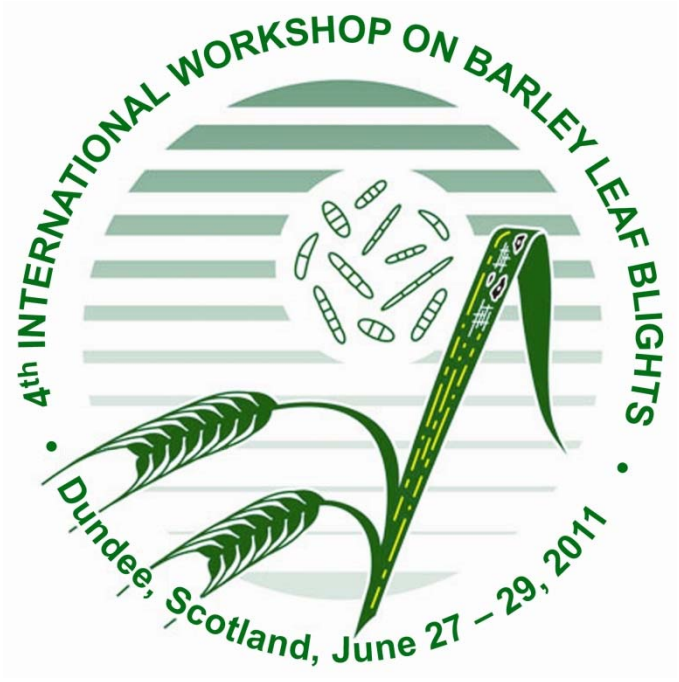


**HOSTED BY**

**THE JAMES HUTTON INSTITUTE, DUNDEE**

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**BRITISH SOCIETY FOR PLANT PATHOLOGY**

**27TH TO 29TH JUNE 2011**

**WEST PARK CONFERENCE CENTRE, DUNDEE**

The James Hutton Institute

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# Programme

**Monday 27th June 2011**

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|---|---|
| 09:30 - 10:00 | Registration in the Sidlaw Auditorium Foyer, West Park Conference Centre |
| 10:00 - 13:00 | Tour of The James Hutton Institute (buses leaving West Park car park once full) |
| 13:00 - 13:30 | Lunch (Henderson's Restaurant, West Park) |

**Session 1: Epidemiology and Disease Management. Chair: Hugh Wallwork**

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|---|---|
| 13:30 - 13:35 | Welcome from Adrian Newton and Brian Steffenson |
| 13:40 | Welcome from the Chair |
| 13:40 - 14:10 | Richard Oliver *Australian Centre for Necrotrophic Fungal Pathogens*<br>**From genomic analyses of cereal Pleosporales pathogens to reduced disease losses** |
| 14:10 - 14:25 | Lise Nistrup Jørgensen *Aarhus University*<br>**Experiences from control of barley diseases using monitoring and decision support systems in Denmark** |
| 14:25 - 14:40 | Bruce Fitt *University of Hertfordshire*<br>**Symptomless infection by *Rhynchosporium commune* in relation to control of barley leaf blotch** |
| 14:40 - 14:55 | Nichola Hawkins *Rothamsted Research*<br>**Triazole sensitivity and cross-resistance patterns in *Rhynchosporium secalis* populations** |
| 14:55 - 15:10 | Dale Walters *Scottish Agricultural College*<br>**Control of *Rhynchosporium secalis* on barley using a combination of resistance elicitors** |
| 15:10 - 15:25 | Hugh Wallwork *South Australian Research and Development Institute*<br>**A recent history of net form net blotch in South Australia and what it tells us about management of the disease** |
| 15:25 - 16:00 | Tea & Coffee Break |

**Session 1: Epidemiology and Disease Management continued**

| | |
|---|---|
| 16:00 - 16:15 | Mark Sutherland *University of Southern Queensland*<br>**Genetic relatedness of *Bipolaris sorokiniana* isolates from different cereal host tissues** |
| 16:15 - 16:30 | Flavio Capettini *ICARDA - International Center for Agricultural Research in Dry Areas*<br>**Virulence spectrum of Rhyncosporium secalis isolates from Syria and Lebanon at adult and seedling growth stages** |
| 16:30 - 16:45 | Olga Afansenko *All-Russian Research Institute of Plant Protection*<br>**An international standard set of barley differential genotypes and experience of using it for populations studies of *Pyrenophora teres* f. *teres*** |
| 16:45 - 17:00 | Mark McLean *Department of Primary Industries, Victoria*<br>**Development of an international differential set for *Pyrenophora teres* f. *maculate*** |
| 17:00 - 17:15 | Kithsiri Jayasena *Department of Agriculture and Food Western Australia*<br>**Understanding climatic and crop management influences on stubble borne inoculum of net blotch in barley in Western Australia** |
| 17:15 - 19:30 | Free Time (Optional dinner in West Park's Henderson's Restaurant, not part of conference package) |

**Special topic: Ramularia. Chair: Dale Walters**

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|---|---|
| 19:30 - 19:35 | Welcome from the Chair |
| 19:35 - 20:05 | Neil Havis *Scottish Agricultural College*<br>**A History of Ramularia research in Scotland** |
| 20:05 - 20:20 | Michael Hess *TU München*<br>**Studying the biology of *Ramularia collo-cygni* for the development of an Integrated Pest Management System to match new challenges from a changing climate** |
| 20:20 - 20:35 | Nazanin Zamani-Noor *Georg-August University*<br>**Comparison of screening methods for resistance of spring barley cultivars to Ramularia leaf spot disease** |
| 20:35 - 20:50 | James Fountaine *Scottish Agricultural College*<br>**Sequencing of the fungal pathogen *Ramularia collo-cygni,* why and how?** |
| 20:50 - 21:15 | Gosia Jedryczka *Institute of Plant Genetics, Polish Academy of Sciences*<br>**DNA polymorphism and rubellin production by *Ramularia collo-cygni* isolates originating from Europe** |
| 21:15 - 21:30 | Posters |
| 21:30 | Finish |

## Tuesday 28th June 2011

**Session 3: Molecular plant-pathogen interactions. Chair: Anna Avrova**

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|---|---|
| 09:30 - 09:35 | Welcome from the Chair |
| 09:35 - 10:05 | Wolfgang Knogge *Leibniz Institute of Plant Biochemistry*<br>***Rhynchosporium* genomics – a strategy towards understanding host specialisation** |
| 10:05 - 10:20 | Amarnath Thirugnanasambandam *The James Hutton Institute*<br>**Investigations of the asymptomatic phase of *Rhynchosporium secalis* and *Ramularia collo-cygni* infection using GFP expressing variants** |
| 10:20 - 10:35 | Anna Avrova *The James Hutton Institute*<br>**Mining the *Rhynchosporium secalis* genome and transcriptomes for pathogenicity determinants** |
| 10:35 - 11:10 | Tea & Coffee Break |
| 11.10 - 11:40 | Amanda Able *The University of Adelaide*<br>**Role of proteinaceous toxins in symptom development of net blotch disease in barley** |
| 11:40 - 11:55 | Tim Friesen *US Department of Agriculture*<br>**Identification of a proteinaceous necrotrophic effector and its corresponding sensitivity locus on barley chromosome 6H** |
| 11:55 - 12:10 | Clara Pritsch *University of the REpublic Uruguay*<br>**Functional diversity on resistance to early infection among barley genotypes inoculated with *Cochliobolus sativus*** |
| 12:10 - 12:25 | Jayne Davis *The James Hutton Institute*<br>**Potassium deficiency and its effect on fungal pathogens of barley** |
| 12:25 - 13:00 | Clement Gravouil *The James Hutton Institute*<br>**The Bad, the Ugly and the Good** |
| 13:00 - 13:30 | Lunch |
| 13:30 - 15:30 | Posters and/or meetings |
| 15:30 - 18:30 | Free Time (tour of Verdant Works or Discovery Point – sign up at Registration. Not part of conference package) |

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|---|---|
| 18:30 - 19:30 | Drinks Reception/Posters cont. in the Sidlaw Auditorium |
| 19:30 - 21:00 | Dinner in the Balbeggie Suite |
| 21:00 - 22:00 | Whisky talk and tasting |
| 22:00 | Finish |

## Wednesday 29th June 2011

**Session 5: Resistance Breeding. Chair: Kithsiri Jayasena**

| | |
|---|---|
| 09:30 - 09:35 | Welcome from the Chair |
| 09:35 - 09:50 | Marja Jalli *MTT Agrifood Research Finland*<br>**Pre-breeding for barley net blotch resistance in Finland** |
| 09:50 - 10:05 | Sanjiv Gupta *Murdoch University*<br>**Mapping and allelic tests depicting recombinants on 6H locus conferring resistance to *Pyrenophora teres* f. *teres* isolates from Australia** |
| 10:05 - 10:20 | Robert Brueggeman *North Dakota State University*<br>**Towards the Identification of Two Recessive Net Form Net Blotch Resistance Genes, rpt.r and rpt.k, in Barley** |
| 10:20 - 10:40 | Michele Stanca *University of Modena & Reggio Emilia*<br>**The CC-NB-LRR *Rdg2a* Resistance Gene Confers Immunity to the Seed-Borne Barley Leaf Stripe Pathogen in the Absence of Hypersensitive Cell Death** |
| 10:40 - 11:10 | Tea and Coffee Break |
| 11:10 - 11:25 | Jerome Franckowiak *Agri-Science Queensland, DEEDI*<br>**Detection of resistance to the net form of net blotch in Australian barley breeding lines** |
| 11:25 - 11:40 | Greg Platz *Agri-Science Queensland, DEEDI*<br>**Mapping resistances to multiple leaf spot diseases in an Australian/American derived doubled haploid population of barley** |
| 11:40 - 11:55 | Brian Steffenson *University of Minnesota*<br>**Multiple Disease Resistance QTL Analysis of Two Wild × Cultivated Barley Populations** |
| 11:55 - 12:10 | William Thomas *The James Hutton Institute*<br>**Detecting and mapping loci for disease resistance in UK elite barley cultivars** |
| 12:10 - 12:25 | Hugh Wallwork *South Australian Research and Development Institute*<br>**The detection of minor genes for resistance to *Rhynchosporium secalis* using seedling assays** |
| 12:25 - 12:40 | Mark Looseley *The James Hutton Institute*<br>**Resistance to *Rhynchosporium secalis* in a cross between winter and spring barley** |
| 12:40 - 13:30 | Lunch |
| 13:30 | Depart for Edinburgh: SAC field tour or drop off in Edinburgh |
| 18:00 | Finish and return to Dundee or drop off in Edinburgh |

# Epidemiology and Disease Management

**From genomic analyses of cereal Pleosporales pathogens to reduced disease losses**

R. P. Oliver[a], C. Moffat[a], R Syme[a], T. L. Friesen[b] and S.R. Ellwood[a],

[a]*Australian Centre for Necrotrophic Fungal Pathogens, Curtin University, WA 6745, Australia and* [b]*United States Department of Agriculture, Agricultural Research Service, Cereal Crops Research Unit, Northern Crop Science Lab, 1039 18th Street North, Fargo, ND 58105*

richard.oliver@curtin.edu.au

The major foliar pathogens of wheat and barley in Australia and in many other areas on the world are necrotrophic fungi from the order Pleosporales. The wheat pathogens are *Stagonospora (syn. Phaeosphaeria or Septoria) nodorum,* causal agent of Stagonospora nodorum blotch and *Pyrenophora tritici-repentis,* cause of tan spot. The barley pathogens are *Pyrenophora teres teres* (net-type net blotch) and *P. teres maculata* (spot-type net blotch). The adoption of minimum tillage practices and climate change are both linked to emergence and increases in these diseases in the last few decades.

Our research group initially focussed on *S. nodorum.* Until recently, this pathogen was treated as an undifferentiated species, which employed a poorly understood arsenal of non-specific toxins and cell-wall degrading enzymes to cause disease. Resistance was partial at best, genetic analysis revealed many weak QTL and no molecular markers were in use. Furthermore, fungicide resistance was reported. A combination of studies based on the genome sequence, released in 2005, revolutionised this picture. *S .nodorum* isolates were found to produce a range of small, secreted proteinaceous effectors (NEs, previously called host specific toxins). This differentiated the pathogen into a large number of races. These effectors interact with wheat sensitivity genes and simplify the interaction into a series of major QTL. The amount of disease is rationalised as a function of the number of effectors produced by the infecting pathogen population that match sensitivity genes in the host.

The presence of necrotrophic effectors in *S .nodorum* aligned this species with the archetypal producers of NEs *Cochliobolus heterostrophus, Alternaria alternata* and *Pyrenophora tritici-repentis,* all species from within the newly recognised class the Pleosporales.

One of the *S. nodorum* effectors was highly similar to a well known NE from *Pyrenophora tritici-repentis, ToxA.* ToxA versions from both species interact with the wheat sensitivity gene *Tsn1*. Purified ToxA is now used by Australian wheat breeders to select cultivars that are insensitive to the effector and thus more resistant to these diseases. The area planted to ToxA-sensitive wheat cultivars has declined by 500,000 ha in the last 5 years, a development that we estimate has already reduced disease losses by A$10m pa. and which promises to grow to A$150m p.a.

The key to this approach is to acquire a series of genomic and genetic resources. These are fungal genome sequences, from which effector gene candidates can be identified and host mapping populations, so that effector/sensitivity gene interactions can be mapped. We have acquired initial genome assemblies of both *P. teres* subspecies. Progress in the use of these assemblies to identify and map effector genes will be described.

**Experiences from control of barley diseases using monitoring and decision support systems in Denmark**

Lise Nistrup Jørgensen[1], Karen Eberhardt Henriksen[1] & Ghita Cordsen Nielsen[2]

*Aarhus University, Faculty of Agricultural Science, Department of Integrated Pest Management, Research Centre Flakkebjerg, Denmark[1], Knowlegde Center for Agriculture, Skejby, Aarhus, Denmark[2].*

Winter barley is grown on app.150.000 ha and spring barley in 4-500.000 ha in Denmark. Brown rust (*Puccinia hordei*) is regarded as the most yield reducing disease, potentially giving losses between 10-20 dt/ha. In spring barley varieties Mlo resistance is common, which keeps the crops free of powdery mildew (*Blumeria graminis*). In winter barley mildew remains a common disease. In years with significant rain events during elongation Rhyncosporium (*Rhynchosporium secalis*) is found to be important in susceptible cultivars giving moderate yield reductions. Net blotch (*Drechslera teres*) is less dependent on rain events but gives moderate to severe yield reductions in susceptible cultivars. Ramularia leaf spot (*Ramularia collo-cygni*) has become common in both spring and winter barley. Although severe attacks are seen, its impact on yields is still regarded relatively low.

