



Oilseed
RapE
Genetic
Improvement
Network:
WP4 & WP5

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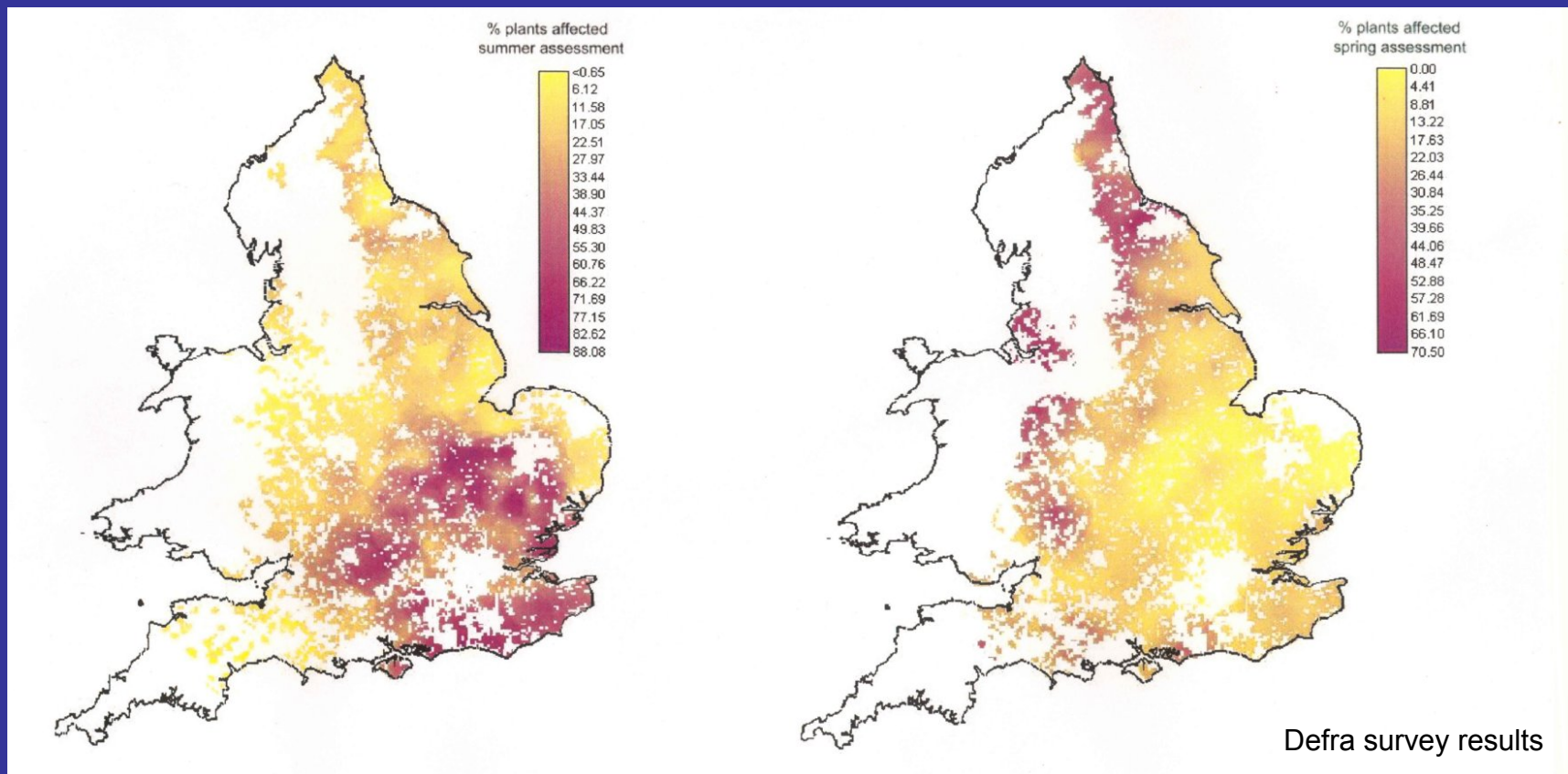
www.oregin.info



Aims of OREGIN:

- to provide focus for UK OSR R&D community
- to deliver accessible tools and genetic resources to UK-based breeding companies
- to create close working relationships between researchers and “end users”
- to improve sustainability/reduce inputs/pollution

Regional oilseed rape disease incidence (1990-1999)



Phoma stem canker

Light leaf spot

WP4 Pathogen isolates

- Culture collections – two main diseases
 - *Leptosphaeria maculans* (stem canker)
 - *Pyrenopeziza brassicae* (light leaf spot)
- “Scientifically important” isolates
 - Cited in peer-reviewed papers
 - Geographically diverse
- Archiving of collections
 - Live cultures
 - DNA
 - Phenotyping by minisatellite and AFLP markers

Why?

