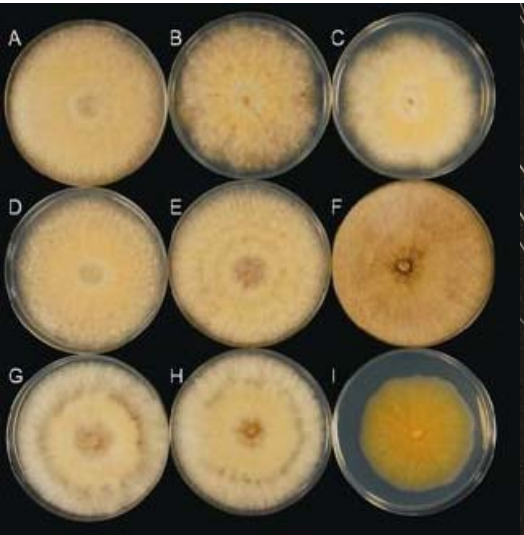
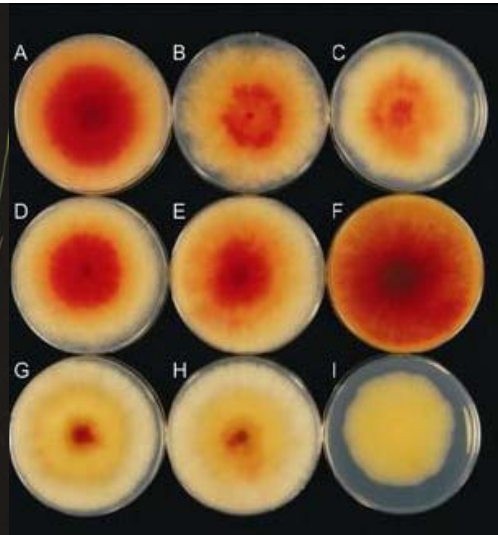
Molecular and Genetic Studies on *Fusarium* Ear Blight disease of Wheat

Kim Hammond-Kosack

BBSRC
bioscience for the future

ROTHAMSTED
RESEARCH





TALK OUTLINE

- **The hyphal infection process:**

  From the initial infected spikelet → rachis

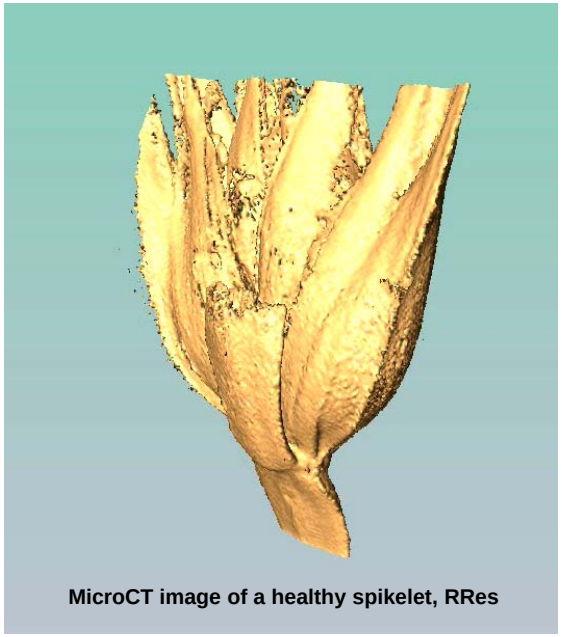
  rachis → the adjoining spikelets

- **Arabidopsis floral Fusarium – pathosystem:**

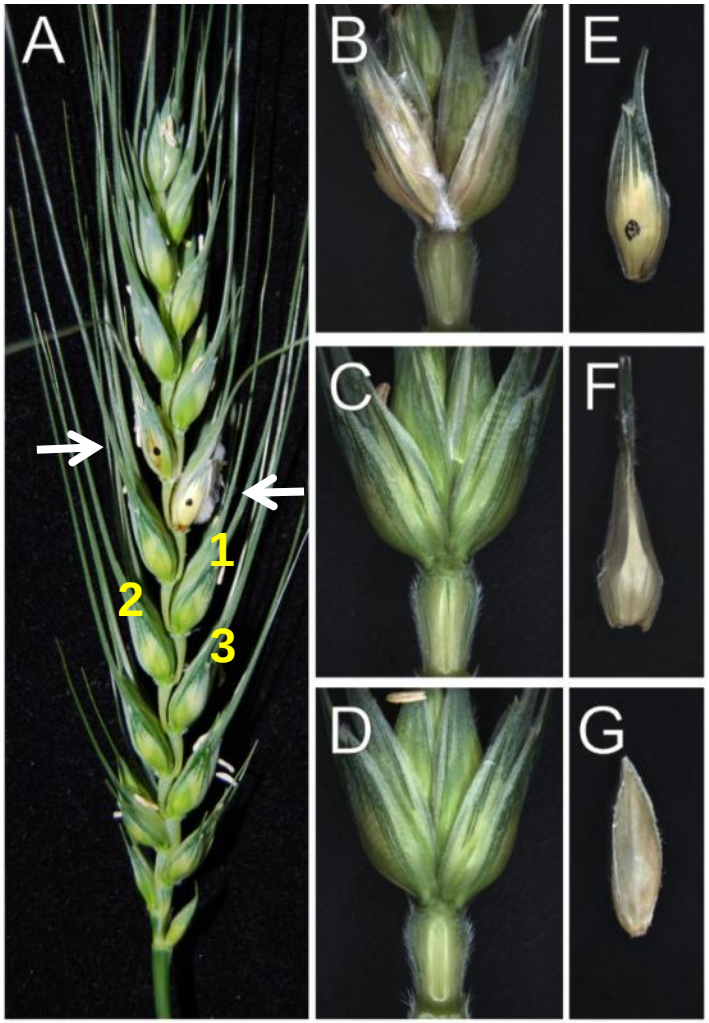
  To identify the pathogen and host components which either restrict or support the Fusarium infection process

- **Establishing a UK facility for VIGS research in wheat**

How does infection escape the spikelet and spread throughout the ear?



MicroCT image of a healthy spikelet, RRes

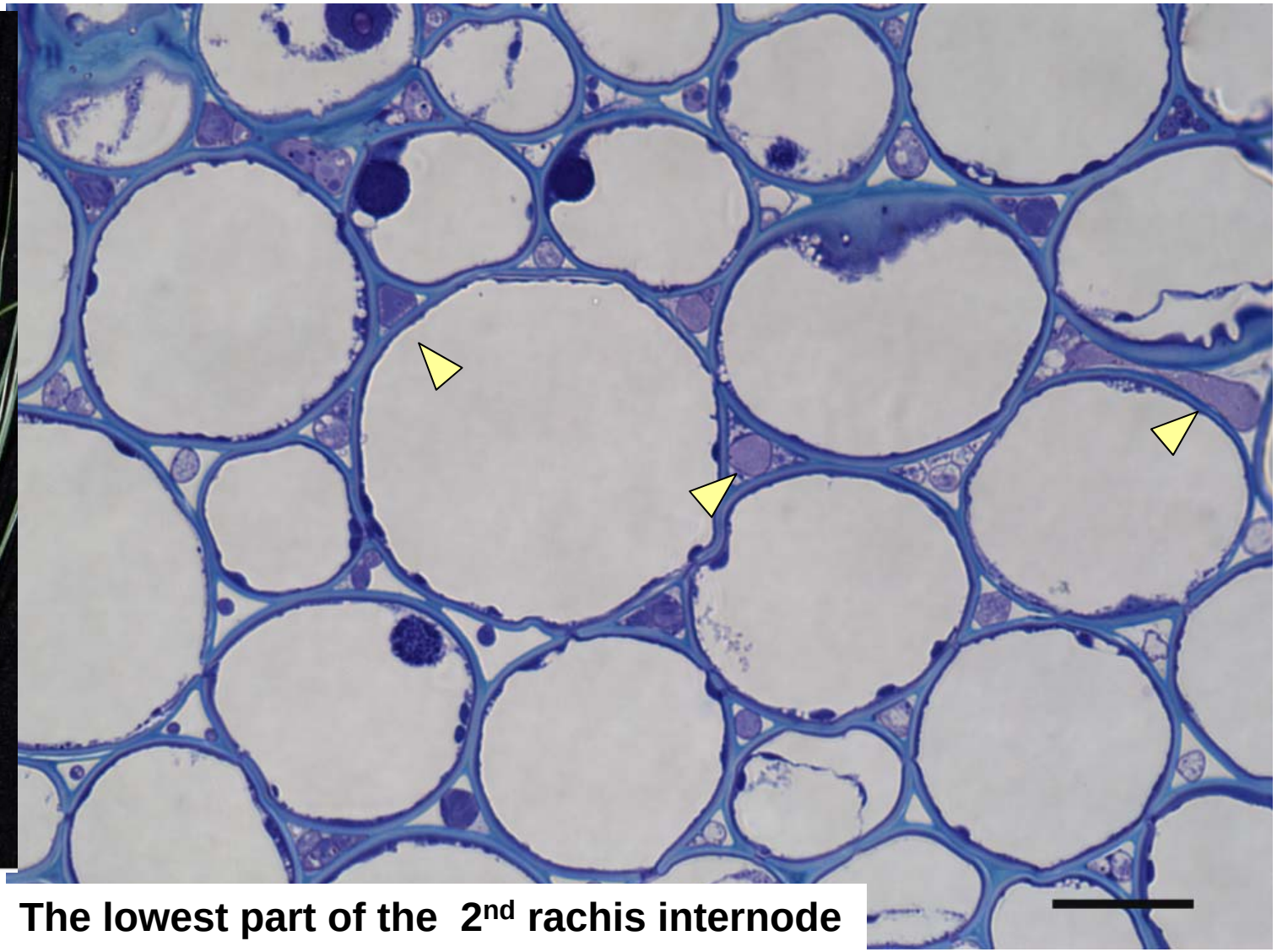


5 days post inoculation (dpi)

Approach:

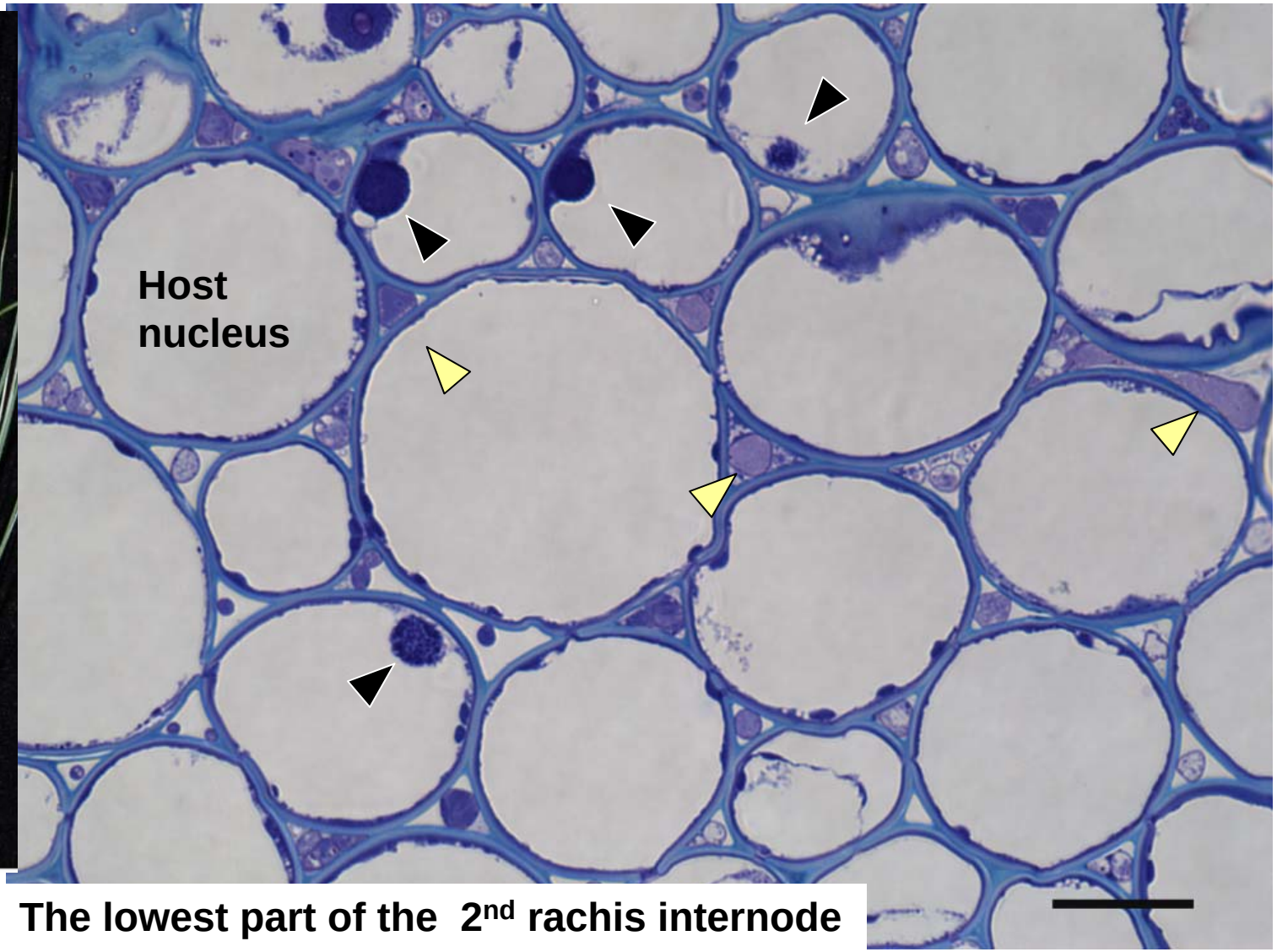
1. Thin sectioning of fixed and plastic embedded ear tissue over a time course (days 2, 5 and 12)
2. Staining sections with Toluidine Blue O
3. Detailed microscopy (~5,000 sections)
4. Cryo SEM analyses

The leading hyphal front is intercellular and advances the infection through the cortex



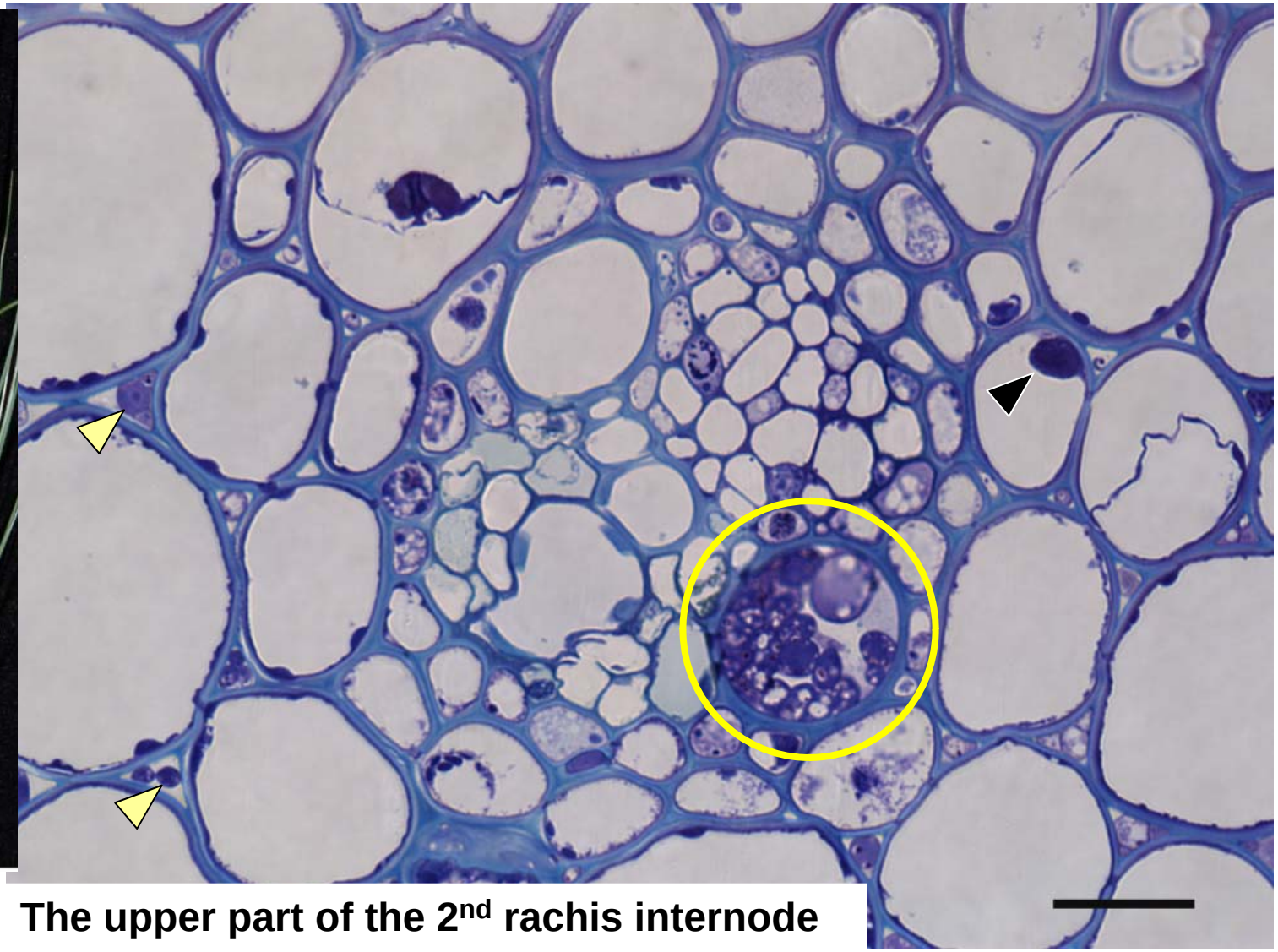
The lowest part of the 2nd rachis internode

Cortical cells of the infected 2nd rachis internode retain their nuclei



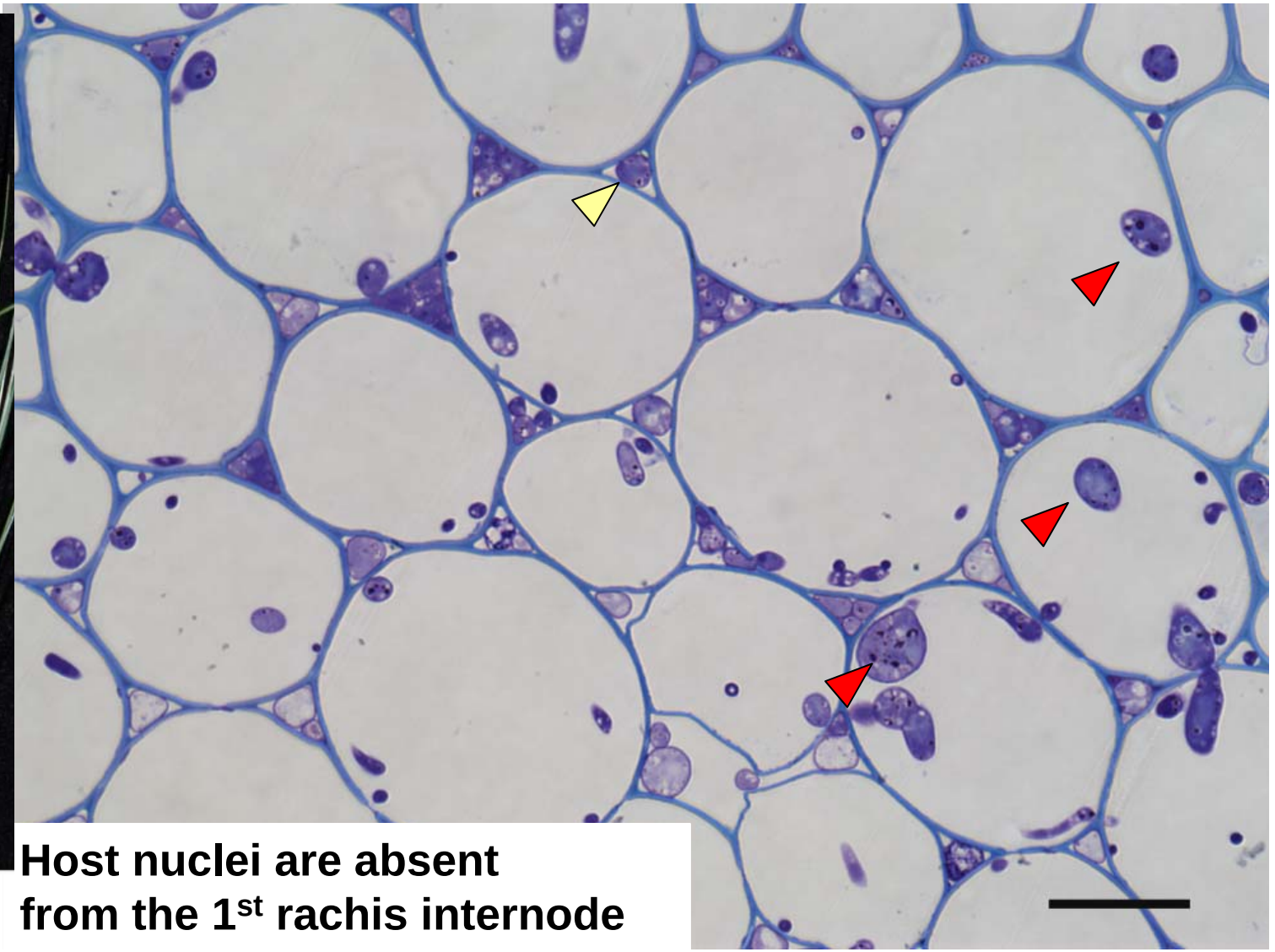
The lowest part of the 2nd rachis internode