National monitoring for diseases in barley is organized by Knowledge Centre for Agriculture and carried out in collaboration with local advisors at weekly intervals in order to support regional risk assessments. Risk evaluation at field level can be carried out using Crop Protection Online (CPO), where risk assessment requires information on cultivar resistance, growth stage, precipitation and level of attack. Validation of CPO in trials has shown that the system provides reliable recommendations with margins in line with the best low dose standard strategies.

Mixtures of triazoles and strobilurins are most commonly recommended and provide effective disease control. In general 25 to 50% of the field rates are used in total which provides the best economical optimum. In very susceptible cultivars a split treatment has been found to be best. Despite widespread problems with strobilurin resistance in the populations of *Drechslera teres* (F129L), *Blumeria graminis* (G143A) and *Ramularia collo-cygni* (G143A) combinations of strobilurins and triazoles are still found to provide reliable control solutions.

**Symptomless infection by *Rhynchosporium commune* in relation to control of barley leaf blotch**

S.D. Atkins[a], A.C. Newton[b], B.A. Fraaije[a], J.A.Lucas[a] and B.D.L. Fitt[a*]

*[a] Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ UK; [b]The James Hutton Institute, Invergowrie, Dundee, DD2 5DA UK: [*]Current address University of Hertfordshire, College Lane, Hatfield, AL10 9AB, UK*

b.fitt@herts.ac.uk

Rhynchosporium leaf blotch (caused by *Rhynchosporium commune*) is the most economically important disease of barley in the UK, but epidemics can be difficult to control with fungicides and the basis of cultivar resistance is not well understood. Data were collected from three seasons of experiments using two susceptible and two resistant cultivars of both winter and spring barley. Amounts of *R. commune* DNA were quantified from samples taken at several growth stages (GS) throughout the growing season and visual symptoms were assessed. There was widespread symptomless infection by *R. commune* in winter barley crops, with extensive colonisation and sporulation on apparently healthy leaves. During a growing season, the pathogen could spread from seed sown (in autumn) through successive leaf layers to seed harvested (the following summer) without causing visible symptoms. There was generally far more symptomless *R. commune* in winter barley than spring barley (sown in spring). Fungicide sprays decreased the amount of symptomless infection but the relationship to their effects on yield was not clear. Leaves of resistant cultivars generally contained less symptomless *R. commune* than those of susceptible cultivars.

**Triazole sensitivity and cross-resistance patterns in *Rhynchosporium secalis* populations**

N. J. Hawkins [a,b], H. J. Cools [a], H. Sierotzki [c], M. W. Shaw [b] and B. A. Fraaije

[a]*Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire, UK;* [b] *School of Biological Sciences, University of Reading, Whiteknights, Reading, UK; and* [c] *Syngenta Crop Protection, Basel, Switzerland*

bart.fraaije@bbsrc.ac.uk

Triazole fungicides are a key component of *Rhynhosporium secalis s.l.* control, but sensitivity shifts have been reported. Thirty isolates collected in 2009 from six European countries were tested for sensitivity to propiconazole, tebuconazole, epoxiconazole and prothioconazole by a microtitre plate based bioassay. Due to partial cross-resistance between triazoles, a Principal Component Analysis was carried out. The first principal component (PC1), encompassing 70% of variation, comprised positive cross-resistance among all isolates tested. Isolates fell into three distinct groups of sensitive, intermediate and less sensitive isolates along this axis. Each group was separated by a tenfold shift in sensitivity to propiconazole and tebuconazole, with correlated but smaller shifts in epoxiconazole and prothioconazole sensitivity. Less-sensitive isolates were collected in all six countries. Too few sensitive and intermediate isolates were obtained to draw conclusions regarding geographic patterns. The second principal component (PC2), encompassing a further 20% of variation, revealed a different pattern of prothioconazole sensitivity compared to tebuconazole and propiconazole. Plotting PC2 against PC1 showed that prothioconazole sensitivity is more variable relative to overall triazole sensitivity among the less-sensitive isolates. Additional isolates were obtained from untreated, propiconazole-treated and epoxiconazole-treated plots at Rothamsted Research, UK, in 2009. All fell within the less sensitive group, suggesting that the initial population at that point was already less-sensitive and no further selection took place over that growing season. However, the possibility remains that new, more resistant strains may emerge in future. Therefore, resistance management and monitoring remain important.

**Control of *Rhynchosporium secalis* on barley using a combination of resistance elicitors**

Dale R. Walters, Neil D. Havis, Linda Paterson and Cecile Sablou

*Crop and Soil Systems Research Group, Scottish Agricultural College, King's Buildings, West Mains Road, Edinburgh, EH9 3JG, UK.*

dale.walters@sac.ac.uk

A cocktail of the resistance elicitors: *cis*-jasmone, β-aminobutyric acid (BABA) and acibenzolar-S-methyl (ASM) was examined for its ability to induce resistance in barley to *Rhynchosporium secalis* and other foliar pathogens in field studies over three seasons. Treatment with the elicitor combination increased expression of *Pr1b*, a marker gene for systemic acquired resistance, but led to greatly reduced expression of *LOX2,* used as a marker for the jasmonic acid signalling pathway. Plants treated with the elicitor combination also exhibited elevated activities of the defence-related enzymes, peroxidase, β-1,3-glucanase, and cinnamyl alcohol dehydrogenase. Although disease control under glasshouse conditions was good, in the field, the efficacy of the elicitor cocktail on its own was poor and variable, depending on both barley variety and season. Greater levels of disease control in the field were obtained using a combination of elicitor and fungicide. However, although a mixture of elicitor and fungicide applied together gave reasonable levels of disease control, most consistent disease control was obtained by treating plants with the elicitor combination at GS24, followed by reduced rate fungicide at GS31 and GS39. Despite the moderate levels of disease control, most elicitor and elicitor + fungicide treatments resulted in increased grain yields.

**A recent history of net form net blotch in South Australia and what it tells us about management of the disease**

H. Wallwork, M. Butt

*South Australian Research and Development Institute, Urrbrae, South Australia 5064, Australia*

hugh.wallwork@sa.gov.au

Net form net blotch (NFNB) was absent from South Australia for many years leading up to 1993, contained by the widespread cultivation of varieties with effective adult plant resistance. The pathogen re-appeared with the release of a susceptible variety, Franklin, in a long season net blotch prone environment. Within a very short period of time a major gene was overcome rendering a second variety, Skiff, highly susceptible leading to a large increase in inoculum and widespread dispersal of the pathogen. Subsequently, there has been an erosion of resistance in South Australia with new virulent pathotypes detected for both seedling and adult plant resistances.

Old and new isolates of *Pyrenophora teres* f *teres* have been tested in adult plant resistance screens using a set of South Australian varieties to verify and monitor the changes in the local pathogen population. Based on these tests in controlled environment rooms, a higher than expected level of pathotype variation has been observed. The rapid evolution of virulence in the pathogen population and the success of earlier containment of the disease suggest that durable multigenic resistance is the most effective strategy for containing NFNB in prone environments. The same methods using specific pathotypes are being used to screen lines for barley breeding programs and in collaborative research projects investigating variation at the molecular level (Linde et al) and variation in toxin production and elicitors (Able et al).

**Genetic relatedness of *Bipolaris sorokiniana* isolates from different cereal host tissues**

[a]M. W. Sutherland, [a]A. Martin, [a]M. Horne, [b]G. Platz and [b]J Franckowiak

*[a]Centre for Systems Biology, University of Southern Queensland, Toowoomba Qld 4350; [b]Hermitage Research Station, DEEDI, Warwick Qld 4370 Australia*

mark.sutherland@usq.edu.au

*Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) is the causal agent in cereals of both the foliar disease spot blotch and the root disease common root rot. While spot blotch of barley is a significant disease problem in northern NSW and Queensland, common root rot is present in all winter cereal growing regions of Australia. In order to investigate the relationship between these disease states, 100 single spore isolates of *Bipolaris sorokiniana* from across the major barley growing regions of Australia were collected during 2009 and 2010. The isolates were sourced from foliar and root materials of wheat and barley showing symptoms of spot blotch and common root rot respectively. Species-specific primers were used to verify the identity of the samples. Cluster analysis of amplified fragment length polymorphisms (AFLP) among these isolates is currently being conducted to investigate correlations with geographic distribution and potential host tissue specialisation. Initial results indicate that the majority of common root rot and spot blotch isolates cluster separately from each other. *B. sorokiniana* has also been suggested as a contributing cause of black point symptoms on barley grain. AFLP analysis of isolates sampled from black-pointed and non-black-pointed grain from six different barley lines failed to distinguish these two groups of isolates from each other or from spot blotch isolates sampled from leaf material. This work is part of a larger program to understand variation in host pathogenicity in *B. sorokiniana* and to develop improved molecular tools for assessing host resistance in breeding lines.

**Virulence spectrum of Rhyncosporium secalis isolates from Syria and Lebanon at adult and seedling growth stages**

Z. Alamdar, M. Al Ahmed, H. Al Khatib, A.Yahyaoui, S. Grando, F. Capettini

*ICARDA - International Center for Agricultural Research in Dry Areas*

F.Capettini@cgiar.org

Scald is a common disease of barley in cooler and semi-humid growing areas of the world, including the Middle Easters countries of Syria and Lebanon where it is considered as a major limiting factor in barley production. Yield loss estimates can be as high as 35-40% (Jenkins and Jemmett, 1967). Infection can occur as early as the seedling stage until to adult plants. In studies carried out at ICARDA it was demonstrated that early infections caused higher losses than infections at adult plant (Yahyaoui, unpublished). Study of race composition would allow better management of the disease. Resistance and susceptibility patterns can be different depending of the isolates present in the crop. The main goal of this research was to determine the race differences among samples collected from different barley growing areas in Lebanon and Syria.

For this study twenty isolates, 10 from Lebanon and 14 from Syria, were collected from farmers' field in different locations and varieties. For field inoculation, samples were mixed by country, creating two inoculum sources for the study – Syria & Lebanon. A set of germplasm consisting of advanced promising and elite lines from the ICARDA breeding program and scald differential were grown in Terbol (Lebanon) and Tel Hadya (Syria), under artificial inoculation and mist irrigation. Seedling tests for race determination were carried out using single spores and bulk inoculum from Syria and Lebanon. Scoring scald reaction was on the basis of 0-4 scale at seedling and 0-9 at adult plant growth stages. Results revealed 80% similarity in severity amongst the Syrian Lebanese isolates. The seedling tests allowed identifying 5 races from Syria and three from Lebanon

**An international standard set of barley differential genotypes and experience of using it for populations studies of *Pyrenophora teres* f. *teres***

O. S. Afanasenko[1], A. V. Anisimova[1], M. Jalli[2], H. O. Pinnschmidt[3], G. Platz[4] , A. Yahyaoui[5] and L. Tvaruzek[6]

olga.afanasenko@gmail.com

*[1]All-Russian Research Institute of Plant Protection, Saint Petersburg, Pushkin 196608 sh. Podbelsky, h. 3 Russia; [2]MTT Agrifood Research Finland, Plant Protection, FI-31600 Jokioinen, Finland; [3] University of Aarhus, Faculty of Agricultural Sciences, Department of Integrated Pest Management, DK-4200 Slagelse, Denmark; [4] Agri-Science Queensland Hermitage Research Station, 604 Yangan Road, Warwick QLD 4370 Australia; [5]ICARDA Box 5466, Aleppo, Syria; and [6] Agricultural Research Institute Kromeriz, Ltd., 767 01 Kromeriz, Czech Republic*

The new differential set for *Pyrenophora teres* f*. teres* was established. It consists of nine barley genotypes and, as a susceptible check cultivar, Harrington. By using this set virulence of nine hundred and eighteen single conidial isolates from different *P. teres* f*. teres* geographic populations from Russia (European, Ural and Siberia), Western Europe, Canada and Syria in 2004 – 2010 was studied. Among these isolates 119 P. teres f. teres virulence phenotypes in different geographic populations were determined. High diversity within each of studied populations was determined with Simpson's diversity indices. The number of virulent isolates to each barley genotype, pathotypes composition, and share of identical phenotypes in different geographic populations were studied. Min similarity was found between European populations and populations from Syria.

**Development of an international differential set for *Pyrenophora teres* f. *maculate***

M.S. McLean[a], T.K. Turkington[b], G.J. Platz[c], A. Martin[d], M.W. Sutherland[d], B.J. Howlett[e] and G.J. Hollaway[a]

*[a]Biosciences Research Division, Department of Primary Industries, Horsham, Vic 3401, Australia. [b]Lacombe Research Centre, Agriculture and Agri-Food Canada, Lacombe, AB T4L1W1, Canada. [c]Department of Employment, Economic Development and Innovation, Warwick, QLD 4370 Australia. [d]University of Southern Queensland, Toowoomba, QLD 4350, Australia. [e]School of Botany, The University of Melbourne, Vic 3010, Australia*

Mark.McLean@dpi.vic.gov.au

Spot form of net blotch (SFNB), caused by the fungus *Pyrenophora teres* f. *maculata*, has become a major foliar disease of barley in many barley growing regions of the world. The development of an international differential set of barley lines for *P. teres* f. *maculata* is essential for breeding resistant barley varieties. Ninety-five barley lines were tested as potential candidates for inclusion in an international differential set for *P. teres* f. *maculata.* Each barley line was tested as seedlings and adults toward multiple isolates in Australia and Canada. The reaction response of each line was used to develop similarity matrices and to perform cluster analysis of responses. A sub-set of 44 lines that represented the major resistances identified in the cluster analysis were then tested as seedlings toward 60 Australian isolates of *P. teres* f. *maculata*. This study confirmed resistance in 92 barley lines toward isolates of *P. teres* f. *maculata*, however variable reaction responses were identified toward different isolates with significant variation between isolates from the same continents as well between isolates from Australia and Canada. Virulent pathotypes were detected toward most resistance sources which indicate that careful selection of resistance sources is required by breeding programs when developing SFNB resistant barley varieties. A preliminary differential set for *P. teres* f. *maculata* is proposed for discussion and further testing toward a more extensive collection of isolates. The authors welcome discussion and input into further development of the *P. teres* f. *maculata* differential set.

**Understanding climatic and crop management influences on stubble borne inoculum of net blotch in barley in Western Australia**

K.W. Jayasena[ad], J. Galloway[b], G.J. Thomas[c], P.L. Payne[b], K. Tanaka[a], T.L. Humphreys[b]; S. Kelly[c], M.U. Salam[c] and W.J. MacLeod[cd].