- SC and LLS cause £millions yield loss annually
- Both pathogens reproduce sexually and are genetically diverse...implications for specific resistance and for effectiveness of fungicides
- Global movement of seed and inoculum increase these problems
- A publicly-owned culture collection accessible to all stakeholders in the UK

Phoma stem canker (*Leptosphaeria maculans*)



Phoma leaf lesion (autumn)



Typical basal canker (summer)

Light leaf spot (*Pyrenopeziza brassicae*)



Leaf lesion
(winter)



Stem lesion
(spring)



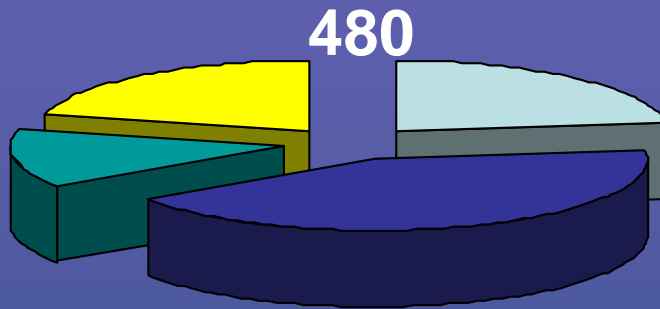
Seed pod
infection (spring)

Leptosphaeria maculans



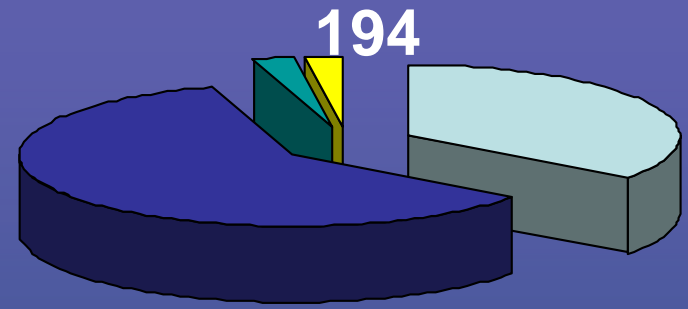
Culture collections

L. maculans (SC)



UK Europe
Americas Australia

P. brassicae (LLS)



UK France
Germany Poland



OREGIN Database of Fungal Isolates

Isolate Name: Other Name: Organism: Alternate State: Country of Origin: Additional Geographical Info: Origin: Host Part: Host Species: Cultivar: Depositor: Date Isolated: Single Spore: Identified by: Mating Type: Biotype: Media: Temperature: PG: Isolate Notes: Other Notes:

Collection reference:

OREGIN IBCN ATCC

STOCKS

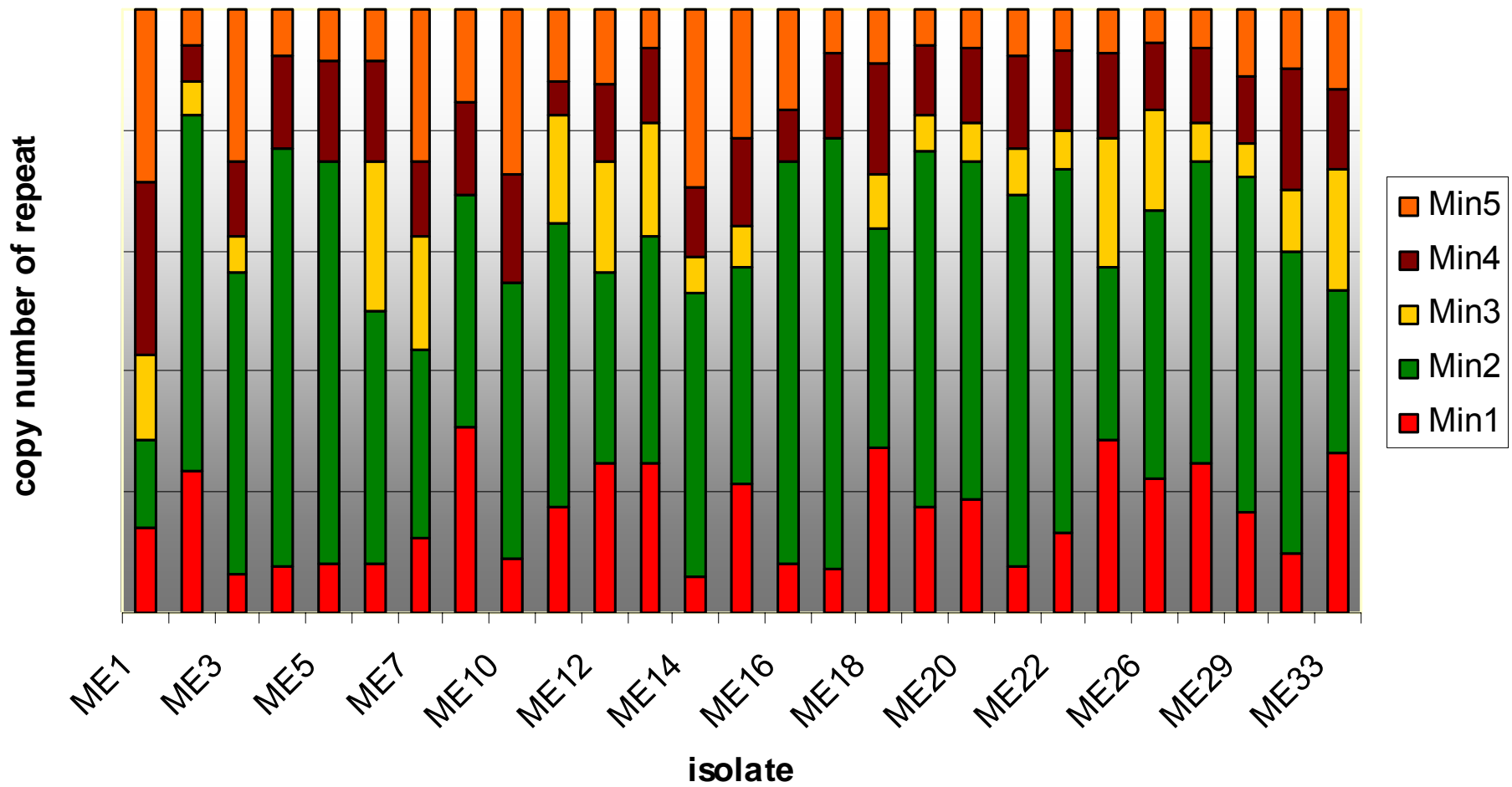
Rothamsted Research

Glycerol Slope DNA

HRI

Glycerol

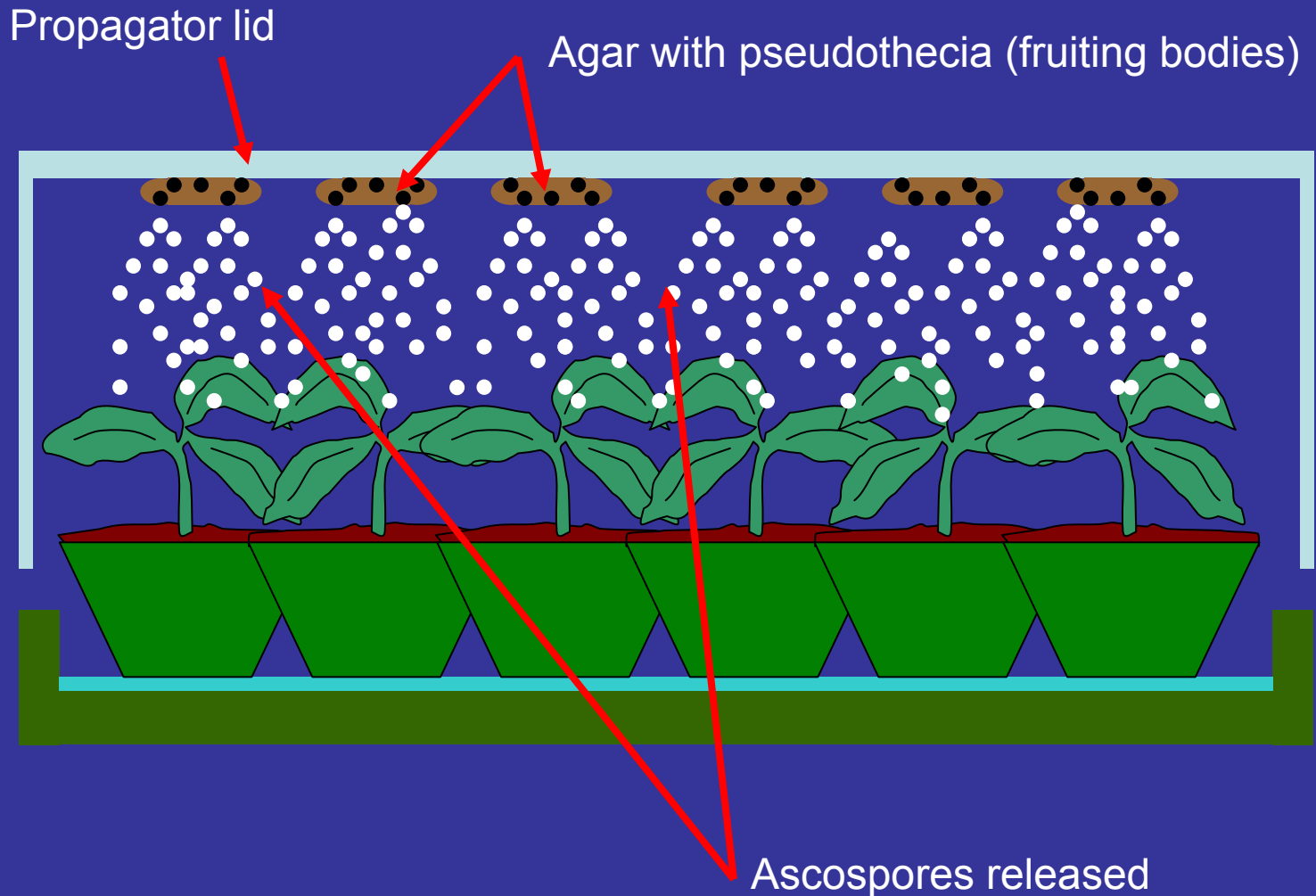
DNA fingerprinting of *L. maculans* using minisatellites



WP5 – Tools for dissecting the interaction

- Ascospore shower inoculation (NIL *L. maculans*)
- GFP- (and DsRed-) expressing transformed isolates of *L. maculans* (and *L. biglobosa*) developed (BBSRC PhD project)
- Allow “visualisation” of infection process
- Use fixed *B. napus* isogenic lines with/without *Rlm6* resistance (INRA)