Infection of the vasculature is slower and initially limited to just one metaxylem vessel



The upper part of the 2nd rachis internode

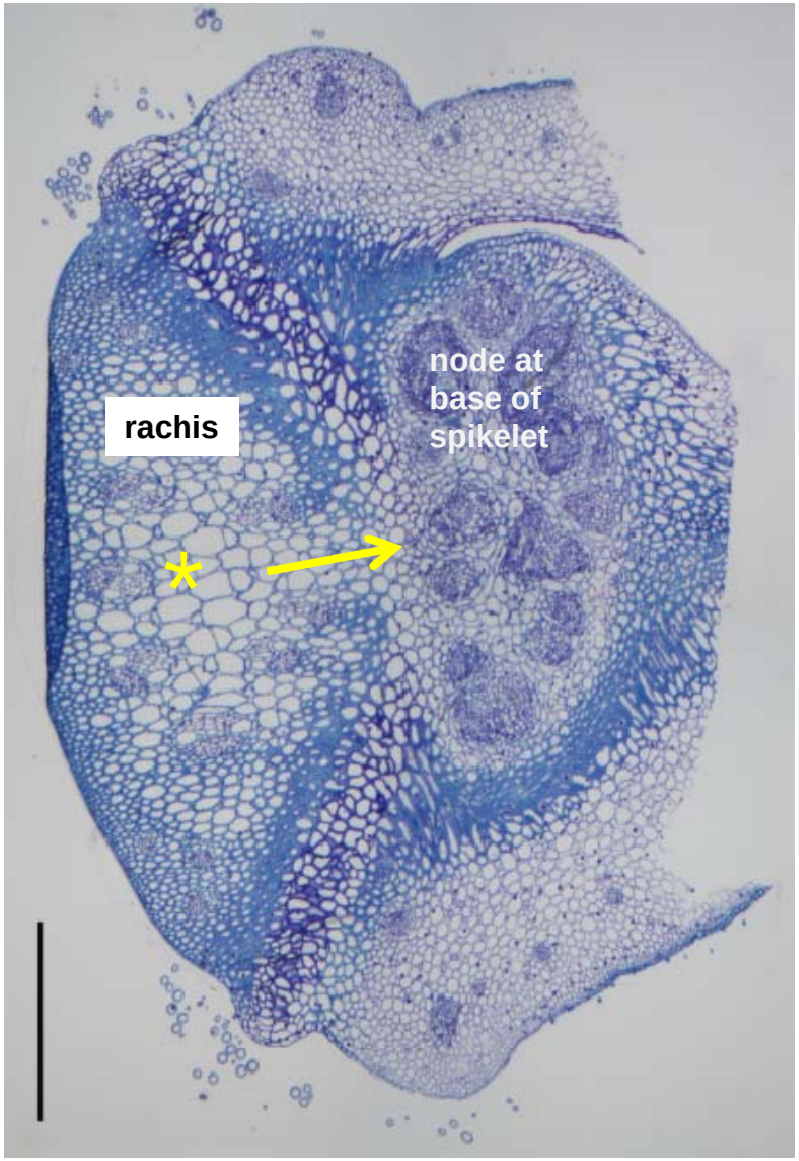
Behind the advancing front intracellular hyphae colonise dead host cells



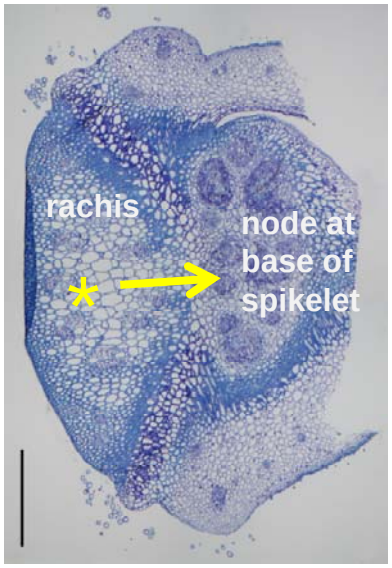
Host nuclei are absent from the 1st rachis internode

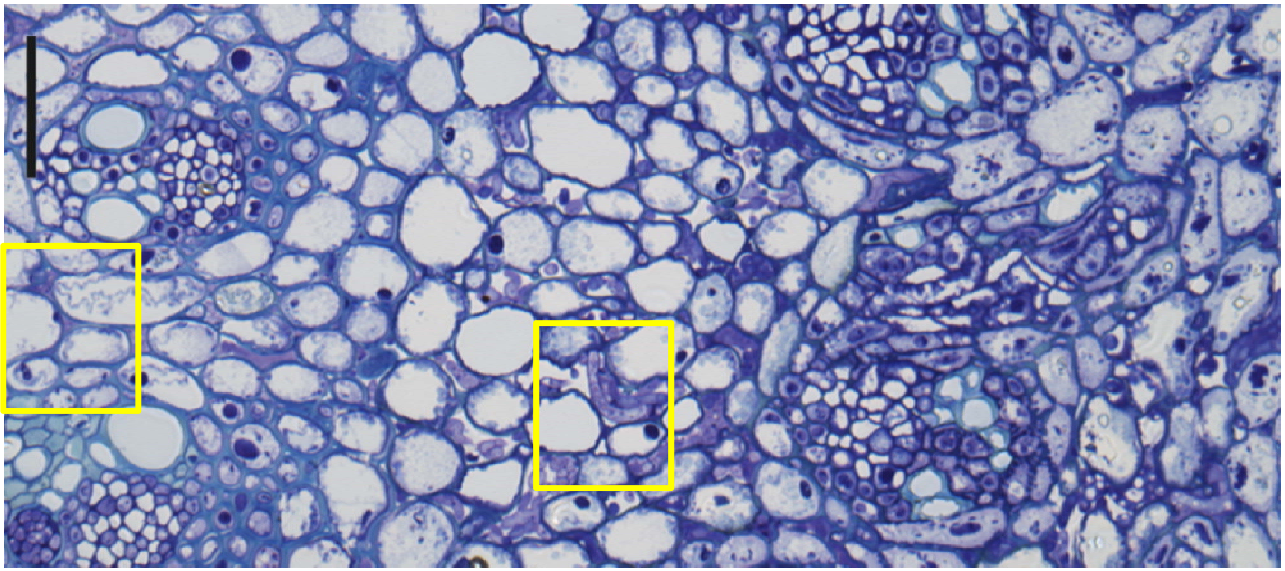
How do hyphae colonise from the rachis into the node of the spikelet ?

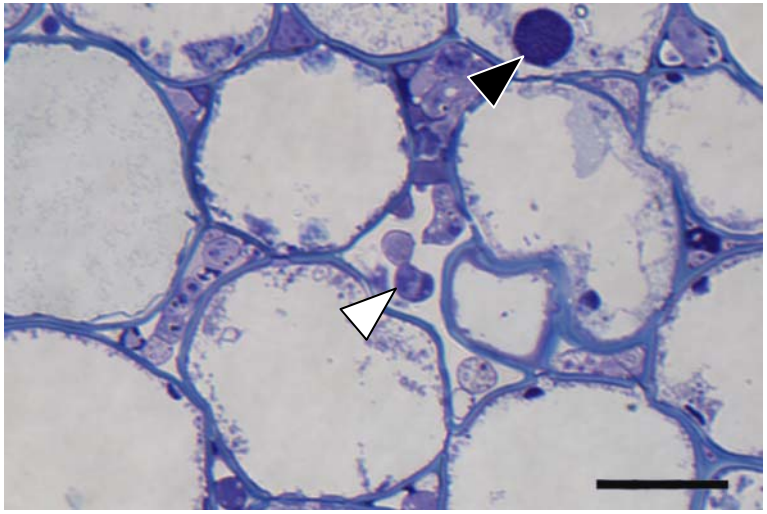
Day 5

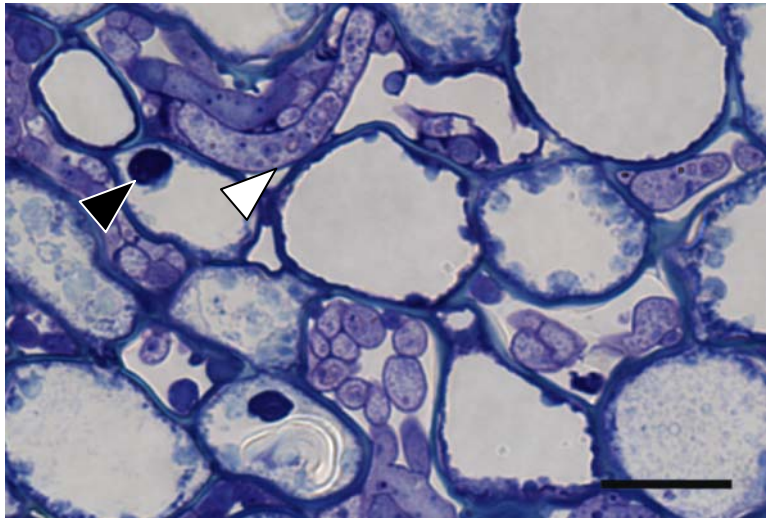


Intercellular hyphae pass from the rachis, between live host cells, into the uninfected node

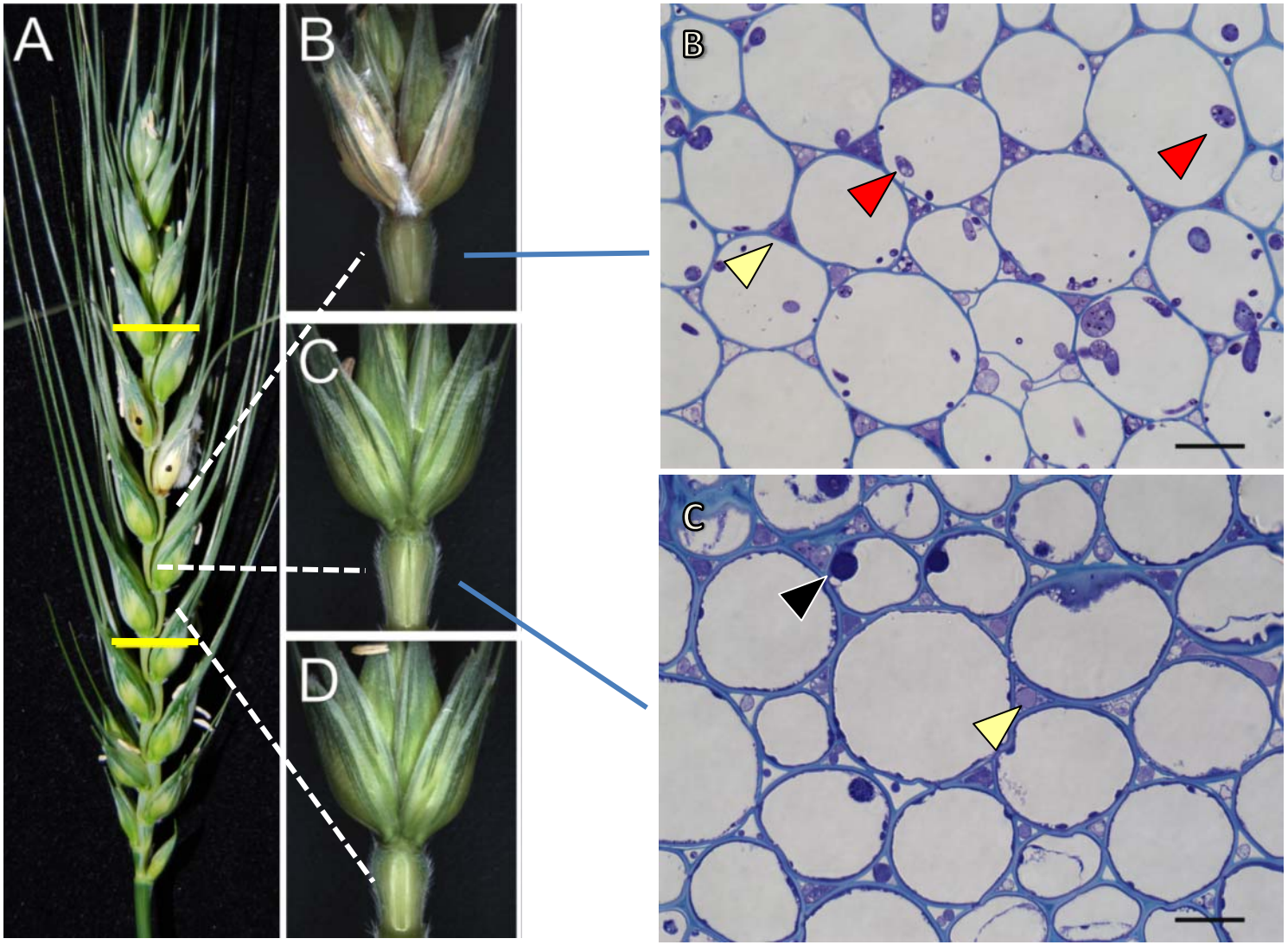








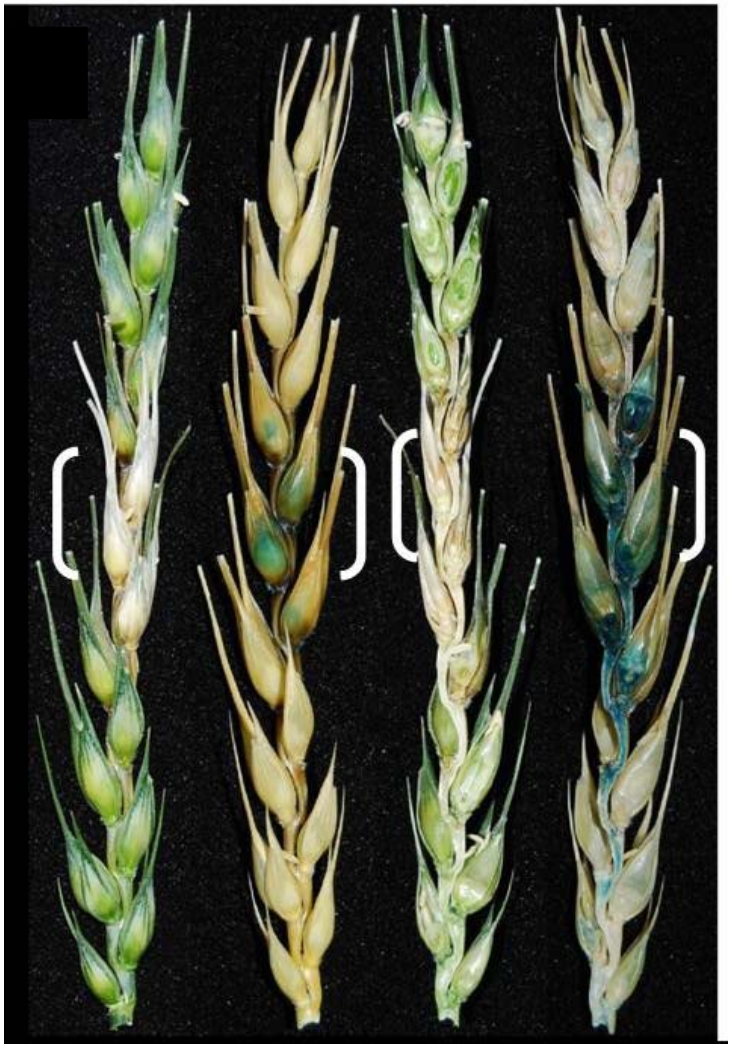
A substantial stage of asymptomatic infection exists



5 days post inoculation (dpi)

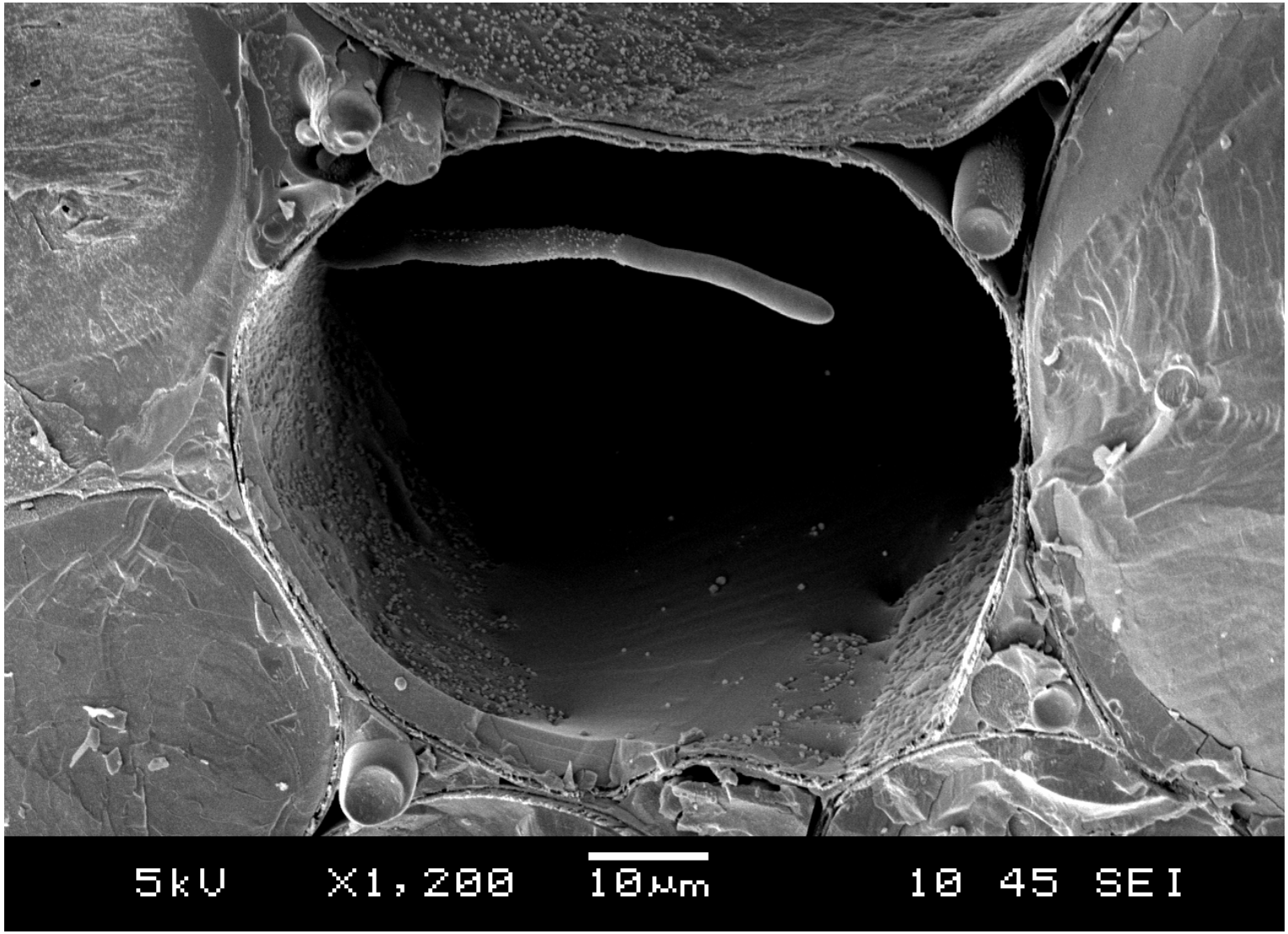
Infection of wheat ears with *Fusarium graminearum* PH-1 constitutively expressing the GUS reporter protein