*[abc]Department of Agriculture and Food Western Australia, [a]Albany, Western Australia, [b]Northam, Western Australia, [c]South Perth, Western Australia; [d]School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA, 6009, Australia*

kithsiri.jayasena@agric.wa.gov.au

Net blotch (*Pyrenophora teres*) mixed infected (NFNB & SFNB) barley stubbles were studied to understand the influence of climatic conditions and crop management variables on stubble borne inoculum at Northam and Albany, Western Australia. Conidia and pseudothecia produced on first year (6 – month old) and second year (18 - month old) stubbles. The amount of inoculum produced varied with the age and cultivar of the stubble. First year stubble produced more conidia, whereas more pseudothecia developed on second year stubble. The susceptible (S) cultivar Baudin produced more conidia than moderately resistant/moderately susceptible (MR/MS) Flagship. Spore trap study revealed that conidia were released when rainfall exceeded 3 mm per day. On a daily average temperature below 15°C, it appears the release of conidia restricted, whereas it was conducive when the temperature remained above this threshold. A two -year field experiment, revealed that net blotch infection and inoculum carryover was higher in cultivar Baudin than Flagship. Net blotch infection and yield loss in the second year barley sown into barley stubble was largely explained with respect to cultivar resistance of both the current season barley, and the stubble thereby the response was the largest when a susceptible cultivar sown over the stubble of susceptible cultivar.

# Special topic – Ramularia

**A History of Ramularia research in Scotland**

N.D. Havis[a] and S.J.P Oxley[b]

[a]*Crop and Soil Research Group, SAC, West Mains Road, Edinburgh, Scotland, EH9 3JG, UK.* [b]*HGCA, Agriculture and Horticulture Development Board, Stoneleigh Park, Kenilworth, Warwickshire, UK, CV8 2TL*

Neil.Havis@sac.ac.uk

In the late 1990's a new, late season spotting complex appeared in spring barley crops in Scotland. The exact cause of the spots remained unclear. However, the fungus *Ramularia collo-cygni* was identified in barley crops in 1998 by Dr Edelgard Sachs from Kleinmachnow, Germany. Funding was obtained from the Home Grown Cereals Authority to investigate the cause of the spotting complex. This project initiated over a decade of research in Scotland into the role of *R. collo-cygni* in the spotting complex. Future projects looked at detection of the fungus within the plant, its relationship with oxidative stress in the host plant, the spread of the pathogen spores and the economic effect of the disease on barley production. Funding has been obtained from the Scottish Government, HGCA and commercial sponsors. This paper will review advances in our knowledge of the pathogen from Scottish research and set this findings in the context of expanding interest in the pathogen within barley growing areas on a global scale.

**Studying the biology of *Ramularia collo-cygni* for the development of an Integrated Pest Management System to match new challenges from a changing climate**

M. Hess[a], M. Nyman [a], H. Hausladen[a], S. Weigand[b]

[a] *Chair of Phytopathology, Center of Life and Food Sciences Weihenstephan, Technische Universität München (TUM), 85350 Freising, Germany;* [b] *Bavarian State Research Center for Agriculture (LfL),Institute for Plant Protection, 85354 Freising, Germany*

m.hess@tum.de

Long term surveys show a shift in pathogen population in Bavaria. As a consequence efficiency of established control strategies has been reduced. Unsatisfying barley yields have been attributed to the occurrence of heavy leaf spotting caused by environmental factors and *Ramularia* leaf spotting (RLS). In a joint project between the Technische Universität München and the Bavarian State Research Center (LfL) the causes of the leaf spotting are being investigated by monitoring different sites in Bavaria and conducting fungicide trials in spring barley and winter barley. Complementary intense studies of the pathogen-biology are conducted. The aim of the project is to integrate the results into an established Integrated Pest Management tool (Gerstenmodell Bayern) for improved control. With the focus on the detection of *Ramularia collo-cygni* in the field and interaction of the epidemics with climatic factors, the project has the advantage of intense observations in a region with high incidence and high agricultural and climatic variability.

Monitoring shows a broad and regular occurrence on all tested sites. The first results with an improved strategy based on the experience from the specific fungicide trials generally gave a positive yield benefit.

To further optimize the tool a better understanding of the pathogen biology is necessary. Our investigations propose a complex interaction between the latency of *Ramularia collo-cygni,* plant development and environmental factors.

**Comparison of screening methods for resistance of spring barley cultivars to Ramularia leaf spot disease**

N. Zamani-Noor, B. Koopmann and A. von Tiedemann

*Department of Crop Science, Plant Pathology and Crop Protection Division, Georg-August University Goettingen, Germany*

atiedem@gwdg.de

*Ramularia collo-cygni* (Rcc) has gained increasing importance as the causal agent of a novel leaf spot disease on barley, Ramularia leaf spot (RLS). RLS disease occurs conspicuously late in the growing season. When the crop has passed the flowering stage, the disease severity in the field often increases dramatically as leaf spots are massively formed and fungal toxins operate sometimes causing complete browning and leaves die off within as few as 12 days. Evidence from observations under controlled conditions suggests, that the development of disease symptoms requires distinctly late developmental stages of the plant and indicates that the disease does not establish on seedlings or juvenile plants (Schützendübel et al., 2008). Generally, there are two main instruments for the control of the disease: (i) chemical control using appropriate fungicides and (ii) cultivation of resistant cultivars. Preliminary work on chemical control has been done, showing that effective fungicides are available. In contrast, the knowledge on cultivar resistance is limited and no systematic studies on Ramularia resistance have been performed. Most barley varieties appear to be susceptible to the pathogen, although there is moderate resistance to *R. collo-cygni* in some varieties of both spring and winter barley (Pinnschmidt *et al*., 2006). Observations of Cromey *et al*. (2002), Greif (2004), Pinnschmidt *et al*. (2009) and McRoberts *et al*. (2009) suggest the existence of genetic variability in RLS resistance which could be used in resistance breeding and disease management. In the present study, thirty-three different spring barley genotypes were evaluated for resistance to RLS. Evaluations were conducted in replicated experiments in growth chambers (with leaf segments) and under greenhouse and field conditions (with whole plants). Plants were evaluated at late growth stages (73-75) for percentage of necrotic leaf area due to RLS on leaf F-1 in the field experiments and whole plant inoculation trials in controlled conditions. In growth chamber experiments, leaf segments were inoculated with $1*10^5$ spores/ml and the numbers of necrotic spots on individual leaflets were counted after 15 days post inoculation. Genotypes displayed significant differences in their response to Rcc infection in the field, greenhouse, and growth chamber experiments. Upon natural infection in the field, the severity of Rcc symptoms depended strongly on local weather conditions. While the cultivar IPZ 24727 showed a moderate resistance to Rcc, the other cultivars were more or less susceptible. A strong correlation was found between leaf segment assays and severity of leaf symptoms in the greenhouse experiments ($P<0.0001$, $R_s=0.68$). A significant correlation was also observed between field experiments in two different years ($p< 0.0419$, $R_s=0.42$). Additionally, a SYBR Green-based quantitative polymerase chain reaction (qPCR) assay for quantification of fungal DNA in plant material was developed. A strong correlation ($p< 0.00179$, $R_s=0.851$) was observed between the visual disease symptoms and fungal DNA concentration in leaf F-1. Furthermore, a novel detection method for the Rcc phytotoxin Rubellin was developed. Results of fungal toxins in diseased leaf tissue correlated strongly ($p<0.00005$, $R_s=0.966657$) with the visual disease symptoms. The results demonstrate the potential for screening barley cultivars for Rcc resistance under controlled conditions.

**Sequencing of the fungal pathogen *Ramularia collo-cygni,* why and how?**

J. M. Fountaine[a], M. Kaczmarek[a], M. Piotrowska[a], F J Burnett[a] and N. D. Havis[a]

[a]*Crop and Soil Research Group, Scottish Agricultural College, Kings Buildings, Edinburgh EH9 3JG, UK*

James.Fountaine@sac.ac.uk

The fungus *Ramularia collo-cygni* is the major biotic agent involved in Ramularia Leaf Spot (RLS). It was first identified as a pathogen of spring barley in Scotland in 1998 and since then has increased in its importance throughout the whole of the UK. Results from testing of the Rothamsted Hoosfield spring barley archive using quantitative real-time PCR indicated a significant increase in pathogen levels since the 1990's (Fountaine and Fraaije, 2009). RLS has recently been re-classsed as a major barley disease in the UK. *R. collo-cygni* is currently classified as a member of the *Mycosphaerella* genera and sequence data derived at SAC suggests a genetic similarity between *R. collo-cygni, Mycosphaerella graminicola* and *M. fijiensis*. These sequences focus pimarily on the genes associated with the target sites for fungicides, such as Beta tubulin, Cytochrome *b* and Succinate dehydrogenase, eburicol 14ά-demethylase (CYP51) genes. This paper will highlight previous sequence work and outline a new project using next generation sequencing by the combined approach of illumiina/solexa and Roche/454 sequencing. The use of these combined approaches will help with the assembly of sequence data which can then be used for comparative genetic studies to address the biology of *R. collo-cygni* in areas such as population genetics, fungicide resistance and pathogenicity. These advances should assist in the development of environmentally sound strategies to control this important disease of barley production systems.

**DNA polymorphism and rubellin production by *Ramularia collo-cygni* isolates originating from Europe**

M. Jedryczka[a], Q. Wen[b], W. Irzykowski[a], P. Kachlicki[a], P. Matusinsky[c], N. Zamani-Noor[d], B. Koopmann[d] and P. Frei[e]

[a] *Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 60-470 Poznań, Poland;* [b]*Department of Seed Science, Faculty of Horticulture, Poznan University of Life Sciences, Baranowo, Szamotulska 28, 62-081 Przezmierowo Poland;* [c]*Agrotest Fyto, Ltd., Havlickova 2787, 767 01 Kromeriž, Czech Republic;* [d]*Division of Plant Pathology and Crop Protection, Department of Crop Sciences, University of Göttingen, Grisebachstrasse 6, D-37077 Göttingen, Germany;* [e]*Research Station Agroscope Changins-Wädenswil ACW, CP 1012, 1260 Nyon, Switzerland*

malgosia_jedryczka@poczta.onet.pl

Barley leaf blotch, caused by the fungal pathogen *Ramularia collo-cygni* has been reported in Europe since the end of the 19th century. It was first found in southern Europe, but it is more associated with its central and northern parts. Apart from Europe, the fungus was also found in New Zealand, Argentina and Canada. The colonies formed on agar and liquid media form a purple colour due to the production of rubellins – secondary metabolites belonging to the anthraquinone family. The aim of this work was to evaluate DNA polymorphism and rubellin production by isolates of *R. collo-cygni*, collected in Europe. The studies were done using 36 isolates, originating from Austria, the Czech Republic, Denmark, Germany, Scotland and Switzerland. The isolates were obtained from barley as well as fescue and annual meadow grass. Fungi were sub-cultured on Potato-Glucose-Agar medium, Czapek-Dox, Czapek-Thom and Fries minimal liquid media and checked for DNA polymorphisms (RAPD) and metabolites, with the special attention to rubellins. The metabolites secreted to the medium were extracted using solid phase extraction (SPE) columns $C_{18}$ and profiles of these compounds were studied using Ultra Performance Liquid Chromatography (UPLC) and High Performance Liquid Chromatography hyphenated to the ion trap mass spectrometer (HPLC-$MS^n$). It was found the secondary metabolites differed between the studied isolates and the time of subculture. DNA polymorphism was studied using 63 decamer primers. Half (54%) of the starters showed polymorphisms among the isolates, with 1-8 polymorphic bands per starter. One third (35 %) of starters produced 2 polymorphic bands.

**B-1**

***In vitro* assessment of QoI and SDHI fungicide sensitivity of *Ramularia collo cygni***

R. Frey, H. Sierotzki

*Syngenta Crop Protection, Research Biology, CH-4332 Stein, Switzerland*

regula.frey@syngenta.com

*Ramularia collo cygni* is a new upcoming disease in barley which can effect up to 70% of yield loss. Major control measures are provided by fungicides. Of these the QoI's were the most efficient. However a strong resistance development has been observed in the last years, reducing the efficacy of the QoI fungicides in controlling this disease.

The new SDHI fungicides (isopyrazam) demonstrate excellent control of *R. collo cygni.* In 2010 a new mixture of cyprodinil and isopyrazam was launched onto the UK market.

Due to the potential risk of resistance to this new fungicide class, monitoring is essential. We developed an *in vitro* sensitivity bioassay, including isolation and cultivation methods. Monitoring results from 3 years are presented and all isolates tested to date are sensitive to isopyrazam.

Based on the data generated a resistance management strategy is proposed.

**B-2a**

## Microscopic study of a dynamic relationship between filamentous fungus *Ramularia collo-cygni* and its host plant *Hordeum vulgare*

M. Kaczmarek[a,b], J. M. Fountaine [a], N. D. Havis[a], K. M. Wright[c], A. C. Newton[c] and N. D. Read[b]

*[a]Crop and Soil Research Group, Scottish Agricultural College, Kings Buildings, Edinburgh EH9 3JG, UK; [b] Fungal Cell Biology Group, Institute of Cell Biology, Rutherford Building, University of Edinburgh EH9 3JH, UK; [c] Plant Pathology Programme, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK*

Maciej.Kaczmarek@sac.ac.uk

The filamentous fungus *Ramularia collo-cygni* causes small necrotic spots, usually with a yellow halo, called Ramularia Leaf Spot (RLS), on a range of host plants e.g. barley, wheat, maize and other *Gramineae* and has now been officially recognized as a major disease of barley in the UK. Although *R. collo-cygni* was first reported as early as the 1890s, there are still considerable gaps in our knowledge of its fundamental biology. Therefore, the complete life cycle, including both, symptomless and symptomatic phase of RLS development, will be observed *in planta* by the combined use of Confocal Laser Scanning Microscopy (CLSM) and transgenic isolates expressing GFP and DsRed fluorescent protein genes as the reporter markers. The microscopic analyses are particularly aimed at determining the time-point, thus the potential triggers, of the transition of fungal life style from endophytic to pathogenic. Our preliminary evidence suggests that *R. collo-cygni* undergoes a change in its behaviour and mode of action to much more aggressive upon stress possibly caused by response to plant physiological growth stages. The current development of reliable barley inoculation assay will allow the observation of the disease progression on whole plant scale at any point of fungal-host interaction and more importantly, this experiment will also help elucidate the nature and importance of the seed borne stage in the *R. collo-cygni* life cycle. These approaches should have a significant impact on the development of new methods in successful RLS management strategies for the future.

