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Cereal Pathosystems

Queen Mary, University of London

16-17 December 2008

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BSPP Presidential Meeting 2008

Cereal Pathosystems

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16th – 17th December 2008

TABLE OF CONTENTS

BSPP Presidential Meeting 2008
- Cereal Pathosystems -

Queen Mary, University of London
16th – 17th December 2008

Programme

16 December 2008

10:30	Registration and coffee
11:30	Ravi Singh CIMMYT (International Maize and Wheat Improvement Center), Mexico **Garrett Memorial Lecture** *Durable resistance to rust diseases of wheat: from theory to breeding application*
12:15	Bruce Macdonald ETH, Zurich **Keynote lecture** *The population and evolutionary biology of fungal necrotrophs*
13:00	Lunch

Disease management with economic and environmental constraints

14:00	Lise Jorgensen (University of Aarhus, Faculty of Agricultural Science, Flakkebjerg) *Controlling cereal disease with reduced agrochemical inputs, a challenge for both growers and advisors*
14:30	Neil Paveley (ADAS, High Mowthorpe) *Traits for reduced fungicide dependence*

The 'Omics world –where is it taking us?

15:00	Robbie Waugh (SCRI, Dundee) *Genomic dissection of phenotypic variation in barley*
15:30	Kim Hammond-Kosack (Rothamsted Research, Harpenden) *How can molecular genetic information of plant pathogens benefit disease management?*
16:00	Tea
16:30-17:00	AGM
17:00-20:00	Posters
20:00	President's Dinner The Octagon, Queen Mary, University of London with music by Oh la la!
23:00	Finish Bus to take delegates to conference hotel (picking up outside the Octagon, transporting delegates to Express by Holiday Inn, London-Stratford)

17 December 2008

8:30 Bus to take delegates to conference venue (picking up outside Express by Holiday Inn London-Stratford, transporting delegates to Queen Mary, University of London)

Intransigent diseases (sponsored by HGCA)

9:00 Richard Gutteridge (Rothamsted Research, Harpenden)

Strategies for control of take-all

9:30 Simon Oxley and Fiona Burnett (SAC, Edinburgh)

Managing rhynchosporium risk in barley

10:00 Frank Ordon (IER, Quedlinburg)

Molecular breeding for resistance to soil-borne viruses in cereals

10:30 Coffee

PH Gregory Prize

11:00 Nicola Powell (John Innes Centre, Norwich)

The genetic characterisation of adult plant resistance to yellow rust in the winter wheat cultivar Claire

11:15 Weiqi Luo (Central Science Laboratory, York)

Predicting annual and regional disease fluctuations for winter wheat — development and application of new approaches

11:30 Siân Deller (Rothamsted Research, Harpenden)

Reactive oxygen species-related genes in the wheat pathogen Mycosphaerella graminicola

11:45 Timothy Simpson (Imperial College London)
The regulation of host-specific differentiation in Blumeria graminis

Presidential address

12:00 Graham Jellis (HGCA, London)

13:00 Lunch

What has the past taught us, and where do we go from here?

14:00 Paul Fenwick and Simon Berry (Nickerson-Advanta, Rothwell)

Marker-assisted breeding for improved resistance to disease in wheat

14:30 John Lucas (Rothamsted Research, Harpenden)
Keynote Lecture

The final straw: crop archives and the recent history of cereal pathosystems

15:15 Awards and Farewell

15:30 Tea

Posters

John Colhoun Poster Prize

P1 **Pathogenicity Determinants of *Fusarium graminearum* on Wheat Ears**
A.M. Beacham, M. Urban, J. Antoniw and K.E. Hammond-Kosack

P2 **Transmission of Barley Yellow Dwarf Viruses by aphids: impact of the primary symbiont *Buchnera***
S. Bouvaine

P3 **The infection biology and transcriptome of wheat pathogen *Fusarium graminearum***
N. Brown, M. Urban, A. van de Meene and K.E. Hammond-Kosack

P4 ***Pch2* resistance to eyespot in wheat**
C. Burt, T.W. Hollins and P. Nicholson

P5 **QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola***
S.F.F. Torriani, P.C. Brunner, B.A. McDonald and H. Sierotzki

Open Poster Submissions

P6 **Resistance of UK wheat cultivars to ergot**
R.A. Bayles, R.J. Birchmore, M. Fletcher and T.W. Hollins

P7 **Population differentiation, spatial aggregation and sexual compatibility in populations of *Fusarium pseudograminearum* from the Australian grain belt**
A.R. Bentley, J.F. Leslie, M.G. Milgroom, B.A. Summerell and L.W. Burgess

P8 **The use of recombinant plant viral antigens for raising of polyclonal antibodies**
N. Cerovska, H. Plchova, T. Moravec and P. Dedic

P9 **Increasing susceptibility of triticale to powdery mildew (*Blumeria graminis*) in Belgium**
V. Derycke and G. Haesaert

P10 **Resistance to root knot nematode in melons is available in the primary gene pool of Indian origin**
N.P.S. Dhillon, A. Roy, S.S. Bal and S. Kaur

P11 **Molecular Characterization of '*Candidatus* Phytoplasma cynodontis' associated with Bermuda grass disease in Rajasthan, India**
R.K.Gaur, R. Raizada, K.P. Sharma,and V. Mishra

P12 **Investigation of the cellular basis for resistance to ergot infection in wheat**
A. Gordon, R.A. Bayles and D.M. O'Sullivan

P13 **Investigating altered fungicide sensitivity in *Rhynchosporium secalis***
N. Hawkins, H. Cools, M. Shaw, H. Sierotski and B. Fraaije

P14 **Providing independent information on fungicide performance in wheat**
G. Jellis and V. Foster

P15 **The development of EURO-Wheat, a wheat-disease based research platform within the ENDURE Virtual Laboratory**
L.N. Jørgensen, M.S. Hovmøller, J. G. Hansen, P. Lassen, C.H. Denholm, I. Denholm, B. Clark and N. Evans

P16 **An investigation of germination pathways, infection and disease development using low inoculum densities of *Phytophthora ramorum* on detached rhododendron leaves**
Y. Kumagai, S. Denman and S. Archer

P17 **Preharvest qPCR diagnostics for better management of phoma stem canker and light leaf spot in oilseed rape**
A.O. Latunde-Dada, K. Downes, J.F. Stonard, S. Rogers, J.S. West and B.D.L. Fitt

P18 **Population structures of global isolate collections of *Leptosphaeria maculans* and *L. biglobosa*, the causal organisms of phoma stem canker and lesion on oilseed rape**
A.O. Latunde-Dada, Z. Liu, K. Downes, B.D.L. Fitt, J.A. Lucas and I.R. Crute

P19 **DON mycotoxin biosynthesis by *Fusarium* species, a metabolomics perspective.**
R. Lowe, J.W. Allwood, M. Urban, M. Beale, J. Ward and K. Hammond-Kosack

P20 **Comparison of cereal eyespot disease levels on winter wheat at two sites in Cambridgeshire, UK**
K. Maguire, R. Birchmore, V. Fanstone, S. Mann and R. Bayles

P21 ***Brachypodium distachyon*: A model species to study cereal-pathogen interactions.**
L.A.J. Mur, A.P.M. Routledge, J.W. Allwood, J.V. Smith, R. Goodacre and D.F. Garvin

P22 **Allele-specific real-time PCR for quantification and discrimination of sterol 14α-demethylation-inhibitor-resistant genotypes of *Mycosphaerella graminicola***
S. Selim, J. Sanssené, C. Roisin-Fichter, V. Hervé and J.-D. Clement

P23 **Sources of ergot infection in wheat**
J. E. Thomas and V. Fanstone

P24 **Closing the blast doors: biological and transcriptional defence responses of wheat to non-adapted and adapted species of the blast fungus, *Magnaporthe***
H.A. Tufan, G.R.D. McGrann, A. Magusin, J.-B. Morel, L. Miché and L.A. Boyd

P25 **Variability in virulence of *Mycosphaerella graminicola* isolates isolated from one blotch**
L. Vechet and L. Burketova

How can molecular genetic information on plant pathogens benefit disease management ?

