

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

James Fountaine

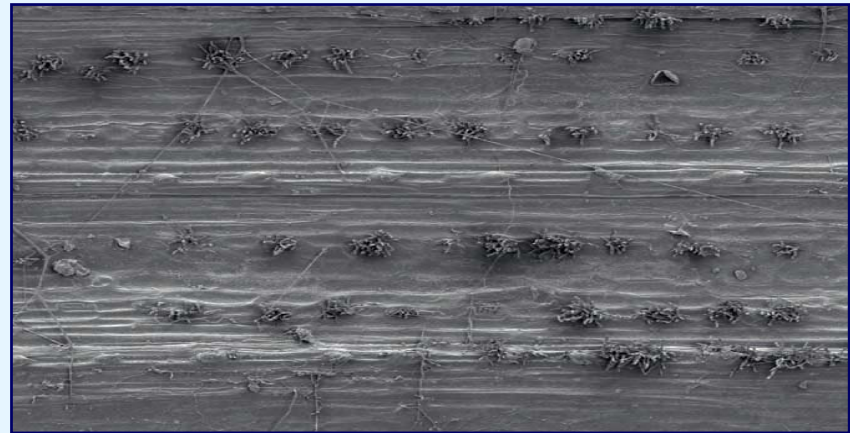
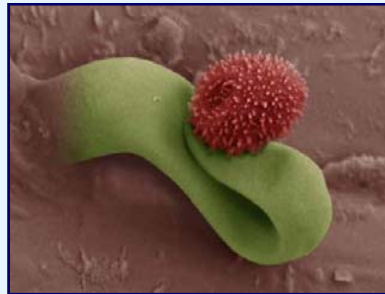
Taxonomy



Ramularia collo-cygni

Collum = neck
Cygnus = swan

Phyllum: *Hyphomycetes*
Order: *Dothideales*
Family: *Mycosphaerellaceae*
Section: ***Mycosphaerella***





SAC



GS0 Ramularia seed-borne
Seed treatments?



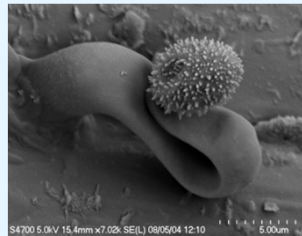
GS 10-13 Ramularia detectable by diagnostics but no visual symptoms



GS25-30 Ramularia spots on dying leaves



Asteromella ?



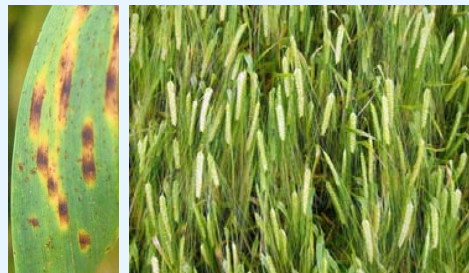
Airborne spores

GS25-30 Fungicides can reduce later disease epidemic

Correlation between Leaf wetness and symptom development In June for spring barley & early April for w barley