Development of the “ascospore shower” method



Plans for 2005

- Make database available on OREGIN website
- Molecular fingerprinting of isolates
- Screen core collection of isolates for (A)virulence genes
- Participate in annotation of genome sequence of *L. maculans*

Major gene *Rlm6* resistance to *L.maculans* is temperature-sensitive

(leaves inoculated with ascospores of *AvrLm6*)

Darmor



DarmorMX (*Rlm6*)



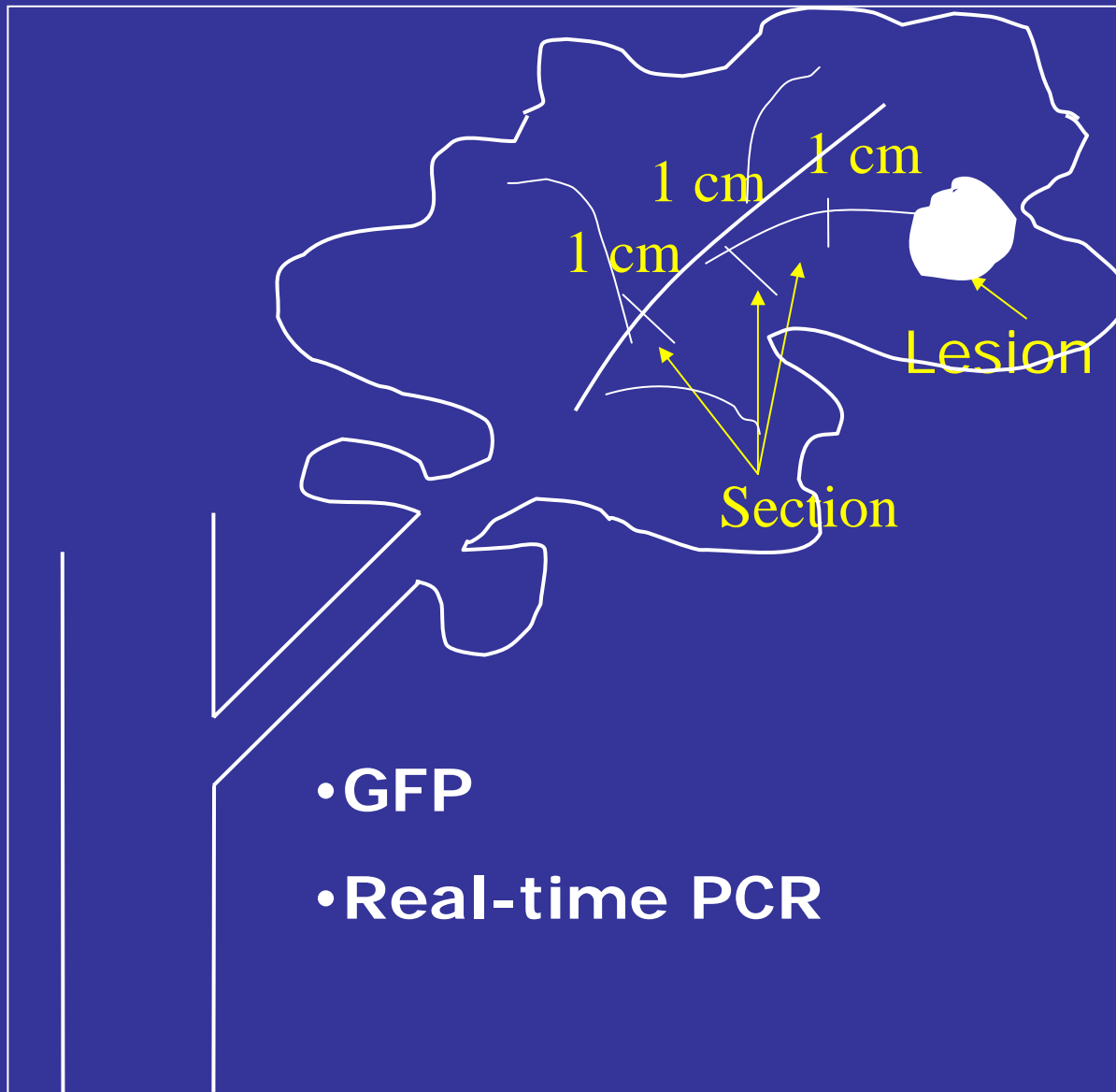
15°C
16 days after
leaf inoculation



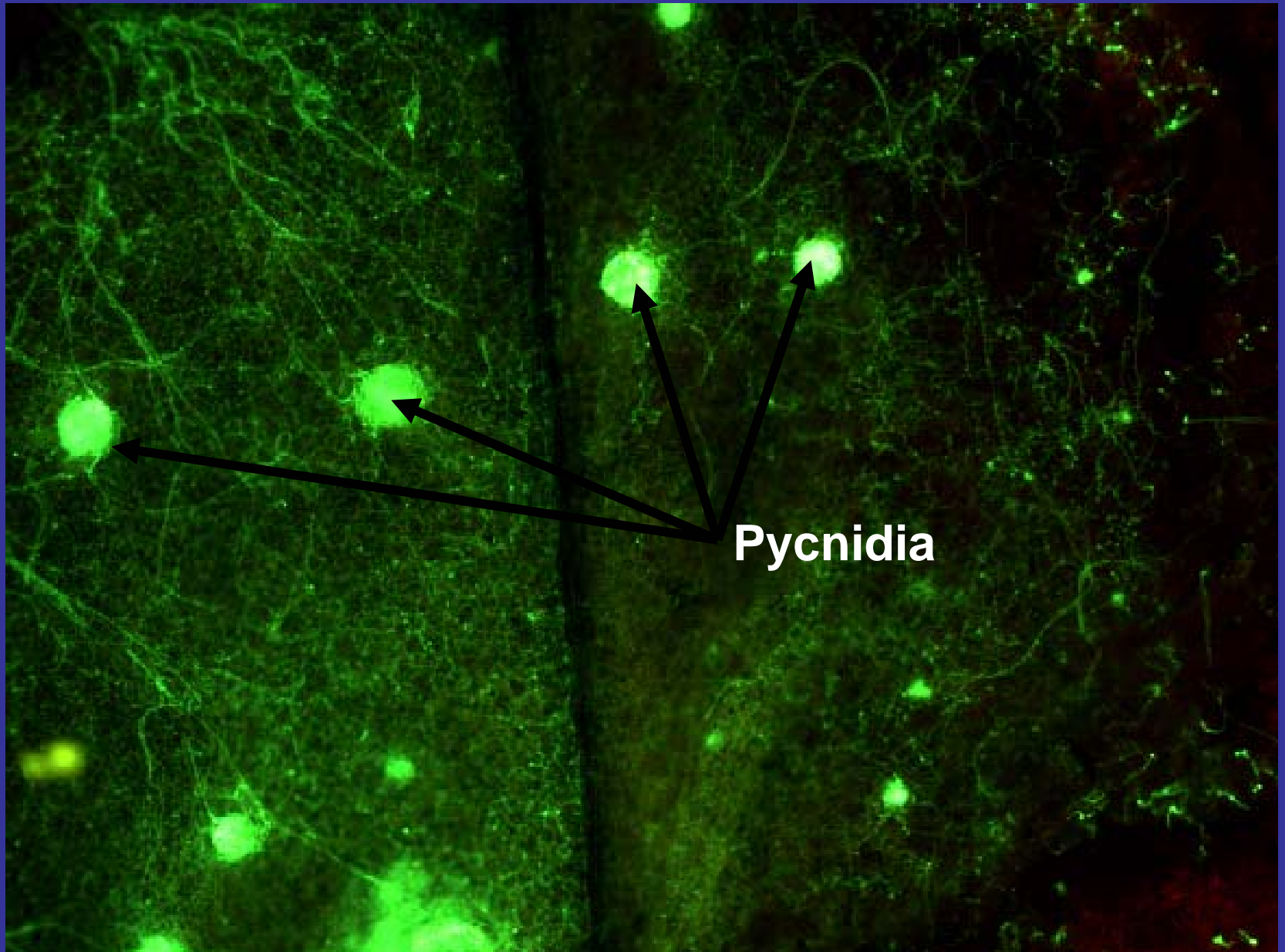
25°C
16 days after leaf
inoculation



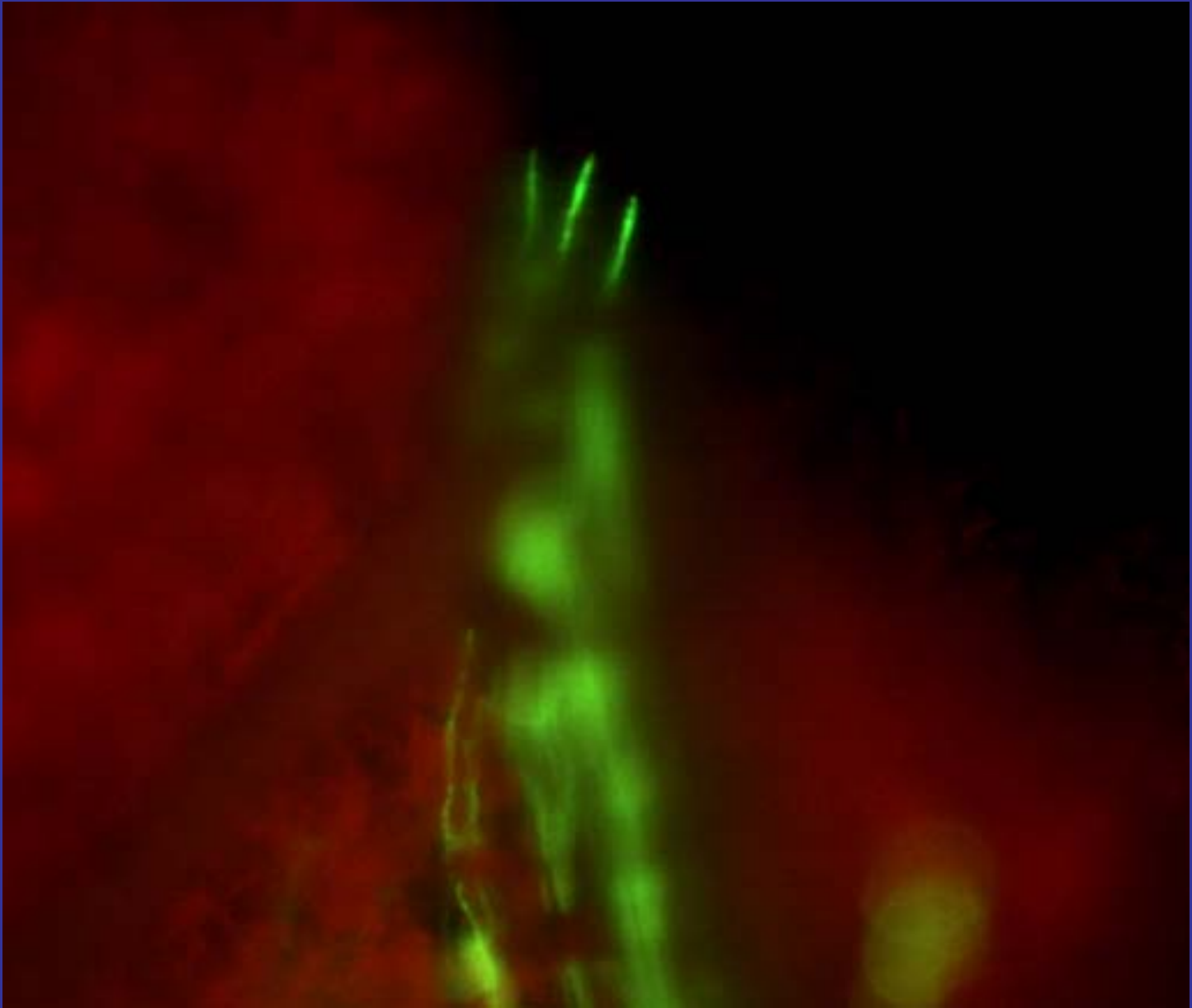
Tools for studying *L.maculans* spread from leaf to stem