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Although disease is rare, in crop monocultures specific pathogenic species and strains can come to dominate populations either locally or over vast areas. Under favourable environmental conditions disease epidemics result which lower crop yields, end – product quality and sometimes product safety. Disease control in crop monocultures relies on an integrated approach which combines good crop husbandry, with the deployment of disease resistant germplasm, based on major resistance genes or quantitative trait loci, and when necessary through the use of appropriate fungicides. However, the durability as well as the sustainability of many disease control options remains an issue as does predicting which specific disease problems will emerge each season.

In the past 25 years many novel types of molecular genetic information have been gathered on a range of plant, animal and human attacking pathogens in an attempt to devise new approaches to achieve both single species as well as broader spectrum disease control. As a result of forward and reverse genetic experiments using transformed strains, many pathogenicity and virulence genes controlling the disease-causing ability of pathogens have been identified. In addition, a smaller repertoire of pathogen toxins and pathogen effectors are now recognised which activate or suppress host defences in specific plant genotypes. In the past 10 years the pace at which novel information on pathogens can be acquired has accelerated dramatically with fully sequenced genomes becoming available for many plant, animal and human-attacking species. This is now permitting comparative genomics analyses to be done to identify which genes and gene families are conserved between pathogens and saprophytes, which are either plant or animal pathogen specific, which are unique to particular taxonomic classes of pathogens and which appear to be specific to a single species.

This increasing wealth of molecular genetic information on plant pathogens can be used to identify novel targets for intervention. These may be plant cell accessible targets because the gene product is secreted outside the pathogen or is located within the plasma membrane and therefore amenable to targeting by conventional plant breeding or through a plant biotechnology approach. Alternatively, if the target is located inside the pathogen this may be accessible for intervention by either existing or novel chemistries.

Molecular genetic information, now framed within the chromosome landscape of each organism, will also become increasing valuable in monitoring pathogen populations. This should identify the changes which occur when a novel disease control option is first introduced as well as the population changes potentially associated with the emergence of new virulent strains which may then threaten the effectiveness and durability of the control achieved.

The final straw: crop archives and the recent history of cereal pathosystems

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The factors influencing the occurrence of epidemics in cereal crops are in many cases well-known, and have been used as the basis for forecasting systems predicting disease risk. Much less is known, however, about longer term changes in disease incidence, such as why certain diseases become more common while others decline in importance. Historical crop archives provide an opportunity to study long term trends, not only in disease occurrence, but also in the genetic properties of major pathogens of interest. The Long Term Experiments (LTEs) at Rothamsted Research are unique in that crop and soil samples were stored from almost every season, and records kept of the crop varieties grown, agronomic practices, and meteorological data. Recent analyses of leaf, stem and seed samples collected from wheat and barley crops over a period of more than 150 years using PCR amplification of host and pathogen DNA and gene sequencing are starting to reveal new clues to evolutionary changes in cereal pathosystems over the period coinciding with the development of modern agriculture. Fluctuations in the predominance of different pathogens, such as those causing the Septoria leaf and glume blotch diseases, have been detected and linked to environmental factors. The recent emergence of new diseases, such as Ramularia leaf spot on barley, is also reflected in the samples, confirming that the archives are representative of national disease trends. Pyrosequencing is now being used to monitor changes in pathogen genes subject to strong selection pressures, such as those encoding pathogen effectors and virulence factors, as well as fungicide targets. These studies have revealed the rapid emergence of resistance to MBC and QoI fungicides in Septoria in the 1980s and since 2002 respectively, as well as more gradual and diverse alterations in the *CYP51* target of azole fungicides. The opportunity now exists to further explore the LTEs for pathogen variation and adaptation on different crop genotypes over many pathogen generations in response to agronomic practice as well as changes in the crop environment such as soil properties, pollution and global warming.

The Genetic Characterisation of Adult Plant Resistance to Yellow Rust in the Winter Wheat Cultivar Claire

Nicola Powell [1], Simon Berry [2], Lesley Boyd [3]

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The winter wheat cultivar Claire was released commercially by Nickerson Seeds U.K. Ltd in 1999. At the time it was given a NIAB rating of 9 for resistance to yellow rust (causal agent *Puccinia striiformis* f.sp. *tritici*). Preliminary analysis shows yellow rust resistance in Claire to be polygenic. A double haploid (DH) population has been constructed between Claire and Lemhi. Lemhi is an American spring wheat which is susceptible to all UK agent *Puccinia striiformis* f.sp. *tritici* isolates. The construction of a genetic linkage map using SSR, NBS-AFLP, DArT and AFLP markers has allowed the estimation of the number, chromosomal position and degree of effect of yellow rust resistance QTLs within the DH population. A total of 436 markers have been mapped to date creating a map of 1350cM. Analysis of the Claire x Lemhi linkage map with phenotypic data collected over 2 years of field tests have identified a number of QTLs associated with adult plant resistance against yellow rust. QTL loci have been identified on chromosomes 2B and 2D.

Predicting annual and regional disease fluctuations for winter wheat — development and application of new approaches

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Under the conditions found in England and Wales, winter wheat is subject to the threat of infection from many pathogens able to cause considerable reduction in grain yield and quality. In recent years, greatest yield loss from foliar diseases has been due to Septoria leaf blotch, which is caused by the fungus *Septoria tritici*. Therefore, control of Septoria leaf blotch has a high priority.

Although there is clearly a relationship between weather and the severity of *Septoria tritici* on winter wheat across England and Wales, it is not understood quantitatively. Various attempts have been made in Britain and elsewhere to produce forecasting systems to model the relationship. However, these forecasting schemes are difficult to implement widely due to the challenges of gaining weather observations at site-specific scales. With the intrinsic limitation of weather data, predicting disease pressure accurately for all the farms at a field scale is probably to be impracticable for commercial management of arable crops. To free wheat growers and crop consultants from collecting in-field meteorological data and to speed up the implementation of disease forecasting, we propose a possible framework for disease modelling at larger scales, which requires less rigorous weather inputs. Cluster analysis has the capability to aggregate homogeneous farms together to allow modelling at a larger scale. After identifying the suitable predictive scale, a novel high dimensional regression technique, Stepwise Partial Least Squares Regression (S-PLSR) is applied for disease modelling. This has advantages of describing and investigating the complex process of disease development, where there are more predictors than observations.