**B-2b**

## An investigation into the possibility of sexual reproduction in the filamentous fungus *Ramularia collo-cygni.*

M. Kaczmarek[a,b], J. M. Fountaine[a], N. D. Havis[a], K. M. Lord[b] and N. D. Read[b]

[a]Crop and Soil Research Group, Scottish Agricultural College, Kings Buildings, Edinburgh EH9 3JG, UK; [b]Fungal Cell Biology Group, Institute of Cell Biology, Rutherford Building, University of Edinburgh EH9 3JH, UK

Maciej.Kaczmarek@sac.ac.uk

The Ramularia Leaf Spot (RLS) has been classified as a major disease of barley in the UK. All currently grown barley cultivars are susceptible to its causal agent, the filamentous fungus *Ranularia collo-cygni*. The infection by this pathogen may lead to substantial barley yield losses in north-western and central Europe where a significant amount on fungicides is applied each year to control the RLS. These factors have contributed to increasing attention being paid towards better understanding of the fundamental biology of this elusive pathogen in order to develop more successful strategies of RLS management. The recently reported development of fungicide resistance by the fungus has instigated research into the drivers of its rapid evolution. Therefore, the characterisation of a potential teleomorph and sexual preference of *R. collo-cygni*, currently classed as asexual, will be attempted by induction of sexual fruiting bodies *in vitro* and the molecular analysis of *MAT* loci. The *Asteromella* stage and its further developmental phases, is examined in detail by Bright Field (BF), Differential Interference Contrast (DIC), Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (CLSM) techniques. The phylogenetic analyses suggest strong relationship of *R. collo-cygni* to *Mycosphaerella* teleomorphs. This will be verified by morphological comparison of the observed structures. Since the sexual reproduction in *Mycosphaerella* species is poorly understood, this study could also help enhance our understanding of sexual life cycles of many major plant pathogens from *Mycosphaerella* clade. Finally, the other potential sources that could drive the genetic diversity of *R. collo-cygni* population such as parasexual recombination by means of vegetative hyphal fusion in mature colony and CAT fusion of spores will also be explored.

**Genetic diversity in *Ramularia collo-cygni* measured by AFLP and sequencing**

R L Hjortshøj[a,b], E H Stukenbrock[c], A R Ravnshøj[a], M Nyman[d], N Havis[d], G Backes[e], J Orabi[e], H Pinnschmidt[f] and J Stougaard[b]

*[a]Sejet Plantbreeding, Nørremarksvej 67, DK-8700 Horsens;[b]Department of Molecular Biology, Aarhus University, Gustav Wieds Vej 10c, DK-8000 Aarhus ;[c]MPI für terrestrische Mikrobiologie, Karl-von-Frisch-Straße 10, D-35043 Marburg, Germany; [d]Crop and Soil Research Group, Scottish Agricultural College, W. Mains Road, Edinburgh, UK; [e] Department of Agriculture and Ecology, Thorvaldsensvej 40, DK-1871 Frederiksberg; [f]Department of Integrated Pest Management, Aarhus University, Forsøgsvej 1DK-4200 Slagelse*

rlh@sejet.com

Knowledge of population genetic structures in pathogens is needed in order to develop sustainable strategies towards control of diseases, This knowledge is furthermore crucial when comparing results from disease nurseries and mapping populations placed in different locations. For breeding programmes, differences in pathogen populations is not a problem if the test location is lies within the target-area for the variety. In contrast, knowledge about the pathogen population at several target sites is important for evaluating the potential of varieties in other areas. This becomes even more important when comparing results in mapping populations, as discovery of resistance genes depends on the virulence of the pathogen populations.

Here an AFLP study was carried out to compare *Ramularia collo-cygni* populations from Denmark and Scotland.

Results showed that the population genetic structure in the four sub-populations, collected in the two countries, were highly similar, and the genetic distance between the populations was small. Further linkage equilibrium tests indicated the clonal reproduction plays a major role in the multiplication of the fungus, despite a high genotypic diversity.

Sequencing of the β-tubulin gene showed a high level of diversity amongst isolates from all over Europe. On the other hand sequences from the ITS region displayed a high degree of conservation, even for isolates collected from other hosts than barley.

**B-4**

## Gene expression profiles in tolerant and susceptible barley cultivars inoculated with *Ramularia collo-cygni*

R. L. Hjortshøj[ab], L. Krusell[b], A. M. Jurkiewicz[b], P. Hedley[c], J. McNicol[c], M. Corbitt[d], J. Brown[d], J. Stougaard[b]

rlh@sejet.com

*[a] Sejet Plantbreeding, Nørremarksvej 67, DK-8700 Horsens; [b] Department of Molecular Biology, Aarhus University, Gustav Wieds Vej 10c, DK-8000 Aarhus; [c] The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland UK; [d] Department of Disease and Stress Biology, John Innes Centre, Norwich, NR4 7UH, England UK.*

Ramularia collo-cygni is a biotroph fungus that can complete its lifecycle inside the host plant without causing any symptoms, thus resembling the behaviour of an endophyte. However, under so far unknown conditions, it can switch to a pathogenic lifestyle. At present the environment and/or signals initiating this shift from endophyte to pathogen behaviour are not known.

In this study two week old seedlings of a tolerant/resistant and a highly susceptible barley cultivar were inoculated with Ramularia collo-cygni. At 3 and 11 days after inoculation samples were taken for RNA extraction and microscopy, in order to study the gene expression of the host plant and the status of infection. The three day sampling point was chosen to mimic the endophytic stage. At the 11 day sampling point weak symptoms were observed and this timepoint resembles the pathogenic stage.

Initial result from the array analysis indicates large differences in gene expression between the cultivars at both time-points. The number of genes regulated by presence of Ramularia collo-cygni is, for example, almost twice as large in the susceptible cultivar compared to the resistant/tolerant cultivar at the first (3 days) time-point. Whereas at the last time-point (11 days), there are significantly more genes regulated in the resistant cultivar.

**B-5**

## Variation in response of winter barley cultivars to Ramularia leaf spot

Pavel Marik[1], Pavel Matusinsky[2], Lenka Stemberkova[1], Martina Hanusova[1]

*[1]Research Centre SELTON, Stupice, Czech Republic; [2]Agrotest fyto, Kromeriz, Czech Republic*

matusinsky.pavel@vukrom.cz

The fungus *Ramularia collo-cygni* (RCC) is increasingly important as the causal agent of Ramularia leaf spot (RLS). The work aimed to identify resources suitable for developing new breeding lines of winter barley with improved resistance. During the first experimental period (2001–2005), RLS incidence was monitored in 711 cultivars and advanced breeding lines. Differences were detected in the intensity of symptomatic expression, but no material showed high resistance. During the second experimental period (2006–2009), response to natural RLS infection was evaluated in 19 winter barley cultivars (12 six-row and 7 two-row) registered in the Czech Republic. Cultivars exhibiting weaker symptomatic expression of infection at milk-waxy to waxy maturity  stand mostly among the late cultivars. Only three cultivars with significantly weaker infection symptoms from the current collection of registered cultivars in the Czech Republic can be recommended for growing in regions with strong RCC occurrence: 2-row medium-early cv. Breunskylie, 6-row late cv. Merlot, and 6-row medium-late cv. Highlight. Nevertheless, none of the cultivars or breeding lines tested so far has proven to be resistant to RLS on such a level that it could be recommended as a donor of resistance to this disease.

**Presence of *Ramularia collo-cygni* in Bavaria in the past 50 years**

M. Nyman[a], M. Hess[a], H. Hausladen[a], S. Weigand[b]

*[a] Chair of Phytopathology, Technische Universität München ,85354 Freising, Germany;[b] Institute for Plant Protection, Bavarian State Research Centre for Agriculture, 85354 Freising, Germany*

m.nyman@wzw.tum.de

*Ramularia collo-cygni (Rcc)* is a fungal pathogen of barley, causing Ramularia Leaf Spot (RLS). *Rcc* was first described about 150 years ago, but has only recently become a problem in barley production. In the past 10 years regular epidemics in Bavaria have significantly reduced yield quantity and quality. To see how long the pathogen has been present here, we screened seed samples of spring and winter barley collected by the Bavarian State Research Centre for Agriculture since the late 1950's. Samples were first tested with nested pcr to see when the pathogen became present, and subsequently by qpcr. It showed that *Rcc* has been present in Bavaria all this time, but apparently without attracting much attention.



**Characterisation of mutations in the succinate dehydrogenase gene for potential fungicide resistance in *Ramularia collo-cygni***

M. Piotrowska[a], F. Burnett[a], J. Fountaine[a], P. Hoebe[a], R. Ennos[b], D. Ranner[c]

*[a]Crop and Soils System Research Group, Scottish Agricultural College, Edinburgh, EH9 3JG; [b]Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, EH9 3JT; [c]Syngenta Crop Protection UK Limited, CPC4, Capital Park, Fulbourn, Cambridge, CB21 5XE*

marta.piotrowska@sac.ac.uk

The fungus *Ramularia collo-cygni* (Rcc) has been present as a major barley pathogen in Scotland since 1998. Since 1999 azoxystrobin (Quinone Outside Inhibitor fungicide) was used and recommended as an effective fungicide treatment but between 2001/2002, after a few seasons of widespread use, the first resistant strains of Rcc developed in the field. Because of this history Rcc is considered a high risk pathogen in terms of resistance to other fungicide groups.
The focus of this study will be on SDHI (Succinate Dehydrogenase Inhibitors) fungicides which inhibit fungal respiration by binding to the mitochondrial respiration complex II. All of the currently available data suggests that Rcc is still sensitive to all SDHI fungicides. However, development of resistance to this chemical group is currently classified by FRAC (Fungicide Resistance Action Committee) as medium to high risk. Rcc has already been exposed to SDHI fungicides for a number of growing seasons. Therefore, the risk of resistance development is high, given that only a single point mutation was required in the target gene to develop high levels of fungicide resistance in other related plant pathogens.
In my PhD project, possible mutations responsible for SDHI resistance development in Rcc will be examined. Mutants will be characterised in the terms of their fitness and pathogenicity. Diversity of the population will be described and their evolutionary potential, in terms of fungicide resistance, will be defined. Finally, an effective assay for monitoring genetic changes that correlate with fungicide resistance will be developed. This will enable detection of *Ramularia* resistant isolates and the production of resistance control strategies.

# Molecular plant-pathogen interactions

**_Rhynchosporium_ genomics – a strategy towards understanding host specialisation**

A. Navarro-Quezada[a], A. Leitner[a], C. Wenzel[a], K. King[b], B.D.L. Fitt[b], M. Münsterkötter[c], M. Felder[d], S. Taudien[d], M. Platzer[d], S. Torriani[e], B.A. McDonald[e] and W. Knogge[a]

*[a] Department of Stress and Developmental Biology, Leibniz Institute of Plant Biochemistry, D-06120 Halle, Germany; [b] Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK; [c] Institute of Bioinformatics and Systems Biology, Helmholtz Centre Munich, D-85764 Neuherberg, Germany; [d] Leibniz Institute for Age Research – Fritz Lipmann Institute, D-07745 Jena, Germany; and [e] Plant Pathology, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland*

wknogge@ipb-halle.de

Based on phylogenetic criteria fungi of the *Rhynchosporium* genus were recently assigned to four species, each also exhibiting a specific host spectrum. *R. secalis* grows on rye (*Secale cereale*) and triticale, *R. commune* on *Hordeum* species including cultivated barley and on brome grass (*Bromus* spp.), and *R. agropyri* on couch grass (*Agropyron* spp.). Previously, these morphologically indistinguishable species were all referred to as *R. secalis*. The fourth species is constituted by *R. orthosporum*, which is characterized by a different spore shape and its host species cocksfoot (*Dactylis glomerata*). DNA sequencing of five isolates from the four *Rhynchosporium* species yielded mitochondrial genomes of 69 kb (*R. orthosporum* 49 kb) and nuclear genomes of 55 Mb carrying about 13,000 genes. Following a comparative genomics approach, we are now aiming at identifying the factors that underlie host colonisation and specialisation. Using molecular and proteomics techniques along with targeted deletion we are currently focusing on genes encoding fungal effector proteins.

**Investigations of the asymptomatic phase of _Rhynchosporium secalis_ and _Ramularia collo-cygni_ infection using GFP expressing variants**

Amarnath Thirugnanasambandam[1], Steve C. Whisson[1], Kath M. Wright[1], Neil Havis[2], Simon D. Atkins[3] and Adrian C. Newton[1]

*[1] The James Hutton Institute, Invergowrie, Dundee, DD2 5DA; [2] Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG, UK; [3] Rothamsted Research, Harpenden, Herts, AL5 2JQ*

*Rhynchosporium secalis* and *Ramularia collo-cygni* are economically important pathogens of barley. *R. secalis* is responsible for scald disease and has been identified globally. It remains one of the most important and prevailing leaf disease of barley in the UK. *R. collo-cygni* causes Ramularia Leaf Spot (RLS) and has increased in importance over the last two decades. Both these pathogens are thought to be spread primarily through man-mediated transfer of infected seeds to various places and RLS continues to be reported in new places. The ability of both these pathogens to survive asymptomatically in and on barley plants and seeds makes assessment of true infection and resistance difficult to assess. The current methods used to quantify resistance of barley cultivars relies on the number of visible symptoms on leaves. However, by quantitative real-time PCR techniques we can quantify the presence these pathogens from leaf and seed samples lacking symptoms.

We have transformed both these pathogens to express green (GFP) and red (DsRed) fluorescent proteins. By confocal microscopic and analysis of the infection pattern of *R. secalis* by calculating colony mass fractal dimensions, we revealed significant differences in the infection pattern on different cultivars. It showed that the difference in the pattern of infection varied between cultivars as early as three days post-inoculation. *R. secalis* showed infection and even sporulation in cultivars that are considered to be resistant. Transformation of *R. collo-cygni* was achieved for the first time ever, thus providing more approaches to study this so far elusive pathogen. The infection cycle was investigated focusing mainly on the asymptomatic period of infection. This revealed several insights into the colonisation process. Likewise sporulation of *R. collo-cygni* during asymptomatic phase was observed nine dpi for the first time on detached leaves.

**Mining the *Rhynchosporium secalis* genome and transcriptomes for pathogenicity determinants**

A. Avrova[a], M. Münsterkötter[b] and W. Knogge[c]

[a]*The James Hutton Institute, Dundee, UK;* [b]*MIPS - Institute of Bioinformatics and Systems Biology, Munich, Germany;* [c]*Leibniz-Institute of Plant Biochemistry, Halle, Germany*

Anna.Avrova@hutton.ac.uk

*Rhynchosporium secalis* is one of the most destructive pathogens of barley worldwide, especially in areas with cool temperate climates. It can lead to yield losses of up to 30-40 % and decrease in grain quality. Despite the damage that *R. secalis* inflicts on barley crops, knowledge of its pathogenicity factors is almost non-existent. The challenge therefore is to gain a greater understanding of novel and essential pathogenicity determinants, as these represent good targets for recognition by host plant genotypes. Some pathogenicity determinants essential for the core biology of the pathogen during infection may also represent potential targets for new environmentally benign fungicides.