Day 8

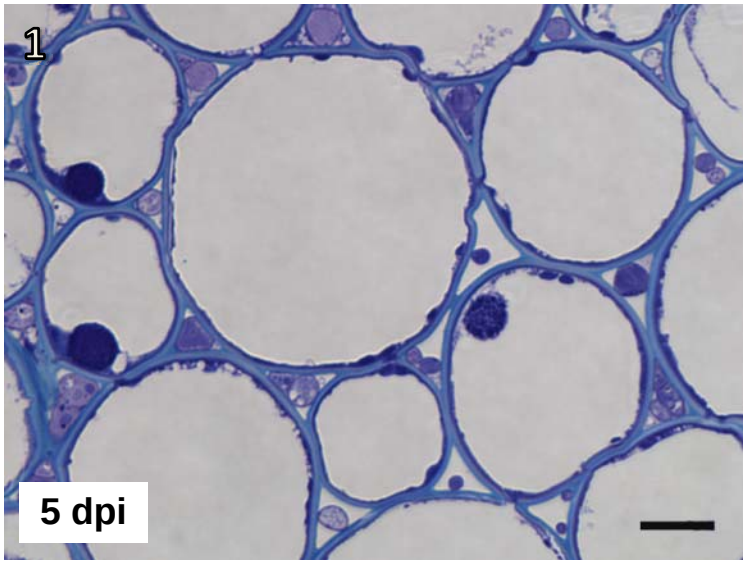


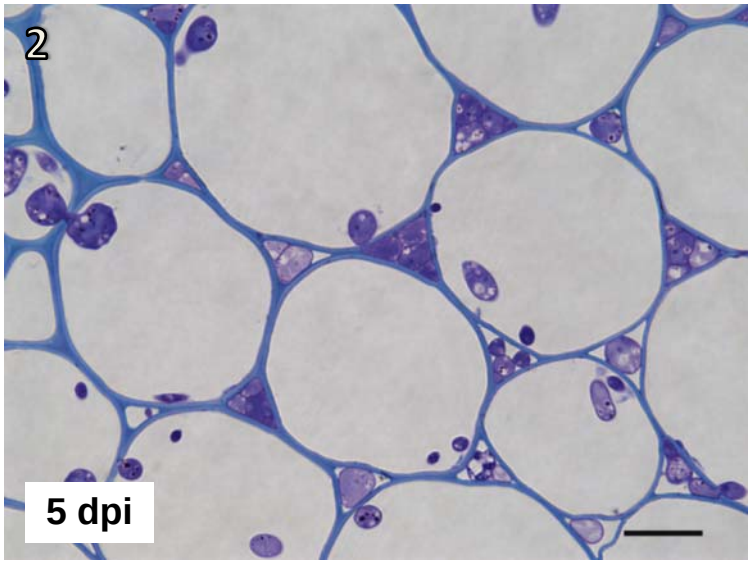
Urban, Baldwin and Bass, unpublished

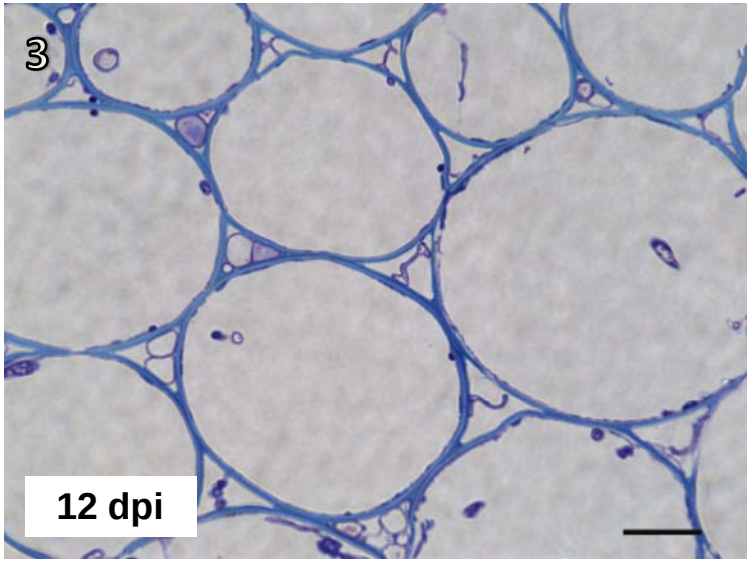
Passing from outside to inside the wheat cell (the inter to intracellular transition)



Development of infection in the rachis cortex: appearance of ‘ghost’ hyphae

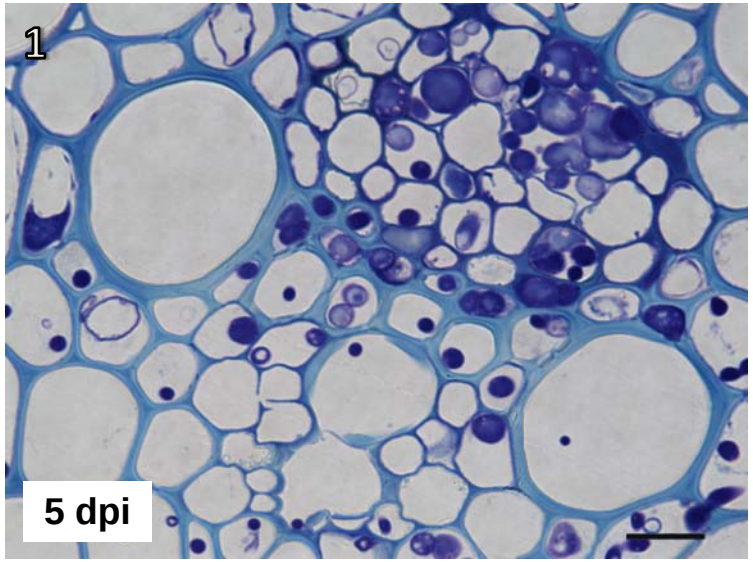


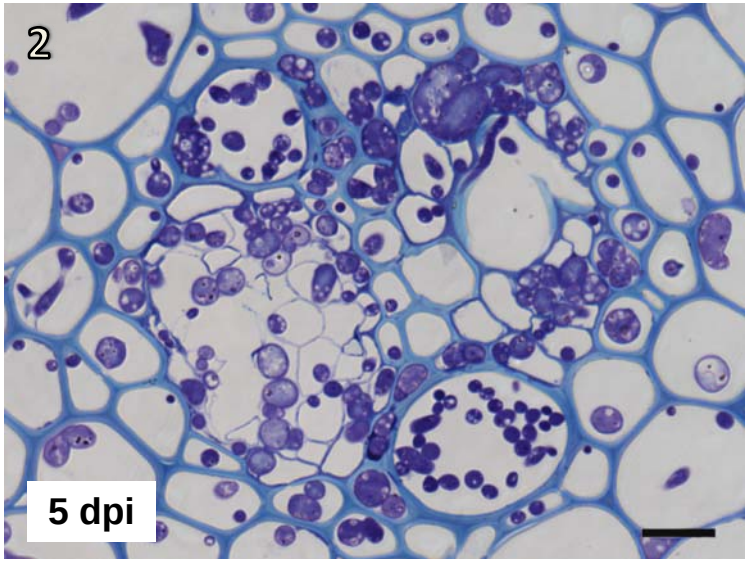


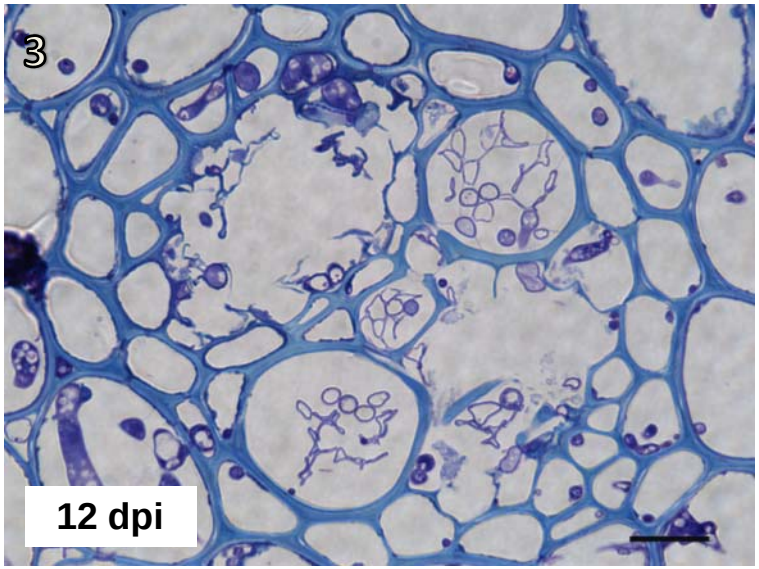


1. Intercellular colonisation, host nuclei
2. Inter- & intracellular colonisation, no host nuclei
3. ‘Ghost’ hyphae behind the infection front

Development of infection in the rachis vasculature – appearance of ‘ghost’ hyphae





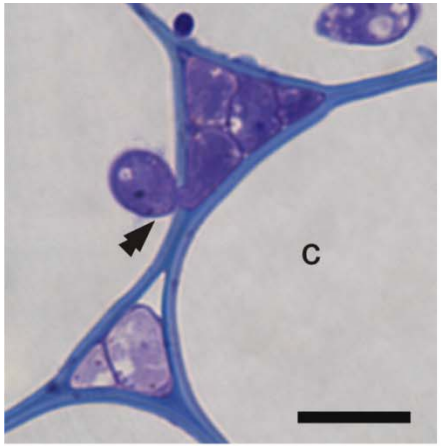


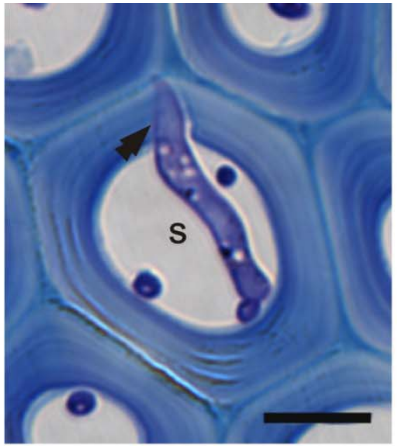
1. Colonise phloem which still contains its fluid content

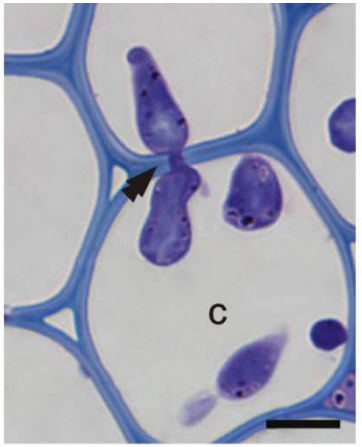
2. Colonise all cell-types, phloem & vascular parenchyma collapse

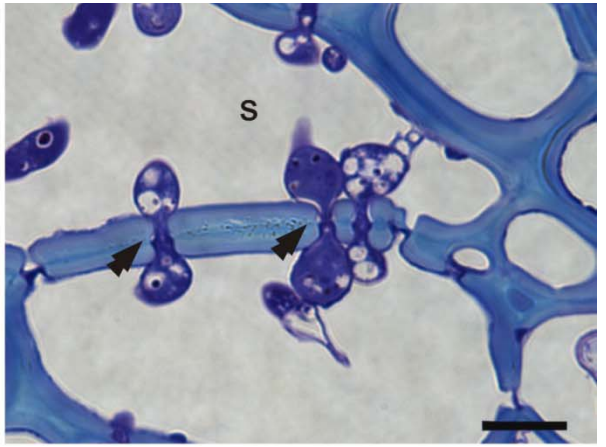
3. Presence of ‘ghost’ hyphae well behind the advancing infection front

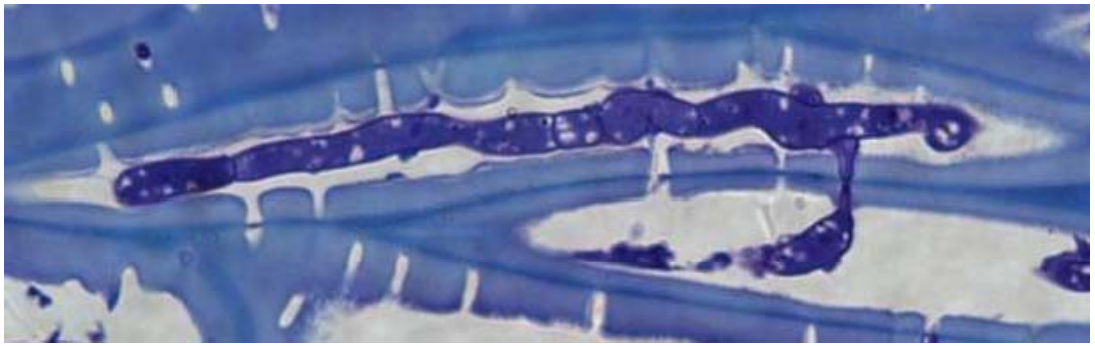
Localised squeezing of hypha through the cell walls

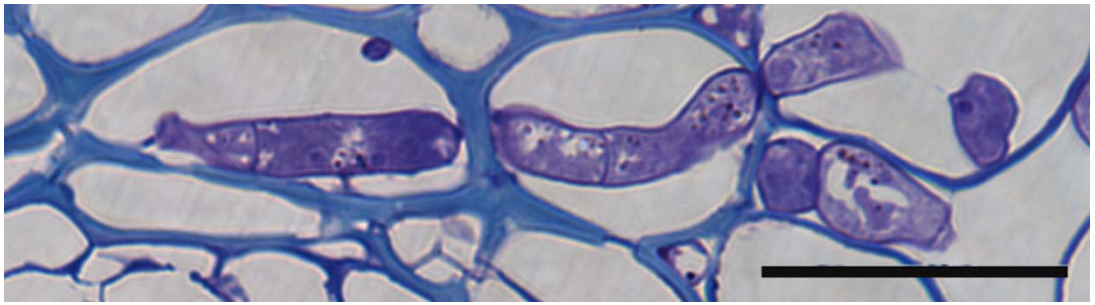


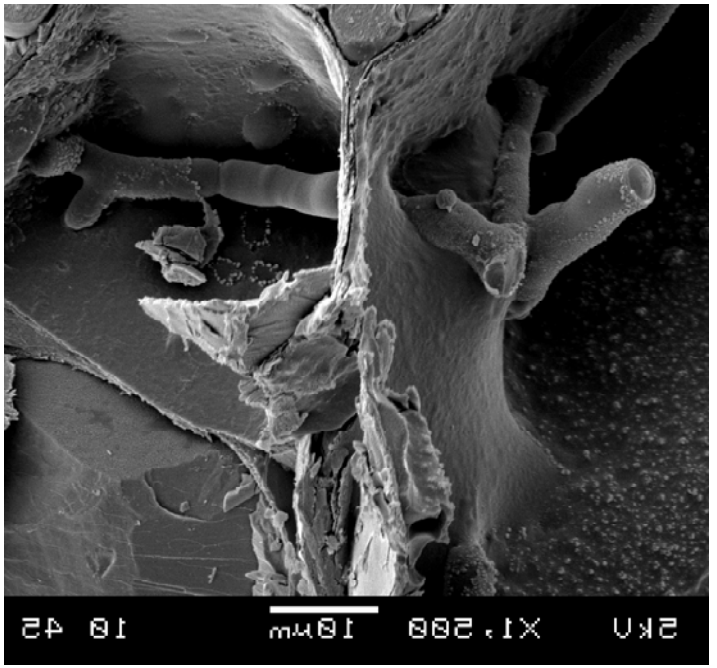




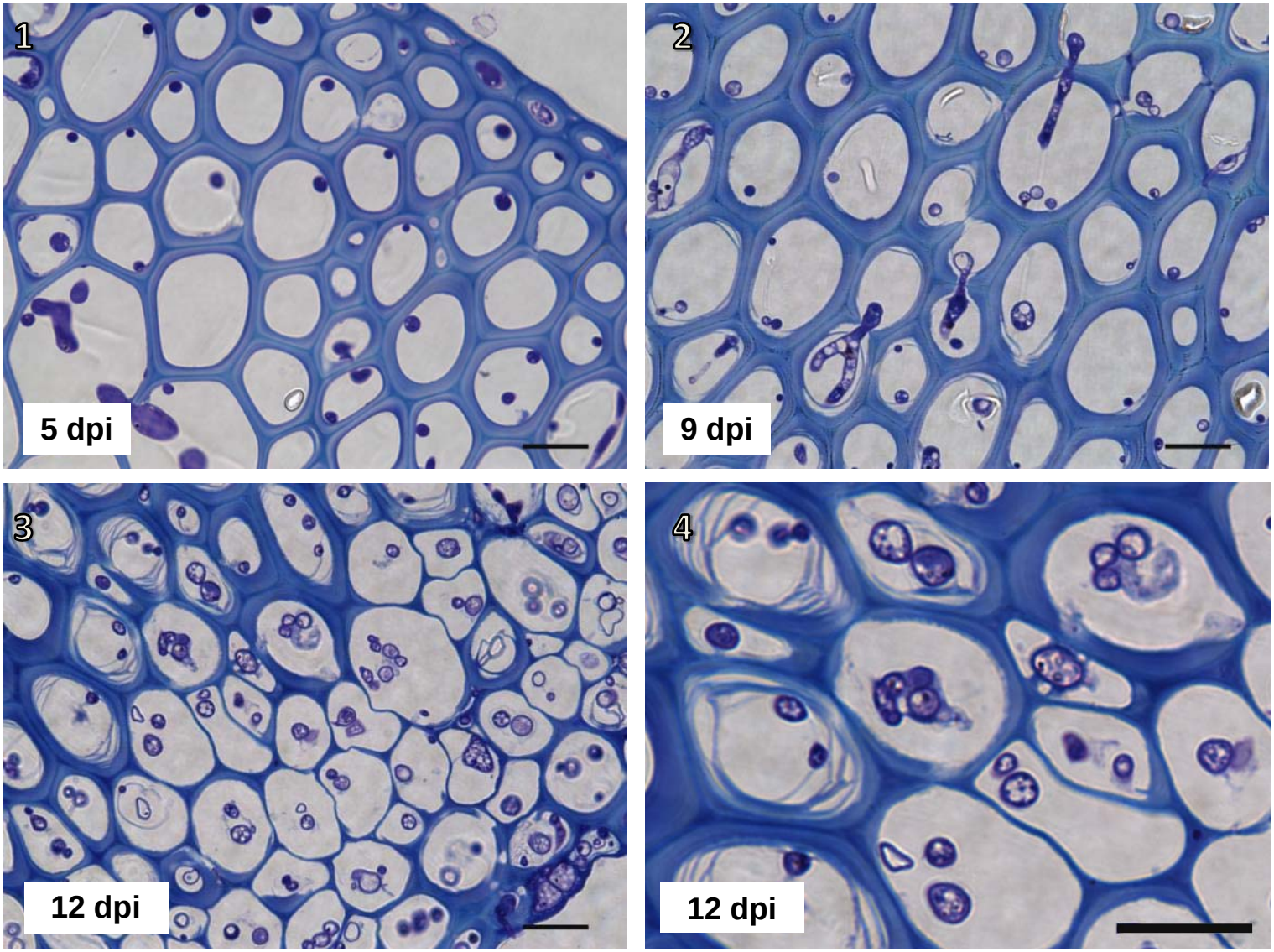






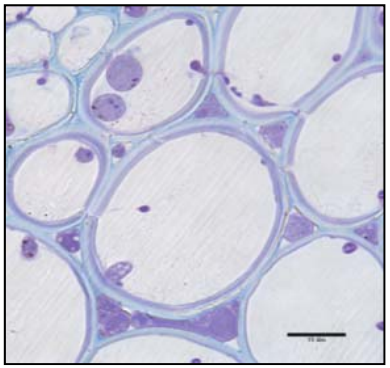


Growth towards the rachis node surface involves the localised internal ‘peeling’ of wheat cell wall



