Developing sustainable strategies to protect wheat roots from take-all disease

Dr. Javier Palma-Guerrero

Take-all Group
Wheat Pathogenomics Team
Biointeractions and Crop Protection Department

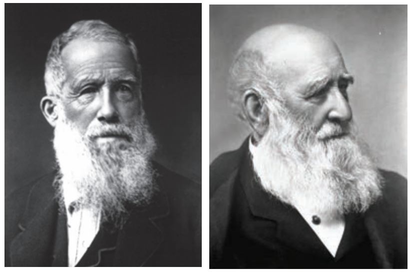
ROTHAMSTED
RESEARCH

UKRI
Biotechnology and
Biological Sciences
Research Council

Lawes
Agricultural
Trust

Rothamsted Research

Longest-running agricultural research institution in the world. **Founded in 1843**



Sir John Bennet Lawes Sir Joseph Henry Gilbert

Research from gene to field

Goals: Secure food production, protect the environment and benefit farmers worldwide

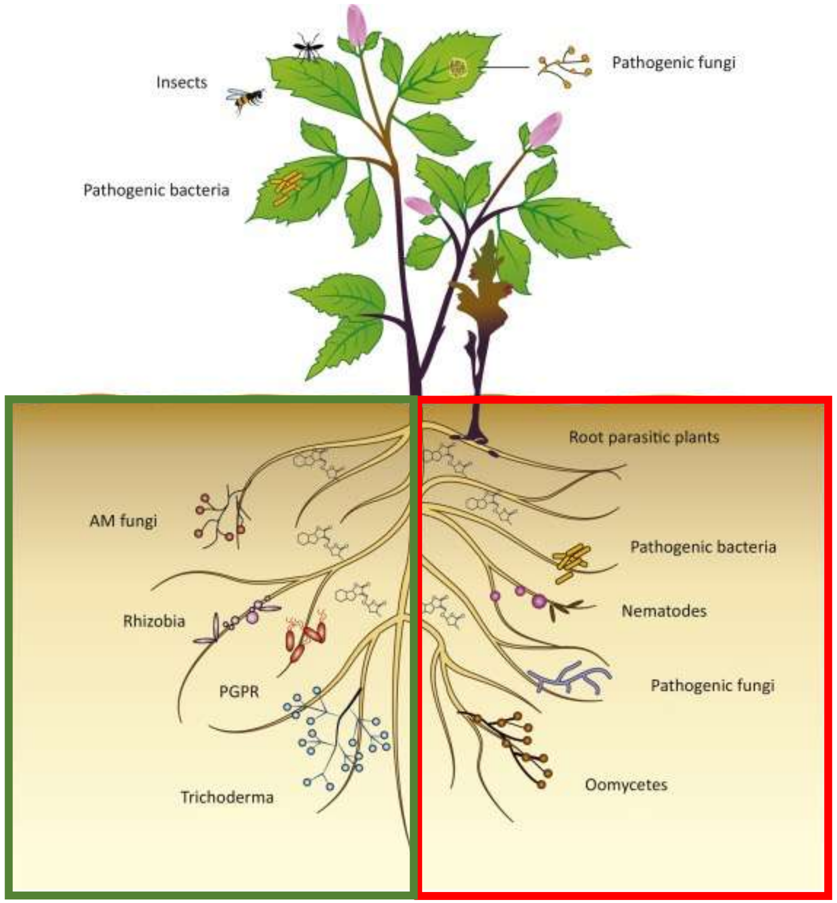
ROTHAMSTED
RESEARCH

Interactions in the rhizosphere

A complex ecosystem

Up to 10^6 –10^9 bacteria, 10^5 –10^6 fungi and 10^1 –10^2 nematodes per gram of soil

(Chuberre *et al* 2018)



Beneficials

Pathogens

Trends in Plant Science (Lopez Raez *et al* 2017)

Impact on yield and quality

ROTHAMSTED
RESEARCH

Take-all disease

Most important root disease of wheat, fungus *Gaeumannomyces tritici*

5 – 20 % yield losses on 2nd and 3rd wheats, up to 60 % when high severity = $60 million in UK