Recent sequencing of *R. secalis* germinated conidia transcriptome revealed enrichment for transcripts encoding potential structural cell wall proteins, adhesion proteins, plant cuticle and cell wall degrading enzymes, signalling proteins, stress response and detoxification enzymes, and nutrient transporters. A subset of transcripts encodes for small secreted proteins, representing putative effectors, including the well-characterised avirulence gene *Nip1*. *R. secalis* genome and interaction transcriptome sequencing provided further information about the extent of gene families, as well as a subset of genes expressed at the onset of *R. secalis* colonization of barley. Comparison of genome sequences from strains with different race specificities will allow rapid prediction of candidate effectors, including those less variable in *R. secalis* populations. *R. secalis* potential pathogenicity determinants will be prioritised for further functional analysis based on their expression profiles.

**Role of proteinaceous toxins in symptom development of net blotch disease in barley**

D.J.C. Wong, I. Ismail, D. Godfrey and A.J. Able

*School of Agriculture, Food & Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, SA 5064 Australia*

amanda.able@adelaide.edu.au

While the fungal pathogen *Pyrenophora teres* f. *maculata* (Ptm) and *Pyrenophora teres* f. *teres* (Ptt) are genetically similar, the symptoms they cause on susceptible barley are different. *Ptm* causes circular or elliptical brown lesions (spot form net blotch) whereas *Ptt* causes distinctive dark-brown, longitudinal lesions (net form net blotch). We have previously shown that the growth patterns of the two forms of fungi *in planta* differ significantly (Lightfoot & Able 2010 *APP* 39: 499-507) and that both forms of the fungus produce a proteinaceous fraction which causes necrosis (Sarpeleh et al 2007 *Phytopath* 97: 907-915; Sarpeleh et al 2008 *PMPP* 72: 73-79). The proteinaceous fraction causes symptoms in a host specific manner suggesting it may contain effectors. We have been using a proteomics approach to isolate and identify individual toxins and effectors as well as to characterise differences between: 1) the net and spot form; and 2) isolates with varying virulence/aggressiveness. Six proteins have been identified as being differentially expressed between net and spot form while a further two proteins have been identified as differentially expressed between low and high aggressive isolates. One protein unique to *Ptt* (NF1) and one protein unique to *Ptm* (SF1) have been characterised further. Patterns of gene expression *in vitro* and *in planta* suggest that the proteins are expressed by actively growing hyphae, and during the development of symptoms. Bioassay of recombinant SF1 and NF1 confirm a role in symptom development and suggest NF1 may be an effector.

**Identification of a proteinaceous necrotrophic effector and its corresponding sensitivity locus on barley chromosome 6H**

T. L. Friesen[a,b], Z.-H. Liu[b], R. S. Brueggeman[b], J. D. Faris[a]

[a]*USDA-ARS, Northern Crop Science Laboratory, Fargo, ND, USA;* [b]*Department of Plant Pathology, North Dakota State University, Fargo, ND, USA*

timothy.friesen@ars.usda.gov

Net blotch of barley is caused by *Pyrenophora teres* which can be divided into two forms, *P. teres* f. *teres* and *P. teres* f. *maculata* causal agents of net form net blotch (NFNB) and spot form net blotch (SFNB) of barley, respectively. A fair amount of research has been done to identify virulence and/or avirulence factors associated with NFNB and SFNB. We have recently developed a barley population from the barley cultivars Hector and NDB112 which are highly susceptible and highly resistant, respectively, to most worldwide net form isolates tested. Interestingly, linkage mapping and QTL analysis in this population show that isolate specific QTL are present on chromosomes 2H, 3HS, 3HL, 5H, and 6H. Additionally, we have identified and partially characterized a necrotrophic effector produced *in planta* that is associated with the disease QTL on chromosome 6H. The effector identified is proteinaceous and based on size exclusion chromatography, the protein is around 10 kDa. Sensitivity to this effector is found in the susceptible parent Hector indicating that it is likely a necrotrophic effector rather than a biotrophic (avirulence) effector. Due to the available tools in the net blotch of barley system, including the presence of host and pathogen mapping populations, the availability of a genome sequence, the genetic tractability of the pathogen and the fact that the pathogen has worldwide importance, we are using net blotch as a model system on barley for studying necrotrophic host-pathogen interactions.

**Functional diversity on resistance to early infection among barley genotypes inoculated with *Cochliobolus sativus***

S. Rodriguez, P. Silva, F. Gamba, C. Pritsch

*Facultad de Agronomía, Universidad de la Republica, Montevideo, Garzón 780, 12900, Uruguay*

clara@fagro.edu.uy

Functional diversity of resistance to spot blotch caused by *C.sativus* present in four barley genotypes was compared. Both the rate of *C. sativus* infection of barley leaf tissues and temporal pattern of Pre-Infection Defense (PID) responses as shown by cell wall apposition (CWA) and HR reaction accumulation in epidermis were assessed at 24 and 48 hai. Progress of infection was rather similar within the first 24 hai for all genotypes, in which most of the interaction sites showed pre-penetrated epidermal cells. However, at 48 hai, resistant NDB112 clearly showed a much lower rate of penetrated epidermal cells as well as lower invasion of mesophyll tissues compared with the remaining genotypes. Similarly, at 24 hai, both CWA and HR reaction were frequently detected in pre-penetrated cells in all genotypes. However, the percentage of postpenetrated epidermal cells expressing HR reaction was much higher in the susceptible genotype. In agreement with previous reports, this HR reaction should be considered as a susceptibility factor. This work provided new hypotheses to be tested related to resistance mechanisms acting during *C. sativus* infection.

**Potassium deficiency and its effect on fungal pathogens of barley**

J. L. Davis[ab], A. C. Newton[a], P. J. White[a] and A. Amtmann[b]

[a] *The James Hutton Institute, Invergowrie, Dundee, DD2 2DA;* [b] *The University of Glasgow, College of Medical, Veterinary and Life Sciences, The Bower Building, Glasgow G12 8QQ*

Jayne.davis@hutton.ac.uk

Increased demand for agricultural products has resulted in more intensive farming methods being employed. This can led to the crop being exposed to a number of stresses, including, nutrient and water availability, access to light and pathogen attack. In the field plants are often subjected to more than one stress at any one time, therefore it is important that we understand how plants respond to multiple stresses. Potassium deficiency is known to affect susceptibility of plants to pathogens, but the nature of this effect is poorly understood, with studies often giving conflicting results. Previous studies have shown increased levels of the stress hormone jasmonate (JA) and related compounds in potassium-starved *Arabidopsis* plants and suggested that this may have a role in defence against pathogens. In order to further investigate this, barley plants were grown in hydroponics, in a full nutrient control or K-free nutrient solution, and the effect of K-deficiency on growth, metabolism, and gene expression, including JA-related genes, was investigated. Detached leaf segments were used to assess the effect of K-deficiency on two fungal pathogens of barley with different strategies for of pathogenesis and therefore nutrient acquisition. I will report the differing effects of K-deficiency on susceptibility of barley plants to the hemi-biotroph *Rhynchosporium secalis* and the biotroph *Blumeria graminis* f.sp. *hordei*.

**The Bad, the Ugly and the Good**

C. Gravouil[ab], J. M. Fountaine[c], M. J. Dickinson[b], I. Hein[a] and A.C. Newton

[a] *The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK;* [b] *Plant and Crop Science Division, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK,* [c] *Scottish Agricultural College, West Mains Road, Edinburgh, EH9 3JG, UK.*

adrian.newton@hutton.ac.uk

The leaf environment is like the “Wild West”: daily temperatures can be extreme, water availability is restricted and the UV exposure is often high. Only adapted, resilient micro-organisms can survive it. ‘The bad, the ugly and the good’ describes three micro-organisms, respectively *Rhynchosporium secalis* (Rs), *Pectobacterium atrosepticum* (Pba) and *Piriformospora indica* (Pi) and their interactions with the leaf.

Rs is a major disease on barley world-wide and the causal agent of the barley leaf blotch. No control approaches have yet proved to be sustainable. The leaf surface is a complex environment, hosting numerous bacteria and fungi. Some of these micro-organisms may enhance Rs establishment, whereas others will hinder it. Understanding the interactions occurring on the leaf between Rs and other micro-organisms may lead to new integrated control approaches. Pba, causal agent of the potato blackleg on stem and soft rot on tubers, can survive on barley leaves and influence disease establishment on Rs in the field. *In vitro* assays help to determine that its toxin producing genes are involved in Rs biocontrol. In contrast, Pi is a root endophyte that increases barley fitness and stress tolerance. This project has demonstrated that Pi also reduces Rs symptoms. However, there is a cultivar-specificity influencing the expression of Pi positive effects.

Both the ugly and the good can be used to improve the control of the bad, by helping in the identification of (i) leaf-associated toxin producing bacteria and (ii) genetic traits facilitating endophyte colonization.

# Resistance breeding

**Pre-breeding for barley net blotch resistance in Finland**

M. Jalli[a], K. Peltoniemi[b], O. Manninen[c] and R. Aikasalo[b]

*[a]Plant Production Research, MTT Agrifood Research Finland, FI-31600 Jokioinen; [b]Boreal Plant Breeding Ltd, Myllytie, FI-31600 Jokioinen; [c]Biotechnology and Food Research, MTT Agrifood Research Finland, FI-31600 Jokioinen*

marja.jalli@mtt.fi

The total agricultural area in Finland is 2.3 million hectares, and barley is cultivated on almost ¼ of the area. *Pyrenohora teres*, the causal agent of barley net blotch, is present in almost 90% of the Finnish barley fields causing in average11% yield reduction when no plant protection is used. Disease resistance based on plant genes is an environmentally friendly way to manage plant diseases. New sources of resistance are sought from genetic resources of plants, especially from landraces and wild species. In Finland, disease resistance breeding programme is based on public-private collaboration between plant breeders and researchers who have developed a pre-breeding tool box by combining the expertise from the research areas of plant genetic resources, plant genomics, plant protection and economics. The tool box carries six working packages: i) characterization of the pathogen, ii) screening for disease resistance sources, iii) characterization of the resistance, iv) development of genetic markers, v) backcrossing programme, vi) pyramiding the resistance genes including the studies on yield penalty caused by the resistance genes. The pre-breeding programme is discussed with the results achieved from the research on *P. teres* pathogen and its interaction with barley.

**Mapping and allelic tests depicting recombinants on 6H locus conferring resistance to *Pyrenophora teres* f. *teres* isolates from Australia**

S. Gupta[ab], C. D. Li[b], M. Cakir[a] and R. Loughman[b]

*[a] State Agricultural Biotechnology Centre, School of Biological Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia; and [b] Department of Agriculture and Food, 3 Baron Hay Court, South Perth, Western Australia 6151, Australia*

S.Gupta@murdoch.edu.au

Net type net blotch (NTNB) is a prominent barley disease in Australia and elsewhere, inducing significant yield reduction. Selecting resistant material against this disease is important against different isolates of the pathogen along with other traits of quality and agronomic value. Based on parental resistance and their differential responses, three doubled haploid populations: WPG8412 x Stirling, WPG8412 x Pompadour and Pompadour x Stirling were phenotyped against two *Pyrenophora teres* f. *teres* isolates 97NB1 and NB73. Bimodal segregation indicated a major gene for resistance was operative in these populations. This major gene was mapped using simple sequence repeat (SSR) markers on chromosome 6H in the centromeric region in all three populations. Ten SSR markers were found to be linked with the resistance gene covering 30 to 40 cM distance of the 6H region. HVM74 and Bmag0173 were found to be the closest markers in these populations. Allelic tests in all possible combinations were conducted on six $F_2$ resistant x resistant (R x R) crosses. These crosses were developed from lines: Pompadour, WPG8412, WA4794 and CI9214 carrying 6H locus imparting resistance to four *P. teres* f. *teres* isolates 97NB1, 95NB100, NB50 and NB81. A small number of susceptible plants were identified in 18 out of 24 allelic tests. No segregation was detected in the remaining six tests. These studies demonstrated that 6H region controlling net type net blotch resistance is a complex region which can provide suite of 6H alleles or linked genes for resistance breeding.

**Towards the Identification of Two Recessive Net Form Net Blotch Resistance Genes, rpt.r and rpt.k, in Barley**

Robert S. Brueggeman[1], Thomas J. Gross[1], Zhaohui Liu [1], Timothy L. Friesen[1, 2]

*[1] Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA; [2] USDA-ARS, Cereal Crops Research Unit, Red River Valley Agricultural Research Center, Northern Crop Science Lab, Fargo, ND 58105, USA*

Robert.Brueggeman@ndsu.edu

Previous research identified two recessive net form net blotch (NFNB) resistance genes, *rpt.r* and *rpt.k*, from the barley lines Rika and Kombar, respectively. The two genes were co-localized to an ~25 cM region of barley chromosome 6H in a Rika x Kombar double haploid population consisting of 118 individuals. Here we report on the generation of a Rika x Kombar high-resolution mapping population consisting of 2,976 recombinant F2 gametes. Recombinants within the *rpt.r*/*rpt.k* region were identified using the flanking SSR markers Bmag0173 and Rbah21g15. Utilizing genome synteny between barley Ch. 6H and *Brachypodium* Ch.3 an ~ 1mb *Brachypodium* sequence was identified spanning the *rpt.r*/*rpt.k* region. Predicted *Brachypodium* genes were used to identify homologous barley ESTs. The EST sequences were utilized to develop PCR based markers specific to the Rika x Kombar population allowing for the marker saturation of the *rpt.r*/*rpt.k* region. Currently we have delimited *rpt.r* and *rpt.k* to an ~0.5 cM region representing ~150 kbp of *Brachypodium* sequence. BAC clones have been identified from the cv. Morex BAC library using barley probes from the *rpt.r*/*rpt.k* region and a barley physical map across the locus is under construction. The delimited *Brachypodium* sequence contains two LRR-receptor-like gene families. Two orthologous barley genes with high homology to one of the *Brachypodium* LRR-receptor-like gene families were identified and shown to cosegregate with *rpt.r* and *rpt.k* in the high-resolution map. The candidate genes are being evaluated by allele analysis and BSMV-VIGS mediated gene silencing followed by infection type assays with NFNB isolates.