GS75-83 Ramularia symptoms on heads and awns



GS65 Fungus detected inside leaves 2-4 weeks before symptoms

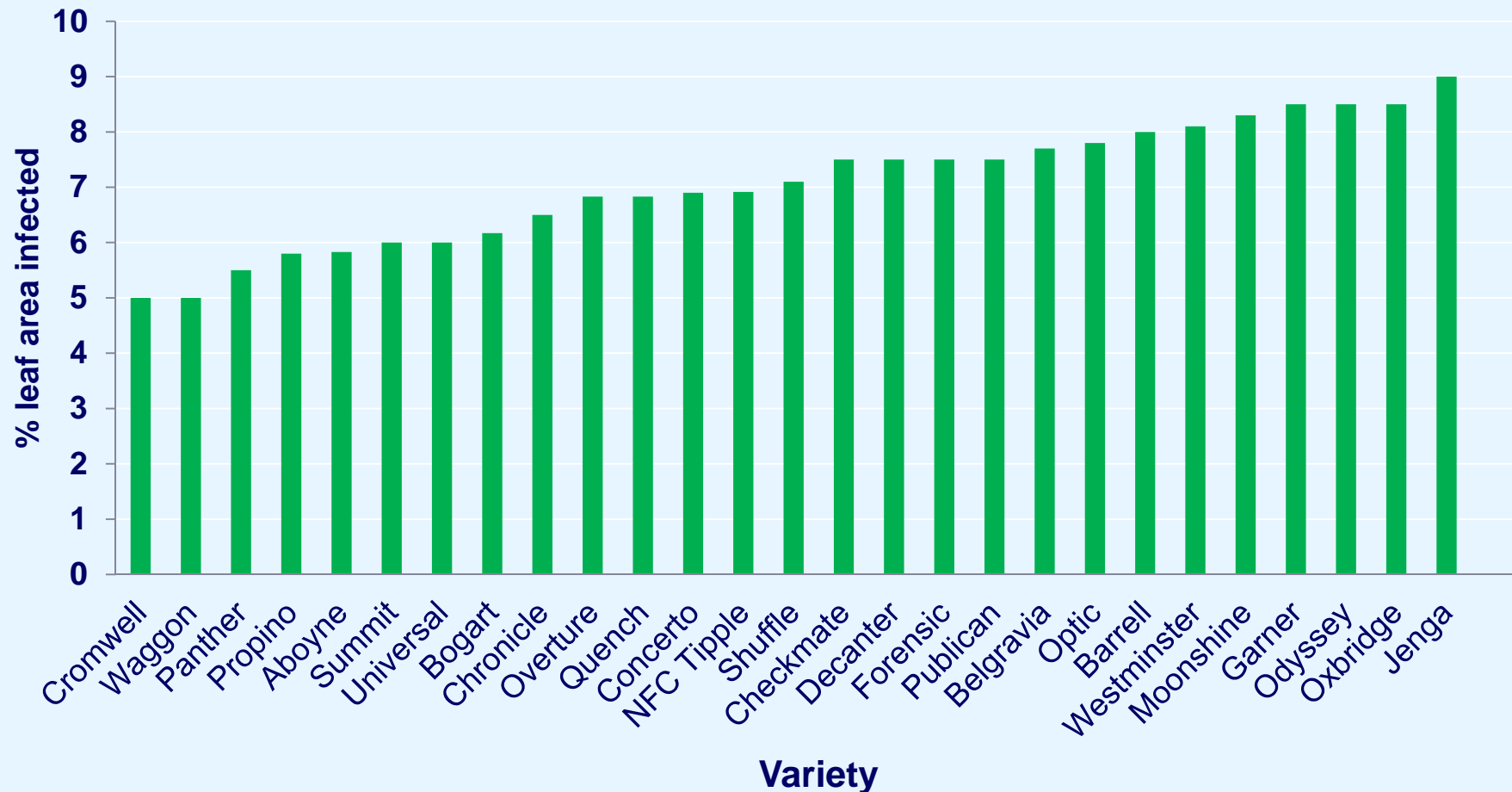


GS45-49 Protect crops with fungicide

No dramatic cultivar resistance



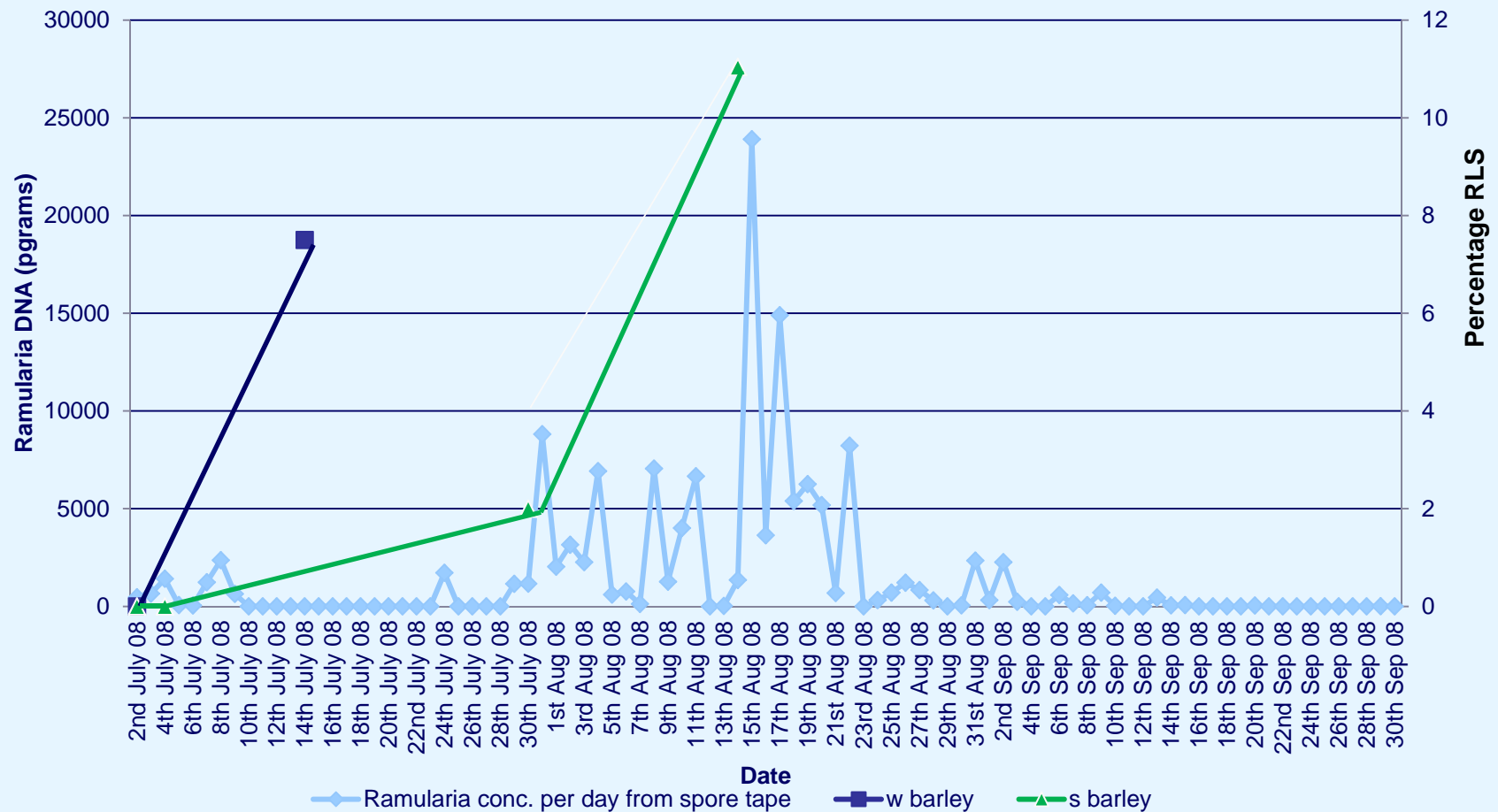
- Varietal Resistance – S Barley 2011