It also infects barley and triticale, but not oats → Avenacin (Osbourn *et al* 1994)

No completely effective fungicide treatment or resistant cultivar → crop rotations

Wheat is an important staple crop produced and consumed globally

Urgent need for new control methods

ROTHAMSTED
RESEARCH

Environmental impact of Take-all disease

- Higher Nitrate leaching by reduced Nitrogen uptake polluting water sources

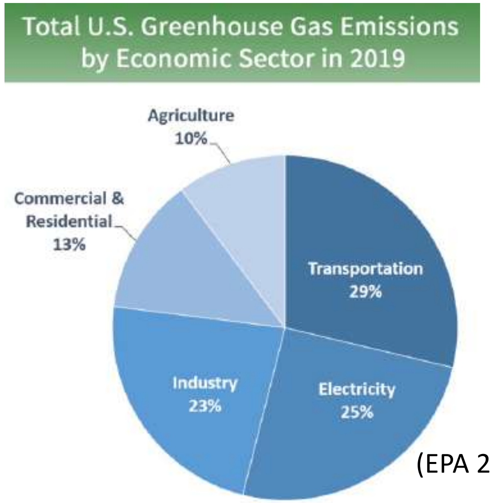
(Macdonald and Gutteridge 2012)

- Higher greenhouse gas emissions

Nitrous oxide from unused fertilizers

Reduced by fungicide treatments

(Hughes *et al* 2011)