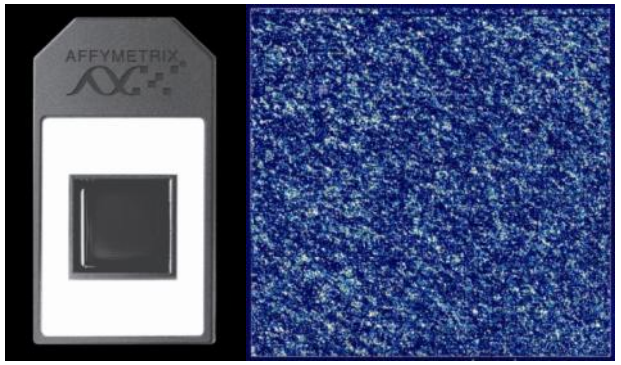
Cell-type specific transcriptomics

Physical progress of infection



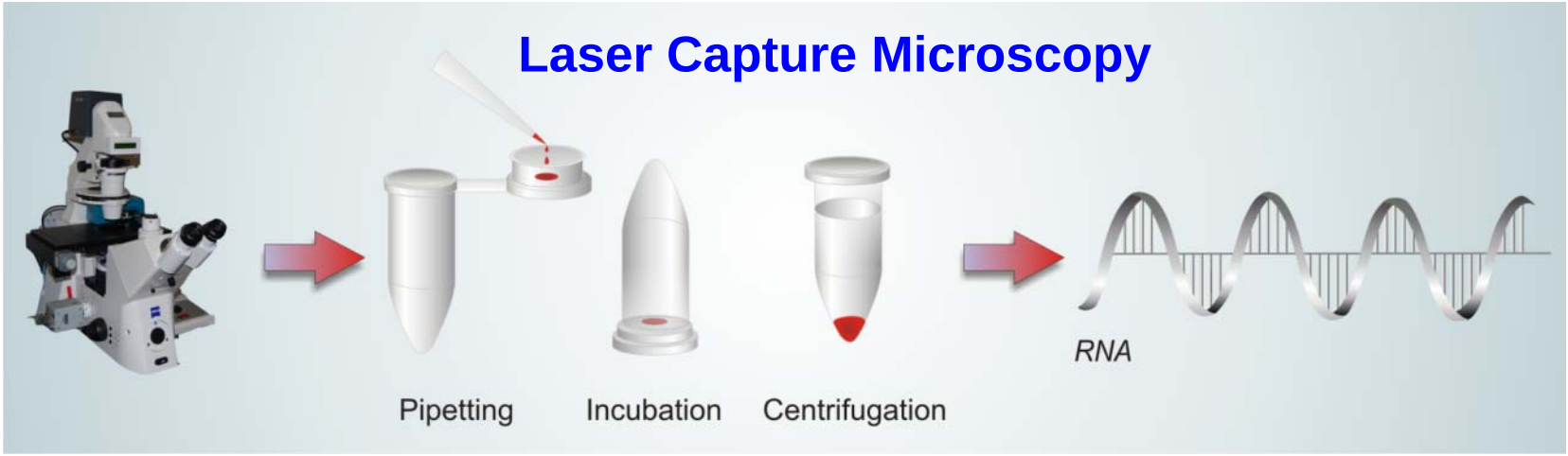
Link

Fusarium Affymetrix Array

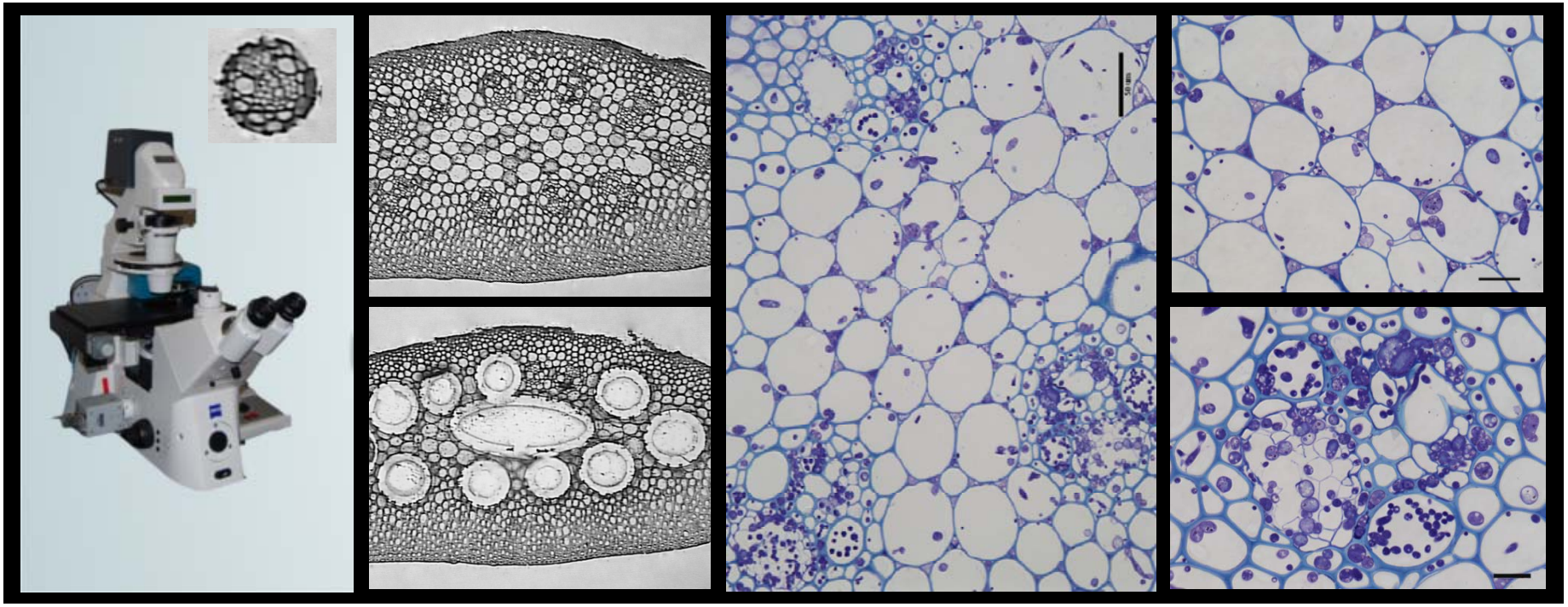


Expression of genes with known & unknown function

Laser Capture Microscopy



Isolation of individual cell-types using Laser Capture Microdissection



Fixation
Cold 75% EtOH:
25% Acetic acid

→

Cryopreservation
Final Sucrose
conc. 0.5M

→

Cryosection
-20^{0}C
10 µm

→

LCM

New knowns and still unknowns

- **Intercellular colonisation leads the infection and not colonisation of the vasculature**
- **Abundant hyphae are present throughout both the symptomless and symptom forming parts of the infection**

A very flexible fungus

Brown et al. (2009) submitted

TALK OUTLINE

- The hyphal infection process:

  From the initial infected spikelet ⟶ rachis

  rachis ⟶ the adjoining spikelets

- **Arabidopsis floral Fusarium – pathosystem:**

  To identify the pathogen and host components which either restrict or support the Fusarium infection process

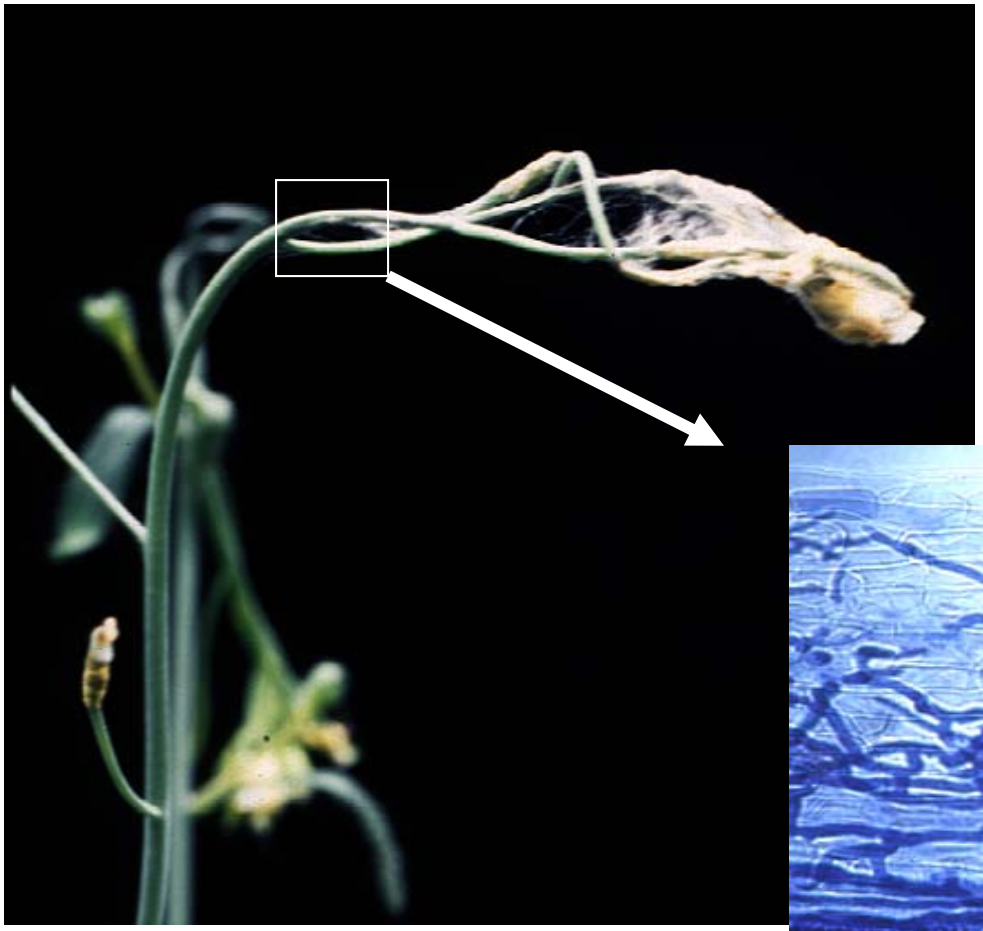
- **Establishing a UK facility for VIGS research in wheat**

Arabidopsis – just in flower

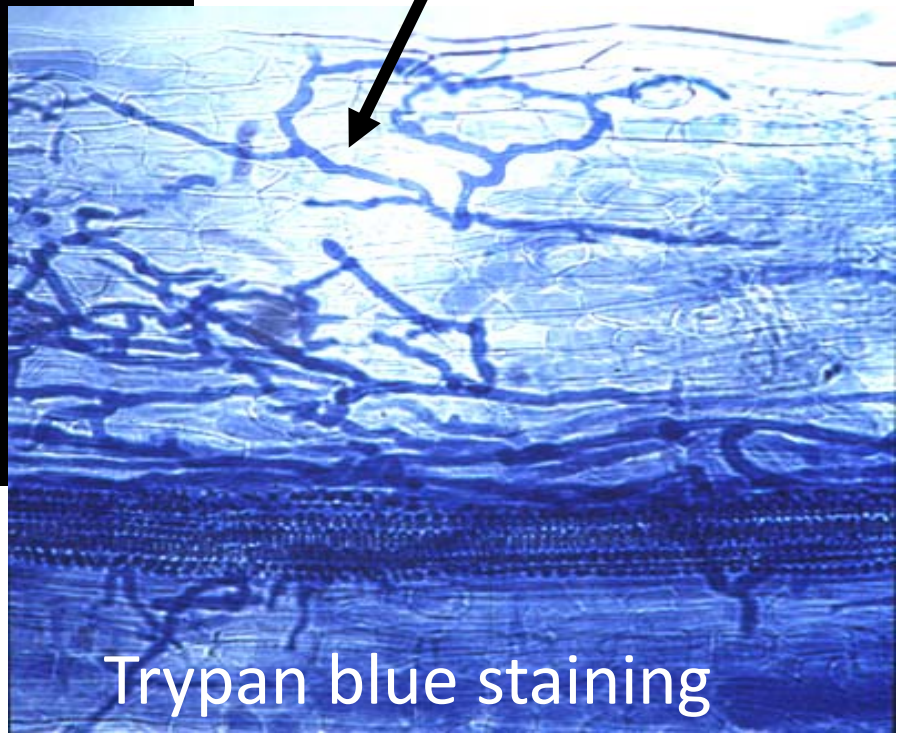
Spray inoculate with fusarium conidia of wheat infecting isolates of either *F. graminearum* or *F. culmorum*

UK field population is currently a 50:50 mix of *Fg* and *Fc* and the majority are 3ADON chemotypes

Arabidopsis - 5 days post floral inoculation



Intercellular hyphae advancing down the stem



HEMIBIOTROPHIC FUNGUS

Urban et al (2002) Plant Journal

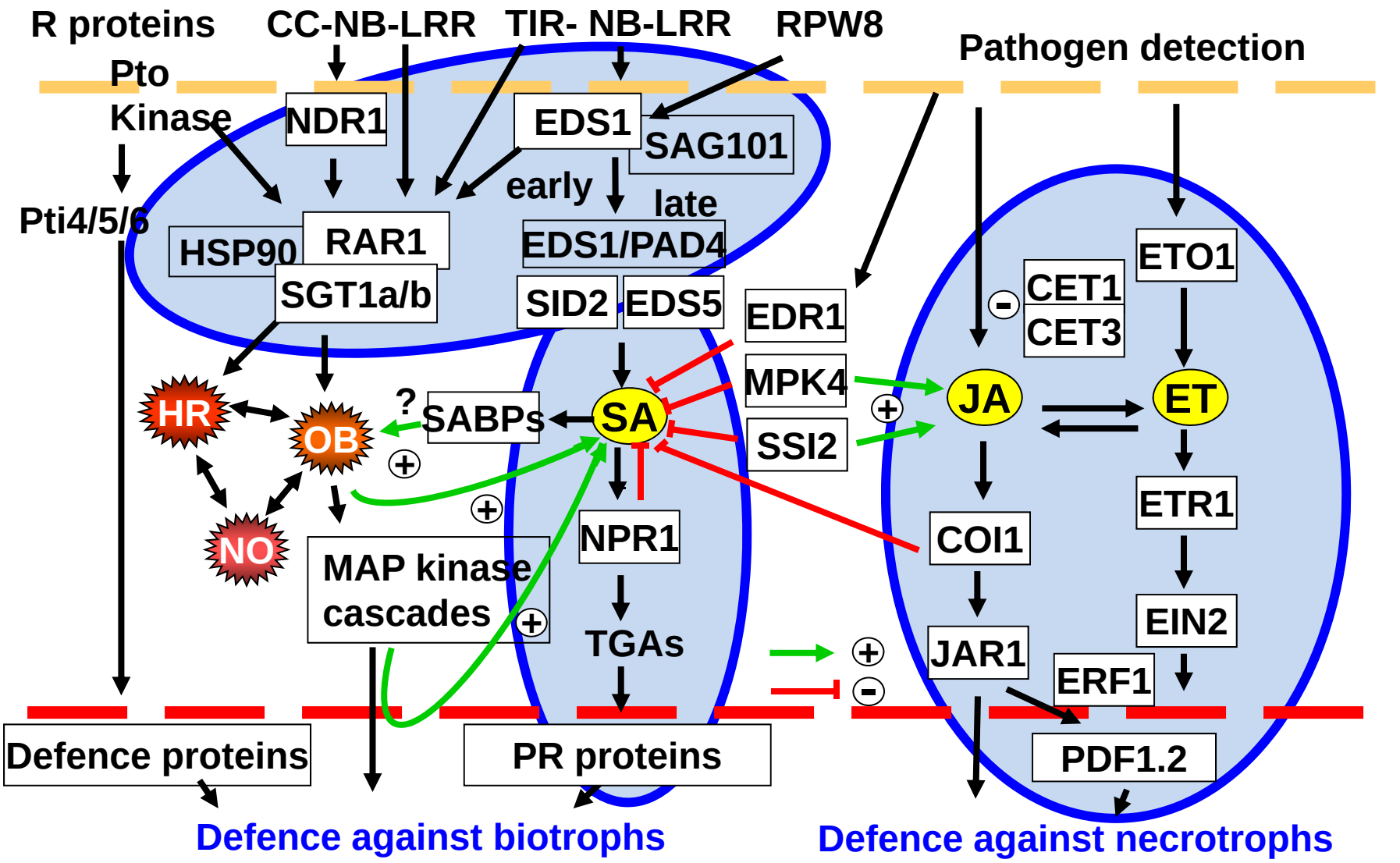
Exploring the Arabidopsis floral – fusarium pathosystem

Screened 240 ecotypes failed to recover extremely resistant or susceptible genotypes

The complete range of defence signalling and defence enhanced mutants to be screened

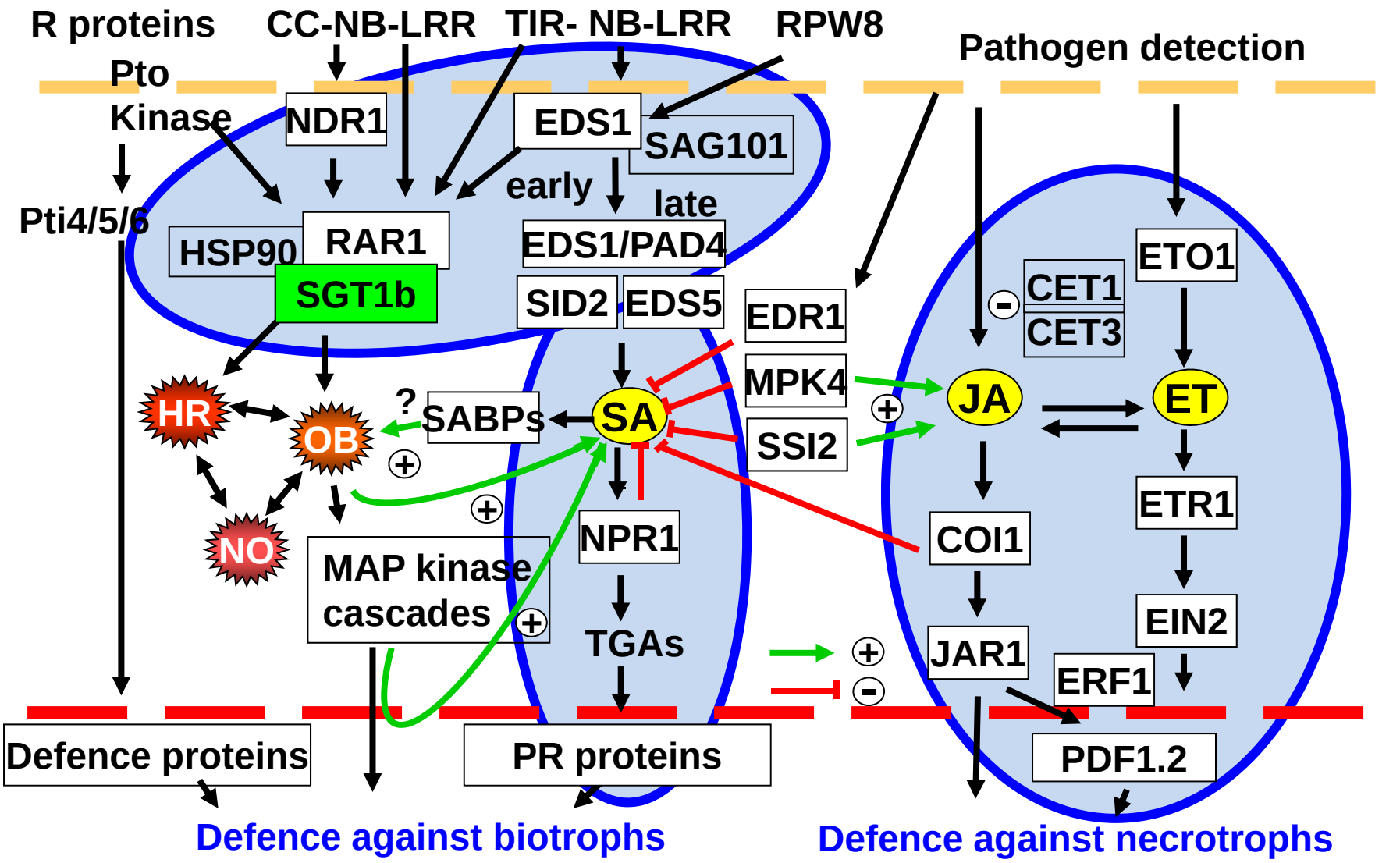
> 140 lines / genes - compromise defence
> 75 lines / genes - enhance defence

Key defence signalling components



Adapted from Hammond-Kosack and Parker, (2003) Curr. Opin Biotechnol. 14, 177-193