Darmor 15°C



Darmor, 15°C, hyphal growth in leaf vein, 16 dpi

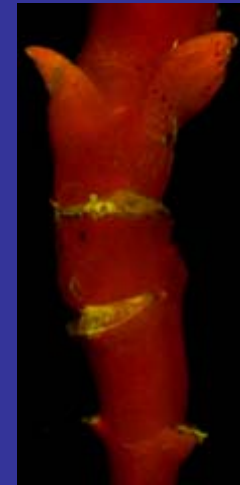
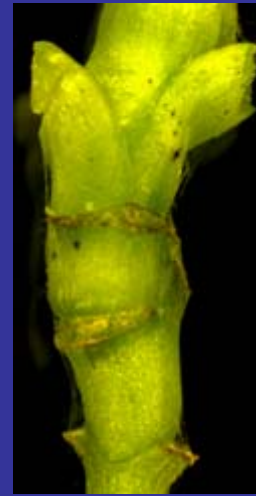
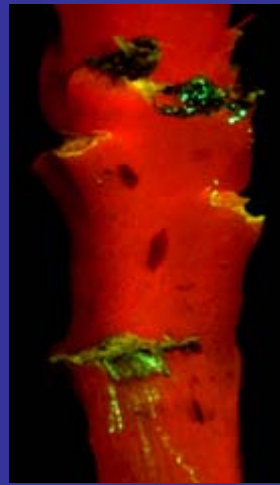
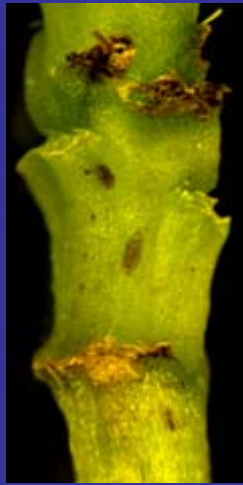


Effects of temperature on *Rlm6* resistance to *L.maculans* (leaves inoculated with conidia of GFP *AvrLm6*)

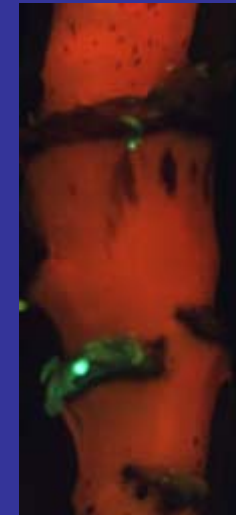
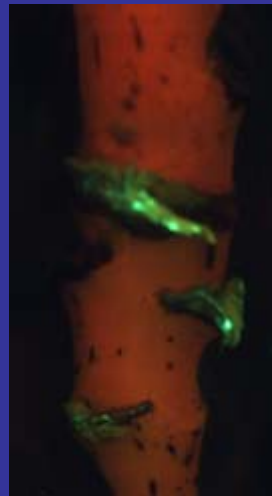
Darmor

DarmorMX (*Rlm6*)