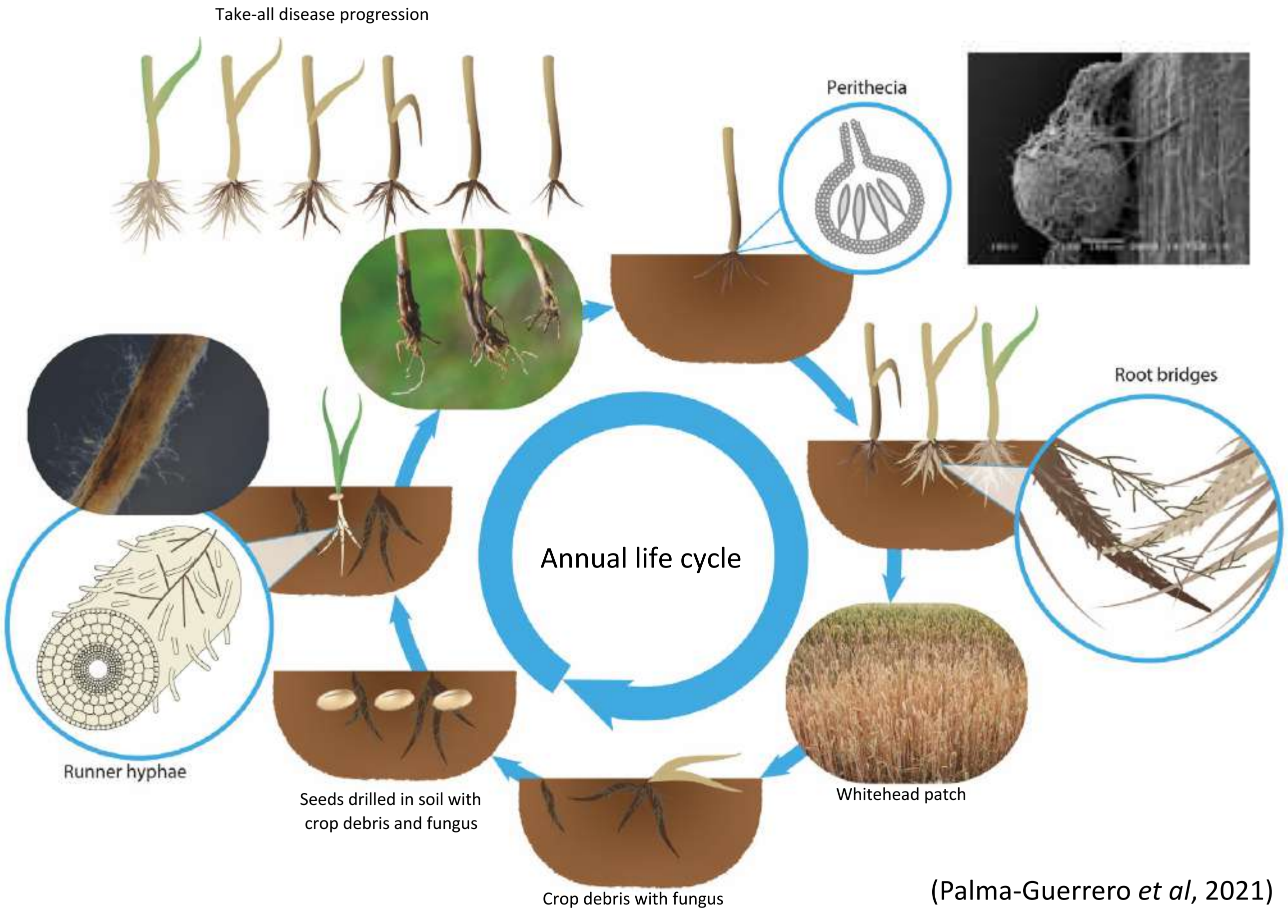


(EPA 2019)

Controlling the disease is important for food security and for the environment

ROTHAMSTED
RESEARCH

Disease cycle



(Palma-Guerrero *et al*, 2021)

ROTHAMSTED
RESEARCH

A distant cousin of *Magnaporthe*

Gaeumannomyces tritici (prev. *Gaeumannomyces graminis* var. *tritici*)

(Hernandez-Restrepo *et al* 2016)

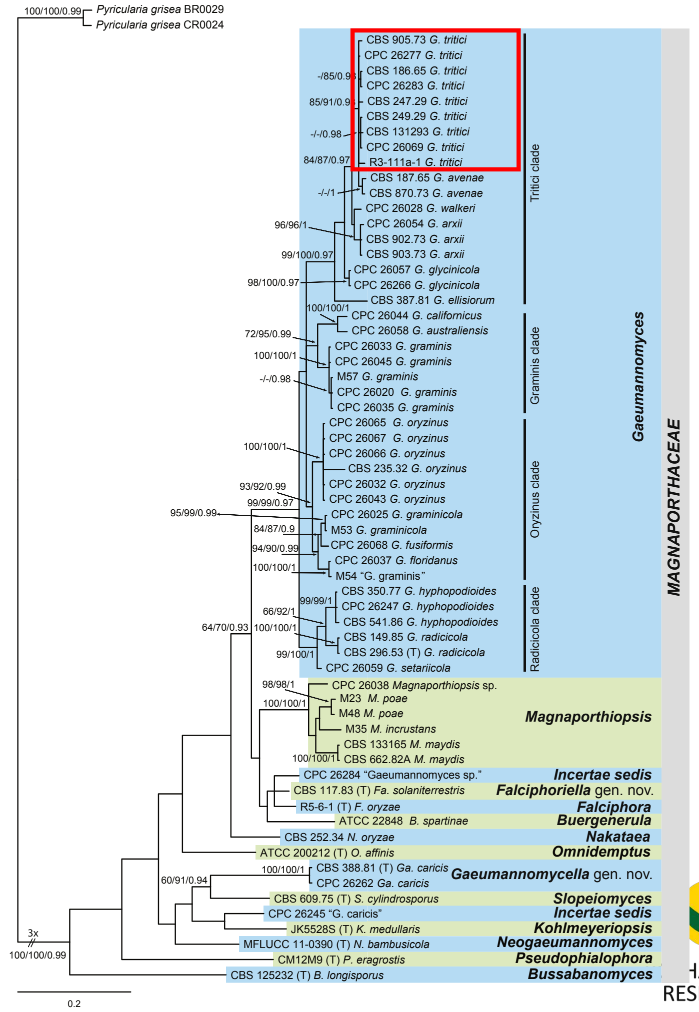
Two genetic groups found by different labs on different populations: A/B, T1/T2, G1/G2, A1/A2, and N/R (based on ability to infect rye)

Correspondence between A, T1, G2, and N isolates (Daval *et al* 2010)

Cryptic species?