**The CC-NB-LRR *Rdg2a* Resistance Gene Confers Immunity to the Seed-Borne Barley Leaf Stripe Pathogen in the Absence of Hypersensitive Cell Death**

A. M. Stanca[a], D. Bulgarelli[a,b], C. Biselli[a], N. C. Collins[c], G. Consonni[d], P. Schulze-Lefert[b], L. Cattivelli[a] and G. Valè[a]

*[a]Genomic Research Center, CRA-GPG, Via S. Protaso 302, I-29017 Fiorenzuola d'Arda, (PC) Italy; [b]Department of Plant Microbe Interactions, Max Planck Institute für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829 Köln, Germany; [c]Australian Centre for Plant Functional Genomics, School of Agriculture Food and Wine, University of Adelaide, Glen Osmond SA 5064, Australia; and [d]DiPROVE, University of Milan, Via Celoria 2, 20133, Milano, Italy*

michele@stanca.it

Leaf stripe, caused by the seed-transmitted fungus *Pyrenophora graminea*, is a common disease in barley districts with a cold sowing season. In susceptible cultivars, the disease causes brown stripes on the leaves, stunted growth and severe yield reductions. Both polygenic partial resistance and race-specific resistance genes have been identified. *Rdg1a* confers complete resistance to a subset of *P. graminea* isolates and was mapped on barley chromosome 2L. *Rdg2a*, conferring complete resistance to the most virulent Italian isolate Dg2, has been mapped on barley chromosome 1. *Rdg2a* containing breeding lines appear to be resistant to the natural field populations of the pathogen, suggesting that *Rdg2a* may have a useful range of activity. Consequently, molecular markers have been developed to facilitate *Rdg2a* selection in breeding populations. Analyses of the changes in gene expression during the resistance response to leaf stripe suggested that this gene activates cell wall reinforcement, generation of ROS, cell protection, jasmonate signalling, and expression of plant effector genes. The positional cloning and functional characterization of *Rdg2a* revealed the presence of three Coiled-Coil, Nucleotide-Binding site, and Leucine-Rich Repeat (CC-NB-LRR) encoding genes at the *Rdg2a* locus. One member of this gene family could confer the same resistance specificity as *Rdg2a* in transgenic plants, demonstrating that this CC-NB-LRR gene is *Rdg2a*. Inducible responses giving rise to physical and chemical barriers to infection in the cell walls and intercellular spaces of the barley embryo tissues represent mechanisms by which the *Rdg2a* gene mediates resistance to leaf stripe in the absence of hypersensitive cell death.

**Detection of resistance to the net form of net blotch in Australian barley breeding lines**

W. R. Lawson[a], G. Platz[a], A. Killian[b], E. S. Mace[a], D. R. Jordan[ac], S. Fletcher[d], S. Neate[d], and J. Franckowiak[a]

*[a]Agri-Science Queensland, Department of Employment, Economic Development and Innovation (DEEDI), Hermitage Research Facility, Warwick, QLD 4370, Australia; [b]Diversity Arrays Technology (DArT) Pty Ltd, 1 Wilf Crane Crescent, Yarralumla, Canberra, ACT 2600, Australia; [c]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Hermitage Research Facility, Warwick, QLD 4370, Australia; [d]Agri-Science Queensland, DEEDI, Leslie Research Facility, Toowoomba, QLD 4350, Australia*

jerome.franckowiak@deedi.qld.gov.au

Durable resistance to the net form of net blotch (NNB, *Pyrenophora teres* f. *teres*) in barley, *Hordeum vulgare*, is a common breeding goal, but it is difficult to achieve due to pathogen evolution. Potentially new sources of adult-plant and seedling resistance were identified in elite breeding material, 369 Australian cultivars and lines, using reactions to isolate NNB330 and association mapping based on whole genome profiling with 1450 DArT molecular markers. Over thirty chromosomal regions were found to be associated with response to NNB. Several resistance factors were located in the *Rpt5* region of chromosome 6H, yet the specific DArT haplotypes in the region were associated with lines originating from several different barley improvement programs. To further study the inheritance of resistance, a doubled haploid (DH) population of 334 lines from the cross ND24260/Flagship was evaluated with isolate NNB50 and DArTs. Although both parents were moderately resistant, the seedling and adult-plant reactions of the DH lines exhibited transgressive segregation. Six QTL were identified on chromosomes 2H, 4H, 5H and 6H with the one on 4H having the strongest effect ($R^2$ = 30 to 55). Since the 4H QTL is closely linked to the blue aleurone gene *Blx1*, its origin could be traced through North Dakota two-rowed barleys to the Canadian six-rowed cultivar Bonanza. Complex interactions among the QTL were noted, therefore both seedling and adult-plant reaction data were needed to identify highly resistant DH lines. Characterization of NNB reaction of lines based solely on DArT haplotypes was ineffective.

**Mapping resistances to multiple leaf spot diseases in an Australian/American derived doubled haploid population of barley**

G. J. Platz[a], D.E. Mather[b], W. Lawson[a], K.J Chalmers[b], J, Franckowiak[a], and R. Graham[c]

*[a]Agri-Science Queensland, Department of Employment, Economic Development and Innovation, Hermitage Research Facility, 604 Yangan Road, Warwick. Queensland 4370. [b]School of Agriculture, Food & Wine, Faculty of Sciences,The University of Adelaide, Plant Genomics Centre, Waite Campus, PMB 1, Glen Osmond SA 5064, Australia. [c]Yanco Agricultural Institute, Industry & Investment NSW, Trunk Road 80, Yanco NSW 2703.*

greg.platz@deedi.qld.gov.au

Resistance to the leaf spot diseases net form net blotch (NFNB, *Pyrenophora teres* f. *teres*), spot form net blotch (SFNB, *P. teres* f. *maculata*), spot blotch (SB, *Cochliobolus sativus*) and scald (*Rhynchosporium secalis*) are important traits in the development of new barley varieties for Australia. Introductions from North Dakota often carry good resistance to NFNB and SB but are deficient for resistance to SFNB and scald. Resistance to SFNB and scald can be found in some Australian varieties. By selecting parents with complementary resistances, progeny with resistance to all four diseases may be produced.

Application of this strategy lead to the production of a doubled haploid (DH) population from a cross between the North Dakotan line ND24260 and the Australian variety Flagship. ND24260 is resistant to NFNB and SB while Flagship has resistance to NFNB, SFNB and scald. Three hundred and thirty-four DH lines were phenotyped for reactions to each of the diseases as seedlings under controlled environments and/or adults in the field. A QTL analysis was performed using phenotypic data and 627 DArT molecular markers. Significant QTL for resistance were detected on chromosomes 1H, 2H, 7H (scald), 3H, 7H (SB), 2H, 4H, 5H, 6H (NFNB) and 4H, 7H (SFNB). Based on phenotypic data and marker haplotypes, DH lines with resistance genes to the four pathogens from both parents in various combinations were identified. These lines will be used as elite parents to continue the improvement of genetic resistance to these diseases and possibly as new varieties.

**Multiple Disease Resistance QTL Analysis of Two Wild × Cultivated Barley Populations**

B. P. Alsop[a], J. K. Roy[a], and B. J. Steffenson[a]

[a]*Department of Plant Pathology, University of Minnesota, St. Paul, MN USA*

bsteffen@umn.edu

Many disease resistance genes have been characterized and successfully deployed from within barley (*Hordeum vulgare* subsp. *vulgare*) germplasm. However, the lack of genetic diversity in cultivated barley is a limiting factor for discovery of novel sources of disease resistance, and therefore searching for resistance in other germplasm is necessary. To identify resistance loci for the major diseases of net blotch (NB), spot blotch (SB), and Septoria speckled leaf blotch (SSLB) in wild barley (*H. v.* subsp. *spontaneum*), we examined two wild × cultivated barley populations (Damon × Harrington and Shechem × Harrington). We identified 3 resistance QTL for each of the three diseases in the Damon × Harrington population and 3, 3, and 4 resistance QTL for NB, SB, and SSLB, respectively, in the Shechem × Harrington population. We compared the location of these QTL with previously identified disease resistance loci in wild and cultivated barley, and reported marker trait associations for marker-assisted selection. Many QTL mapped to regions of the genome containing major genes for resistance, such as *Rcs5*, *Rsp1*, and *Rsp2*/*Rsp3*. Also of potentially high value are several QTL (on chromosomes 4H, 6H, and 7H) for resistance to SSLB that have not been previously identified. The QTL identified in this study are good targets for marker-assisted introgression of novel disease resistance traits into cultivated barley.

**Detecting and mapping loci for disease resistance in UK elite barley cultivars**

W. Thomas[1], J. Comadran[1], L. Ramsay[1], P. Shaw[1] D. Marshall[1], A. Newton[1], D. O'Sullivan[2], J. Cockram[2], I. Mackay[2], R. Bayles[2], J. White[2], R. Waugh[1] and the AGOUEB Consortium

[1]*The James Hutton Institute, Invergowrie, Dundee, DD2 5DA UK;* [2]*NIAB, Huntingdon Road, Cambridge, CB3 0LE UK*

william.thomas@hutton.ac.uk

We have utilised the high throughput SNP genotyping platform Barley Oligo Pooled Array 1 (BOPA1) to genotype 1000 elite barley lines with 1536 genic markers to characterise the variation that exists amongst UK elite barley varieties and associate sequence variants with differences in performance and morphological characters. Over 500 of these lines had been evaluated in spring and winter barley National and Recommended List trials between 1988 and 2006 and thus an extensive body of performance data (yield, height, disease resistance, quality etc.) and morphological data used in assessing Distinctness, Uniformity and Stability (DUS) already existed for these lines. Additionally we grew a subset of lines representing market successes and failures over our survey period to both provide an unambiguous estimate of breeding progress and additional data, including disease resistance, to improve the prediction of means of varieties that generally were not grown in the same trials. We have combined the genotypic and phenotypic data in Genome Wide Association Scans to find that resistance to the main UK foliar pathogens of barley is controlled not only by the major genes identified in the UK Cereal Pathogen Virulence Survey but also at other loci. The latter have frequently been identified in QTL studies of non-UK elite material and indicate the widespread interchange of germplasm amongst breeders in their search to improve disease resistance.

**The detection of minor genes for resistance to *Rhynchosporium secalis* using seedling assays**

H. Wallwork, M. Grcic

*South Australian Research and Development Institute, Urrbrae, South Australia 5064, Australia*

hugh.wallwork@sa.gov.au

Specific isolates of *Rhynchosporium secalis* have been identified which enable minor genes for resistance to scald to be detected in barley seedlings. These minor genes go undetected in most seedling assays and have therefore been thought of as genes for adult plant resistance. The isolates lack virulence on the known major genes but produce typical scald lesions without delay on susceptible check varieties so do not appear to have reduced pathogenicity or aggressiveness in the absence of minor resistance genes. The isolates are being used to screen barley breeding lines for field resistance where major genes are absent and to identify chromosome locations (QTL) of minor genes in mapping populations. These isolates form part of a large collection made in Australia in recent years as part of a survey of the *R. secalis* population aimed to identify new sources of resistance in barley, to determine which resistances are likely to be more durable to a diverse pathogen population and to identify stable and prolific isolates for use in screening barley breeders germplasm.

**Resistance to *Rhynchosporium secalis* in a cross between winter and spring barley**

M. E. Looseley[a], A. C. Newton[a], S. D. Atkins[b], B. D. L. Fitt[b], B. A. Fraaije[b], W. T. B. Thomas[a], R. Keith[a], M. Macaulay[a], J. Lynott[a], [c]D. Harrap.

*[a]James Hutton Institute, Invergowrie, Dundee, DD2 5DA UK; [b]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ UK; [c]KWS UK Ltd, 56 Church Street, Thriplow, Nr. Royston, Hertfordshire, SG8 7RE UK*

mark.looseley@hutton.ac.uk

Winter barley types shows higher levels of field resistance to *R. secalis* compared to spring barley varieties. In addition, barley leaves have been shown to tolerate relatively high levels of *R. secalis* colonisation in the absence of visible symptoms, suggesting that suppression of symptom expression may be an alternative mechanism of resistance. This study examined resistance in 191 DH lines from a cross between spring variety Cocktail, and a winter parent derived from a cross between Leonie and Pearl. Field resistance was scored by measuring visible symptoms and also by R. secalis specific qPCR of sampled leaves. Two novel resistance QTL (on chromosomes 2H and 7H) were identified, which affected both pathogen colonisation and visible symptoms. These were not associated with genes controlling winter/spring growth habit. A further QTL (on chromosome 5H) was identified as affecting relative expression of disease symptoms.

# Poster Abstracts

A-1

**Virulence spectrum in isolates of *Cochliobolus sativus* in Uruguay**

F. Gamba, C. Pritsch[a] and M. Ziminov[b]

[a]*Departamento de Biología Vegetal, Facultad de Agronomía, Universidad de la República Oriental del Uruguay, Avda. Garzón 680, Montevideo, Uruguay;* [b]*Departamento de Protección Vegetal, Facultad de Agronomía, Universidad de la República Oriental del Uruguay, Ruta 3 k 363, Paysandú, Uruguay*

fgamba@fagro.edu.uy

Spot blotch induced by *Cochliobolus sativus*, is one of the most devastating leaf diseases in Uruguay due to yield and quality losses. The use of cultivars with genetic resistance is the basis of an integrated disease management however; its effectiveness may be affected by changes in virulence of the pathogen population. The virulence spectrum of 32 monosporic isolates of *C. sativus* was evaluated on 17 barleys genotypes under controlled temperature and photoperiod conditions. Eight isolates were identified with virulent reactions, but they were different considering the other two reaction types. The maximum avirulence level was detected in two isolates that induced 76% of the reactions with this trait. Resistance reactions varied from 39,3 % to 0% with a very wide continuous range of different reactions. Studies with more extensive pathogen and barley samples would contribute to develop more focused breeding strategies, to obtain cultivars with more effective and durable resistance.

A-2

**Virulence profiles of *Pyrenophora teres* f. sp. *teres* in Uruguay**

F. Gamba and M. Ziminov[a]

[a]*Departamento de Protección Vegetal, Facultad de Agronomía, Universidad de la República Oriental del Uruguay, Ruta 3 k 363, Paysandú, Uruguay*

fgamba@fagro.edu.uy

Net blotch, induced by *Pyrenophora teres* f.sp. *teres*, is an important disease of barley. The use of resistance is the best means to manage diseases in an integrated management approach. In this study 33 isolates were inoculated in 18 barley genotypes at seedling stage under controlled temperature and photoperiod conditions. Fifteen isolates induced maximum virulence levels on all barley genotypes. It was not found a single isolate completely avirulent and all barley genotypes were susceptible to these isolates, in varying degrees. It was not possible to identify different virulence groups nor barley genotypes with differential resistance. The high variability found in these *P. teres* f. sp. *teres* isolates and barley genotypes, suggests that more complete studies will improve the understanding on virulence spectrum and therefore will contribute to improve barley germplasm with more valuable resistance.

A-3

**Physiological and morphological characterization of *Pyrenophora teres* f. sp. *Teres***

F. Gamba, A. Yahyaoui[a] and F. Capettini[b]

[a]*Departamento de Protección Vegetal, Facultad de Agronomía, Universidad de la República Oriental del Uruguay, Ruta 3 k 363, Paysandú, Uruguay;* [b] *International Center for Agricultural Research in the Dry Areas(ICARDA), Aleppo, Syria, Box 5466*

fgamba@fagro.edu.uy

Net blotch of barley, induced by *Pyrenophora teres* f.sp. *teres*, is an important disease of barley worldwide. The study was conducted to describe and compare morphology and virulence profiles in a collection of 36 single spore isolates originated on different sites in major barley growing areas of Syria, Eritrea, and Morocco. Shape, color and size of each of the colonies were registered. Different virulence profiles were determined upon inoculations at seedling stage under controlled conditions. An international differential barley set was used. Virulence groups were established based on phenotypic characterization of the isolates. More extensive isolate evaluations will allow establishing the association between virulence groups, distribution and geographical origin. This knowledge will improve the collaborative exchange of more effective and lasting resistant germplasm in the region and could be eventually expanded to other countries in Central, West Asia, and North Africa (CWANA).