Epidemiology of *R. collo-cygni*



Spore release and RLS - Bush 2008

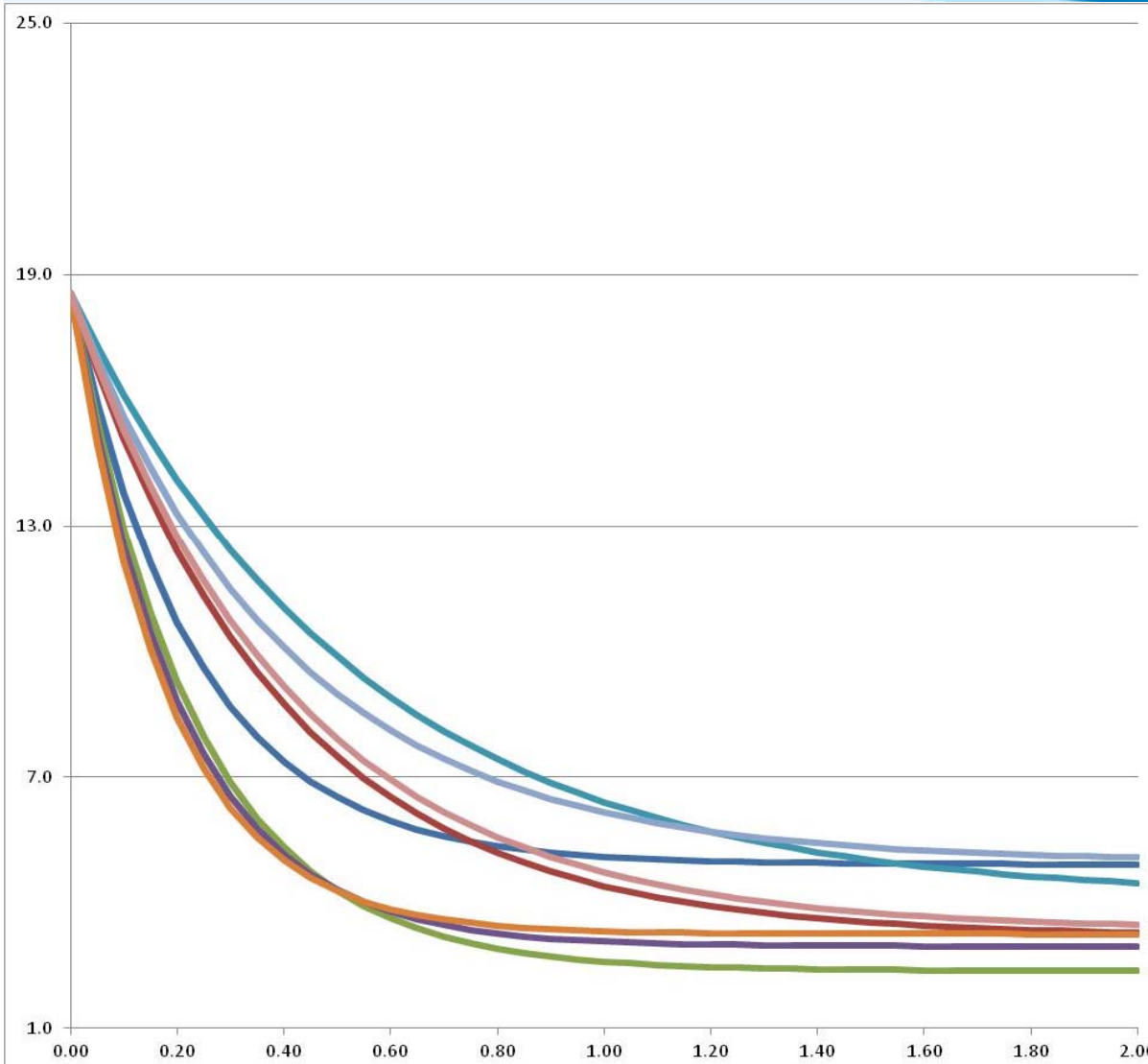


Control measures



- 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



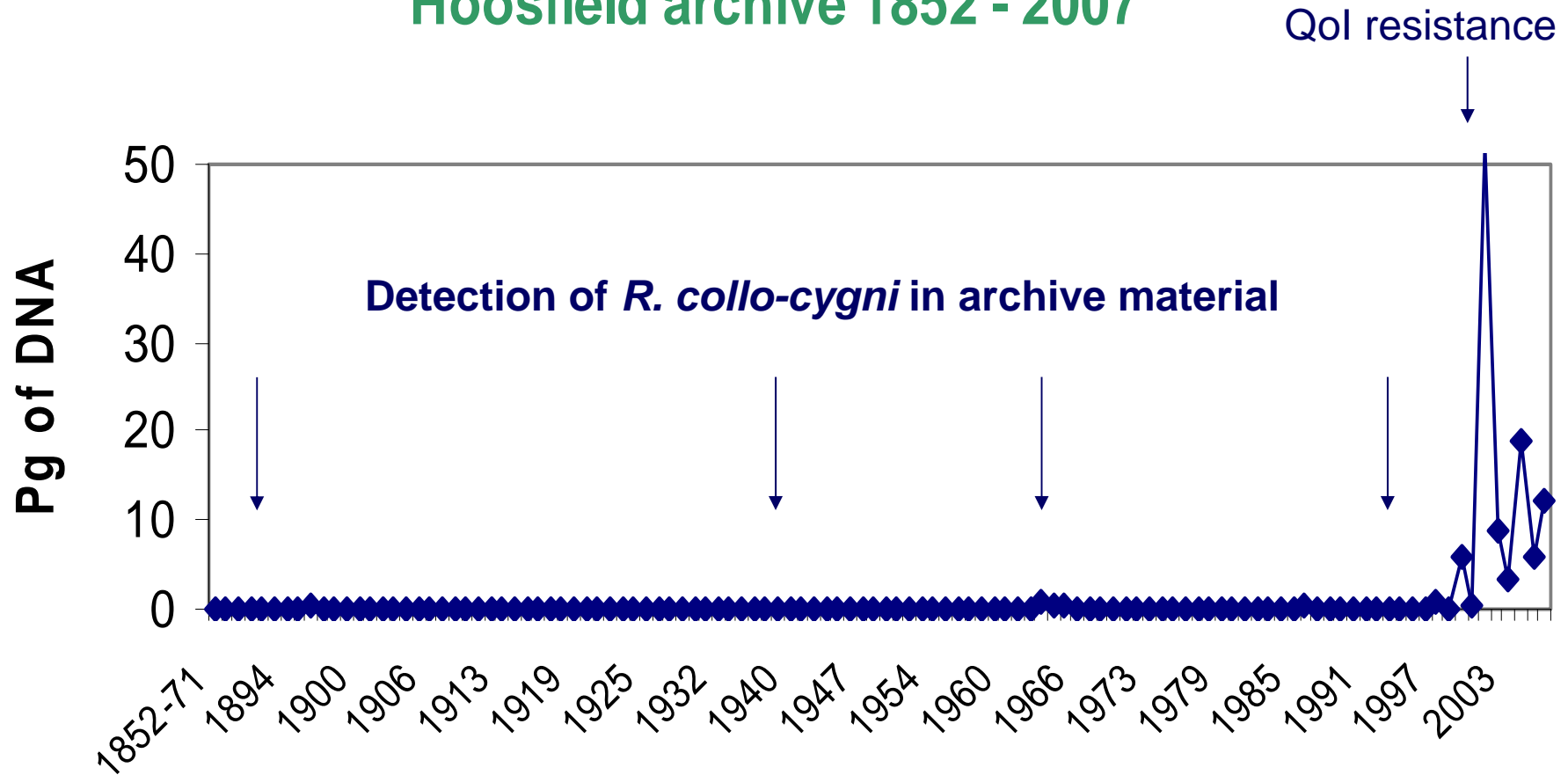
Agriculture & Horticulture
DEVELOPMENT BOARD