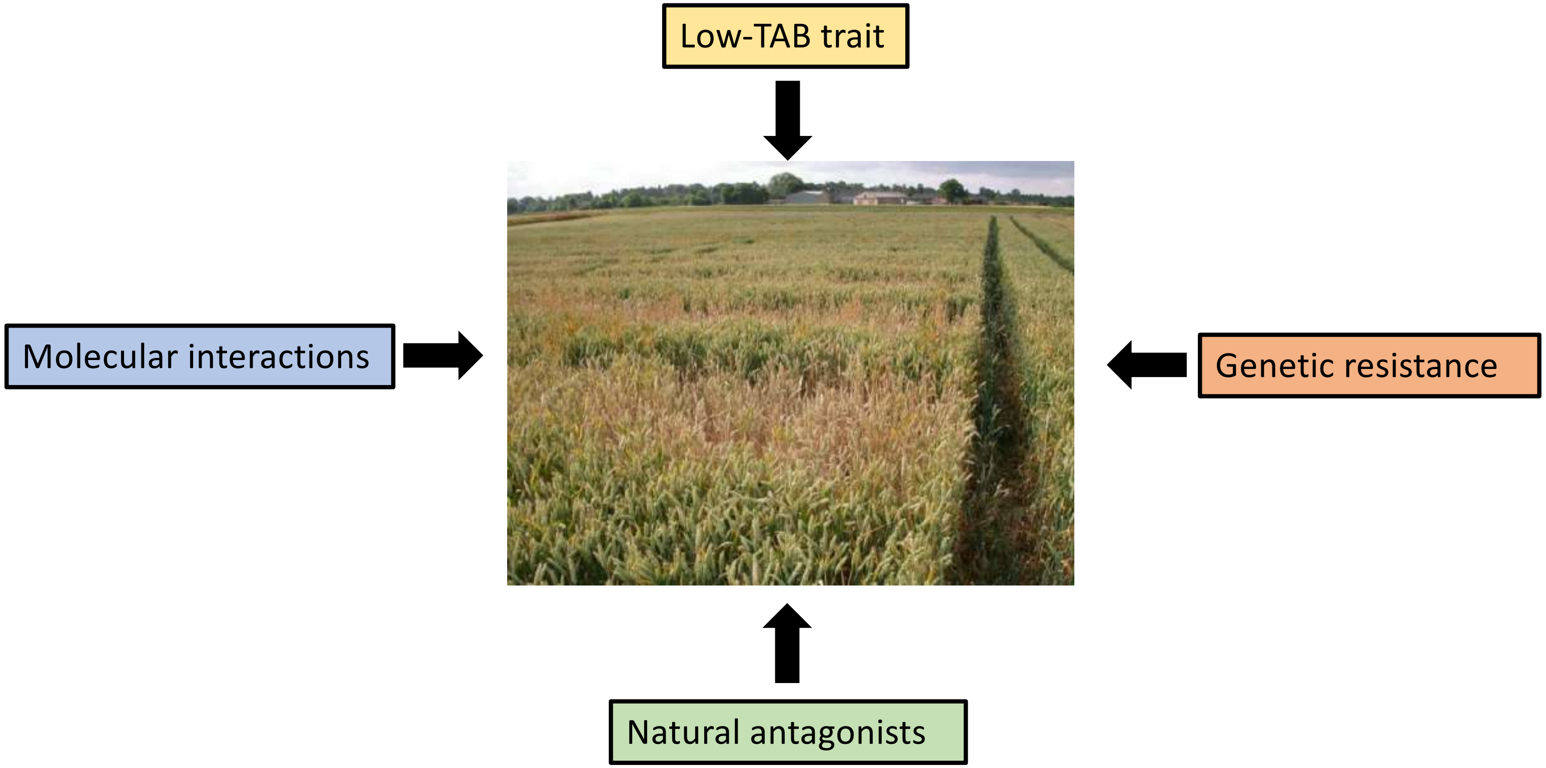
A/G2 show higher virulence (Lebreton *et al* 2007)