A-4

**Leaf spot diseases of barley in Hungary during 2006-2010**

Klára Manninger[1], J. Fodor[1], I. Murányi[2]

[1] *Plant Protection Institute Hungarian Academy of Sciences, Budapest;* [2]*Károly Róbert University, Rudolf Fleischmann Research Institute, Kompolt*

sman@nki.hu

Barley leaf spot diseases were surveyed in Hungary during 2006-2010. The weather conditions were particularly favourable for development of leaf spot diseases in 2006. *Pyrenophora species* were the dominant fungal pathogens. In 2007, no necrotic spots were found on the upper leaves of winter barley in experimental plots until flowering. Interestingly, unusual small necrotic spots were detected on the upper leaves of winter barley after flowering in West-Hungary (Röjtökmuzsaj). The pathogen was identified as *Ramularia collo-cygni* by microscopic observation of typical conidiophores and conidia. That was the first record of Ramularia leaf spot in Hungary. Further spread of *R. collo-cygni* was confirmed in 2008. Both winter and spring barley leaves were heavily infected with *R. collo-cygni* in West-Hungary, however, sporadic cases were found in East-Hungary. As compared to 2008, the same barley genotypes were investigated in 2009 and 2010 in East- and West-Hungary. The severity of leaf spot diseases was consistently lower in 2009 due to drought, but incidences varied by environment, region and cultivar. In 2010, leaf rust, brown leaf spot, Ramularia leaf spot were found in West-Hungary, while net blotch, barley leaf stripe, brown leaf spot, Ramularia leaf spot appeared in East-Hungary. In general, *Puccinia hordei, Bipolaris sorokiniana* and *Pyrenophora species* were the most important pathogens in this year. In 2009-2010 *Ramularia leaf spot* disease was of small importance. All examined genotypes were susceptible to both net blotch and barley leaf stripe in East-Hungary. Furthermore, most genotypes were infected *by Puccinia hordei, Bipolaris sorokiniana, R. collo-cygni* in West-Hungary.

A-5

**Managing spot-type net blotch through cultivar resistance and fungicides in Uruguay**

S. A. Pereyra[a] and M. Castro [a]

[a] *INIA Instituto Nacional de Investigación Agropecuaria, La Estanzuela, Colonia, CP 70000, Uruguay*

spereyra@inia.org.uy

Spot-type net blotch (STNB) caused by *Pyrenophora teres* f. sp. *maculata* Smed.Pet., has become a predominant disease of barley in Uruguay since it was first reported in 2004. Major factors that contributed to this had been no-till and cultivar susceptibility. In order to optimise disease control measures, cultivar resistance and fungicides were investigated under Uruguayan conditions. Commercial cultivars and advanced lines were characterised under intermediate to high disease pressure in nurseries and field trials during 2008 to 2010. Few commonly grown cultivars had high levels of resistance. Cultivars INIA Arrayán and INIA Ceibo that comprised 40 to 50 % of the barley area in 2009 and 2010 had intermediate to low susceptibility and represented the best commercial cultivars for this trait. Optimum timing for fungicide application for STNB control in a susceptible cultivar with large amount of infected residue on the soil surface was at stem elongation (Zadoks GS 31 to 39) when disease thresholds of 5-8% were attained. Pyraclostrobin + epoxiconazole, trifloxystrobin + tebuconazole, azoxystrobin + tebuconazole and kresoxim-methyl + epoxiconazole were the most effective fungicides in controlling STNB in a single application, improving yield and grain quality. These results suggest that it may be possible to manage STNB by cultivar resistance and timely fungicide applications.

A-6

**Use of arabinoxylan polymers for plant defence**

Jaan Rätsep[a], Dr Neil Havis[a], Dr Gary Loake[b], Prof. Dale Walters[a]

[a] *Crop & Soil Systems, Scottish Agricultural College, Edinburgh EH9 3JG;* [b] *Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh EH9 3JH*

Jaan.Ratsep@sac.ac.uk

Successful control of plant diseases is a key priority in agricultural research. Effective control, however, might require multiple applications of toxic substances, which often become ineffective due to development of resistance by pathogens. On the other hand, every plant possesses the ability to defend itself, and employing the plant's own defence mechanisms in combating the infection is possible. A range of biotic and abiotic factors, including certain substances, is known to elicit plant resistance to disease.

The current project focuses on use of plant-derived arabinoxylan polymers to control foliar pathogens of barley (*Hordeum vulgare*). The emphasis in this project is on one of the major fungal pathogens responsible for substantial yield losses, the barley scald (leaf blotch) caused by *Rhynchosporium secalis*. The work is being carried out to examine the potential of polymers as elicitors that might induce local or systemic resistance, as well as for disguising the leaf surface, thus disrupting spore adhesion and germ tube growth and creating a physical barrier to penetration.

Initial, preliminary research demonstrated a significant influence of the arabinoxylan polymer on disease severity. Confirmation and further characterisation of the resistance-inducing properties of the polymer would be of great benefit to agriculture, minimising environmental impact by using non-toxic substances to induce the plants' own defences, and providing a cost-effective measure, due to production of the polymer from a readily available and renewable source.

**Evaluating the *Pyrenophora teres* international standard barley differential set with Canadian isolates of the pathogen**

A. Tekauz[a], M. Desjardins[b] and F. Kleiber[c]

*[a] Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg MB, R3T 2M9; [b] Crop Diagnostic Centre, Manitoba Agriculture, Food and Rural Initiatives, Winnipeg MB, R3T 5S6; [c] Ecole Nationale d'Ingénieurs des Travaux Agricoles de Bordeaux, 33175 Gradignan, CEDEX, France*

andy.tekauz@agr.gc.ca

An 'international' standard set of 18 barley differential lines (IBDS) to assess pathogenic variability in *Pyrenophora teres* f. *teres* (*Ptt*), causal agent of 'netted net blotch' (NNB), has been published for research use. The IBDS was developed to evaluate and compare virulence in the pathogen globally, and identify sources of resistance relevant to local breeding programs. The IBDS was used in an exploratory study to evaluate the virulence of 15 Canadian isolates of *P. teres* obtained from affected barley crops in 2009. Conidial suspensions of both *Ptt* and *P. teres* f. *maculata* (*Ptm*), causal agent of 'spotted net blotch' (SNB), were used to inoculate IBDS seedlings. These were rated for infection phenotype on a 1-10 scale; values 1 to 5 and >5 to 10, represented 'resistant' and 'susceptible' reactions, respectively. The 9 isolates of *Ptt* and 6 of *Ptm* all had unique virulence spectra. Resistance to *Ptt* was present in differentials CI 9819 and CI 5791, and to *Ptm* in 'Manchurian' and CI 9214. Five IBDS lines were susceptible to all *Ptt* isolates. An isolate of *Ptt* from Manitoba had the highest mean infection phenotype score (7.6), and may represent a new level of virulence for the region. While not developed for *Ptm*, the IBDS may also be useful in assessing virulence in this pathogen form.

**C-1**

## Effects of rhynchosporium on leaf physiology beyond the visible lesion

I.J. Bingham and C. Crawford

*Crop and Soil Systems Research, SAC, West Mains Road, Edinburgh, EH9 3JG, UK*

ian.bingham@sac.ac.uk

Predicting the impact of disease epidemics on crop yield requires an understanding of the effects of disease on leaf function and an ability to scale those effects up to the canopy level. It is recognised that pathogens can impair photosynthetic activity in green tissue beyond the visible lesion, but it is not clear whether the location of lesions on the leaf influences the response. Rhynchosporium lesions often form within the leaf axil and it has been suggested that these may inhibit transport of material into and out of the leaf and promote premature leaf senescence. Two experiments were conducted on spring barley plants grown in a controlled environment and inoculated with *R*. *secalis* to test the effects of general and localised disease symptoms on rates of photosynthesis and leaf senescence. Following an overall inoculation of the leaf, photosynthesis was reduced in regions expressing disease symptoms. The ratio of virtual lesion to visible lesion size (Bastiaans L., 1991, Phytopathology 81, 611-615) was 2.7. Inoculation directly into the leaf axil resulted in severe necrosis across the entire base of the leaf. However, rates of photosynthesis and stomatal conductance measured in non-symptom expressing central regions of the leaf were not significantly different from controls. The rate of leaf senescence was also unaffected. The results suggest that rhynchosporium impairs leaf function in green tissue immediately surrounding the visible lesion but not more in distant regions and that lesions in the leaf axil may be no more damaging than those located elsewhere on the leaf.

**C-2**

## Rhynchosporium secalis cell wall proteins

A. E. Mackenzie[a,d], M. Münsterkötter[b], W. Knogge[c], A. C. Newton[a], C. A. Munro[d] and A. Avrova[a]

[a] *The James Hutton Institute, Dundee, UK;* [b] *MIPS - Institute of Bioinformatics and Systems Biology, Munich, Germany:* [c] *Leibniz-Institute of Plant Biochemistry, Halle, Germany;* [d] *University of Aberdeen, Aberdeen, UK*

Ashleigh.Mackenzie@hutton.ac.uk

*Rhynchosporium secalis* is one of the most destructive barley pathogens worldwide. It can cause yield loss of up to 30-40%. *R. secalis* grows symptomlessly under the cuticle before producing new conidia and finally, visual symptoms. It may also complete its infection cycle asymptomatically. *R. secalis* populations can change rapidly leading to new resistance genes and fungicides become ineffective after only a small period of use. Therefore the development of long term, sustainable strategies to manage Rhynchosporium is dependent on an improved understanding of *R. secalis* and its interaction with the barley host.

The cell wall and especially cell wall proteins (CWPs) play a major role in the establishment of pathogenesis in fungi. The cell wall is a structure protecting the fungus from the host defence mechanisms. It is involved in initiating the direct contact with the host cells by adhering to their surface. Fungal cell wall also contains important antigens and other compounds modulating host immune responses.

*R. secalis* germinated conidia and interaction transcriptome annotations combined with genome annotation allowed to generate a list of over 50 different CWPs. This list includes structural CWPs, proteins involved in cell wall organization, adhesion as well as cell wall biosynthesis and modification enzymes. *R. secalis*-barley interaction transcriptome sequencing also provided a subset of genes expressed during barley colonization by *R. secalis*. Transcription profiling of *R. secalis* CWPs during the infection development allowed their prioritisation for functional characterisation. Targeted disruption of individual genes will be followed by pathogenicity tests, complementation and biochemical studies.

**C-3**

## Gene expression in barley – *Pyrenophora teres* interaction

T. Tenhola-Roininen[a], J. Tanskanen[a], T. Tiirikka[a], M. Serenius[ab] and O. Manninen[a]

[a] *Biotechnology and Food Research, MTT Agrifood Research Finland, Myllytie 1, FI-31600 Jokioinen, Finland;* [b] *Current address: Agricultural Foundation of Trade, K-maatalous Experimental Farm, Hahkialantie 57, FI-14700 Hauho, Finland*

o.m.manninen@mtt.fi

Net blotch (caused by *Pyrenophora teres*) is a worldwide barley disease causing lower yields and decreased grain weight. Several resources for resistance have been recognized, one of them being the Ethiopian landrace CI 9819, which carries a major resistance gene *Rpt5* against the net type of net blotch on chromosome 6H. We have earlier developed the resistant doubled haploid line C40 from the cross between the Finnish susceptible variety Rolfi and CI9819. We have collected leaf samples of barley to study gene expression levels of infected vs. uninfected plants of resistant and susceptible host in a time series from 0 to 72 h after infection. Early gene expression in barley – *P. teres* (net and spot types) interaction was studied in four time points: 0, 6, 12, 24 h after inoculation, on the Agilent barley microarray 4*44 K with three differentially labeled samples. Barley lines, Rolfi and C40, were inoculated with either net type *P. teres* isolate p549, spot type isolates p1332 or p51, or with Mock (water) as a control. Gene expressions between resistant (C40) and susceptible (Rolfi) lines to net type isolate and between different spot types within C40 (resistant to p1332, susceptible to p51) were analyzed and compared. Results will be shown in the poster.

**C-4**

## Pathotype Diversity and Distribution of *Pyrenophora teres* f. *teres* in Australia

R. A. Fowler and G. J. Platz

*Agri-Science Queensland, Department of Employment, Economic Development and Innovation, Hermitage Research Facility, Warwick, QLD 4370, Australia*

ryan.fowler@deedi.qld.gov.au

*Pyrenophora teres* f. *teres* (PTT) which is the causal agent of the foliar disease net form of net blotch (NFNB) is a serious pathogen in all barley growing regions of Australia. It is currently estimated to cause yield losses of $19million annually and has the potential to cost the industry $117million if no control measures are practiced. Internationally, NFNB is of high importance with many countries investing heavily in resistance breeding and pathology research.
The most recent national survey was conducted in 1999 since then varieties have changed and new virulences have occurred. In 2010, forty-nine isolates were pathotyped on a differential set relevant to the Australian population and contained 31 lines, 23 of which were used in the 1999 survey with the addition of 8 recently released cultivars.
Analysis was conducted with HaGiS: Spreadsheet for Automatic Habgood-Gilmour Calculation V.3.1 using a selection of 18 lines which gave clear differentiation. 26 pathotypes were identified, with 9 of these containing more than one isolate. The largest group (Skiff virulent - NFNB50) contained 10 isolates and originated from Queensland, New South Wales, Victoria and South Australia. The second largest group (Prior virulent, Covrette avirulent) contained six isolates and originated from South Australia and Western Australia.

This survey is currently 50% complete, with the remainder of accessions to be pathotyped in 2011. This study will ultimately benefit the breeding companies as it will identify isolates with the virulences most suited to be used in disease screening nurseries used to assess advanced germplasm.