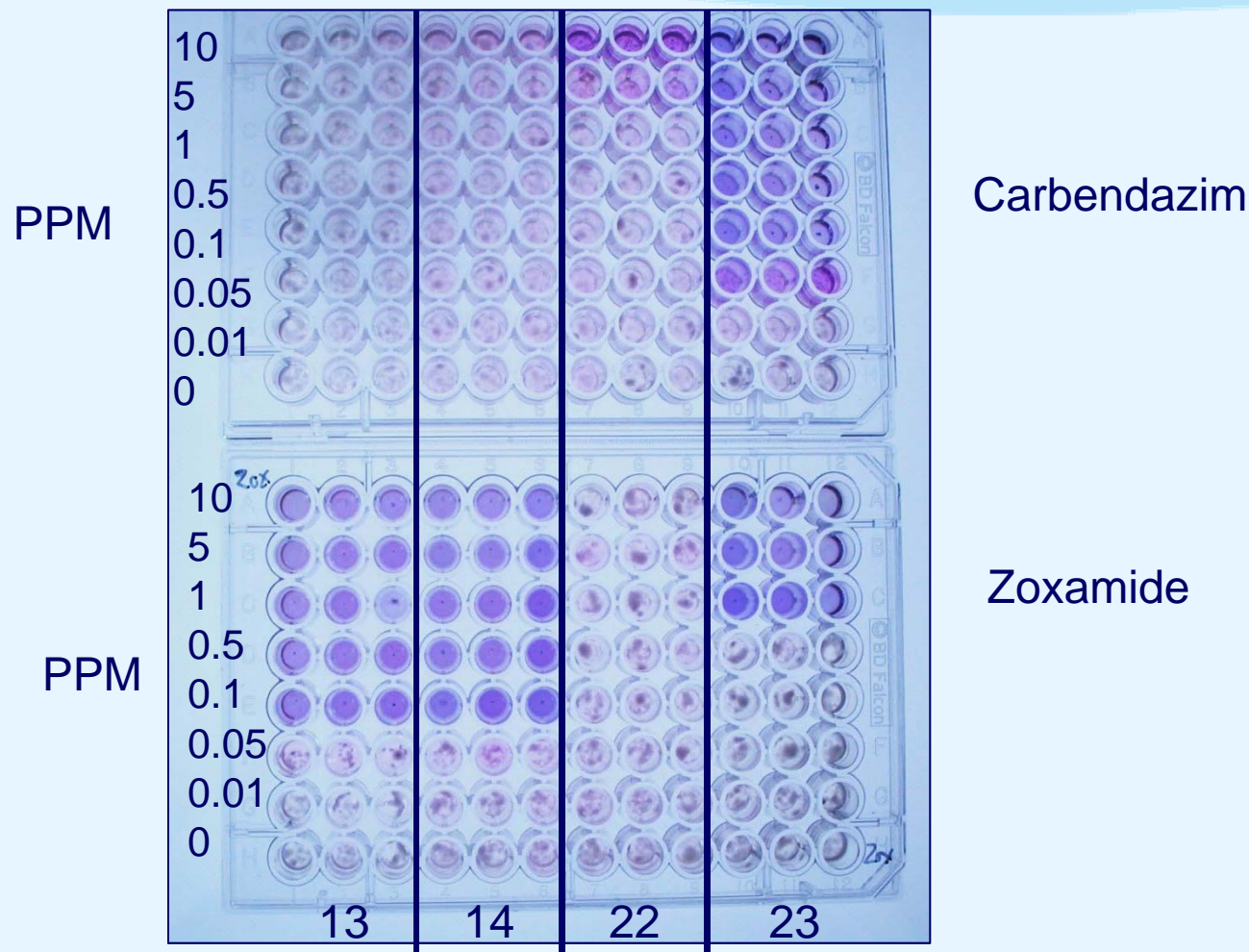
Historical archive samples



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



Bioassay results MBC's



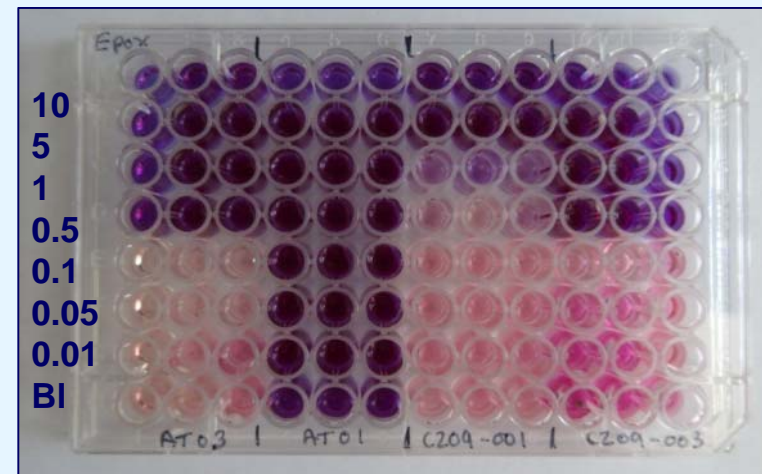
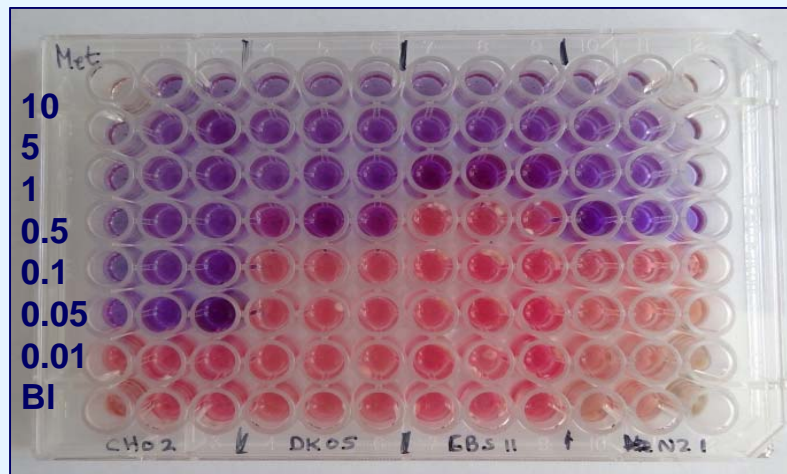
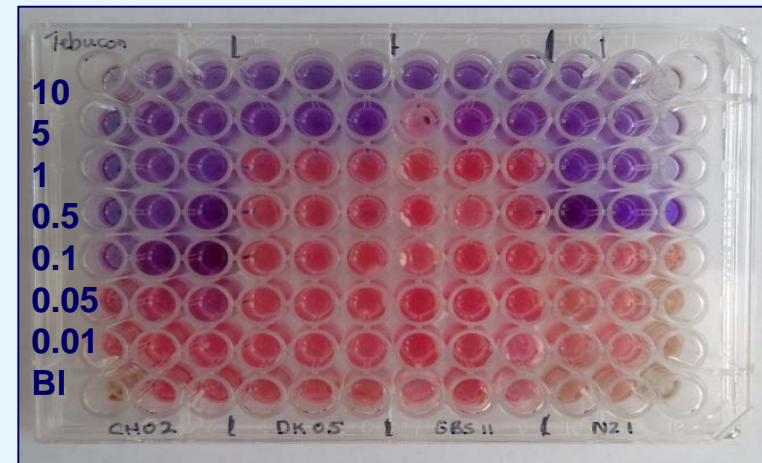
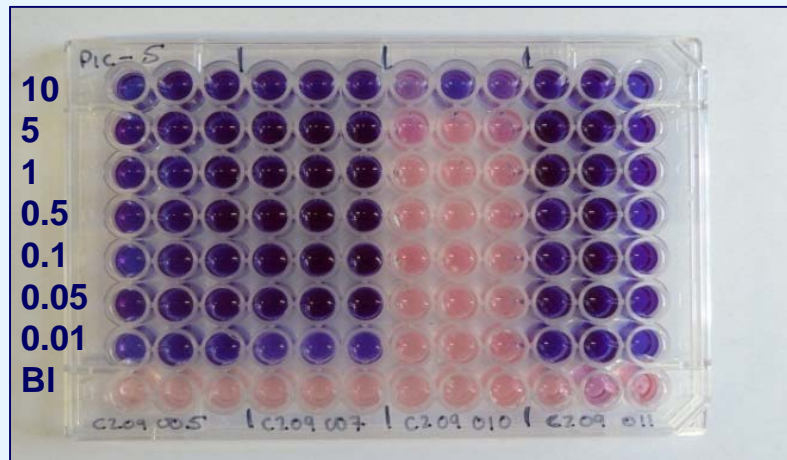
Negative cross resistance is observed when a mutation is found at codon 198

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- subunits A, B, C & D

Sdh B



Replacement
of **Histidine**



High level of
fungicide resistance
in related
pathogens



| | 1 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 | 130 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| RccSdhB | M | A | L | R | L | A | T | R | R | F | T | P | L | V | F | R | R | G | M | A | T | P | T | T | T | N | E | L | T | N | A | - | A | G | S | T | T | A | A | A | T | P | I | P | A | S | K | T | T | P | A | Q | E | P | A | M | D | A | E | A | K | T | K | T | F | Q | I | Y | R | W | N | P | D | Q | P | A | E | K | P | R | H | Q | S | Y | T | L | D | L | N | K | T | G | P | M | L | D | A | L | I | R | I | K | N | E | V | D | P | T | L | F | F | R | S | C | R | E | G | I | C | G | S | C | A | M | N | I | D |