This presentation will:

(1) Provide a strategic overview of the temporal and regional variation in disease severity.
(2) Evaluate the performance of various cluster analysis algorithms;
(3) Identified cluster regions that can be used as an informative predictive scale for disease forecasting;
(4) Describe the effects of key meteorological parameters such as temperature and rainfall on the development of *Septoria tritici*;
(5) Show how these results have been used to develop new disease warning system, with an estimate of reliability, for crop managements.

Reactive oxygen species-related genes in the wheat pathogen *Mycosphaerella graminicola*

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Mycosphaerella graminicola (anamorph *Septoria tritici*) is a fungal pathogen of wheat leaves causing lesions of chlorotic and dead plant cells. In the early stages of infection the fungus grows in the leaf intercellular spaces without causing visible disease symptoms. In the later infection stages fungal biomass increases, hyphal nutrition becomes necrotrophic and localised host programmed cell death occurs (1). Reactive oxygen species (ROS)-specific stains have shown that hydrogen peroxide and superoxide are present in infected leaves, that levels increase with the appearance of disease symptoms (1, 3) and that ROS are closely associated with the fungal asexual fruiting bodies.

Expression profiling of *M. graminicola* genes during infection of susceptible wheat genotypes has shown a number of ROS scavengers have greatly increased expression as disease symptoms become visible (1, 2, 3). Candidate genes have been selected for functional analysis based either upon literature or their transcriptional up-regulation *in planta* during disease symptom formation. The sequenced genome of *M. graminicola* has enabled complete deletion of genes through targeted *Agrobacterium*-mediated transformation.

Results will be presented describing the functional characterisation of fungal genes involved in the production of ROS and the oxidative stress response. Deletant strains have been characterised for pathogenicity, sensitivity to ROS, altered gene expression and protein activity.

1. Keon *et al.* (2007) MPMI, **20,** 178-193
2. Keon *et al.* (2005) Fungal Genetics and Biology, **42,** 376-389
3. Keon *et al.* (2005) Molecular Plant Pathology, **6,** 527-540

This project receives financial support from Syngenta and the Biotechnology and Biosciences Research Council (BBSRC) of the UK. Rothamsted Research receives grant aided support from the BBSRC.

The regulation of host-specific differentiation in *Blumeria graminis*

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Blumeria graminis f. sp. *hordei* (Bgh) is an obligate biotrophic pathogen that causes barley powdery mildew. Bgh relies on host stimuli for correct development leading to successful infection. We compared development on barley (the host), wheat (a non-host), and on two artificial substrates (cellulose membrane and glass). We found that there was no obvious difference in differentiation on the two plant surfaces during early-prepenetration infection; however, on the artificial substrates development would terminate and show aberrance. This is reflected in the results of investigations in transcriptome dynamics that accompany development on these surfaces.

We have produced modified glass surfaces with 16-hydroxyhexadecanoic acid (HHD) and rough-cutin extract that mimic the plant's epidermis in an attempt to recover normal Bgh development. Results indicate HHD does spur limited recovery of development.

In order to test the potential of *Magnaporthe oryzae* (Mgo) as a surrogate model to study gene regulation in Bgh, we selected DNA sequences 5' to 23 genes that are differentially expressed during development on host and artificial surfaces. We fused these Bgh promoters to *GFP* then transformed them into Mgo. We are currently systematically screening these transformants during growth and development on onion epidermis and barley.

P1

Pathogenicity Determinants of *Fusarium graminearum* on Wheat Ears

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Using a novel bioinformatics approach, we have identified a micro-region of verified pathogenicity gene homologues in the important crop pathogen *Fusarium graminearum*. This region is now being analysed by a combination of bioinformatic and reverse genetics approaches to ascertain its role in pathogenicity of this species with the aim of locating novel virulence determinants.

Comparative genomics has been used to investigate conservation of the micro-region in other closely and less closely related species. Targeted deletion of single genes has allowed the determination of the role of micro-region genes in *F. graminearum* pathogenicity.

Deletion of the neutral trehalase gene *NTH1* appears to slow infection of wheat ears, while deletion of the *SNF1* protein kinase or *PKAR* cAMP-dependent protein kinase regulatory subunit inhibits pathogen spread in wheat. In addition, a conserved hypothetical gene in the micro-region has also been shown to be required for full pathogenicity and so represents a new type of virulence factor.

This micro-region appears to be distinctly different from the virulence-associated biosynthetic and secreted protein clusters identified so far in pathogenic fungi. Further investigation will reveal more about the evolution of this small genomic region.

P2

Transmission of Barley Yellow Dwarf Viruses by aphids: impact of the primary symbiont *Buchnera*

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The Barley Yellow Dwarf disease is one of the most widely distributed viral disease of cereals. It affects economically important crop species such as barley, oats, wheat, maize, triticale and rice. The agents responsible for this disease are a group of viruses ('BYDV') belonging to the family Luteoviridae. The BYDV virions are obligatorily transmitted by aphids through a persistent circulative process. At least 25 aphid species are known to be vectors, and the most important ones are *Rhopalosiphum padi, Sitobion avenae* and *Schizaphis graminum.* A very large intraspecific variation for the virus transmission has been reported. Almost all the aphid species harbour bacterial symbionts called *Buchnera* involved in nutritional interactions. Those symbionts have been shown to release in the aphid body a homologue protein of the *Escherichia coli* GroEL chaperon, able to bind in vitro specifically to luteoviruses. The aim of this study is to investigate the nature of this interaction and its role in the luteovirus transmission process. Monoclonal antibodies specific to the *Buchnera* GroEL have been developed and offered a tool to study the distribution of this protein in the aphid body and to characterise its abundance in the different aphid populations.

P3

The infection biology and transcriptome of wheat pathogen *Fusarium graminearum*

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The global re-emergence of Fusarium ear blight on wheat and other cereal crops has both economic and health implications. In the UK the filamentous ascomycete *F. graminearum* is one of the main causal agents of the disease. Since the *F. graminearum* genome was sequenced, an increasing volume of transcriptomic, proteomic and metabolomic data has been generated. However, the mode by which the pathogen establishes infection and colonises the entire ear is unknown. A controversy also remains over whether the pathogen invades wheat floral tissue via necrotrophic or hemibiotrophic means. This investigation is concentrating on the interaction between the sequenced *F. graminearum* strain PH-1 and the susceptible wheat cultivar Bobwhite.

A detail microscopic investigation into fungal colonisation of a wheat ear has revealed two distinct stages to infection. Wheat ears were inoculated with a droplet of a conidial suspension into the floral cavity at anthesis. Towards the advancing front of fungal infection, hyphae adopt the form of intercellular spaces and intracellular hyphae pass between host cells via the plasmodesmata. Despite a high level of inter and intracellular fungal colonisation host cells appear healthy. Behind the infection front fungal colonisation resembles a necrotroph, with host cells being heavily degraded. This may suggest the existence of a switch between the subtle and destructive modes of nutrition.

The use of the vascular system as a means to establish and colonise throughout the ear is supported by the preferential hyphal colonisation of the phloem. Again, initial subtle colonisation was followed by the rapid destruction of the phloem elements. This preferential colonisation of the phloem may also sustain the pathogens subtle radial growth from the vascular network throughout the other tissues in the ear.