C-5

**Both mating types are present in *Rhynchosporium commune*, *R. agropyri* and *R. secalis* populations**

K. M. King[1, 2], J. S. West[1], T. S. Stefansson[3], B. A. McDonald[3], W. Knogge[4], P. S. Dyer[2], B. D. L. Fitt[5]

*[1]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK; [2]School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, UK [3]Institute of Integrative Biology, ETH-Zurich, LFW, Universitätsstr. 2, 8092, Zürich, Switzerland, [4]Department of Stress and Developmental Biology, Leibniz-Institute of Plant Biochemistry, Weinberg 3, D-06120, Halle, Germany, [5]School of Life Sciences, University of Hertfordshire, Hatfield, Herts, AL10 9AB*

kevin.king@rothamsted.ac.uk

*Rhynchosporium commune* is the causative agent of leaf-blotch of barley. In recent years, several lines of evidence have suggested that *R. commune* may possess a cryptic sexual cycle, e.g. presence of mating-type (*MAT*) loci consistent with a heterothallic mating system and the equal distribution of isolates of *MAT1-1* and *MAT1-2* genotypes in field populations. It is now demonstrated that populations of two related species (*R. agropyri* and *R. secalis*, causing disease on couch-grass and rye, respectively) are also composed of isolates that produce either a *MAT1-1* or a *MAT1-2* amplicon in PCR diagnostic tests, and that isolates of both genotypes are present in natural populations of each species. These findings suggest that *MAT* gene organization appears to be conserved within these three *Rhynchosporium* species, and it is possible that crossing of isolates of *R. agropyri* and *R. secalis* of complementary mating type could successfully induce a sexual cycle within the respective species.

C-6

**Distribution of *Ptr ToxA* gene in Central European *Pyrenophora teres* isolates genome**

L. Leisova-Svobodova[a] and V. Minarikova[b]

*[a] Department of molecular biology, Crop Research Institute, Drnovska 507, Praha 6-Ruzyne, Czech Republic; [b]Agricultural Research Institute, Havlickova 2787, 767 07 Kromeriz, Czech Republic*

leisova@vurv.cz

Ptr ToxA is proteinaceous, necrosis-inducing toxin identified and cloned from the wheat pathogen *Pyrenophora tritici-repentis*. Toxin A acts as a pathogenic factor and was transferred from another wheat pathogen, *Stagonospora nodorum*. Recently, *Ptr ToxA* gene was detected in isolates of *Pyrenophora teres* being predominantly a barley pathogen. A study on the distribution of *Ptr ToxA* gene in *P. teres* isolates genome within its Central European population showed a proportional representation of the both *P. teres* forms (*P. teres* f. *teres* and *P. teres* f. *maculata*) within *Ptr ToxA+* isolates. Higher occurrence of *Ptr ToxA* gene was detected in genome of isolates collected in last ten years from warmer part of the region. As higher occurrence of *P. teres* in wheat is supposed, its distribution in wheat fields is now studied.

**Genetic diversity of *Pyrenophora teres f. teres* populations on wheat in the North-West of the Russian Federation**

N.V.Mironenko, L.A.Mikhailova, I.G.Ternuk, N.M.Kovalenko

*All Russian Institute for Plant Protection, Saint-Petersburg-Pushkin, Russia*

nina2601mir@mail.ru

In 2007 *Pyrenophora teres*, the causal agent of barley net blotch, was detected on wheat in the North-West of Russia. Fungal populations of the common geographical origin were isolated from spring and winter wheat, and from barley. All fungal isolates were identified as *P. teres f. teres* using PCR primers specific to spot and net forms (Williams et al. 2001). Genetic differences were found between *P. teres f. teres* populations isolated from winter and spring wheat ($F_{st}$ = 0,41), winter wheat and spring barley ($F_{st}$ = 0,21), and spring wheat and spring barley ($F_{st}$ = 0,18) using RAPDs,. On wheat leaves *P. teres* isolates induced necrotic spots without chlorosis that are similar to the symptoms caused by *P. tritici-repentis* (races 2 and 4). They were compared with *P. tritici-repentis* isolates for virulence on 14 wheat samples. Indexes of virulence of *P. teres* isolates showed higher variation, than those of *P. tritici-repentis* isolates. *P. teres* isolates exceeded *P. tritici-repentis* isolates in infection type and frequency of occurrence on some wheat samples. In our opinion these results testify that *P. teres* is in process of genetic and physiological specialization to colonize wheat.

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**D-1**

**Mapping of major net-type net-blotch resistance loci in Ethiopean barley line c-23874 and the Russian barley cultivar Zernogradskij 813**

O. Afanasenko[1], E. Potokina[1], A. Kozyakov[1], A., O. Manninen[2], M. Jalli[2], P. Hedley[3]

*[1] All-Russian Research Institute of Plant Protection, Saint Petersburg, Pushkin 196608 sh. Podbelsky, 3, Russia; [2]MTT Agrifood Research Finland, Plant Protection, FI-31600 Jokioinen, Finland; [3]The James Hutton Institute, Dundee, DD2 5DA*

olga.afanasenko@gmail.com

Two newly barley doubled-haploid (DH) populations to identify quantitative trait loci (QTLs) associated with seedling resistance to Russian and Finnish isolates of *Pyrenophora teres* f. *teres* were developed. The first population (A) combining 44 DHLs was derived from the cross of susceptible cultivar Pirkka and resistant Ethiopian line (c-23874). The second population "B" including 114 DHLs was derived from a cross between susceptible cv. Rannij 1 and resistant cv. Zernogradskij 813. The 384 SNP set employed for genotyping of the both DH populations was selected at the JHI and covered all seven barley chromosomes. All 384 SNP markers provided good quality genotype calls for the DHLs, 108 and 164 SNP markers showed polymorphism in population «A» and «B» respectively and were used for mapping. In greenhouse test in all four replications of inoculation of "A" population by Finnish isolate the highest LOD score (2,9) was associated with the SNP marker 11_11067 located on chromosome 6H (58 cM). Major resistance genes effective against *P. teres* f. *teres* have previously been identified on chromosome 6H by at least 3 other groups using different sources of resistance. In "B" population an identical marker interval associated with resistance to pathogen (detached leaf technique) was identified for two Russian isolates on short arm of chromosome 5H explaining 8-12% of the total variation.

**D-2**

**Mapping of quantitative trait loci for net blotch resistance in Tunisian barley**

M. Cherif[a], S. Rezgui[a], M. Von Korff[b], M. Baum[b], M.Harrabi[a] and A. Yahyahoui[b]

*[a] Department of crop science, Institut National Agronomique de Tunisie, 43, Av. Charles Nicolle, 1082 Cité Mahragène, Tunis, Tunisia; [b] Biotechnology laboratory. International Center for Agricultural Research in the Dry Areas. Box 5466, Aleppo, Syria*

cherif_majda@yahoo.fr

Net blotch, caused by *Pyrenophora teres*, is a prevalent foliar disease of barley (*Hordeum vulgare* L.) in Tunisia. The identification of QTLs conferring resistance from local germplasm is not studied yet. Fifty nine doubled haploid barley population derived from a cross between the Tunisian cultivar 'Roho' and the local line '90' was used for mapping potential QTLs associated with net blotch resistance at seedling and adult plant growth stage using simple sequence repeat markers. The population was evaluated with three isolates at seedling growth stage and one at adult growth stage. At seedling growth stage, three to six QTLs were identified depending on the isolate used and the form of *P. teres*. For the two isolates of *P. teres* f maculata, comparable QTLs were mapped on chromosomes 3H, 4H and 7H. These QTLs explained 5 to 20% of the phenotypic disease variation. For *P. teres* f *teres*, others QTLs located on chromosomes 4H and 6H, explaining 10 to 31% of the phenotypic variation, were mapped. At adult growth stage, three QTLs located on chromosomes 3H and 6H contributed 4 to 17% of the disease severity were mapped. This investigation indicated that two QTLs conferred resistance to *P. teres* f *teres* are expressed at both seedling and adult plant growth stage. These QTLs are located on chromosome 6H and are linked to Bmac0500 for the former QTL and to HVM31 as well as EBmac0806 for the latter QTL. Further investigations are needed to use this tool in marker assistant selection for field resistance to net blotch.

**D-3**

**Spot blotch QTLs in barley germplasm from Latin America**

[1]Lucía Gutiérrez, [1]Natalia Berberian, [2]Flavio Capettini, [3]Darío Fros, [4]Silvia Germán, [4]Silvia Pereyra, [5]Carlos Pérez, [6]Sergio Sandoval-Islas, [3]Ariel Castro

*[1]Departamento de Biometría, Estadística y Computación, Facultad de Agronomía, UDELAR, Uruguay; [2]ICARDA, Syria; [3]Departamento de Producción Vegetal, Facultad de Agronomía, UDELAR, Uruguay; [4]Instituto Nacional de Investigación Agropecuaria, Uruguay; [5]Departamento de Protección Vegetal, Facultad de Agronomía, UDELAR, Uruguay; [6]COLPOS, Chapingo, Mexico*

spereyra@inia.org.uy

Spot blotch (caused by *Cochliobolus sativus*) is one of the most important diseases that attack barley in Latin America. Chemical control is both economically and environmentally inappropriate, rendering the development of durable resistant varieties a priority for breeding programs. However, the availability of new resistance sources is a limiting factor. In order to identify genomic regions associated with quantitative resistance to this disease we determined the associations between plant disease severity measured in several environments across the Americas and 1536 SNPs (belonging to the Barley OPA1), using a population of 378 genotypes from ICARDA and national breeding programs. We used association mapping with mixed models incorporating structure and kinship matrixes (Q+K). This model considers the structure of the population (Q) and identity by descent through pedigree information (K). Preliminary results show significant marker-trait associations for spot blotch in chromosomes 1H (at 50 cM), 2H (at 39.1 and 129.3 cM), 3H (at 55 and 69.6), 4H (at 23.1 and 69.5 cM), 5H (at 50, 69.5 and 151.4 cM), 6H (at 55.6 cM), and 7H (at 21.1 and 79.6 cM). Some of these associations are close to previously mapped QTL. Better models are being developed to further study these associations.

**D-4**

**Mapping of net blotch resistance locus in barley line c-8755**

T. Tenhola-Roininen[a], M. Jalli[b], M. Erkkilä[a], O. Afanasenko[c] and O. M. Manninen[a]

*[a] MTT Agrifood Research Finland, Biotechnology and Food Research, FI-31600 Jokioinen, Finland; [b] MTT Agrifood Research Finland, Plant Production Research, FI-31600 Jokioinen, Finland; [c] VIZR, All-Russia Institute of Plant Protection, Pushkin St. Petersburgh, 189620 Russia*

o.m.manninen@mtt.fi

Net blotch of barley (*Hordeum vulgare* L.), caused by the fungal phytopathogen *Pyrenophora teres* Drechs. f. *teres* Smedeg., constitutes one of the most serious constraints to barley production world-wide. Several barley lines with major gene resistance to net blotch have been identified. Recently a concise set of barley genotypes for differentiating virulences in *Pyrenophora teres* f. *teres* was formulated (Afanasenko et al. 2009). Here we present results from mapping the resistance genes in one of the barley genotypes included in the differential series, namely c-8755 (Ethiopian landrace).

The doubled haploid progeny between Harrington (susceptible) and c-8755 (resistant) was genotyped using Barley OPA SNP markers. Linkage groups were constructed using JoinMap and QTL analyses were done with NQTL. Barley plants were disease tested by infecting two week old seedlings at greenhouse and scoring the symptoms after ten days according to Tekauz scale. Mean for four seedlings was used as the phenotypic value in QTL mapping.
With the net type isolate V276 a major resistance gene was located on chromosome 3H, explaining up to 38% of the phenotypic variation of infection response in the progeny.

Afanasenko O, Jalli M, Pinnschmidt OH, Filatova O & Platz GJ, 2009. Plant Pathology 58: 665-676.

D-5

**Genetic of resistance of Tunisian Barley to *Rhynchosporium secalis***

W. Fériani[a], M. Cherif[a] ,S. Rezgui[a] and A. Yahyahoui[b]

[a]*Department of crop science, Institut National Agronomique de Tunisie, 43, Av. Charles Nicolle, 1082 Cité Mahragène, Tunis, Tunisia ;* [b]*Biotechnology Laboratory, International Center for Agricultural Research in the Dry Areas. Box 5466, Aleppo, Syria*

wissalferiani@yahoo.fr

Leaf scald (*Rhynchosporium secalis*) of barley (*Hordeum vulgare* L.) is a serious disease in many barley-growing areas. Doubled-haploid progeny from a cross between the two-rowed barley cultivar 'Roho' and the six-rowed line `90' was evaluated for resistance at the seedling stage to three isolates (Bousalem, Krib and Teboursouk) of *R. secalis*. Doubled-haploid frequencies' distributions of reaction to Bousalem and Krib isolates were continuous suggesting a polygenic genetic control. However, the frequency distribution of the doubled-haploid reaction to Teboursouk isolate was bimodal indicating a qualitative genetic control. Analysis of variance showed significant genotype, isolate and genotype x isolate interaction effects. The GLM procedure showed that spike type had no effect on scald resistance. Heritability of the resistance to the three isolates ranged from 40 to 60% and heritability of resistance to the three isolates was about 64%. The reaction to Bousalem, Krib and Teboursouk isolates was positively correlated. Three QTLs localised on chromosomes 3H, 4H and 6H were identified using the individual marker analysis.

D-6

**Barley genotype interaction with three virulent scald (*Rhynchosporium secalis*) pathotypes from western Canada**

J. R. Tucker[a *], T. K. Turkington[b] and W. G. Legge[a]

[a] *Agriculture and Agri-Food Canada, Brandon Research Centre, Brandon, MB R7A 5Y3; and* [b] *Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, AB T4L 1W1, Canada*

James.Tucker@agr.gc.ca

Scald, incited by *Rhychosporium secalis* (Oudem.) J. J. Davis, is a major foliar disease of barley (*Hordeum vulgare* L.) in the cooler, moister regions of western Canada. High pathogenic variability combined with limited rotational intervals between barley crops increases the risk of breakdown of cultivar resistance. Previous molecular genetic investigations demonstrated that resistance in western Canadian barley cultivars is predominantly derived from the *Rrs1* locus (*Rh/Rh3/Rh4* complex) on chromosome 3 (3H) or the *Rrs2* locus (*Rh2*) on chromosome 1 (7H). Single-spore isolations of *R. secalis* were produced from infected leaf tissue of resistant cultivars: CDC Earl (*Rrs1*), Kasota (*Rrs2*) and Seebe (undefined resistance; different from the former two). Over 2007 and 2008, reactions of these virulent pathotypes were evaluated on a diverse set of 57 resistant genotypes, under growth chamber and field conditions at Lacombe, AB. Cultivars CDC Earl, Kasota and Seebe demonstrated susceptible reactions when infected with their corresponding isolate. Significant genotype by pathotype interactions were observed for the tested genotype and pathotype combinations. Kasota exhibited a moderate level of resistance against the CDC Earl pathotype under both field and growth chamber conditions, and although CDC Earl exhibited a resistant reaction against the Kasota pathotype in growth cabinets, it was overcome under field conditions. Seebe exhibited resistant reactions when challenged with both the Kasota and CDC Earl pathotypes, whereas the resistance sources in Kasota and CDC Earl were ineffective against the Seebe pathotype. However, several genotypes of differing parentage showed promising resistance to all pathotypes.

# NOTES