| MgSdhB | M | A | L | R | L | A | T | R | R | F | A | P | I | A | F | R | R | G | M | A | T | I | E | H | T | K | E | P | I | S | A | T | A | E | A | L | S | A | S | R | P | P | I | K | E | T | K | T | S | T | V | K | E | P | Q | M | D | A | D | A | K | T | K | F | H | I | Y | R | W | N | P | D | Q | P | T | D | K | P | R | H | Q | S | Y | T | L | D | L | N | K | T | G | P | M | L | D | A | L | I | R | I | K | N | E | V | D | P | T | L | F | F | R | S | C | R | E | G | I | C | G | S | C | A | M | N | I | D | |
| Consensus | M | A | L | R | L | A | T | R | R | F | a | P | i | a | F | R | R | G | M | A | T | p | i | e | h | T | n | E | L | i | n | A | - | A | e | a | L | s | A | a | a | P | i | k | a | S | K | T | s | p | a | Q | E | p | A | M | D | A | # | A | K | T | K | T | f | q | I | Y | R | W | N | P | D | Q | P | a | # | K | P | R | H | Q | S | Y | T | L | D | L | N | K | T | G | P | M | L | D | A | L | I | R | I | K | N | E | V | D | P | T | L | F | F | R | S | C | R | E | G | I | C | G | S | C | A | M | N | I | D |