Following the identification of key points in the infection process specific tissues will be isolated at the host-pathogen interface via laser capture micro-dissection. This will enable the exploration and comparison of fungal and / or host gene expression profiles in different temporal and spatial sequences. When used in combination, the measurable development of fungal infection and the transcriptomic analyses would validate one another, by providing a link between the physical infection process and the expression of genes coding for proteins of known function. This approach should increase our understanding of how this destructive pathogen modulates its development throughout the infection cycle.

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK. Neil Brown is supported by a BBSRC industrial CASE studentship awarded to Syngenta.

P4

Pch2 resistance to eyespot in wheat

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Eyespot is an important fungal stem base disease of cereal crops in northern Europe caused by two species; *Oculimacula yallundae* and *Oculimacula acuformis*. The aim of the work was to further understand the partial resistance conferred by the gene *Pch*2, which derives from the variety Cappelle Desprez (CD). Evidence from the present study suggests that *Pch2* is more effective against *O. acuformis* than *O. yallundae* and furthermore is mostly effective at the seedling stage. We mapped *Pch2* to the distal end of chromosome 7AL and closely linked SSR markers were identified. Expression studies have isolated a set of cDNA-AFLP fragments differentially expressed between the susceptible wheat line Chinese Spring (CS) and the *Pch2* containing chromosome substitution line CS/CD7A. These fragments are currently being investigated to further understand the *Pch2* resistance response, to identify potential candidate genes for *Pch2*, and to develop further markers for the selection of *Pch2*.

P5

QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*

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QoI fungicides or quinone outside inhibitors (also called "strobilurins") have been widely used to control agriculturally important fungal pathogens since their introduction in 1996. QoIs block the respiration pathway by inhibiting the cytochrome bc1 complex in mitochondria. Several plant pathogenic fungi have developed field resistance. The first QoI resistance in *Mycosphaerella graminicola* was detected retrospectively in UK in 2001 at a low frequency in QoI treated plots. During the following seasons resistance reached high frequencies across northern Europe. The aim of this study was to clarify the evolutionary mechanisms behind the emergence and spread of QoI resistant isolates in *M. graminicola*. The main hypotheses tested were i) the G143A mutation occurred only once or very few times, potentially in only one mtDNA haplotype and/or one region, and was subsequently distributed to other regions by migration, and ii) the G143A mutation occurred independently in several different mtDNA haplotypes and/or geographic regions.

The G143A mutation causing QoI resistance was first detected during 2002 in all tested populations and in eight distinct mtDNA sequence haplotypes. By 2004, 24 different mtDNA haplotypes displayed the G143A mutation. Phylogenetic analysis showed that QoI resistance was acquired independently through at least four recurrent mutations at the same site of cytochrome b. Estimates of directional migration rates showed that the majority of gene flow in Europe had occurred in a west-to-east direction.

This study demonstrated that recurring mutations independently introduced the QoI resistance allele into different genetic and geographic backgrounds. The resistant haplotypes then increased in frequency due to the strong fungicide selection and spread eastward through wind dispersal of ascospores

P6

Resistance of UK wheat cultivars to ergot

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Ergot, caused by the fungus *Claviceps purpurea*, is a sporadic disease in wheat, but one of great significance to the grower as the presence of sclerotia in a load of grain will result in rejection at the mill intake. Since cleaning to remove ergots is costly and rarely completely successful, emphasis must be on reducing the risk of infection in the field. Cultivar resistance, if available, could make a major contribution to this.

The risk of infection is usually assumed to be greatest for cultivars with florets which gape open during, and for some time after, pollination. Florets that open less widely, or for a more limited period, may provide a mechanical barrier to entrance of spores and thereby escape infection. In addition, there is the possibility that cultivars may differ in 'tissue' or 'post-infection' resistance, something about which very little is known. The aim of the research described here was to investigate differences between 46 winter wheat cultivars in their 'field resistance' to ergot and to examine the contributions of tissue resistance and 'escape'.

'Field resistance' was estimated by exposing cultivars to inoculum generated by artificially infected blackgrass spreader plots. This revealed a high degree of variability in ergot infestation between sites and years and some inconsistency in cultivar effects. Despite this, it was possible to identify a number of cultivars which never suffered more than slight infection and others which were consistently heavily infected.

Investigation of tissue resistance was by direct introduction of ergot inoculum into individual florets in order to bypass possible 'escape' mechanisms. This approach provided clear evidence of consistent cultivar differences in tissue resistance. No variety was immune, but some expressed a significantly greater degree of partial resistance than others.

Escape was examined by attempting to quantify flowering traits associated with 'open-ness'. The results pointed to an over-riding effect of environment, with no evidence of a consistent relationship between open-ness of flowering and field resistance / susceptibility.

Our data suggests that growers have the opportunity to reduce the risk of ergot contamination through cultivar choice, although testing to a wider range of *C. purpurea* strains and further commercial experience would be desirable. Selection for the level of resistance expressed by current genotypes would be possible in inoculated nurseries similar to those developed here. However, for more rapid progress, selection at an early stage in the breeding process is desirable. This would require the development of molecular markers for resistance, or susceptibility.

P7

Population differentiation, spatial aggregation and sexual compatibility in populations of *Fusarium pseudograminearum* from the Australian grain belt

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The nature of the genetic relationships within and between regional, field and micro-scale populations of the crown rot fungus *Fusarium pseudograminearum*, together with the potential processes responsible for their generation, were assessed. Population genetic parameters were evaluated using AFLP fingerprinting and mating type analysis of *F. pseudograminearum* isolates collected in major winter cereal producing regions of the Australian grain belt in northeastern, south central and southwestern Australia. From the 217 isolates examined, 176 haplotypes were identified and grouped into two main clusters. One of these clusters consisted exclusively of isolates from northeastern Australia. In contrast, the other cluster included isolates from both south central and southwestern Australia, which were not clearly distinguishable from one another and may be a single population. It is hypothesized that the *F. pseudograminearum* populations from northeastern and southern Australia are historically independent, which could result from different founding events or their differentiation due to geographic isolation and the accumulation of genetic differences due to genetic drift and/or selection.

Additional studies to determine population genetic parameters and the presence of physical and/or genetic spatial aggregation on a micro-scale were undertaken on three intensively sampled 1-m-row section populations from the Australian grain belt. The calculations of population genetic parameters based on AFLP fingerprinting indicated that genetic diversity on a micro-scale described a large proportion of the diversity recorded for corresponding field and regional populations. Matrix comparison tests showed significant aggregation of clonal haplotypes and subsequent spatial autocorrelation of physical distance and both mating type and genetic distance. The presence of non-random physical and genetic spatial structuring in at least two of the three 1-m-row section populations is hypothesized to be a reflection of the fact that infested crop residues are the primary inoculum source in no-tillage farming systems, conferring aggregated patterns of disease and maintaining *F. pseudograminearum* haplotypes in the field over a number of ephemeral cropping cycles.

Studies on the role of sexual recombination in the lifecycle of *F. pseudograminearum* revealed inherently low levels of female fertility in field populations despite the observation of naturally occurring perithecia of the teleomorph *Gibberella coronicola* at two sites. Female fertility levels in field isolates could only be increased to produce female fertile tester strains after four generations of single and multi-stage crossings between sibling progeny derived from fertile laboratory crosses. These tester strains represent a significant advancement in the understanding of *G. coronicola*, and have utility for numerous further applications, including construction of a genetic linkage map for *F. pseudograminearum* and characterisation of various biological and epidemiological parameters.