Key defence signalling components



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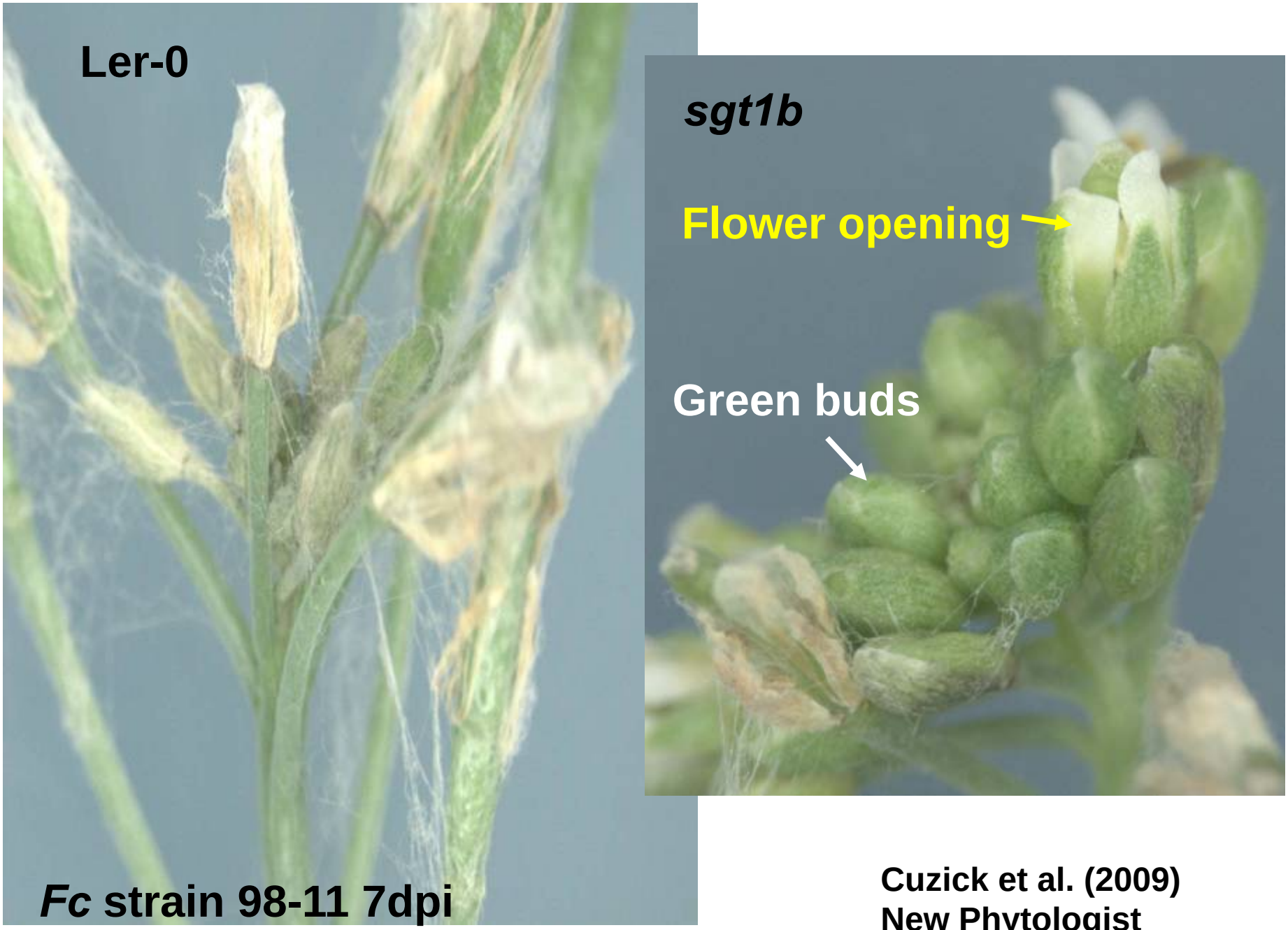
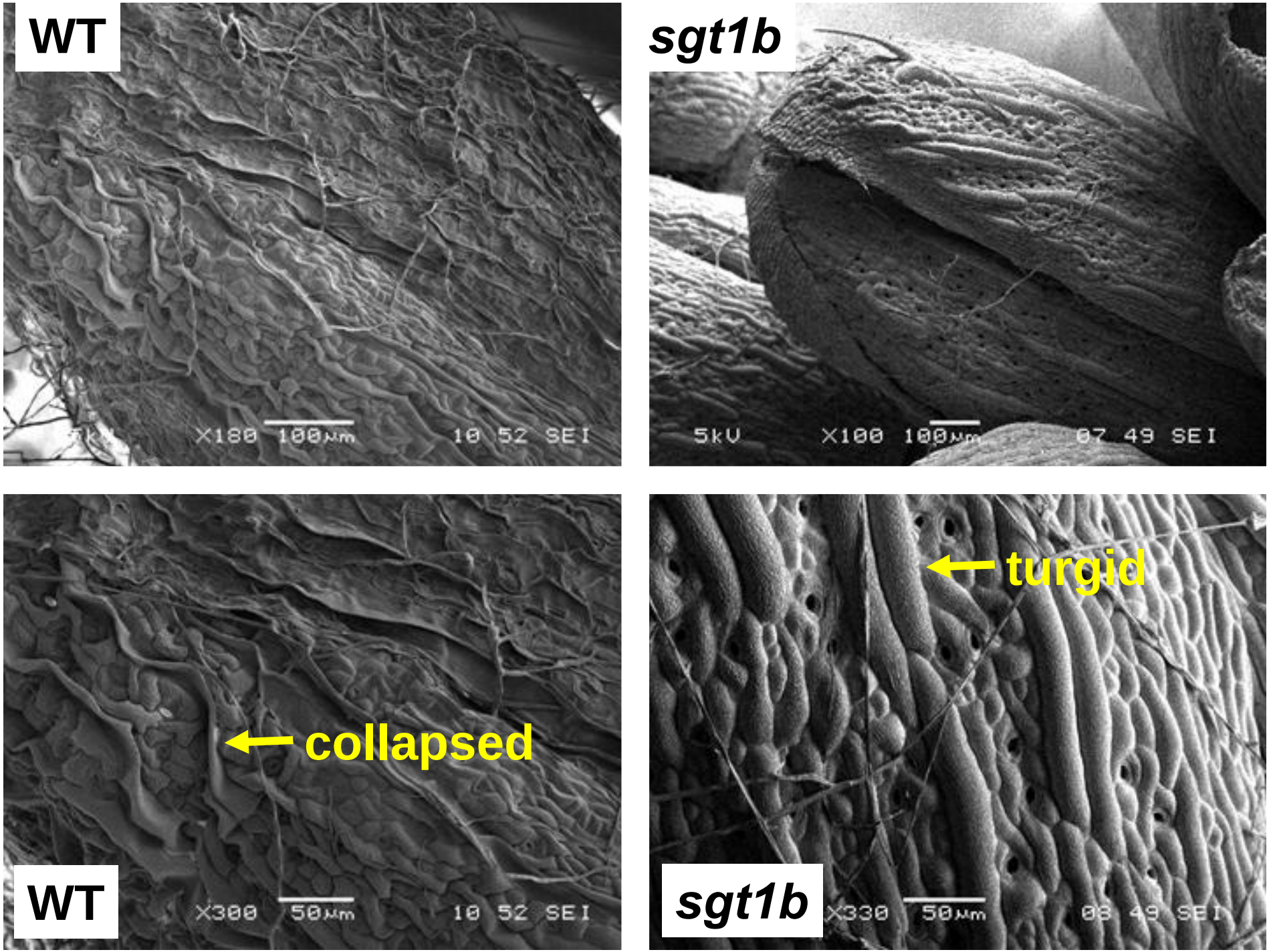
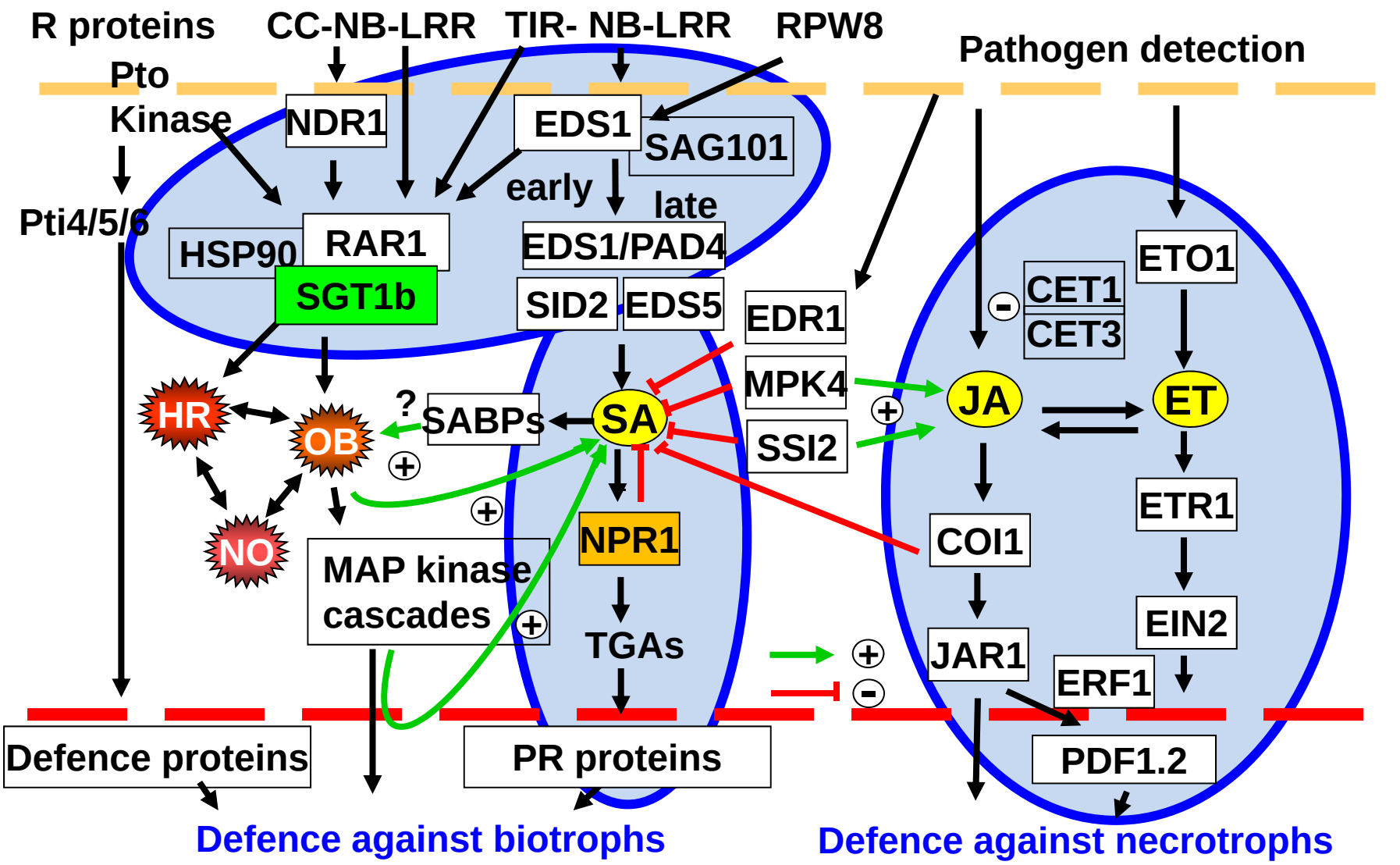


Cuzick et al. (2009)
New Phytologist


WT
X180 100µm 10 52 SEI
sgt1b
5kV X100 100µm 07 49 SEI
collapsed
WT
X300 50µm 10 52 SEI
turgid
sgt1b
X330 50µm 08 49 SEI

Key defence signalling components



Adapted from Hammond-Kosack and Parker, (2003) Curr. Opin Biotechnol. 14, 177-193

NPR1 is important for wheat ear defence

Genetically Engineered Resistance to Fusarium Head Blight in Wheat by Expression of *Arabidopsis NPR1*

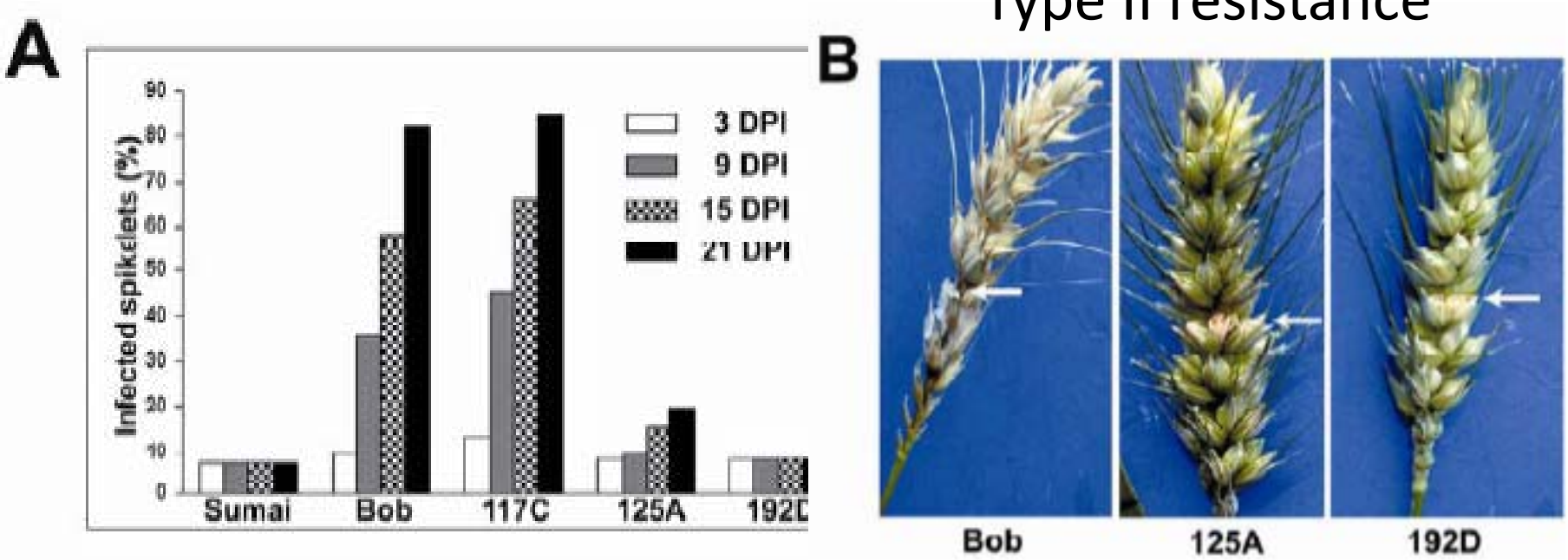
Ragiba Makandar,[1] Juliane S. Essig,[2] Melissa A. Schapaugh,[2] Harold N. Trick,[2] and Jyoti Shah[1,3]

[1]Division of Biology, [2]Department of Plant Pathology, and [3]The Molecular Cellular and Developmental Biology Program, Kansas State University, Manhattan 66506, U.S.A.

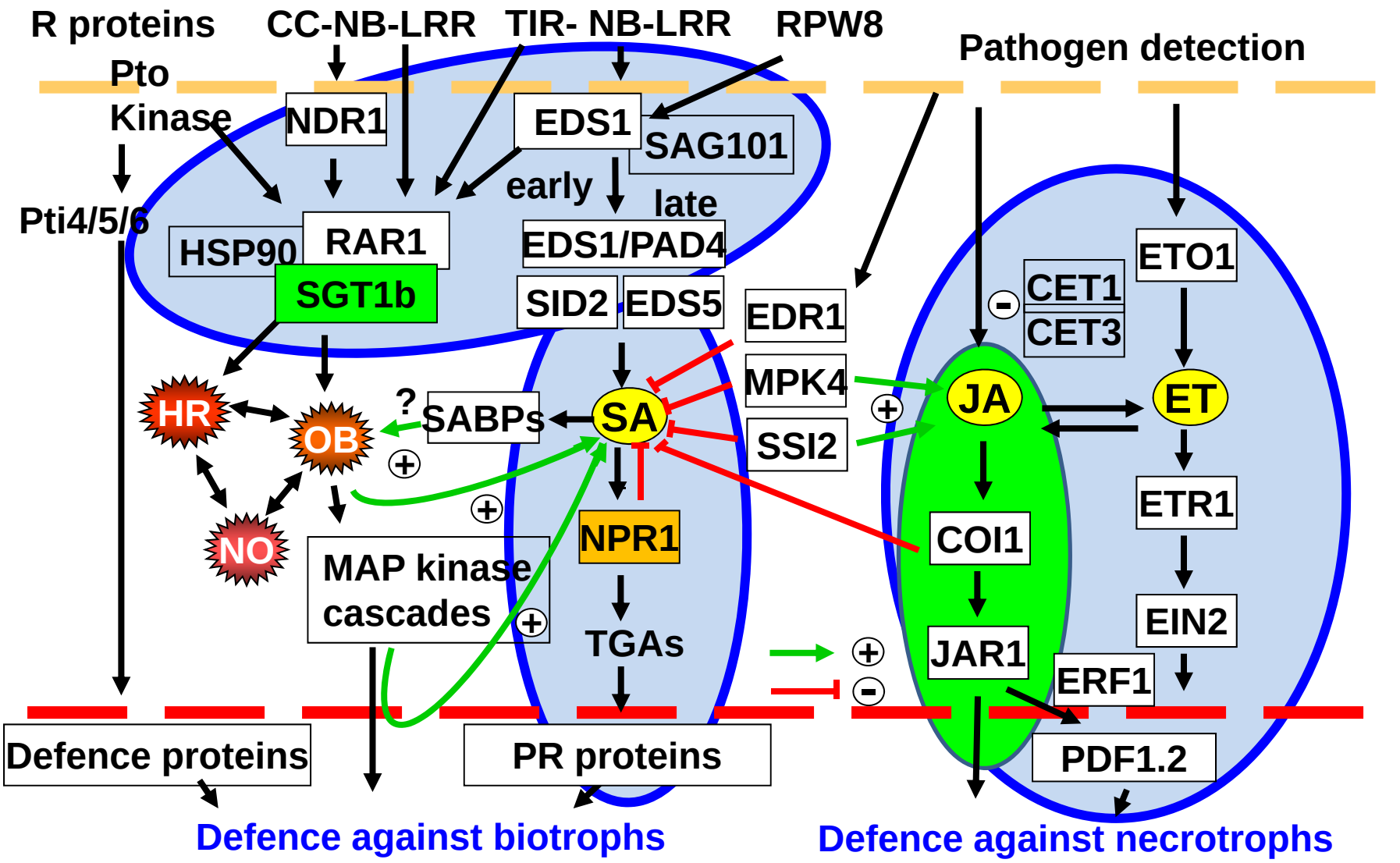
Submitted 11 August 2005. Accepted 11 October 2005.