Also classified by silthiofam sensitivity



HAMSTED
RESEARCH

Sustainable approaches to control Take-all



Low-TAB trait

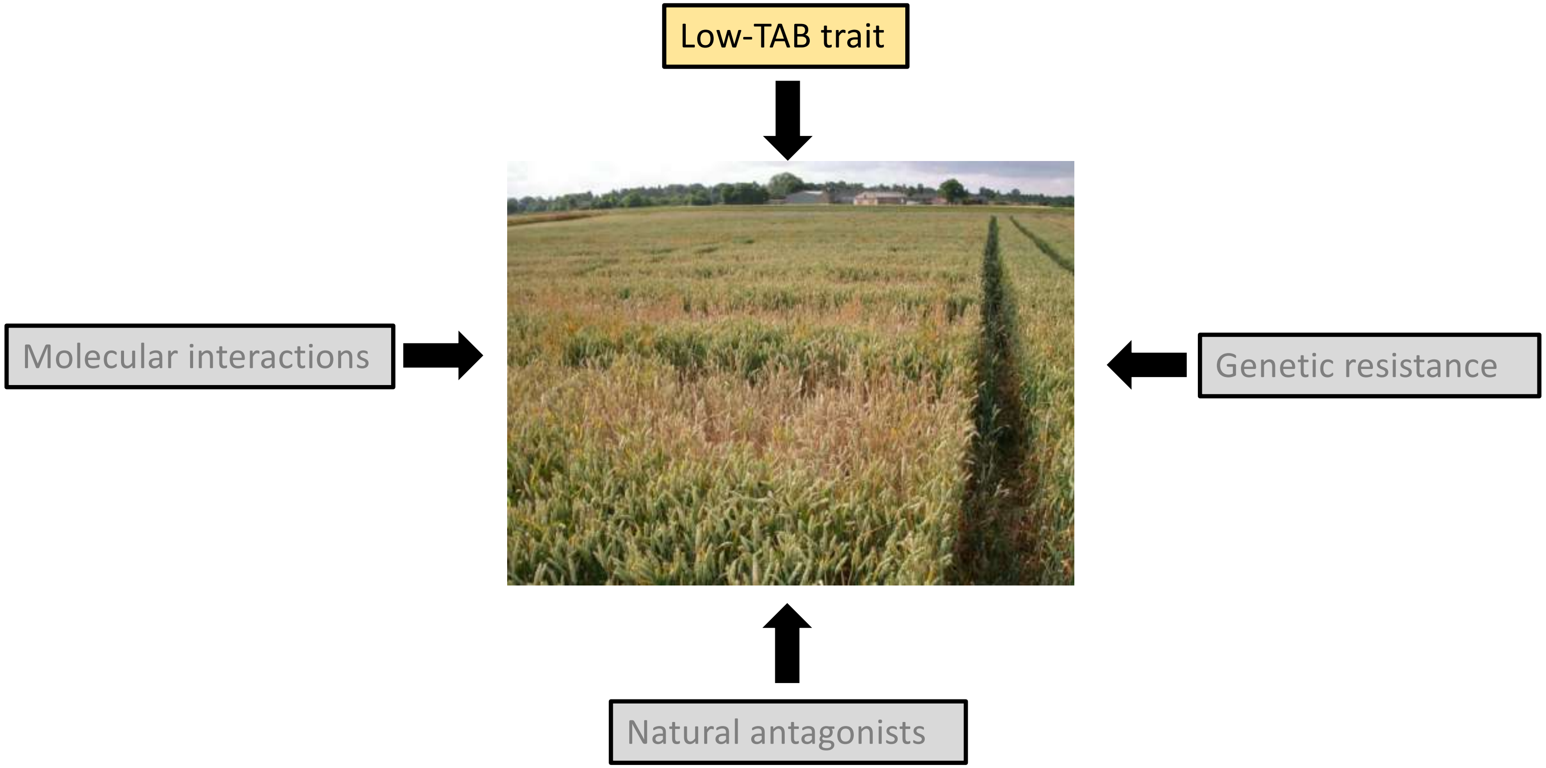
Molecular interactions

Genetic resistance

Natural antagonists

ROTHAMSTED
RESEARCH

Sustainable approaches to control Take-all



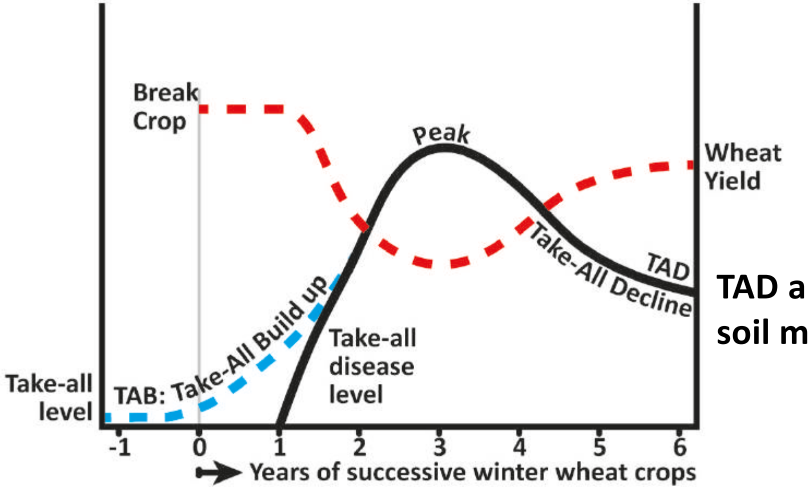
Low-TAB trait

Molecular interactions

Genetic resistance

Natural antagonists

Take-all Build-Up



TAD associated to soil microorganisms

1st wheat

Yield average 12.69t/ha