15°C
36 days
after leaf
inoculation

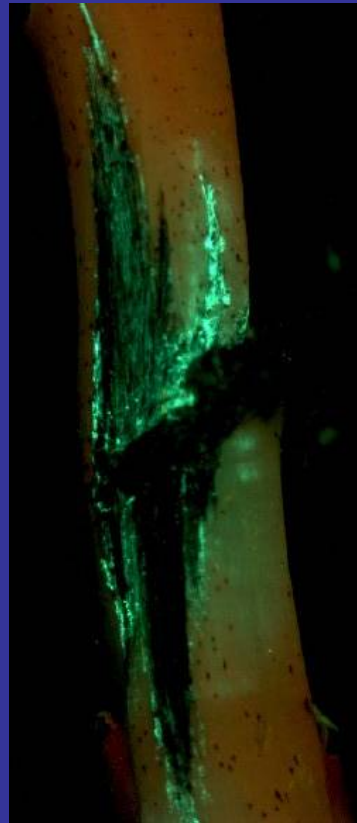


25°C
30 days
after leaf
inoculation

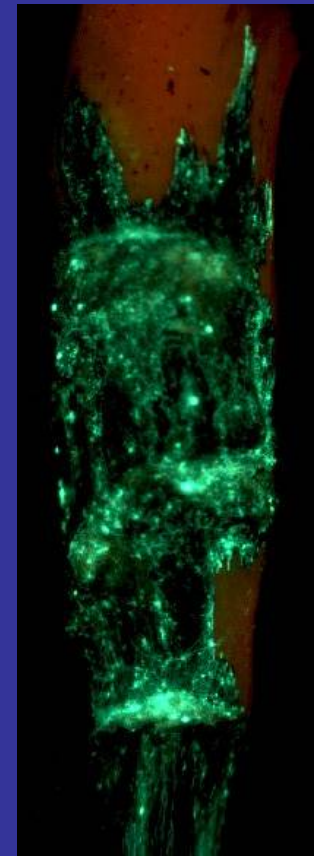
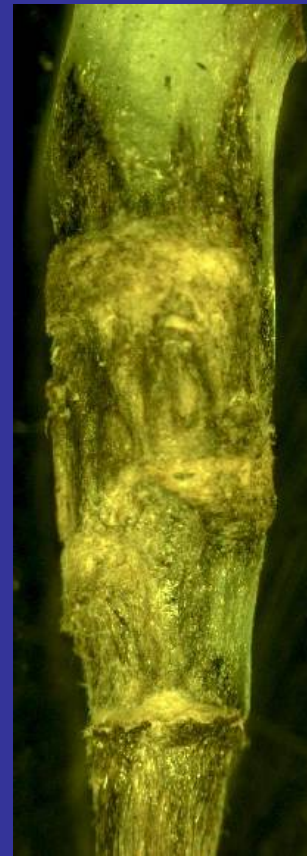


Phoma stem canker, 60 days at 25°C

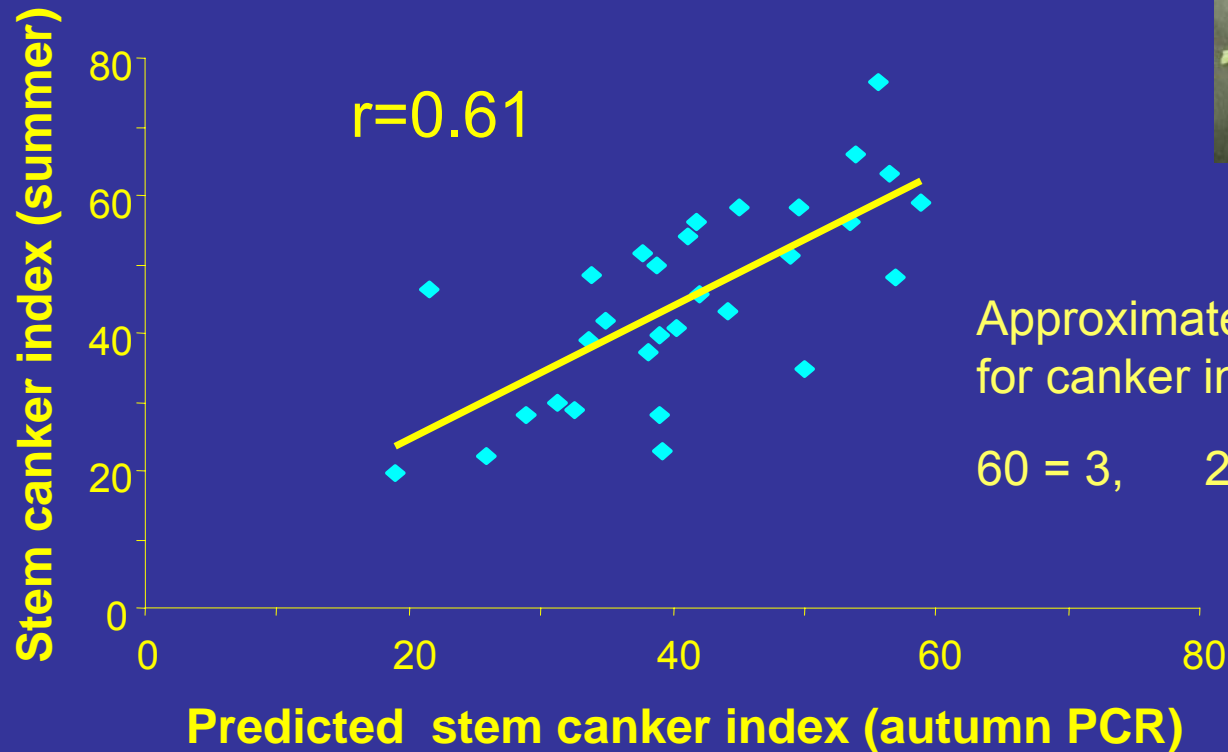
Darmor



Darmor MX (*Rlm6*)