Mol. Plant Microbe Interact. (2006) 19, 123-129

Type II resistance

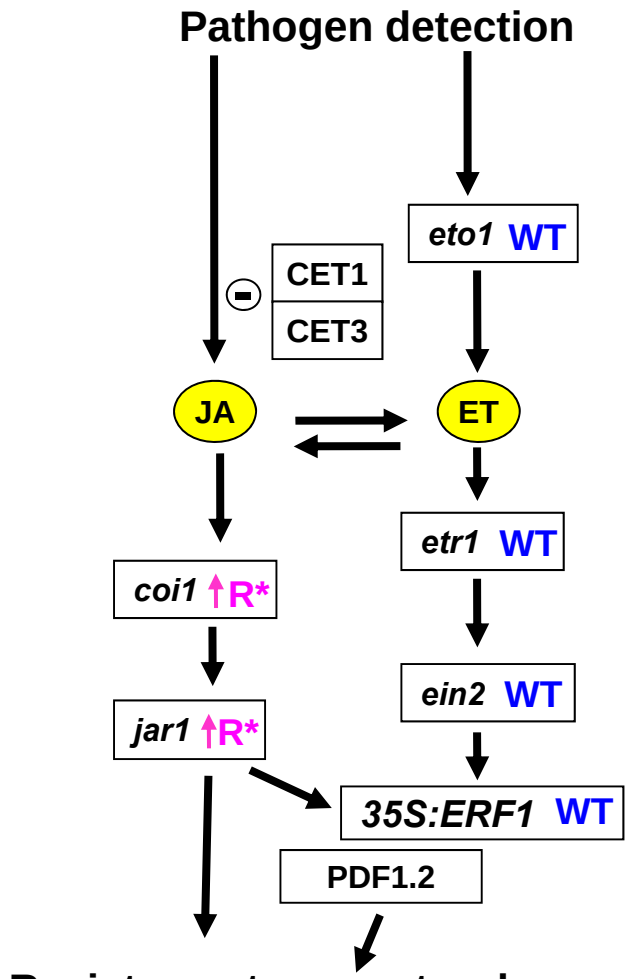


Key defence signalling components



Adapted from Hammond-Kosack and Parker, (2003) Curr. Opin Biotechnol. 14, 177-193

Neither the ethylene defence signalling pathway or combined ET / JA pathways are involved



Cuzick et al (2008) Mol Plant Path

REML data analysis

Arabidopsis	Flower Mean (SEM)	P-value	N
WT (Col-0)	1.85 (0.27)	-	244 (19)
coi1	0.15 (0.57)	0.006	43 (4)
jar1	0.83 (0.46)	0.039	77 (5)

WT *coi1-16*

↑R*

- JA mutants often male sterile
- Long inflorescence →disease escape

JA alone results so far inconclusive

The unknown-*eds11*

- **Forward genetics 'look-see' experiment**

→ ***eds11* more disease than wt (Col-0)**

- **EMS mutant selected for enhanced basal disease susceptibility to virulent bacteria (Volko *et al*., (1998) *Genetics*, 149, 537-548)**

REML data analysis

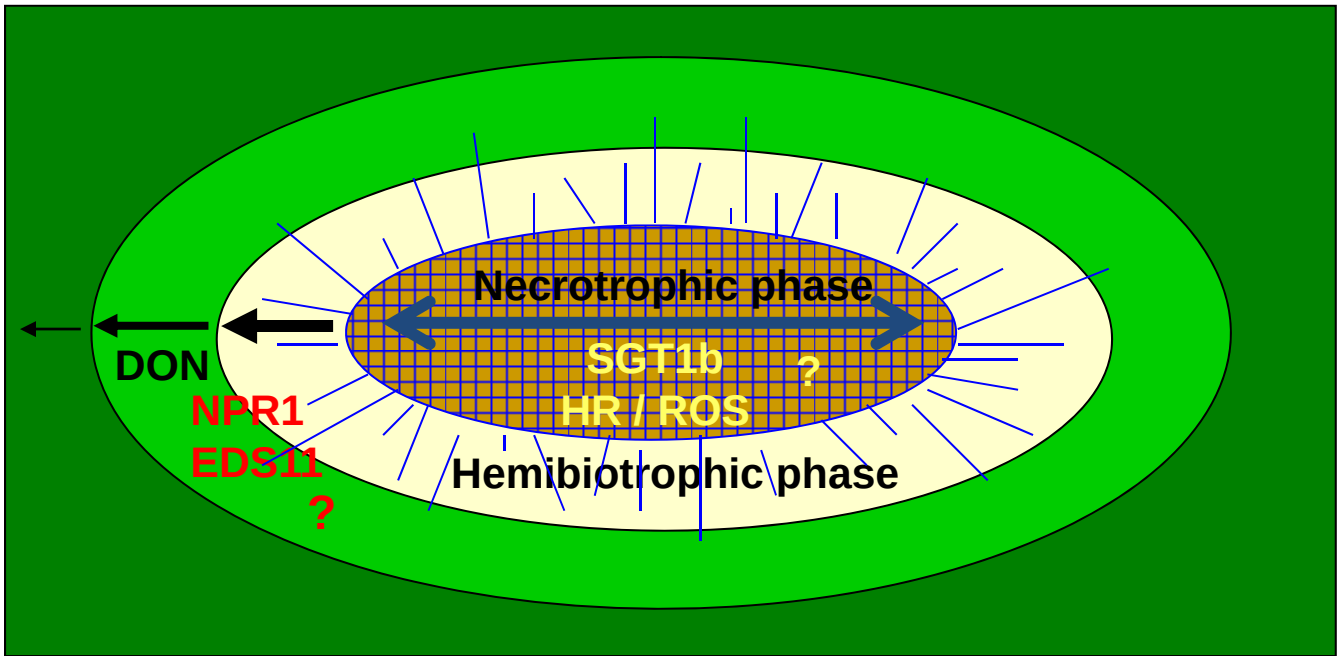
Organ	Mean (SEM)		P-value
	WT (Col-0)	*eds11*	
Flower	1.85 (0.27)	3.62 (0.48)	0.0008
New silique	1.90 (0.24)	2.74 (0.41)	ns
N	244 (19)	92 (5)	

Cuzick et al (2008) Mol Plant Path

SUMMARY and WORKING MODEL

- Both infections cause severe necrosis
- Hyphae are in front of the necrosis in both species
- DON in advance of hyphae in wheat
  - Arabidopsis not known

Working model



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  rachis → the adjoining spikelets

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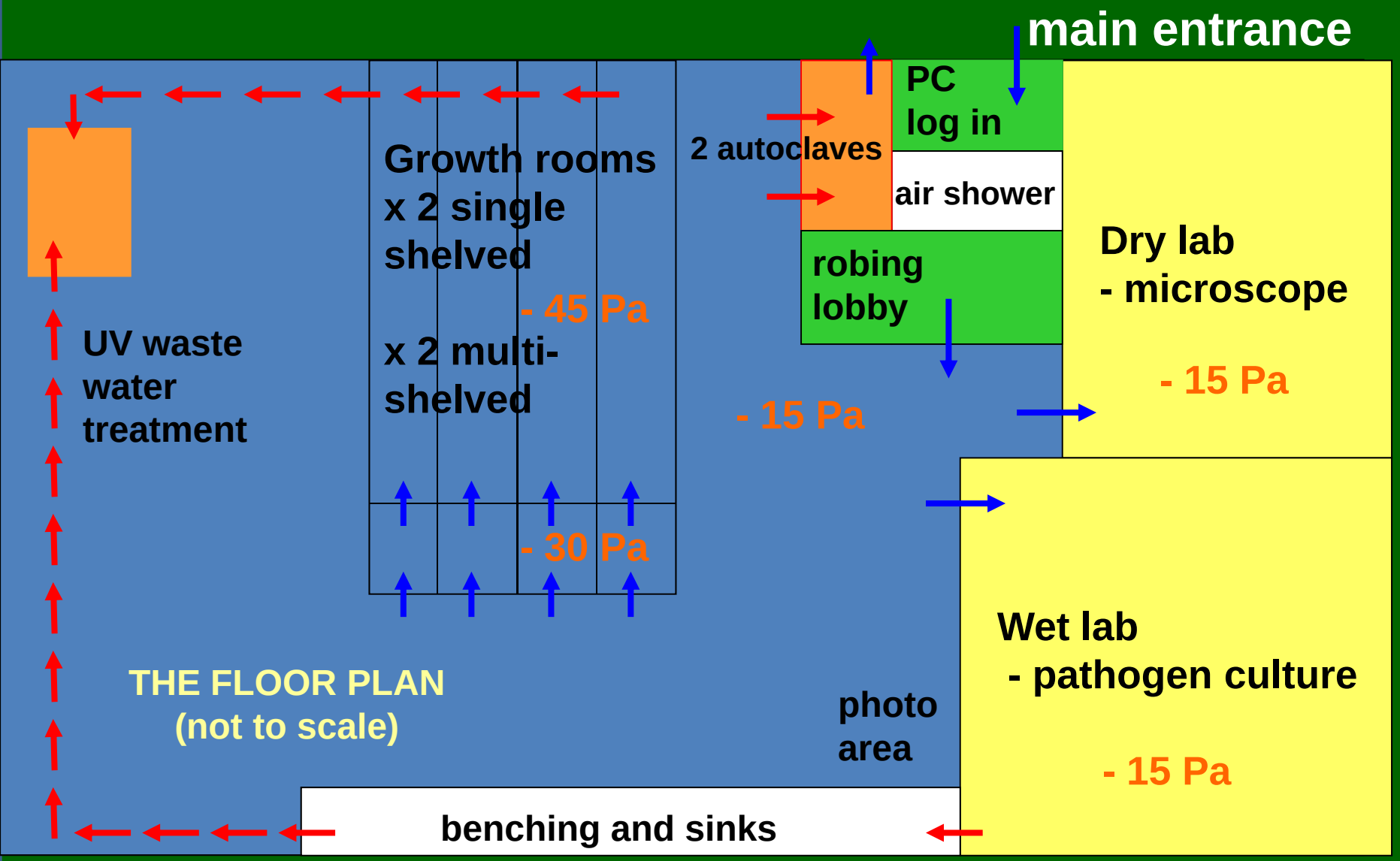
- **Establishing a UK facility for VIGS research in wheat**

July 2007

THE JENKINSON BUILDING (B63)

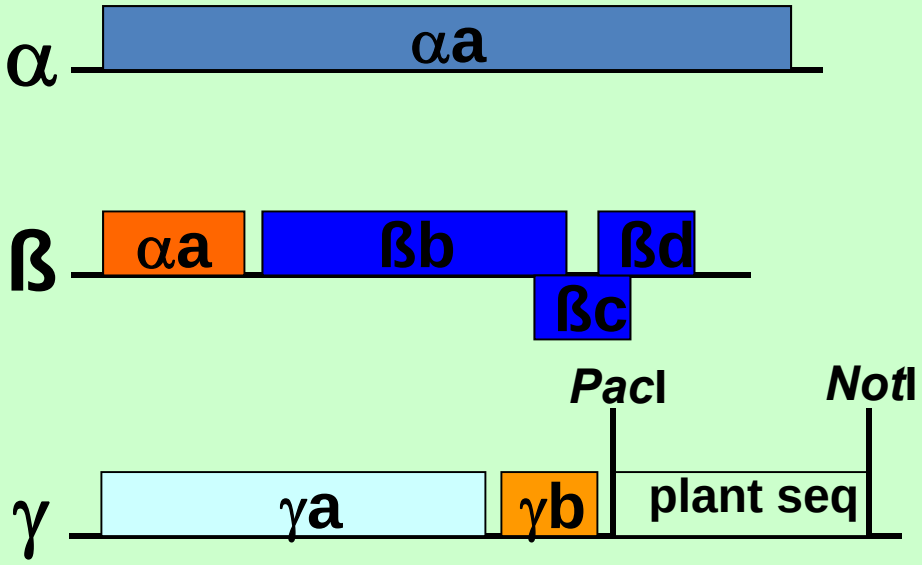
Rothamsted Research – 15 miles north of London, England

A Category 3 Containment Facility



(Hammond-Kosack, Urban, Jing and Kanyuka *et al*. (2009) 5 page pdf
+ peer reviewed manuscript in preparation for Letters in Applied Microbiology

Virus-induced RNA silencing: to assess the function of gene families, single genes and allele variants



Barley stripe mosaic virus

Barley - Hein et al. (2005) *Plant Physiology* 138: 2155–2164
Wheat - Scofield et al. (2005) *Plant Physiology* 138: 2165–2173

VIGS in wheat floral tissue to explore Fusarium fungal infection

Inoculation of flag leaf

Scofield (unpublished)

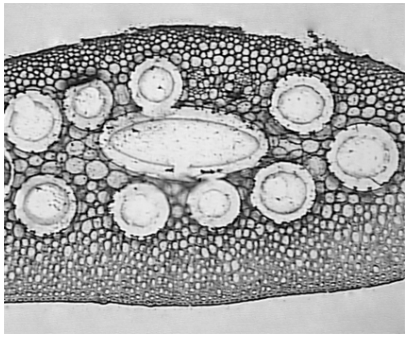
Susceptible

Resistant

Assess gene function
in the ear

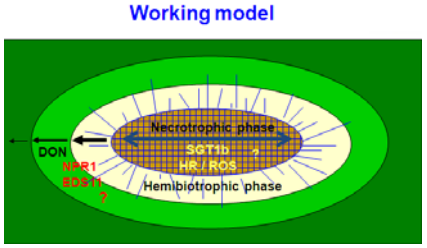
Current Research Approach

Arabidopsis leads



LCM captured wheat ESTs + bioinformatics

Other gene candidates from the literature



↓

VIGS testing

↓

For the best gene leads – generate and test stable transgenic wheat lines +/- *Fhb1* gene

Many thanks to

ROTHAMSTED RESEARCH

Fusarium research

Martin Urban
Neil Brown
John Antoniw
Amy Freeman
John Baker
Jane Ward

Former lab members

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Noemie Desmouceaux
Kerry Maguire
Thomas Baldwin
Chris Bass
Will Allwood
Sarah Holdgate
Arsalan Daudi

Cat3 - VIGs

Martin Urban
Juliet Motteram
Sam Lee
Kostya Kanyuka

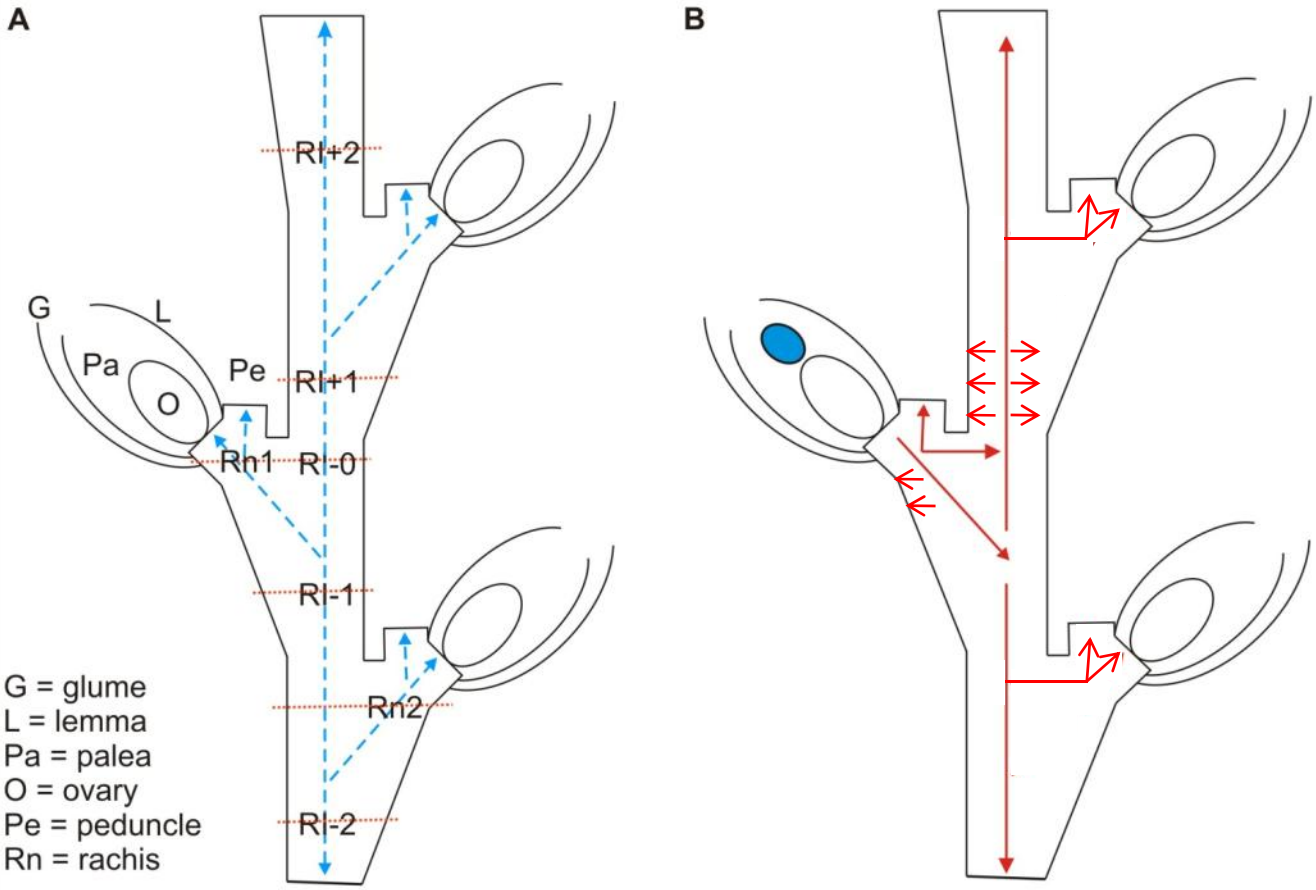
MeT-RO

The many members of global Fusarium community for providing isolates for metabolomics analyses and for bioinformatics analysis of the various fusarium genomes

BBSRC bioscience for the future

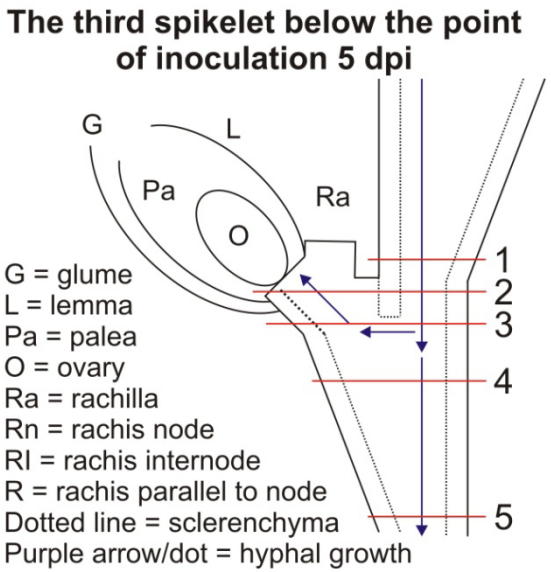
syngenta

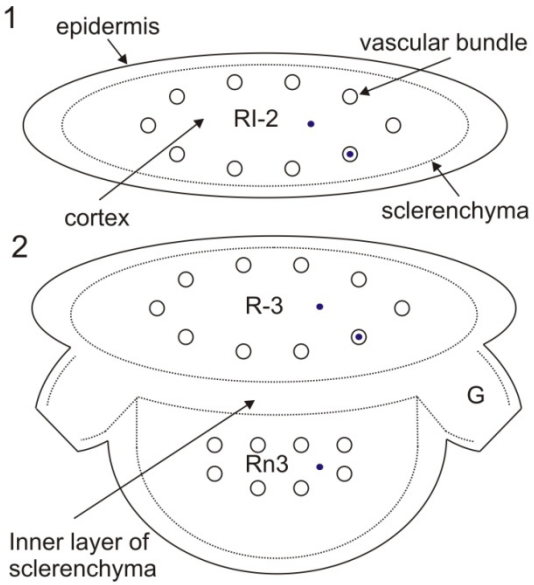
Spikelet to spikelet spread of *F. graminearum* infection

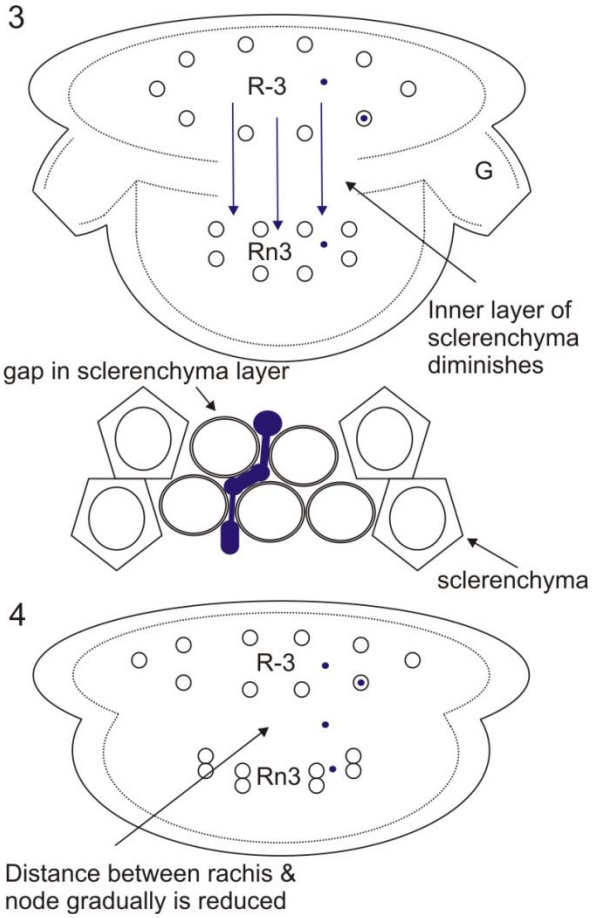


Brown *et al.*, 2009. Fungal Biology (Submitted)