1st and 3rd wheat field trials; 45 elite winter wheat cultivars

3rd wheat

Yield average 7.64t/ha

ROTHAMSTED
RESEARCH

Measuring take-all build up

Soil core bioassay method

1. Soil core taken angled underneath row
2. Core inverted into plastic cup
3. Ten bait wheat (cv Hereward) seeds sown
4. Growth room for 5 weeks

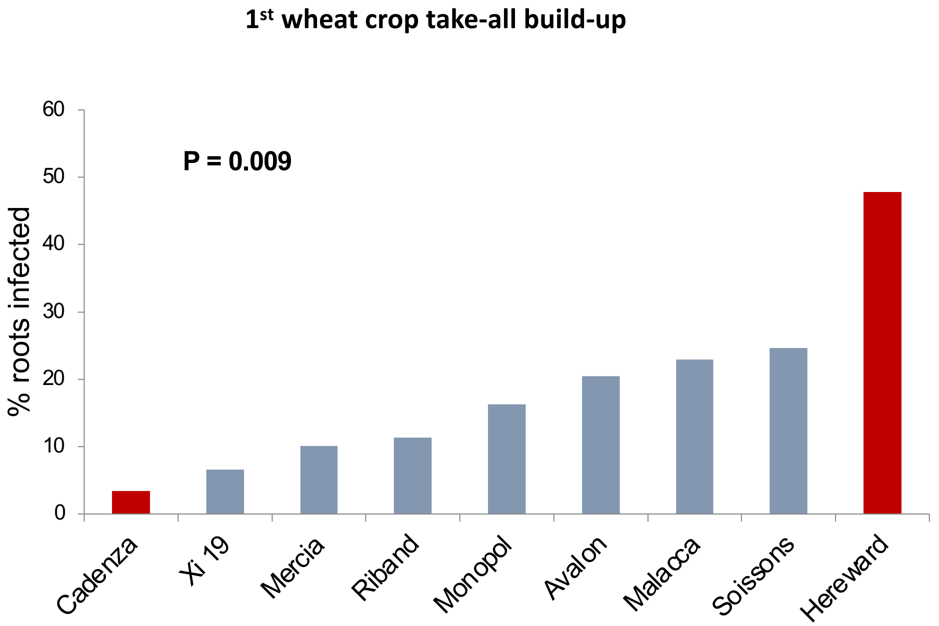
Developed by Richard Gutteridge

Severe take-all infection

Slight take-all infection

ROTHAMSTED
RESEARCH

Wheat cultivars differ in TAB levels



(McMillan *et al* 2011)