| | 131 | 140 | 150 | 160 | 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 | 250 | 260 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| RccSdhB | G | V | N | T | L | A | C | L | C | R | I | P | T | D | T | A | K | E | S | R | I | Y | P | L | P | H | T | Y | V | V | E | D | L | V | P | D | M | T | Q | F | Y | K | Q | Y | K | S | I | K | P | Y | L | Q | R | D | T | A | P | P | D | G | K | E | N | R | Q | S | V | A | D | R | K | K | L | D | G | L | Y | E | C | I | L | C | A | C | C | S | T | S | C | P | S | Y | W | N | S | E | E | Y | L | G | P | A | V | L | L | Q | S | Y | R | W | I | N | S | R | D | E | R | T | E | Q | R | K | D | A | L | N | N | S |
| MgSdhB | G | V | N | T | L | A | C | L | C | R | I | P | T | D | T | A | K | E | T | R | I | Y | P | L | P | H | T | Y | V | V | K | D | L | V | P | D | M | T | Q | F | Y | K | Q | Y | K | S | I | K | P | Y | L | Q | R | D | T | A | P | P | D | G | K | E | N | R | Q | S | V | A | D | R | K | K | L | D | G | L | Y | E | C | I | L | C | A | C | C | S | T | S | C | P | S | Y | W | N | S | E | E | Y | L | G | P | A | V | L | L | Q | S | Y | R | W | I | N | S | R | D | E | K | T | A | Q | R | K | D | A | L | N | N | S |
| Consensus | G | V | N | T | L | A | C | L | C | R | I | P | T | D | T | A | K | e | s | R | I | Y | P | L | P | H | T | Y | V | e | D | L | V | P | D | M | T | Q | F | Y | K | Q | Y | K | S | I | K | P | Y | L | Q | R | D | T | A | P | P | D | G | K | E | N | R | Q | S | V | A | D | R | K | K | L | D | G | L | Y | E | C | I | L | C | A | C | C | S | T | S | C | P | S | Y | W | N | S | E | E | Y | L | G | P | A | V | L | L | Q | S | Y | R | W | I | N | S | R | D | E | r | T | a | Q | R | K | D | A | L | N | N | S | |

| | 261 | 270 | 280 | 290 | 297 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-----------|-----|-----|-----|-----|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RccSdhB | M | S | L | Y | R | C | H | T | L | N | C | S | R | T | C | P | K | G | L | N | P | A | L | A | I | A | E | I | K | K | S | M | A | F | T | |
| MgSdhB | M | S | L | Y | R | C | H | T | L | N | C | S | R | T | R | P | K | G | L | N | P | A | L | A | I | A | E | I | K | K | S | M | A | F | T | G |
| Consensus | M | S | L | Y | R | C | H | T | L | N | C | S | R | T | r | P | K | G | L | N | P | A | L | A | I | A | E | I | K | K | S | M | A | F | T | . |

Courtesy of Marta Piotrowska

Sequencing of genome



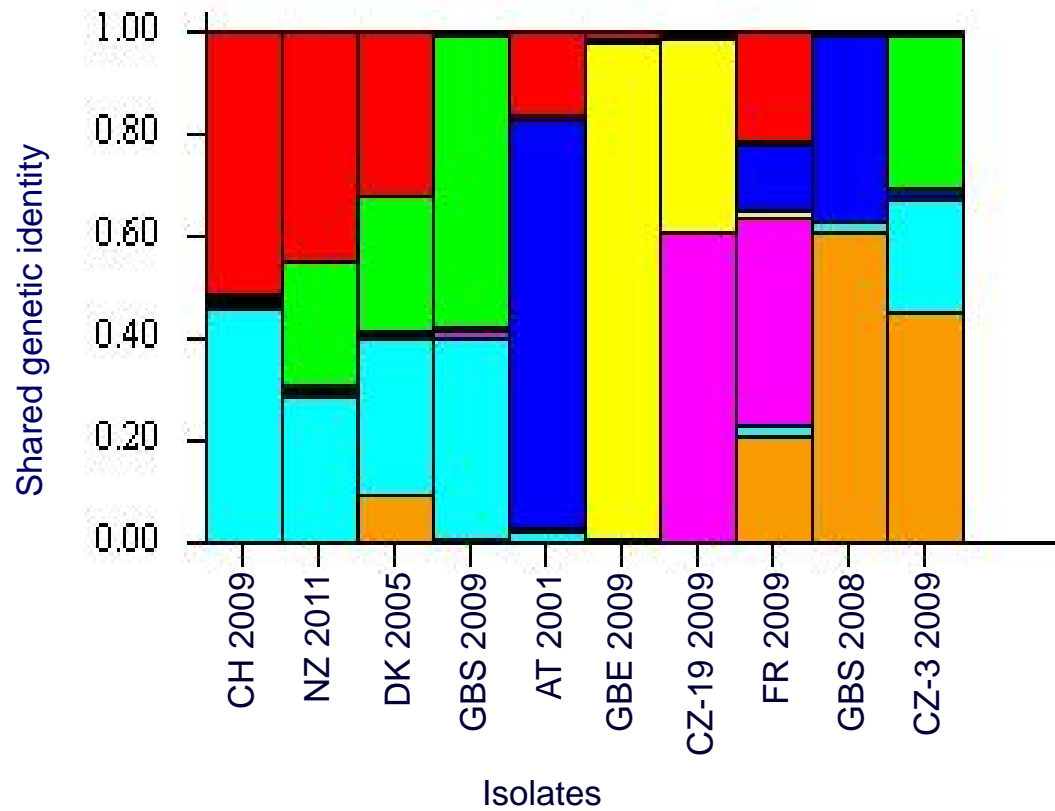
- Illumina/solexa base sequencing
 - Standard paired end library: 80x (4 Gb)
 - 3 kb and 6 kb mate-pair library (10x)
 - RNA seq library (80x)
- 454 Titanium sequencing
 - cDNA library to yield 180,000 reads
 - Genome library to yield 360,000 reads

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 *Mycosphaerella* spp.