The gap is the primary route of entry from the rachis into the next spikelet

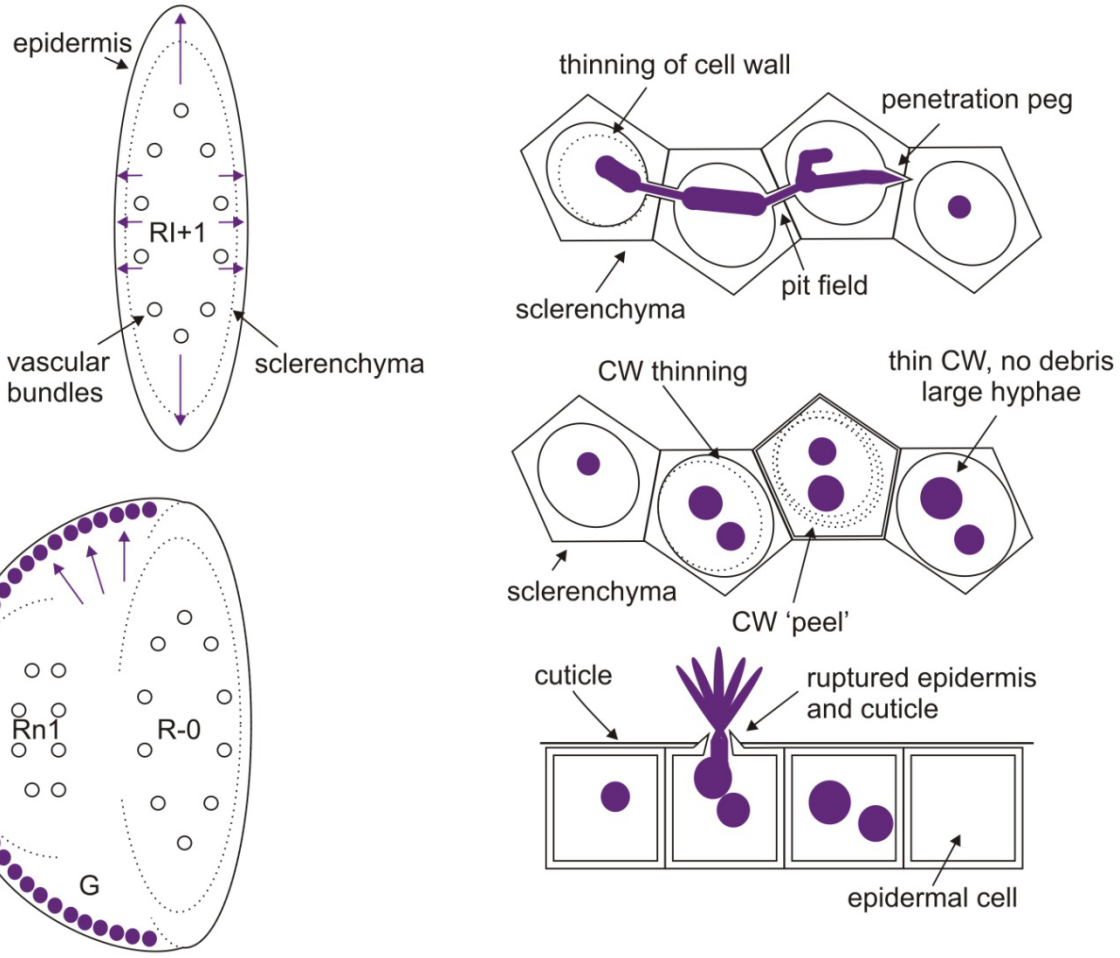






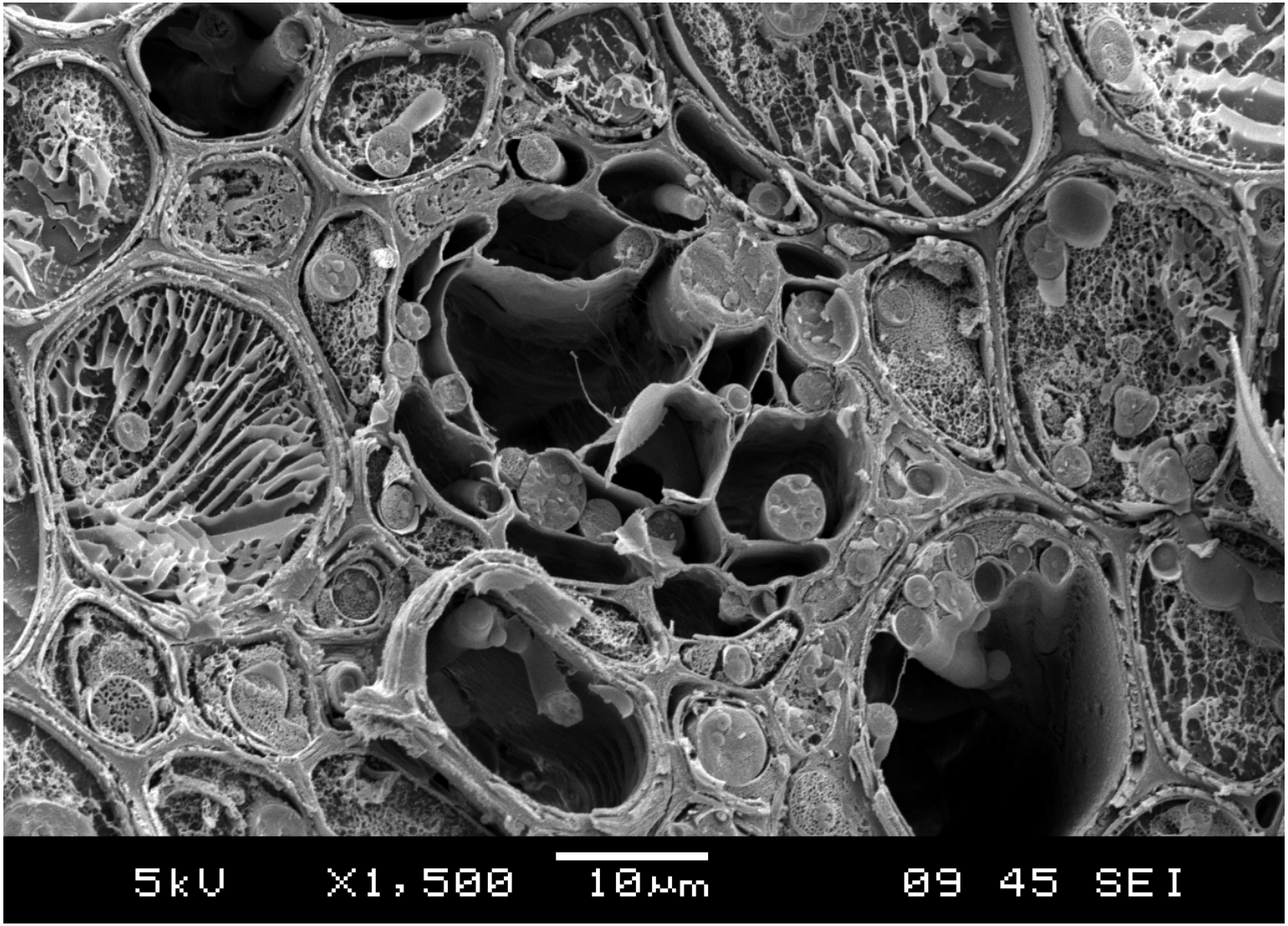
Brown *et al.*, 2009. Fungal Biology (Submitted)

F. graminearum increases radial growth and ruptures the surface

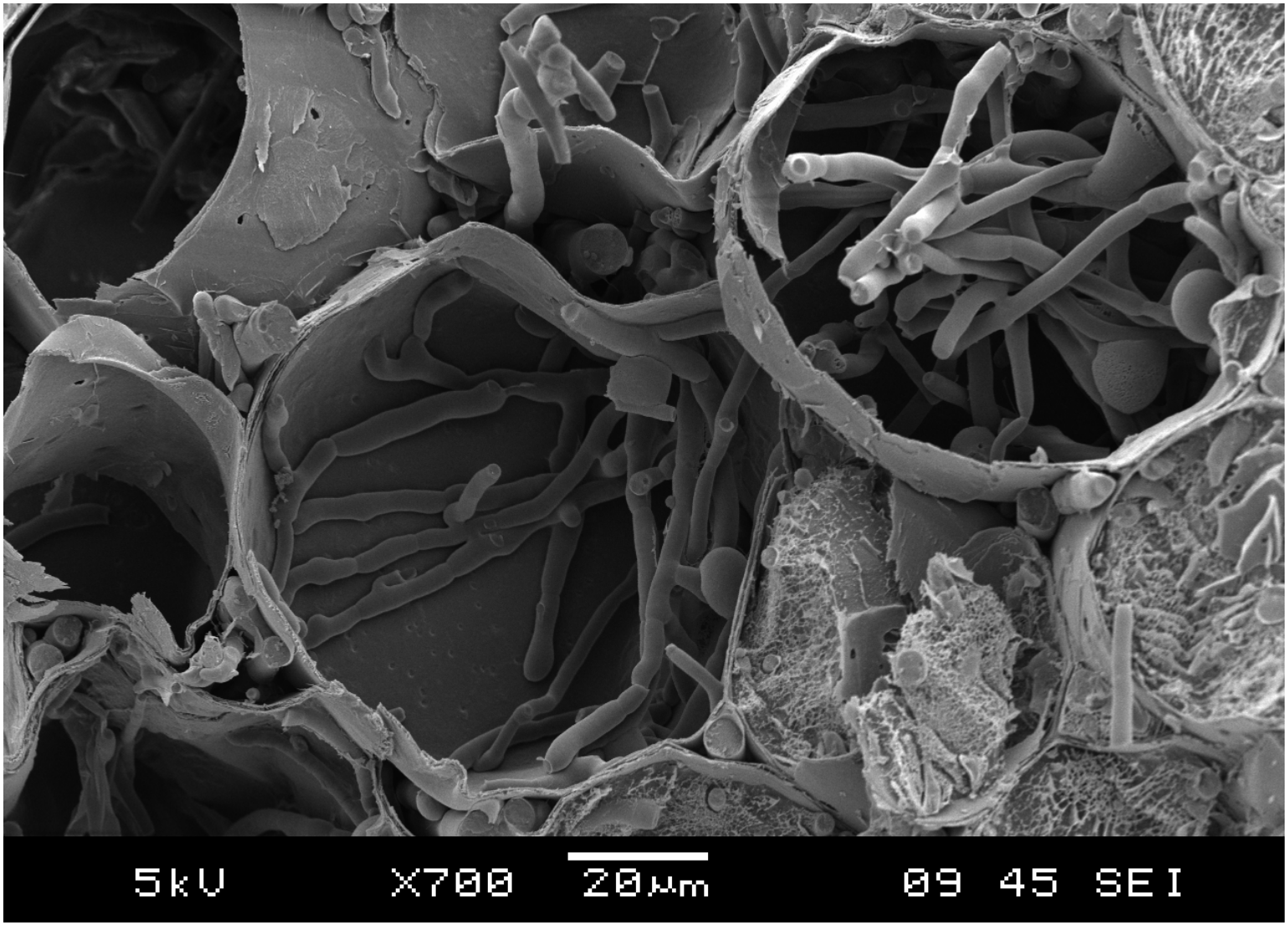


Brown *et al.*, 2009. Fungal Biology (Submitted)

The collapsed phloem lost its fluid content

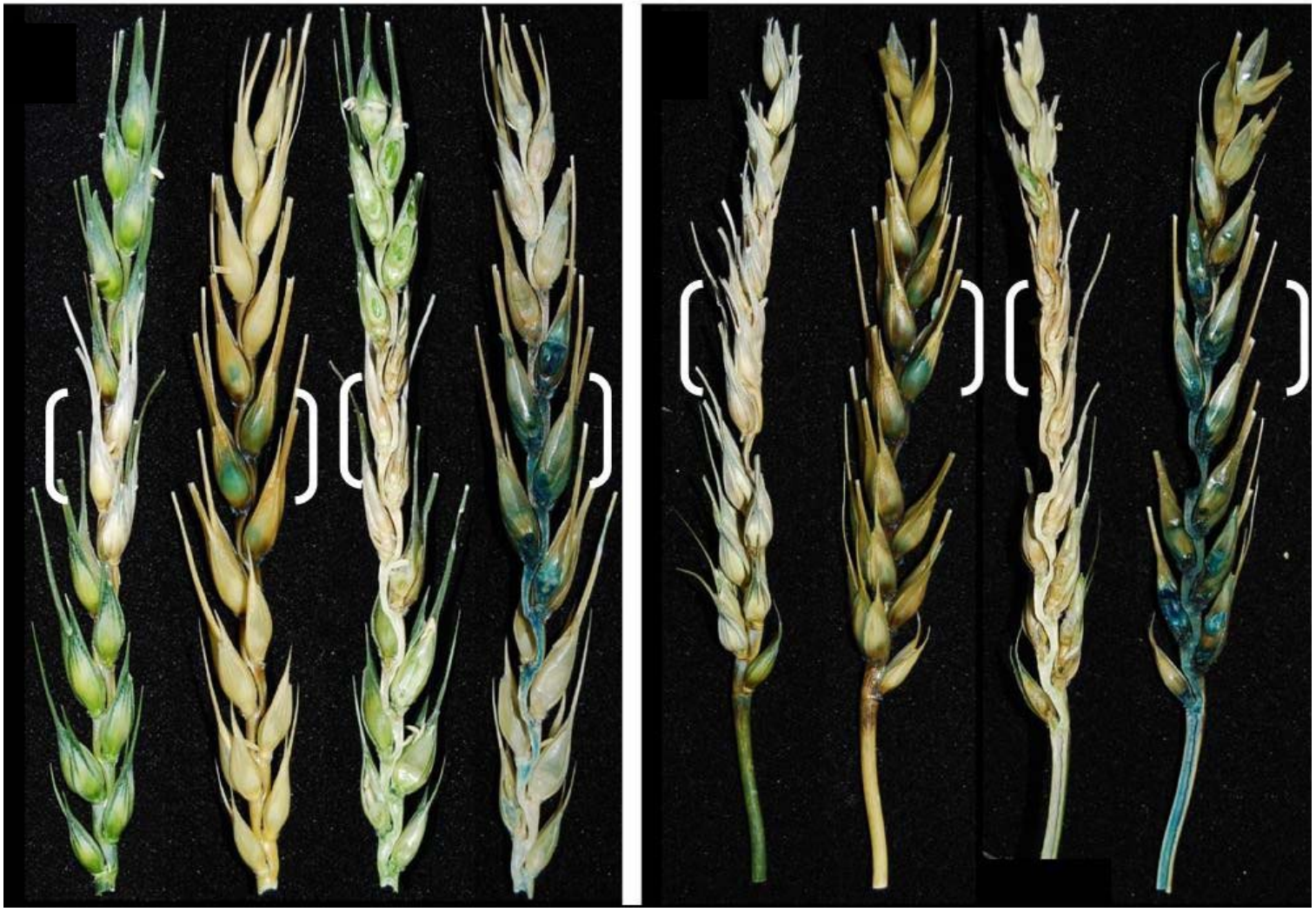


A network of hyphae destructively colonise dead cortical cells



Infection of wheat ears with *Fusarium graminearum* PH-1 constitutively expressing the GUS reporter protein

Day 8 **Day 16**



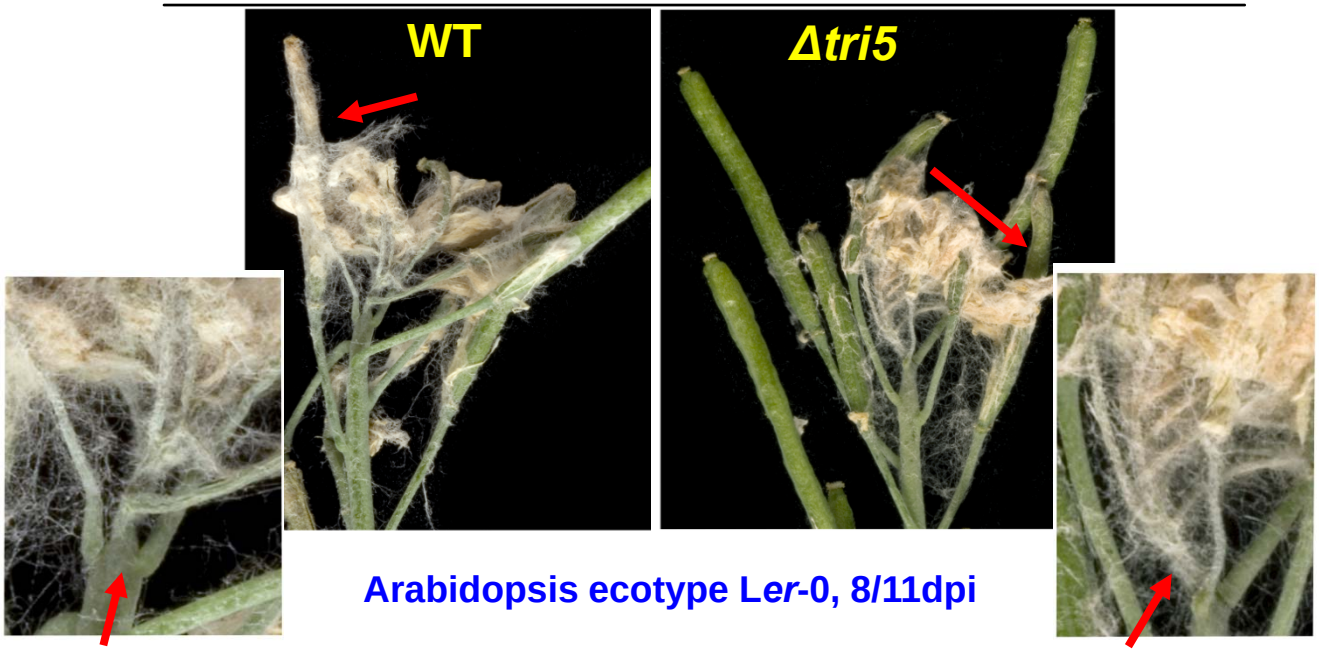
Urban, Baldwin and Bass, unpublished

The *tri5* mutant which produces no DON mycotoxin exhibits wild - type disease on Arabidopsis

Reduced virulence on wheat ears

Eye - shaped lesions and no rachis colonisation

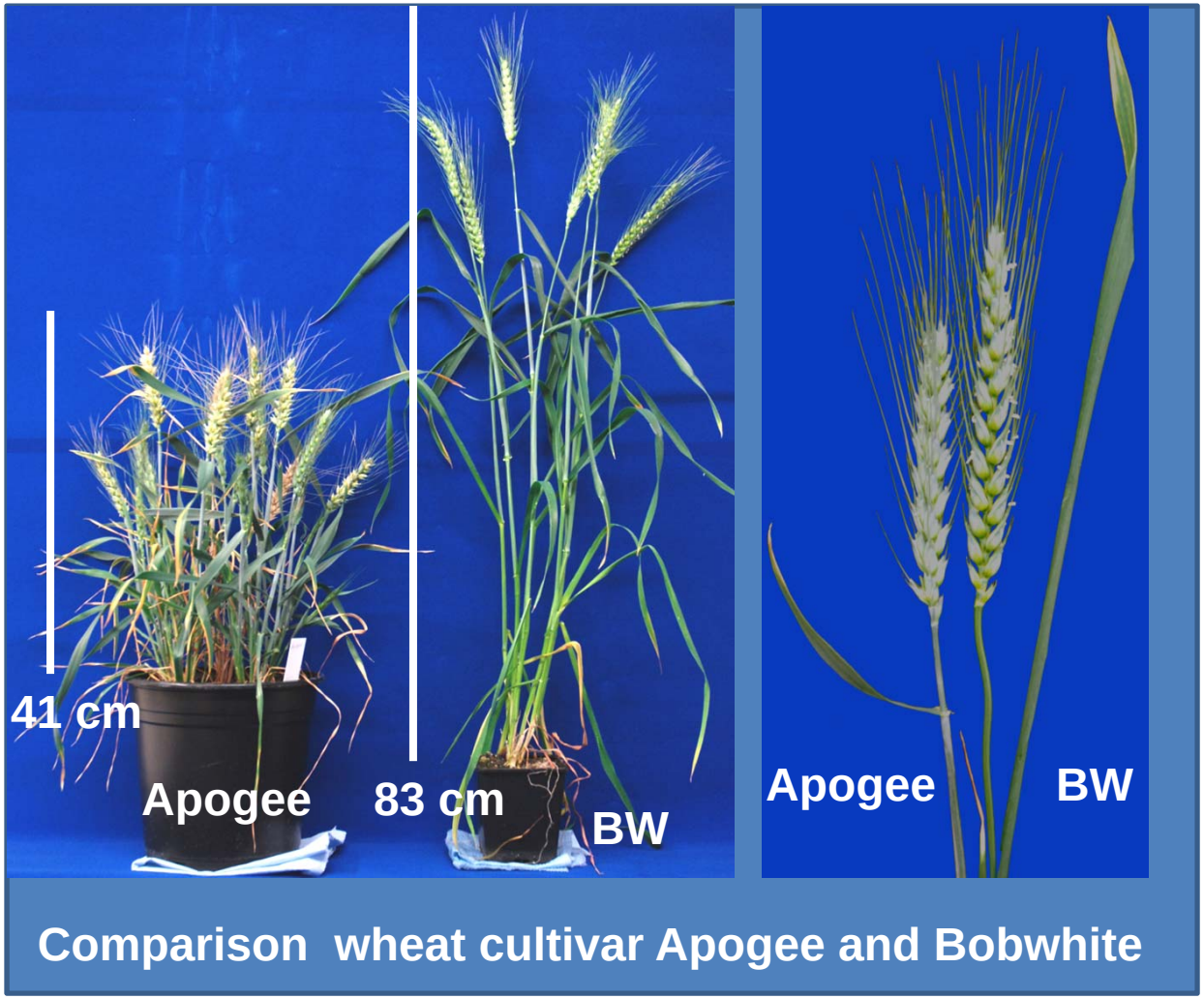
Organ	Fusarium genotype WT	*Δtri5*	SEM	P-value
Flower	2.43	3.00	0.174	0.554
New silique	3.19	3.42	0.251	0.060
N	72	72		