Predicted stem canker index (PCR on petiole segment) vs observed canker index



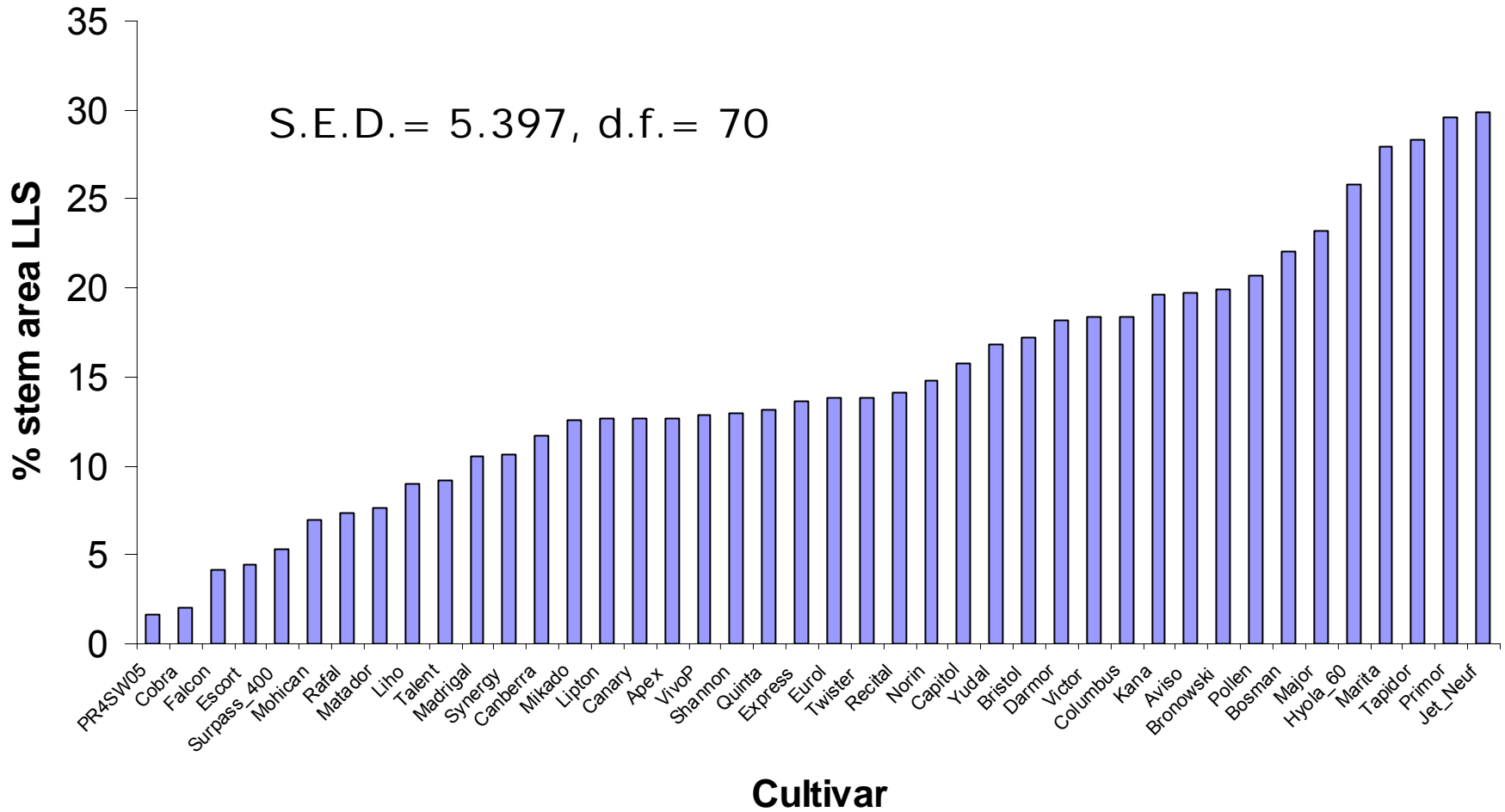
Approximate resistance ratings
for canker indices:

60 = 3, 20 = 8

2004/05 Field experiment

- 42 lines sown 3 Sept 2004
- Weekly assessments
- % plants affected phoma/LLS
- % leaf area affected

Range of severity of LLS (% stem area affected), OREGIN experiment, Rothamsted, 7 July 2004



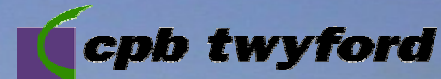
2004/05 Field experiment – latest information

- Phoma leaf spotting now less
- Infected “old” leaves shed
 - Severe stem cankers expected due to warm, wet autumn
- Light leaf spot observed early
 - Moderate/ high incidence for Jan

Plans for 2005

- Develop GFP and real time PCR for assessing resistance in petiole
- 2005/06 field experiments
 - include some lines from the DFFS
 - test real time PCR on sampled petioles

Components of Field Resistance to Diseases in Winter Oilseed Rape Cultivars (CORDISOR)



NIAB
ADAS
Rothamsted
SAC

HGCA
Saaten Union
CPB Twyford
Nickerson
Elsoms
Syngenta Seeds
Monsanto