Population genetics (SSR's)

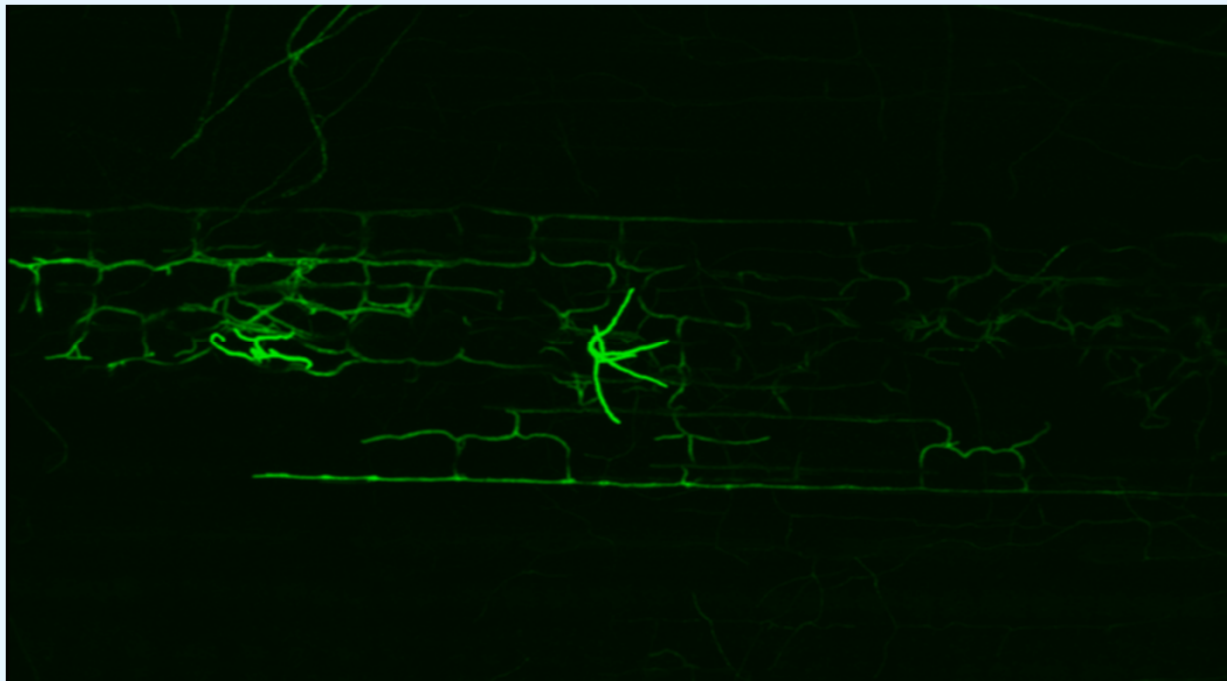


| SSR nr. | SSR type | Alleles per locus |
|---------|----------|-------------------|
| 1 | p5 | 4 |
| 2 | p5 | 2 |
| 3 | p5 | 4 |
| 4 | p5 | 5 |
| 5 | p5 | 2 |
| 6 | p5 | 4 |
| 7 | p5 | 4 |
| 8 | p5 | 4 |
| 9 | p5 | 4 |
| 11 | p5 | 2 |
| 12 | p5 | 6 |

