Understanding *Ramularia collo-cygni* in the past, present and future

James Fountaine
**Taxonomy**

*Ramularia collo-cygni*

*Collum* = neck

*Cygnus* = swan

Phyllum: *Hyphomycetes*

Order: *Dothideales*

Family: *Mycosphaerellaceae*

Section: *Mycosphaerella*
The spread of Ramularia Leaf Spot (RLS)

- Other known locations include: New Zealand, Australia, North and South America
GS45-49 Protect crops with fungicide

GS65 Fungus detected inside leaves 2-4 weeks before symptoms

GS0 Ramularia seed-borne

GS0 Ramularia detected by diagnostics but no visual symptoms

GS10-13 Ramularia detectable by diagnostics but no visual symptoms

GS25-30 Ramularia spots on dying leaves

GS75-83 Ramularia symptoms on heads and awns

GS25-30 Fungicides can reduce later disease epidemic

Leaf wetness and symptom development

Correlation between leaf wetness and symptom development

Spring barley in June for spring barley & early April for w barley

Asteromella?

Airborne spores

Asgard SAC
No dramatic cultivar resistance

- Varietal Resistance – S Barley 2011
Epidemiology of *R. collo-cygni*

Spore release and RLS - Bush 2008

- Spore release and RLS for *R. collo-cygni* over the period from 2nd July 2008 to 30th September 2008.
- The graph shows Ramularia DNA (in pograms) over time, with a peak around 20th August 2008.
- Percentage RLS is also indicated on the y-axis.
Control measures

- Chemical fungicides are the only option available at present
  - In the UK, a mixture of Prothioconazole + SDHI + Chlorothalonil at GS45-49 is recommended
    - Many of these chemicals are under threat from new EU legislation
    - SDHI fungicides, give excellent control
  - Significant resistance issues
    - Development of QoI and MBC resistance
      - MBC have not been used for RLS control
Ramularia protection 2009 – 2011 three year mean

- Epoxyconazole
- Izopyrazam + Cyprodinil
- Bixafen + Prothioconazole
- Xemium + Epoxyconazole
- Prothioconazole
- HGCAB2
- Boscalid + Epoxyconazole
- Chlorothalonil
Historical archive samples

Ramularia DNA on leaves and stems from the Hoosfield archive 1852 - 2007

Detection of *R. collo-cygni* in archive material

QoI resistance
Bioassay results MBC’s

Negative cross resistance is observed when a mutation is found at codon 198
Risk of fungicide resistance

- Relatively high risk

- DMI fungicides showing decline in efficacy
  - Older fungicides

- QoI and MBC showing high level of resistance in most populations

- SDHI resistance a real risk!
  - SAC and Syngenta have a joint project
Fungicide efficacy tests
Sequence of Sdh gene

- Sdh gene- sub-units A, B, C & D

RccSdhB

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Replacement of Histidine

High level of fungicide resistance in related pathogens

Courtesy of Marta Piotrowska
Sequencing of genome

- Illumina/solexa base sequencing
  - Standard paired end library: 80x (4 Gb)
  - 3 kb and 6 kb mate-pair library (10x)
  - RNA seq library (80x)
- 454 Titanium sequencing
  - cDNA library to yield 180,000 reads
  - Genome library to yield 360,000 reads
Why sequence and initial data

• Comparative genetics with other related pathogens, to develop understanding of the plant-pathogen interactions

• CLC assembly of illumina and 454 data gives a genome size of 30 Mb in 355 supercontigs

• Close match to both *Mycosphaerella graminicola* and other *Mycospharella* spp.
Population genetics (SSR’s)

|----------|---------|---------|---------|----------|---------|----------|------------|---------|----------|-----------|

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R. collo-cygni biology

• Using GFP isolate to understand development during the whole growing season

Photos courtesy of Maciej Kaczmarek
R. collo-cygni biology

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R. collo-cygni biology

- Location of R.cc in seed

Thick layer of hyphae present under the seed coat, outside the aleurone layer of the endosperm

Photos courtesy of Maciej Kaczmarek
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