P8

The use of recombinant plant viral antigens for raising of polyclonal antibodies

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The use of recombinant proteins is an attractive strategy for the production of antibodies against viruses, which are present in low concentrations in infected plants, or are difficult to purify. On the other hand, the use of antibodies against recombinant structural proteins in diagnostic tests seems to be impeded by their inefficiency in recognizing native epitopes.

The recombinant viral structural and non-structural proteins expressed in bacterial cells have great potential as a source of antigens for raising specific antibodies.

We compared different ways of antigens expression, purification and immunization. We prepared polyclonal antibodies to a recombinant coat protein of *Potato virus* Y^N– Wilga (PVY^N-W), *Potato virus X (*PVX*), Potato mop-top-virus (*PMTV*)* and to a recombinant non-structural protein TGB1 of PMTV.

The obtained sera and antibodies were tested for the detection of mentioned pathogens in laboratory hosts (tobacco species) and natural host *Solanum tuberosum* by ELISA as well as by Western blots. The obtained antisera have been used successfully for plant virus detection by Western blot analysis and indirect PTA ELISA, but they have failed in DAS ELISA.

According to our expectation the failure to get positive results when the obtained antibodies were used for coating ELISA plates (DAS ELISA) suggests that our antibodies may not recognize native epitopes, but only epitopes which are affected by some denaturation steps (e.g. binding to the surface of ELISA plates in binding buffer with high pH or SDS-PAGE in case of Western blot analysis), as this binding may affect their conformation.

The obtained polyclonal antibodies could be a very good tool not only for virus detection but also for the study of their life cycle.

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P9

Increasing susceptibility of triticale to powdery mildew (*Blumeria graminis*) in Belgium

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Triticale (x *Triticosecale* Wittmack) was created as a hybrid from species of *Triticum* and *Secale*. In this way the high yield capacity of wheat was combined with the better disease resistance of rye. This disease resistance has still been considered as one of the most important and durable advantages of triticale. This is especially the case for leaf blotch (*Mycosphaerella graminicola*), but the excellent resistance of triticale to mildew (*Blumeria gramininis*) is mostly also praised in literature.

Triticale is - 30 years after its introduction - cultivated on ca. 2.5 million hectares, especially in Poland, Germany and France. The triticale growing area has stabilized in Belgium during the last ten years at 10.000 ha. In Belgium triticale is used in mixed farming systems and organic farming.

Since 1984 disease observations in triticale varietal experiments were performed on the experimental farm of the University College Ghent. In 1997 the observations were extended with 6 other locations spread over the country from west to east and with different soil types. These experiments fitted in the network of the 'Agriculture centre for small cereals' (LCG vzw). The observed genotypes were all commercial varieties. Normal crop husbandry measures were taken: fertilisation based on soil analysis, adequate chemical weed control and growth regulation of small cereals of at least 1 to 3. No fungicide treatments or artifical infection were done. The experimental design was always a completely randomized block design with 3 or 4 replications. Disease symptoms were scored on 10 tillers per plot using a 1-9 scale with 1: no symptoms and 9 extensively infected. The 10 tillers were chosen at random. The disease assessments were done at regular times in relation to the presence of the diseases but normally between growth stage 32 (second node) and 83 (early dough).
In 1998 the response of triticale varieties to fungicide treatments were also studied with additional experiments on the experimental farm. Fungicides were applied in growth stage 'all ears visible". Disease observations were carried out at the same way as for the varietal experiments.

Mildew (*Blumeria graminis*) was not present on any commercial variety during 17 successive growing seasons; all varieties presented an excellent resistance for mildew. In the growing season 1999-2000 a few varieties became infected by mildew. From then on the problem with mildew expanded: more varieties became infected and the intensity of infection became much higher.

A possible explanation for these extensive susceptibility of triticale to mildew is probably the increasing cultivation time and the expanded cultivated area through which probably new physiological races of *Blumeria graminis* have appeared.

P10

Resistance to root knot nematode in melons is available in the primary gene pool of Indian origin

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Forty accessions of wild melon (*Cucumis melo* var. *agrestis;* $2n = 2x = 24$), collected from the various agro-ecological regions of Punjab State of India, were evaluated for resistance to root knot nematode (*Meloidogyne incognita*). The assessment was carried out in infested potted soil using three replications and five plants per replication. For analysis, 6 week old seedlings were uprooted, washed and scored. A 1-5 root galling index scale was used for scoring the plants: (1) 1-10% galling = resistant (R), (2) 11-25% galling = moderately resistant (MR), (3) 26-50% galling = moderately susceptible (MS), (4) 51-75% galling = susceptible (S), (5) 76-100% galling = highly susceptible (HS). Variability for root knot nematode resistance in wild melon accessions was recorded. The wild melon accessions WM 8 and WM 16 (root galling index of 1.0) were observed to be segregating for nematode resistance. These accessions are being used to understand the genetic control of resistance and to achieve homozygosity through selfing. High levels of resistance to root knot nematode occur in *Cucumis metuliferus* but it cannot be exploited because this species belongs to the tertiary gene pool. Crosses between *C. melo* and *C. metuliferus* are not successful. Wild melon accessions WM 8 and WM 16 belong to the primary gene pool and thus provide an excellent opportunity to breed root knot nematode resistant melons. These two accessions originated in two different agro-ecological regions and were found genetically divergent through Simple Sequence Repeat (SSR) analysis.

P11

Molecular Characterization of '*Candidatus* Phytoplasma cynodontis' associated with Bermuda grass disease in Rajasthan, India

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Bermuda grass showing symptoms of white leaf disease has been observed in the area of Rajasthan, India. The diseased plants showed typical white leaf symptoms, proliferation of axillary shoots, bushy growing habit, small leaves and shortened stolons.Using polymerase chain reaction (PCR) amplification with P1/P6 univeral primer followed by R16F2n/R16R2 primers, all symptomatic plants tested postiviel; whereas no amplification product obtained from nonsymptomatic plants. RFLP analysis of PCR products with *Hae*III, *Rsa*I or *Alu*I endonuclease generated fragment profiles that were identical for all white leaf samples. The phytoplasma 16/23S intergenic region was sequenced directly with primers P4 and P7 and compared by BLAST analysis with those of other phytoplasma rDNA sequences archived in GenBank. The highest sequence homology (95%) obtained was with that of '*Candidatus* Phytoplasma cynodontis' (GenBank Accession No.Y14645), the type member of the BGWL (16SrXIV) group. This is the first molecular identification of '*Ca.* Phytoplasma cynodontis'(16SrXIV group) associated with Bermuda grass white leaf disease in West India (Rajasthan).

P12

Investigation of the cellular basis for resistance to ergot infection in wheat

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The aim of this project is to gain a cellular and molecular understanding of the interaction between the mycotoxin producing plant pathogen *Claviceps purpurea* and its host hexaploid wheat (*Triticum aestivum*). *Claviceps* forms a fungal body called an ergot in the place of a wheat grain following infection of the flower. Ergots contain a set of extremely toxic alkaloids and pose a risk to humans and animals when ingested hence a zero tolerance level for wheat entering the human food chain and strict levels allowed for sale of grain for feed.

