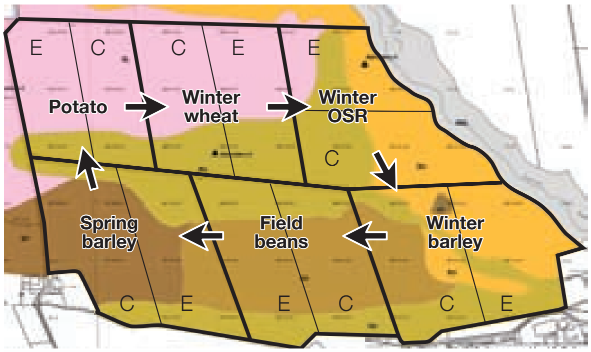


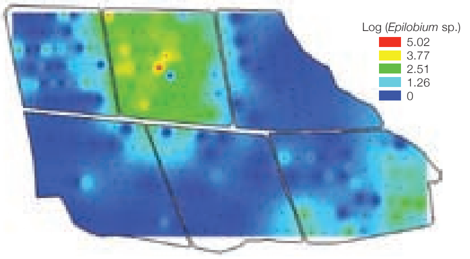
Figure 13 Layout of fields and treatment (conventional [C] and experimental [E]) management methods within the sustainability platform at Balruddery Farm.

40 ha in the south-east corner of Balruddery Farm. Each field will be divided into two halves according to topography, boundary vegetation and soil type. The conventional and experimental management treatments will be randomly allocated to each half field. Four to five different varieties of the six crops in the rotation will then be sown across both treatments, providing a comparison of varietal differences in performance (Fig. 13). Background on the farm and the design of the platform was given in the SCRI Annual Report for 2008 and further details can be found at www.scri.ac.uk/sustainability

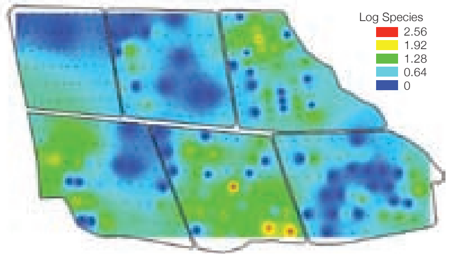
The experiment will start with the sowing of winter wheat, barley and oilseed rape varieties in autumn 2010 and beans, barley and potatoes in spring 2011. Maize was sown in April 2009 to provide a C_4 input to the existing C_3 based system that will be used as a signal to trace carbon fluxes and turnover under the two contrasting management systems. The maize crop grew well during 2009 and was cut at cob formation in September when the crop was between 1.2 and 1.8m high (Fig. 14). The entire crop residue was chopped and incorporated in the autumn. A second crop will be grown and incorporated in the same way in 2010 to boost C_4 levels in the soil and extend the period over which the pulse can be detected during the first rotation. This signal will provide a tool to characterise the flux of carbon from plants to soils, the processing of these inputs through components of soil food webs and their return to the atmosphere through biological activity.

Figure 14 Maize at Balruddery grown to provide a C_4 signature for measuring carbon dynamics.

a) *Epilobium* sp



b) *Viola arvensis*



c) Species number

d) Topography

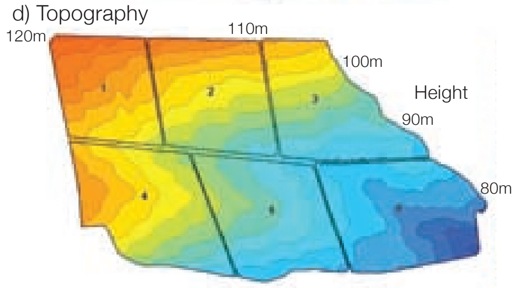


Figure 15 The distribution, abundance and diversity of arable weeds in the seedbank as assessed in spring 2009.

Baseline data is being collected on a wide range of system properties including arable plant and invertebrate biodiversity, soil microbial diversity and function, soil chemistry and biophysics, hydrology, nutrient and carbon fluxes and greenhouse gas emissions. The distribution, abundance and diversity of arable weeds in the seedbank was assessed in spring 2009 by identification of emerged weed seeds germinating from soil samples collected from a grid of 60 sample points across each field. The data shown include the weeds from the first flush of emergence only (Fig. 15). This is a good representation of the arable seedbank diversity, but does not provide an estimate of absolute abundance. The full dataset will be reported in 2010. However, these data indicate a high degree of spatial variation across the site. *Epilobium* sp. (willowherb) is concentrated in one field (Fig. 15a), whereas *Viola arvensis* (field pansy) is more widely distributed (Fig. 15b). Species richness (Fig. 15c) is lowest in the north-west corner and increases towards the south-east, following the NW-SE slope across the Centre (Fig. 15d). Explanations for these patterns from field histories and erosion patterns will be sought and trends away from this baseline under conventional and experimental management will be measured annually. The data collected during 2009 and 2010 will provide the baseline against which to assess future changes in soil health, biodiversity and system functions under experimental and conventional field management.

A major obstacle to the adoption of more sustainable farming practices in Scotland is the short-term economic situation of farmers which provides a strong incentive for intensive crop production methods. Maximising yield is therefore often prioritised over management for environmental sustainability, particularly as the latter is generally ill-defined. The aim of the Centre is to quantify the longer-term costs and benefits of new ‘sustainable’ crop varieties and management practices at a scale that is relevant to commercial farmers. This will provide the data necessary to identify a balance between the risk of potential short-term loss for long-term environmental and economic gain.