R. collo-cygni biology

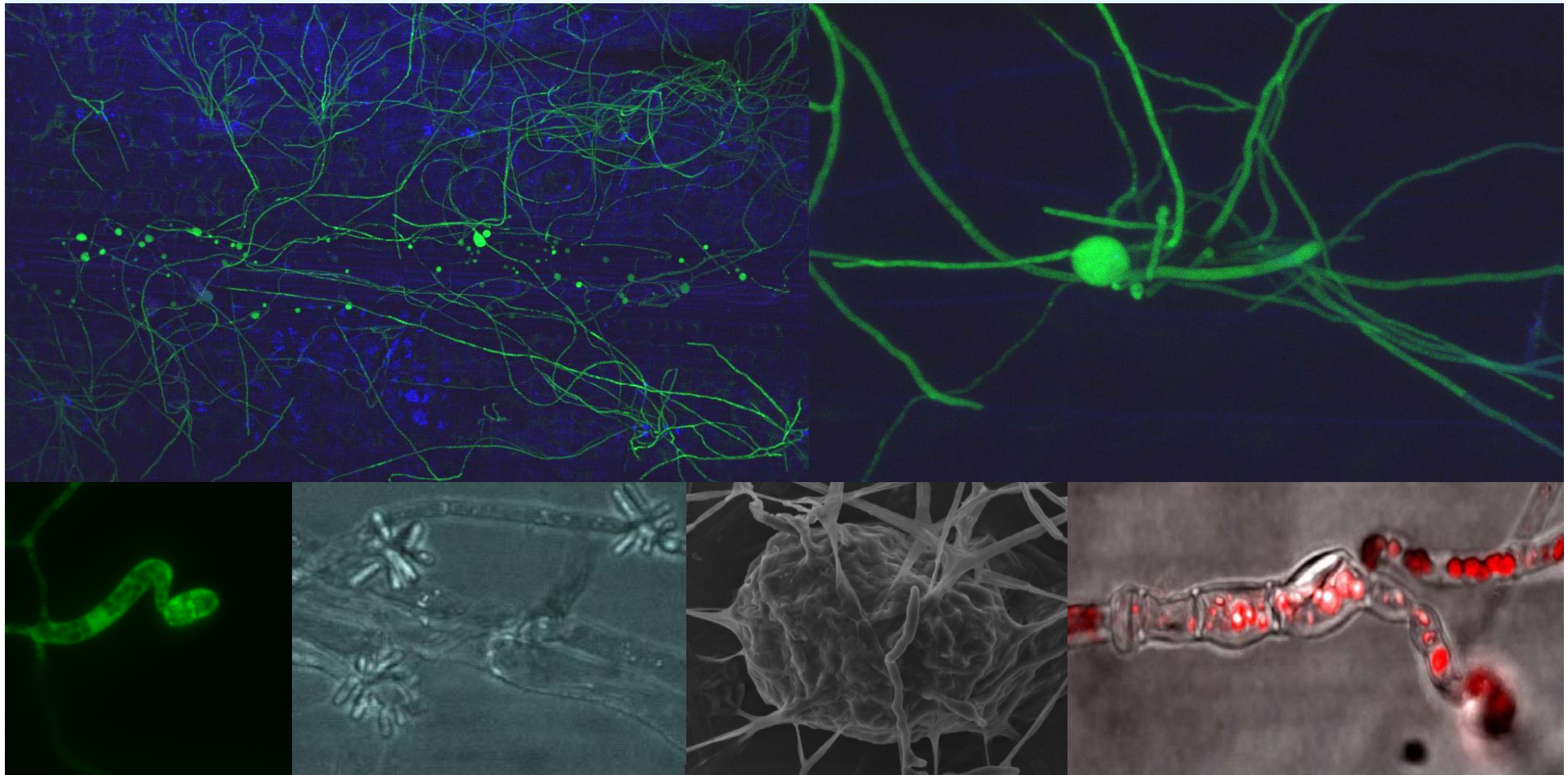


- Using GFP isolate to understand development during the whole growing season



Photos courtesy of Maciej Kaczmarek

R. collo-cygni biology

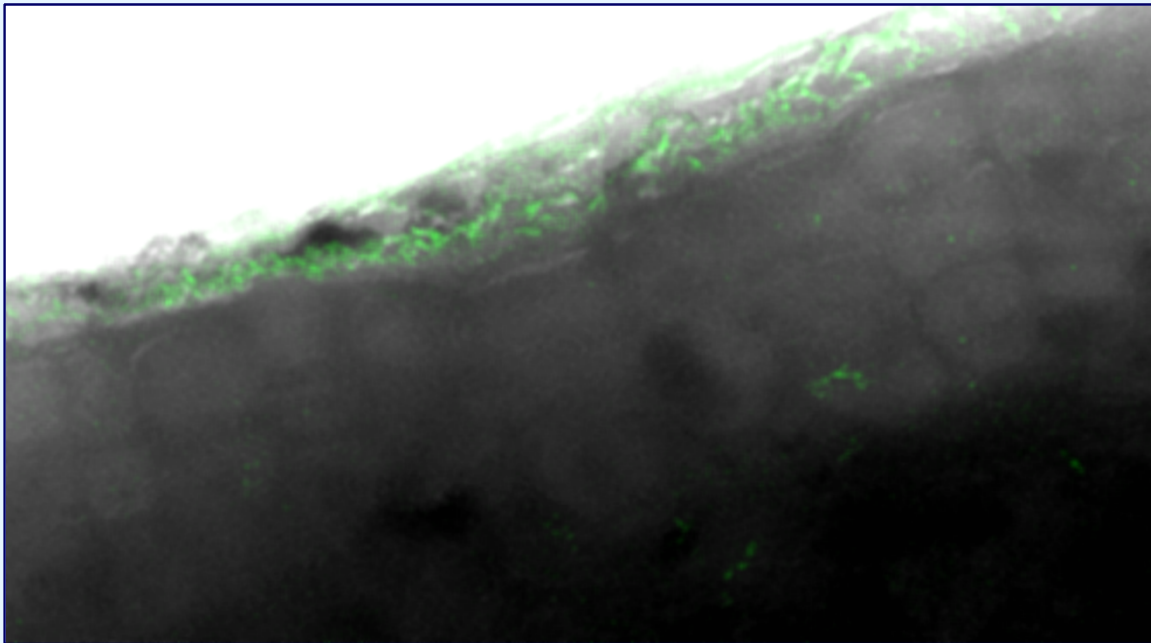


Photos courtesy of Maciej Kaczmarek 18

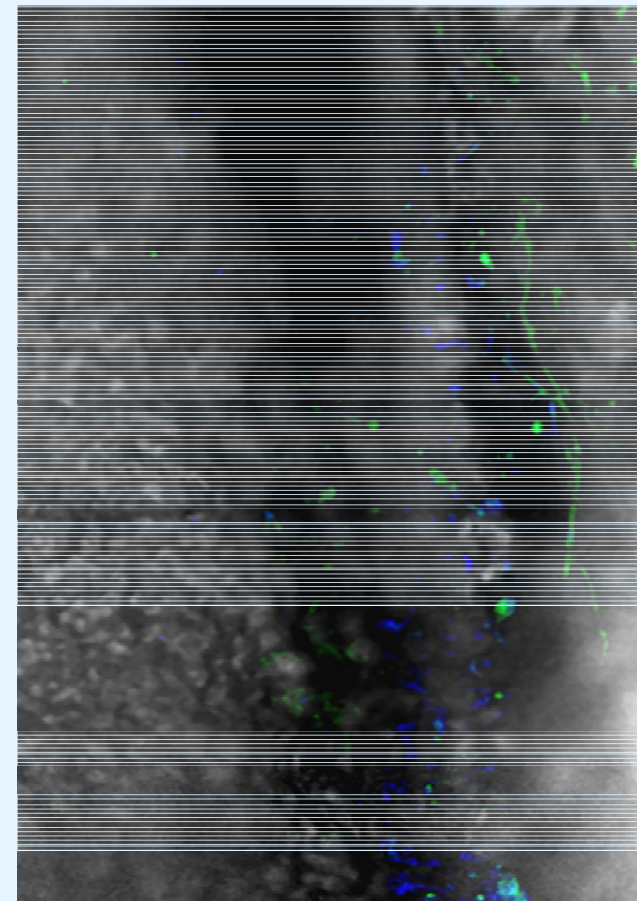
R. collo-cygni biology



- 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

Acknowledgments



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Dr. Bart Fraaije, Rothamsted Research

And both

Marta Piotrowska & Maciej Kaczmarek for being enthusiastic Ph.D. students

The Syngenta logo features the word 'syngenta' in a blue, lowercase, sans-serif font, with a small green leaf icon above the letter 'n'.

The BASF logo features the word 'BASF' in a bold, white, sans-serif font on an orange background, with a small white square icon to the left. Below it, the text 'The Chemical Company' is written in a smaller, white, sans-serif font.