Although detailed studies have been carried out on ergot of rye, we want to understand the fundamental biology of the interaction with wheat during the infection process and to this end we will present the results of our microscopy studies covering early events in the infection of a susceptible wheat variety. *Claviceps* spores germinate on the stigma hairs and grow through tissues of the wheat flower enveloping the ovule within three days. By six to seven days the whole wheat ovary is colonised and the fungus enters its sphacelial stage whereby conidiospores are exuded from the plant in a sticky phloem sap residue known as honeydew. The structures observed at each of these stages will be presented.

There is no chemical that can be deployed to defend against infection by *Claviceps* which drives the need to exploit natural resistance to this pathogen for a long-term sustainable solution. Partial resistance has been identified for a UK wheat variety by NIAB as part of a LINK-funded project and having established the timing and nature of the susceptible interaction at the cellular level, our next objective is to determine which stages in the development of a Claviceps infection are delayed or reduced in severity in resistant backgrounds.

In the future, transcript profiling techniques will be used to look at changes in gene expression in response to various stages of Claviceps colonisation and to compare the transcriptomic response between resistant and susceptible varieties at key stages in the interaction.

P13

Investigating altered fungicide sensitivity in *Rhynchosporium secalis*

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Barley leaf blotch or scald, caused by the fungus *Rhynchosporium secalis*, is the most economically damaging foliar disease of barley in the UK, and fungicides are a major component of control programmes for this disease. However, fungicide use can exert a strong selective pressure for the development of fungicide resistance. Resistance has already rendered the MBC fungicides ineffective against *R. secalis*, and a reduction in sensitivity to some triazole fungicides has been found in the field. Strobilurin resistance has been reported in many pathogens, but not yet in *R. secalis*.

Understanding the genetic basis of fungicide resistance enables improved detection through molecular diagnostics, facilitating a better understanding of the spread and management of resistance. This project aims to identify genetic changes responsible for reduced fungicide sensitivity in *R. secalis*, and to study their occurrence and spread in populations.

A fungicide sensitivity bioassay has been developed for *R. secalis*. This has revealed a hundred-fold reduction in sensitivity to some triazoles over the last 10-15 years, and the molecular basis of this reduction is now being investigated, initially looking for target-site mutations.

P14

Providing independent information on fungicide performance in wheat

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The HGCA, a public body funded through a levy on growers, processors and the trade, provides growers and agronomists with independent information on the performance of fungicides against the prevalent diseases of winter wheat. At a time when there have been significant shifts in fungicide performance due to resistance, HGCA research has enabled resistance to strobilurin and triazole fungicides to be detected, quantified and managed. Through collaboration with agrochemical companies, who provide new products for testing, the programme enables growers and agronomists to gain considerable insight into the performance of new products, enabling strategies to be developed based on knowledge of the spectrum of activity and effective dose rate, against a background of shifting activity due to changes in sensitivity in the target pathogens. The information is primarily delivered through activity rating 'star charts' and a fungicide dose tool which enables comparison of fungicide performance at various dose rates. Each year, major changes are also highlighted through workshops and the press. The fungicide performance work links in to other important sources of information, providing effective integrated management of disease. These include Crop Monitor, which assesses disease incidence and risk based on regularly monitored field sites and provides updates via email and the web, the HGCA Recommended Lists which include up-to-date resistance ratings for wheat varieties, and the UK Pathogen Virulence Survey, which monitors the virulence of important pathogens on current varieties. All the information is available via the HGCA website www.hgca.com.

P15

The development of EURO-Wheat, a wheat-disease based research platform within the ENDURE Virtual Laboratory

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The ENDURE project (European Network for the Durable Exploitation of Crop Protection Strategies, FP 6, Network of Excellence, project number 031499) brings together more than 300 researchers in the fields of agronomy, biology, ecology, economics and the social sciences from 18 organisations in 10 European countries. The objectives of ENDURE are to define research priorities on pesticide reduction at the European level, to pool knowledge, facilities and human resources according to the needs of the agricultural extension, industry and non-profit sectors and to become a source of reference satisfying farmers' needs and societal expectations.

Within the ENDURE network, a "virtual laboratory" (VL) has been developed to bring together resources, collections, knowledge and data held by the ENDURE partner organisations with the VL designed to underpin research activities within the network. For example, the "collections" database of the VL provides ENDURE partner organisations with direct access to over 600,000 isolates, organisms or samples which can be used in collaborative research. The VL also contains a number of "Research Platforms" that are designed to bring together information and resources on specific research areas. The most recent addition to the Research Platforms was the development of EURO-Wheat (www.eurowheat.org). EURO-wheat is an Internet based platform aiming at collating and displaying host - and pathogen characteristics, and pesticide efficacy on a European scale. EURO-Wheat brings together existing information from national programs and ensures that data are in a format which can be readily understood trans-nationally in order to provide significant added value on a European scale. New disease - and resistance data are published on the platform as soon as possible to support effective disease control, deployment of host resistance and breeding programs. Current information available on the platform are:

- Virulences in the yellow rust population.
- Effectiveness of fungicides in different countries
- Information on disease thresholds and DSSs used in Europe
- Documents and up-to-date information on disease management at the national scale.

The main focus of research over the coming 24 months will be the further development of EURO-Wheat to include all available information on Septoria leaf blotch (Mycosphaerella graminicola), yellow rust (*Puccinia striiformis*) and Fusarium head blight (*Fusarium* spp.).

P16

An investigation of germination pathways, infection and disease development using low inoculum densities of *Phytophthora ramorum* on detached rhododendron leaves

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Phytophthora ramorum is severely impacting the ornamental plant nursery industry in Europe and North America and is the cause of 'sudden oak death' in native forests in California and Oregon. On ornamentals *P. ramorum* causes leaf blight and shoot-dieback, but it causes lethal bleeding stem cankers on trees. Asymptomatic foliage infection reported recently may be a consequence of low levels of inoculum. We investigated germination behaviour of sporangia and effects of low inoculum density on infection and disease expression of detached Rhododendron 'Cunningham's White' leaves. Ten sporangia and zoospores were separately placed on water agar blocks, which were transferred to lower leaf surfaces, covered with 10 µL of water and incubated at 20°C, with 12 hours light for 7 d in a moist chamber. Sporangia were also placed on glass slides and treated similarly. Microscopic observations revealed that direct germination of sporangia was rare but when present mycelial branches developed from the apex of the sporangium beneath the papillum. Direct germination generally led to the formation of secondary sporangia. Infection was confirmed by back-isolation and occurred on 26% of leaf inoculation points. Sporangia caused significantly more symptomatic infections than zoospores which caused significantly more asymptomatic infections. The positive effect of sporangial inoculum on disease expression was interpreted as the effect of increased inoculum due to zoospore release. Our study has developed the technology to induce asymptomatic infection *in vitro*. Asymptomatic infection has important implications for management of *P. ramorum* in the nursery trade where the failure of visual inspections to detect asymptomatically infected plants is thought to contribute to spread of the pathogen.