Arabidopsis ecotype L*er*-0, 8/11dpi

Cuzick, Urban (2008) New Phytologist

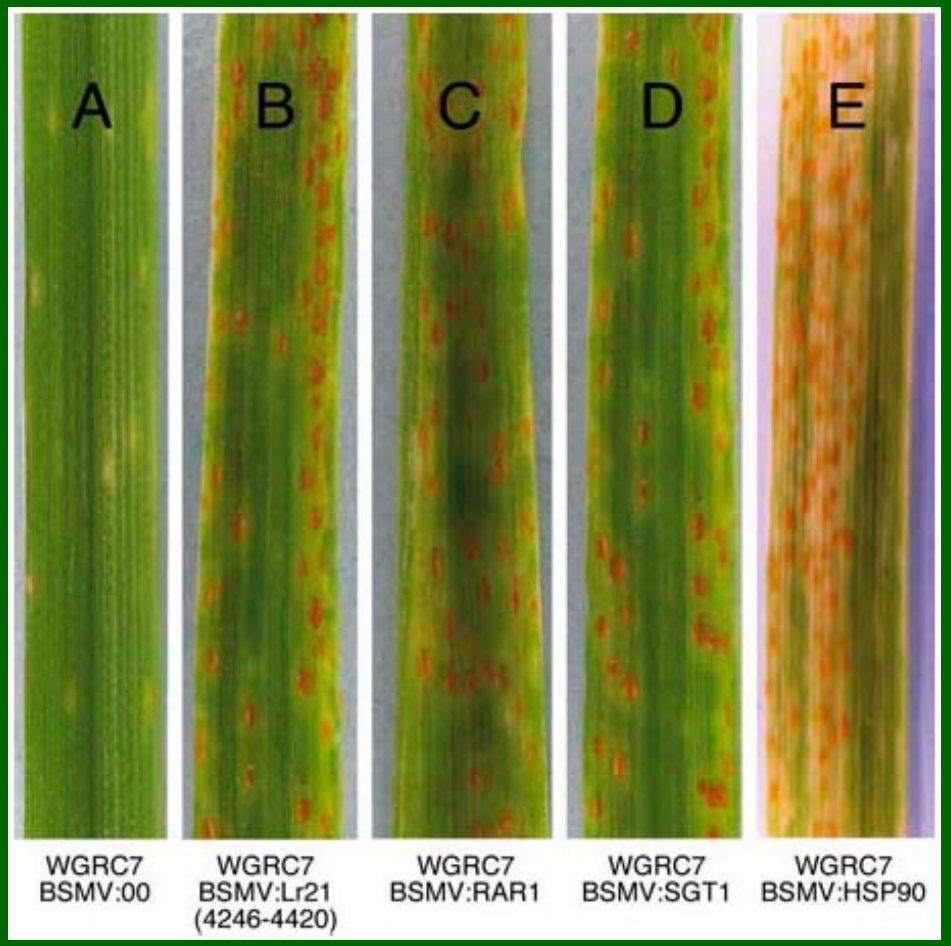
In the Cat3 facility at RRes, wheat cultivar Apogee has reduced flowering time of 35 days, reduced size and is fully susceptible to *F. graminearum*



Comparison wheat cultivar Apogee and Bobwhite

Fg point inoculated Apogee

Virus-induced RNA silencing: Functional analysis of the *Lr*21-mediated leaf rust resistance pathway in wheat



Scofield et al. (2005) *Plant Physiology* 138: 2165–2173

PDS triggered silencing of phytoene desaturase by BSMV.*Hv*PDAas in wheat

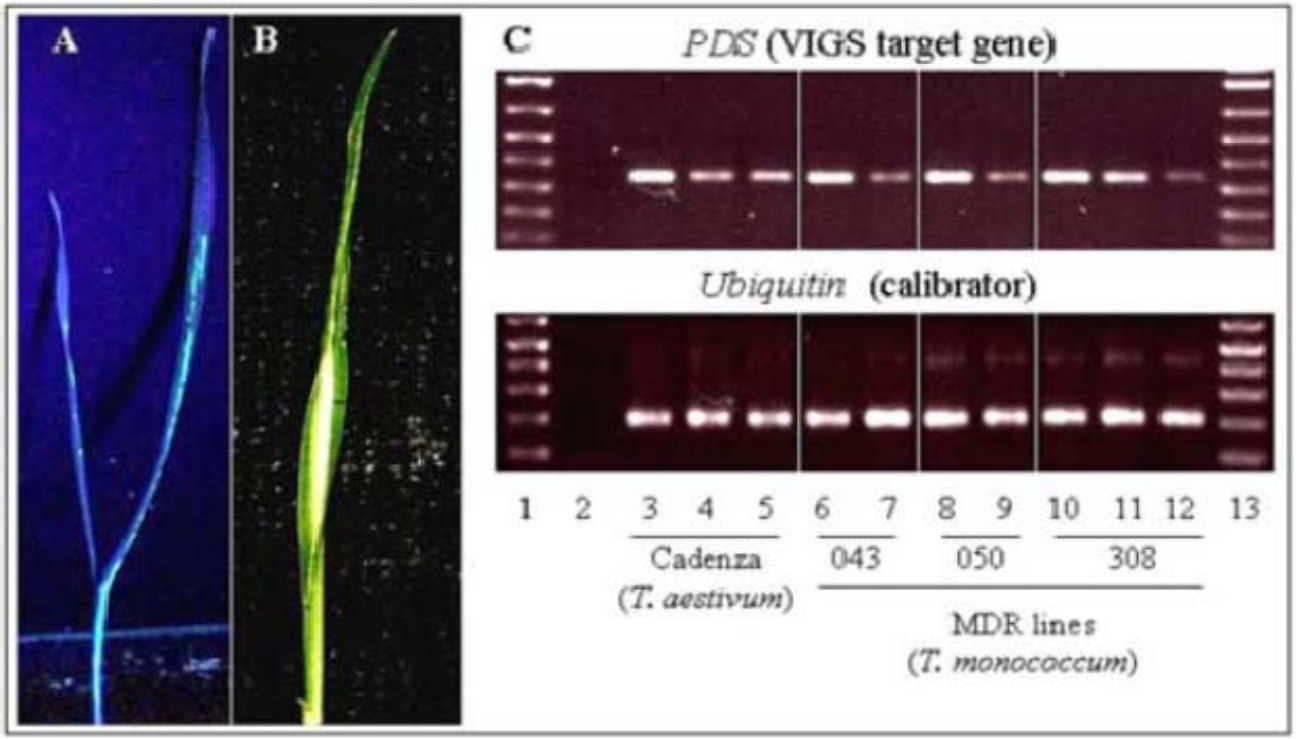
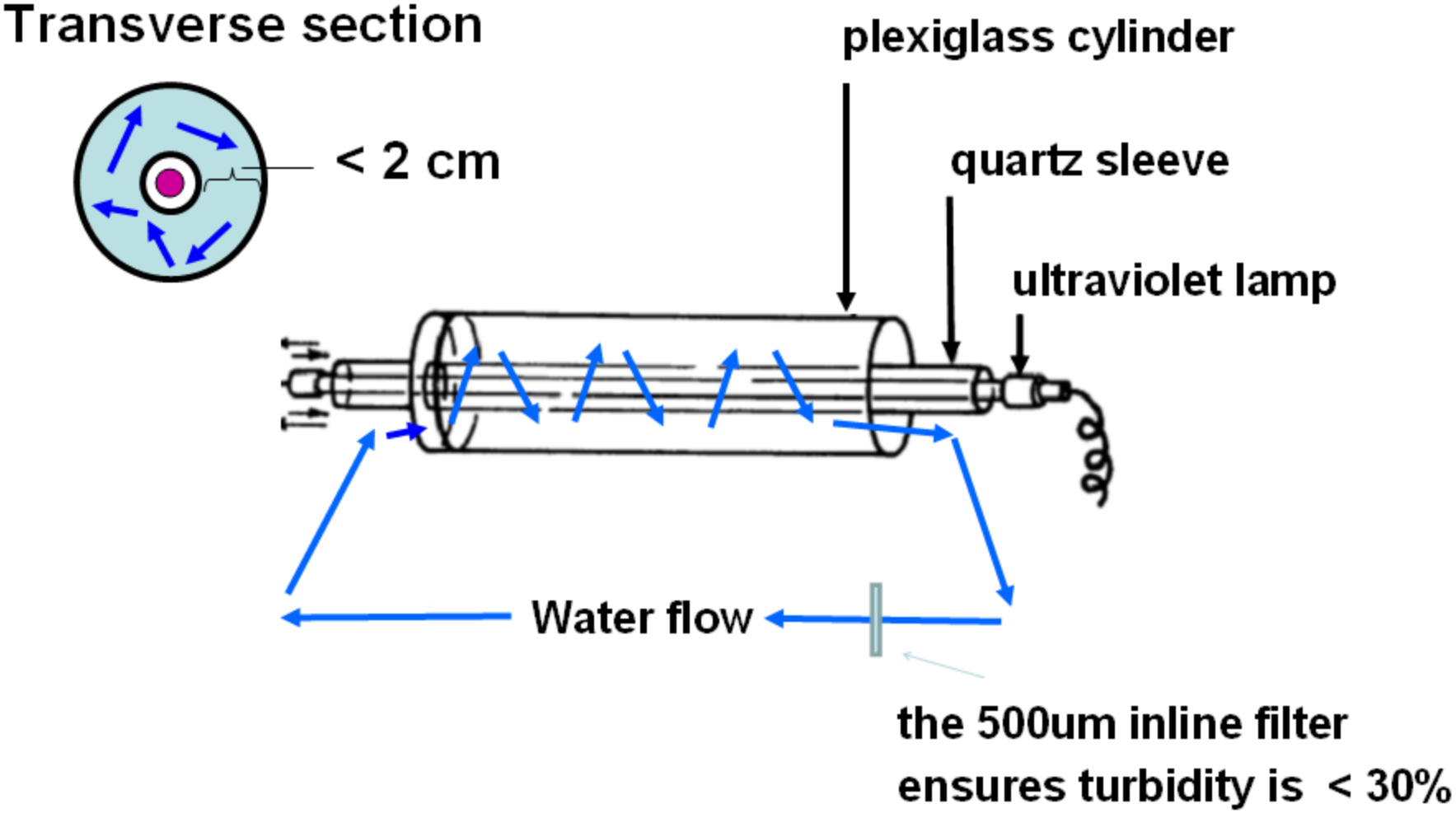


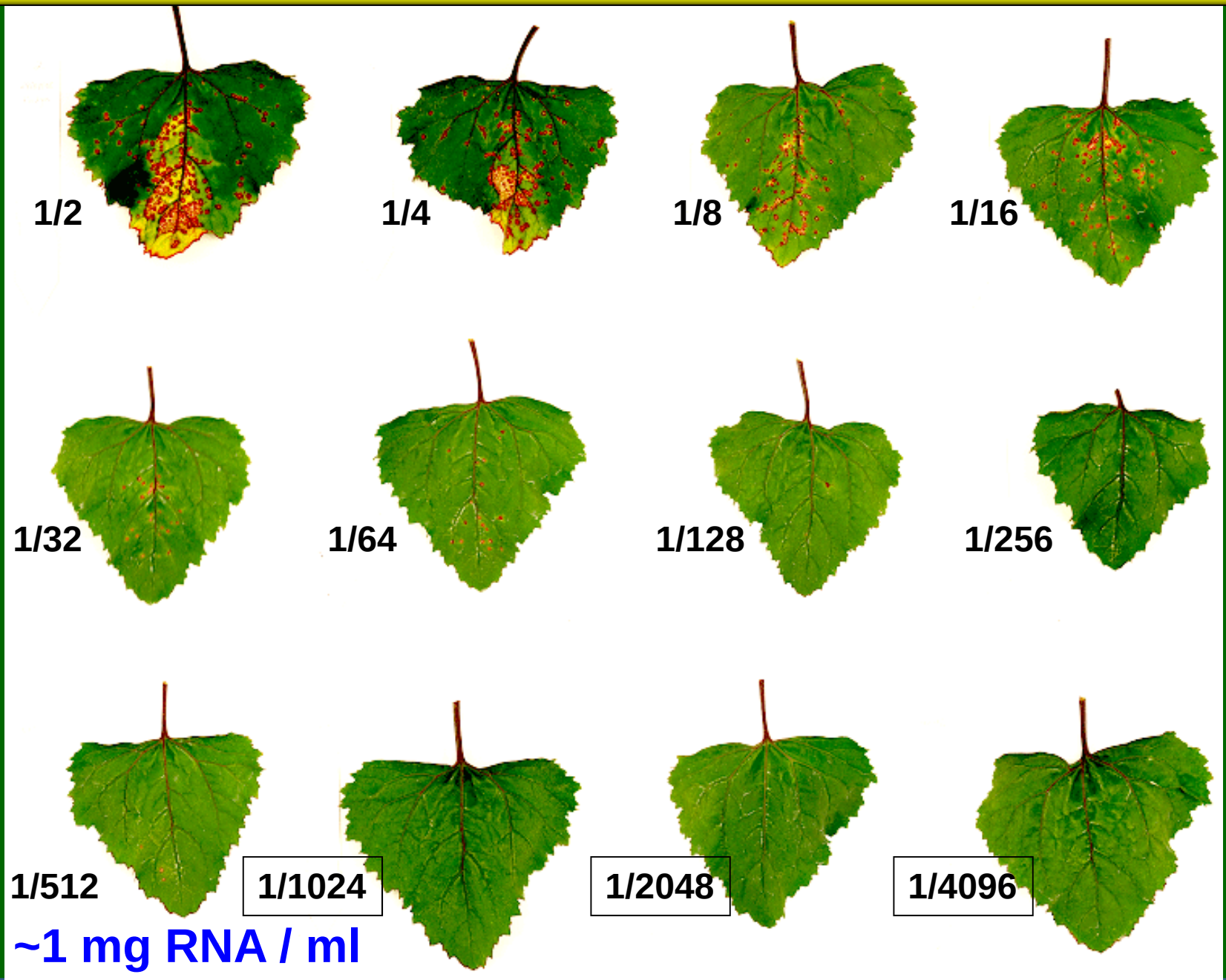
Figure 1: BSMV vector infects and triggers VIGS in wheat (cv Cadenza) and *T. monococcum* (MDR lines). (A) Systemic GFP fluorescence observed on wheat following BSMV.GFP infection at 6 dpi. (B) Systemic photobleaching as a consequence of *PDS* silencing triggered by BSMV.*Hv*PDSas in wheat at 10 dpi. (C) Semi-quantitative RT-PCR characterisation of *PDS* silencing in silenced (BSMV.*Hv*PDSas-infected: lanes 4, 5, 7, 9, 11, 12) and control (BSMV.GFP-infected: lanes 3, 6, 8, 10) wheat cv Cadenza, and *T. monococcum* MDR line 043 (lane 6 – control, lane 7 – silenced), 050 (lane 8 – control, lane 9 – silenced), and 308 (lane 10 – control, lanes 11 and 12 – silenced). Lanes 1, 13: molecular weight markers; lane 2: control RT-PCR without RNA template. Amplification of *PDS* gene-specific PCR product is reduced in silenced leaves (upper panel); *ubiquitin* cDNA (housekeeping gene) used and an internal calibrator. **J Shaw, C Lacomme & K Kanyuka, unpublished.**

The waste water passes along the entire length of the ~ 80 UV lamp contained within a quartz sleeve



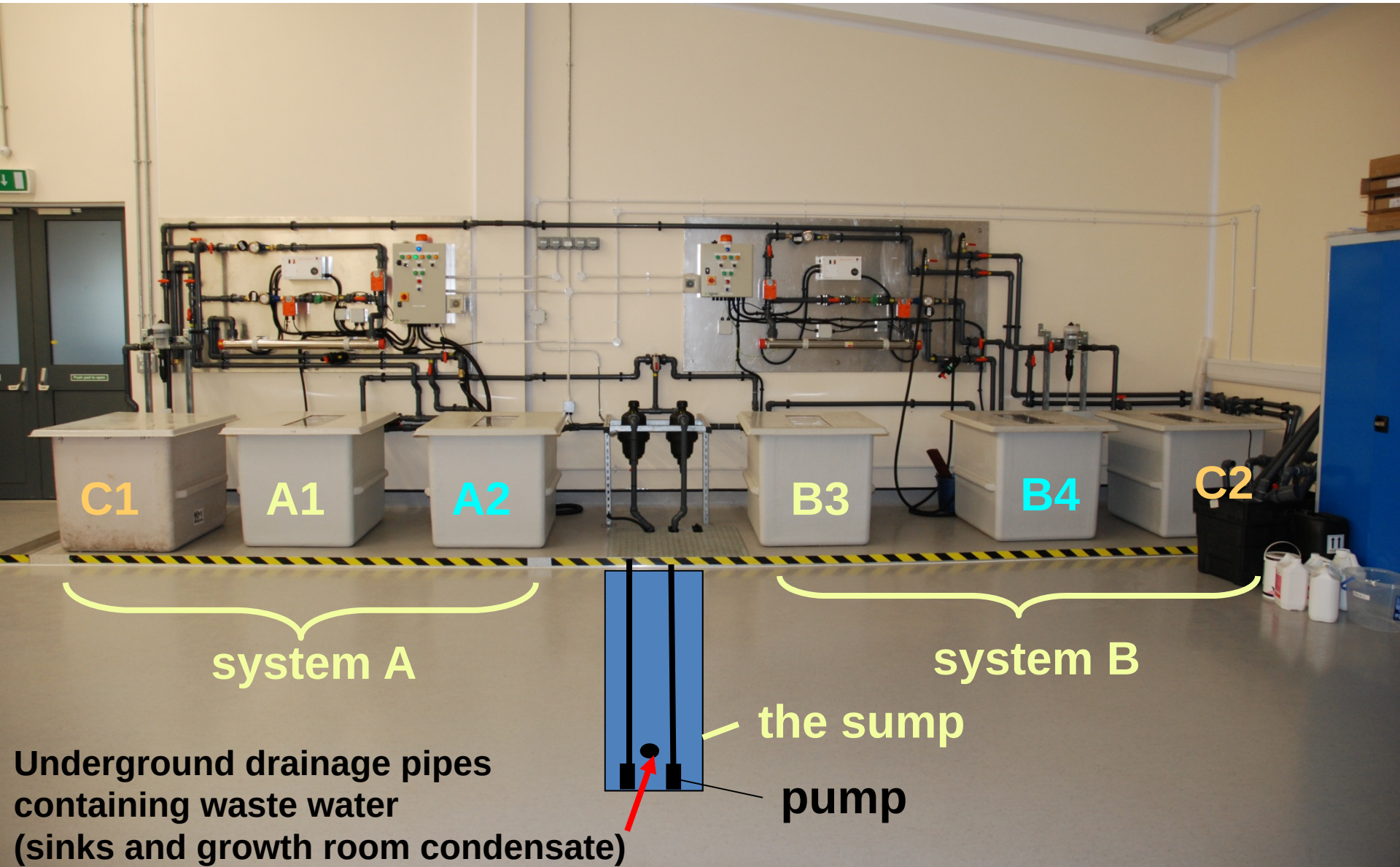
Up to 400 litres of waste water treated in a batch

BSM virus - Dilution experiments



~1 mg RNA / ml

The waste water treatment system – UV irradiation for 24 h followed by 12 h chemical disinfection



Category 3 containment facility @ RRes

The issue

The 2007 Foot and Mouth Epidemic

Source – Institute of Animal Health, Purbright
A BBSRC Institute

with defra[A] and HSE approved SOPs

1. **Water treatment – UV (need to publish method in a peer reviewed journal – Letters in Applied Microbiology**
2. **Drainage pipes – are they intact ?**

3. **Building design - Is it really fit for purpose?**
4. **Are the facts supplied to the two UK licencing bodies (HSE and defra) for each pathogen species correct ?**