P17

Preharvest qPCR diagnostics for better management of phoma stem canker and light leaf spot in oilseed rape

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Oilseed rape (*Brassica napus*) features prominently as the choice break crop in the cereal-based agriculture of the UK. Over 500,000 ha are sown to the crop annually and economic returns from vegetable oil as well as benefits to soil structure and bee farming are invaluable. Fungal diseases caused by species of *Leptosphaeria* (phoma stem canker) and by *Pyrenopeziza brassicae* (light leaf spot) are major constraints to UK winter oilseed rape production and currently, azole fungicides and RL cultivars are the sole means of control. Studies on the biology and lifestyles of the fungal pathogens and recent advances in diagnostic techniques offer opportunities for better deployment of these control methods. Monitoring of asymptomatic, endophytic growth of fungal mycelia within selected organs of field-sown juvenile plants has yielded data that are predictive of final disease outcome. In addition, disease control can be further improved by precise sampling of air followed by differentiation and quantification of pathogen propagules by real-time PCR. These results will enable a better understanding of both the population dynamics and race structures of these fungal pathogens.

P18

Population structures of global isolate collections of *Leptosphaeria maculans* and *L. biglobosa*, the causal organisms of phoma stem canker and lesion on oilseed rape

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Although UK agriculture is largely cereal based, oilseed rape (*Brassica napus*) features prominently as a break crop in wheat and barley rotations. The dothideomycete fungi *Leptosphaeria biglobosa* (cause of phoma stem lesions) and *L. maculans* (cause of phoma stem canker, the more serious disease) are major constraints to oilseed rape production worldwide and account for significant annual yield losses in the UK. Extensive culture collections comprising over 700 isolates of both pathogens have been assembled and are now held securely at Rothamsted Research. Minisatellite and AFLP markers have enabled molecular fingerprinting of isolates and the estimation of global and regional genetic variability and relatedness within populations. More significantly, routine evaluation is being conducted of the virulence allele diversity within a subset of *L. maculans* populations using *B. napus* differential lines and cultivars with known *LepR* or *Rlm* resistance genes against stem canker. Implications of these findings on selection and breeding for disease resistance in oilseed rape are discussed.

P19

DON mycotoxin biosynthesis by Fusarium species, a metabolomics perspective.

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Many Fusarium species are fungal plant pathogens causing disease on both cereal and non-cereal hosts. Infection of the wheat ear typically results in bleaching and a subsequent reduction in grain yield. In addition, an increasing proportion of the harvested grain may be spoiled by trichothecene mycotoxins, such as deoxynivalenol (DON). The biosynthesis of DON in the wheat ear is a critical event, because infection of approximately one ear per 600 is sufficient to prevent usage of grain consignments under EU legislation. Much progress has been made in the elucidation of genes required for trichothecene production most of which are clustered at a single locus in the Fusarium genome.

This project seeks to extend this field of research by describing the metabolic characteristics associated with DON biosynthesis. As part of a new BBSRC metabolomics initiative, this project will examine a wide range of well characterised wild-type Fusarium laboratory strains and single-gene deletion mutants under controlled DON-inducing conditions *in vitro*. A variety of analytical techniques are being employed to analyse the metabolome, including 1H-NMR, electrospray mass-spectroscopy (ESI-MS) and GC-TOF-MS. Initial results have shown that both NMR and ESI-MS techniques are sufficient to discriminate a range of wild-type isolates. Principal components analysis identified metabolic differences between the wild-type strains, and was able to resolve *F. graminearum*, *F. culmorum*, *F. pseudograminearum* and *F. venenatum* isolates after growth in minimal medium. In addition, several single-gene deletion strains that are reduced in pathogenicity exhibit large shifts in primary metabolism relative to their parent strains. Future work will attempt to find correlations between observed metabolic trends and DON biosynthesis and then confirm these by targeted methods.

P20

Comparison of cereal eyespot disease levels on winter wheat at two sites in Cambridgeshire, UK

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Cereal eyespot caused by the closely related fungi *Oculimacula yallundae* and *O. acuformis* is an important disease of cereals in temperate regions. Initial infection of the coleoptile occurs in the autumn. The fungi then infect the subsequent leaf layers and the stem base. An eye-shaped lesion develops on the stem base, weakening the stem and giving the plant a predisposition to lodge as well as reducing grain yield. Resistance genes to eyespot have been identified and bred into commercial varieties, which currently show a range of susceptibilities to the disease.

Two inoculated field trials were set up in Cambridgeshire at different locations to compare disease levels in winter wheat recommended list varieties. Both trials were sown at the same time. The trials were inoculated with oat grain infected with isolates of *O. acuformis* and *O. yallundae* in a 1:1 ratio of the two species. Inoculation of the two trials was carried out on the same day. Both trials were assessed three times at fortnightly intervals starting in June 2008. The disease index (DI) illustrated that the recommended list varieties express a range of resistance to eyespot without any variety being completely resistant. The results revealed that the DI for a particular variety varied by both site and assessment period. The DI of some varieties was very similar at the two sites however the DI of other varieties was different at the two sites. The varieties were then ranked in order of DI at each assessment time point. Some varieties maintained their rank position across the assessment period while others varied by up to 20 places. This appeared to be due to contrasting patterns of epidemic development over time. Some varieties had a high disease index at the initial assessment point but this value did not increase over the period, whilst others started with a low disease index which then increased rapidly. This data illustrates that eyespot disease levels not only depend on the genetics of the variety but also the environmental conditions at the trial site and the timing of assessments.

P21

Brachypodium distachyon: A model species to study cereal-pathogen interactions.

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Brachypodium distachyon is rapidly emerging as model grass species for temperate cereal crops. Due to its undemanding growth requirements, small stature, inbreeding reproductive strategy and particularly its small genome (~ 320 Mbp; 2x = 2n = 10), this species is being adopted by a large number of researchers. Since it was first proposed as a model species in 2001, genetic stocks have been derived from single seeds, and a high throughput transformation system based on *Agrobacterium has* been developed, which will eventually lead to a considerable collection of T-DNA tagged lines. Most importantly, the US Department of Energy Joint Genome Institute has completed a checkpoint assembly of the *Brachypodium* genome sequence. This is complemented by a deep EST-mining initiative which will generate 180,000 ESTs to facilitate the annotation of the *Brachypodium* genome. Further, in the near future recombinant inbred lines will be available to community. Thus, there is now a clear imperative to exploit these emerging *Brachypodium* resources to answer biological questions.

Losses in cereal crops from diseases are estimated at 13.3%, and so *Brachypodium* holds great promise as a surrogate for understanding resistance to a variety of major cereal diseases. *Brachypodium*-pathogen interactions where different levels of resistance have been described include rice blast (*Magnaporthe grisea)* and the rusts. This includes brown rust (*Puccinia recondita*) and yellow rust (*P. striiformis*). More recently, compatible interactions between *Brachypodium* and crown rust (*P. coronata*) and stem rust (*P. graminis*) have been reported. We have yet to isolate a powdery mildew fungus which is virulent on *B. distachyon*, though susceptibility to this pathogen has been reported in the literature. Therefore, *Brachypodium* can be used by the community of cereal pathologists to study multiple pathosystems.

An example of the potential of *Brachypodium* to reveal key aspects of responses to pathogens is its interaction with *M. grisea,* which has been extensively characterised. Metabolomic approaches have revealed key non-polar changes linked to resistance to *M. grisea* in *Brachypodium*, and implicated octadecanoid products as key mediators of resistance. Screening of subtractive cDNA libraries identified early changes in host gene expression which suggested a rapid induction of phenylpropanoid biosynthetic genes. The expression of some of these genes proved to be influenced by the octadecanoid product jasmonic acid. These initial successes suggest that *Brachypodium* is poised to serve as a powerful system for exploring resistance to many other cereal diseases as well.

P22

Allele-specific real-time PCR for quantification and discrimination of sterol 14α-demethylation-inhibitor-resistant genotypes of *Mycosphaerella graminicola*

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BACKGROUND: *Mycosphaerella graminicola* resistance level to sterol 14 α-demethylation inhibitors (DMIs) is characterized by point and deletion mutations in the *CYP51* gene that encodes the sterol 14 α-demethylase. Rapid and presymptomatic detection of these mutations is required for effective control by fungicides.

RESULTS: In this study, an allele-specific real-time PCR method was developed. An additional mismatched nucleotide at the third position from the SNP at the 3-prime end of each allele-specific primer (IMP) has abrogated non specific PCR amplification. A minor groove binding (MGB)-specific probe was designed to quantify general strains of *M. graminicola*. Isoleucine at position 381 of the protein sequence of *CYP51* gene was the target of the MGB probe that was used to quantify DMI-sensitive and low-resistant strains. Reproducible specific amplification of the target alleles was observed. A high level of discrimination between genotypes using pure fungal DNA was confirmed *in vivo*, based on leaf samples collected from different wheat growing regions in France. A high level of DMI moderate-resistance genotypes (>70%) was observed in all samples.

CONCLUSION: Allele-specific real-time PCR allows presymptomatic and accurate quantitative detection of DMI-resistant genotypes of *M. graminicola*, with a shorter turnaround time compared to conventional methods. The simplicity and effectiveness provided by intentional mismatch primers offers a broad range of applications for laboratory and field analysis.

P23

Sources of ergot infection in wheat

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A large number of grass species are susceptible to infection by the ergot fungus, *Claviceps purpurea*. The arable environment in the UK contains a wide range of areas where grasses are the predominant plant type, including field margins which are either deliberately sown with seeds mixtures containing grasses, or which are allowed to regenerate naturally with local grass species. Allowing margin grasses to flower is a critical part of the environmental benefits that they can deliver, though it also allows infection by ergot. There has been growing concern among cereal farmers that the increased prevalence of grasses in or around arable fields may be one reason for increased ergot contamination in grain.

Previous studies on potential sources of outbreaks of ergot in wheat showed that several grass species did become infected by *C. purpurea* which was also capable of infecting wheat. However, ergots from other species were found to have low infectivity towards wheat (Mantle, Annals of Applied Biology, **86**, 339-551, 1977).The survey work which lead to these conclusions was carried out in the early 1970s, and thus in a very different arable environment to the present. The work reported here was carried out to investigate the infectivity of ergots collected from grass species in a range of arable environments, including sown margins, and to determine whether some grass species could be regarded as low risk in terms of ability to produce wheat infecting ergots.

Ergot sclerotia were collected from grass species in field margins and the arable environment during a four year monitoring period. Inoculum from single sclerotial cultures was used to infect wheat plants in the field or glasshouse. Infectivity was assessed by counting ergot numbers produced. There was wide variation in infectivity of ergots from within a single host grass species and between species. Cocksfoot (*Dactylis glomerata*) is commonly used in sown grass margins, and is widespread in the arable environment. Over sixty samples of ergot were received from cocksfoot, and while some produced low numbers of ergot on wheat, others were highly infective. This grass species should be regarded as presenting a potential source of inoculum for wheat crops, and its use in field margins could increase the level of infective inoculum. Ergots from *Holcus lanatus* (Yorkshire Fog) which is also used in field margins, had consistently low infectivity on wheat and could be used to reduce the risk of grass margins as sources of ergot inoculum. No ergots were found on Crested Dog's Tail (*Cynosurus cristatus*) during the monitoring period, despite its presence in grass margins, and this may also be a useful low risk species.

P24

Closing the blast doors: biological and transcriptional defence responses of wheat to non-adapted and adapted species of the blast fungus, *Magnaporthe*.

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Rice blast, caused by *Magnaporthe oryzae*, is one of the most economically important diseases worldwide. The *Magnaporthe* species complex infects over 50 graminaeceous plant species. *M. oryzae* tends to colonise cultivated grasses whilst *M. grisea* attacks wild grass species. Recently in Brazil, *M. oryzae* has become a problem to local wheat production and the epidemic is beginning to spread in South America and could potentially become a threat to global wheat production. We have investigated the resistance present in the wheat cultivar Renan against species of *Magnaporthe* that are either adapted or non-adapted to wheat. Confocal microscopy demonstrated that the early defence response against both adapted and non-adapted species involves the production of a diffuse autofluorescent HALO structure around the site of attempted fungal penetration. A high proportion of HALO-like structures were associated with penetration events over time in response to the non-adapted *M. grisea*, and very few infection attempts were able to progress further. In contrast the adapted *M. oryzae* was frequently able to infect past the HALO-like structure and develop further into the leaf. In these cases whole cell autofluorescence was often observed, indicative of a hypersensitive response to prevent further pathogen colonisation. Microarray analysis of the defence response 24 hours post inoculation indicated that wheat undergoes extensive transcriptional reprogramming during interactions with both adapted and non-adapted species. Comparison between the identified differentially expressed transcripts responding to the adapted and non-adapted *Magnaporthe* species revealed levels of both conservation and diversification in the type of transcripts that are regulated. This suggests some common mechanisms in the defence response against adapted and non-adapted *Magnaporthe* species, whilst highlighting potential differences that may result in the observed biological phenotypes. Functional genomic approaches are currently being used to examine the roles of candidate transcripts in innate immunity of wheat against different species of the blast fungus.

P25

Variability in virulence of *Mycosphaerella graminicola* isolates isolated from one blotch

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Septoria tritici bloch *Mycosphaerella graminicola* (Fuckel) Schröter (anamorph *Septoria tritici*) is a pseudothecial ascomycete that causes serious damages in wheat worldwide. A number of molecular studies has shown the presence of high levels of genetic variability in this pathogen. We demonstrate variability in virulence of *M. graminicola* isolated from different pycnidia in one leaf blotch. The differential set of thirteen winter wheat cultivars was tested in laboratory experiments for their reactions to twenty-five isolates of *M. graminicola* by the method of detached leaf segments. In the assortment of tested cultivars ten commercial cultivars and three cultivars possessed the specific genes of resistance were included. Isolates of the pathogen were collected from different cultivars of wheat in three areas of the Czech Republic. Mono-pycnidia isolates were prepared and cultivated on potato dextrose agar. After subsequent pycnidia induction, wheat seedlings were sprayed by spore suspension, cut into 3 cm segments and placed on benzimidazole water agar. The symptom development was evaluated twice during the period of 20-26 days after inoculation. The aggressiveness of the isolates was expressed as a disease severity based on the percentage of leaf area covered by necrotic lesions bearing pycnidia.

All isolates under study showed a high variation in virulence patterns to the set of testing cultivars.

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