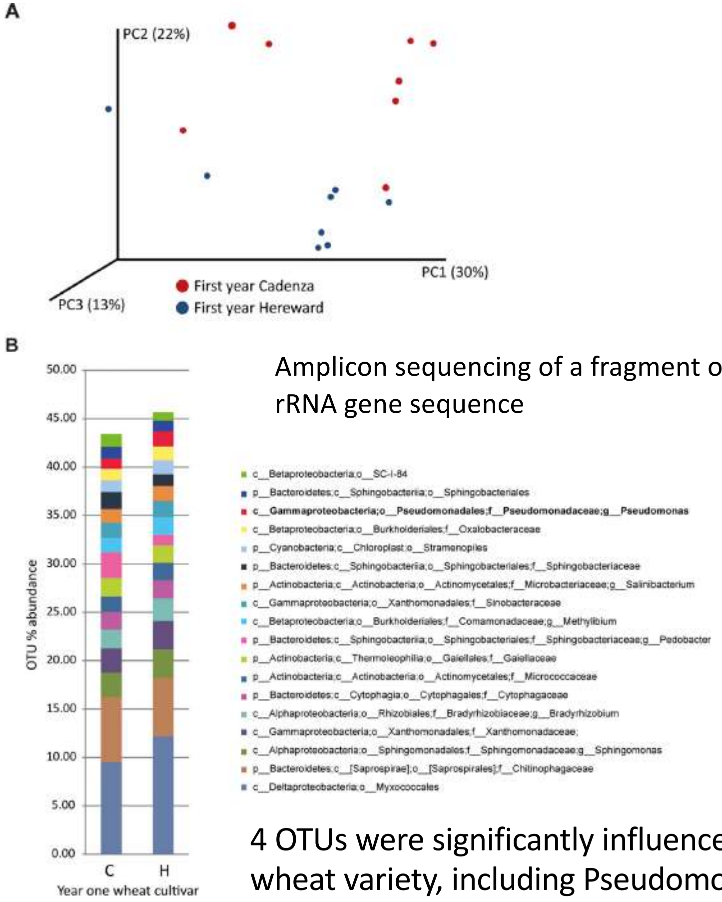
44% difference between Hereward and Cadenza

Associated to changes in soil microbiome

ROTHAMSTED
RESEARCH

Rhizosphere microbiome changes

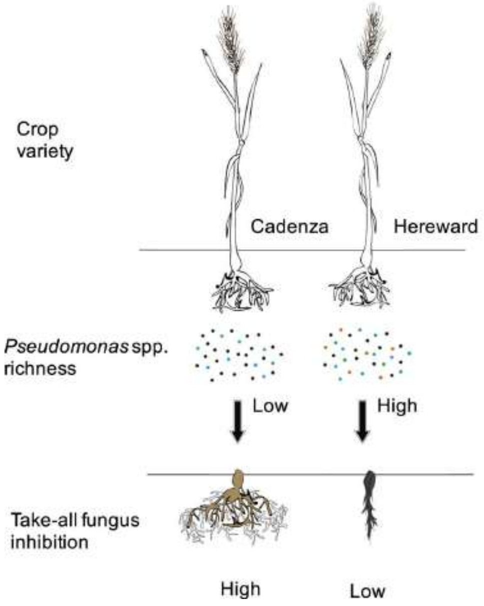
Second year soil shows different microbiome depending on the wheat cultivar in the first year



Amplicon sequencing of a fragment of the 16S rRNA gene sequence

4 OTUs were significantly influenced by first year wheat variety, including Pseudomonas spp.

(Mauchline *et al* 2015)



Pseudomonas spp. diversity is negatively associated with take-all suppression

(Mehrabi *et al* 2016)

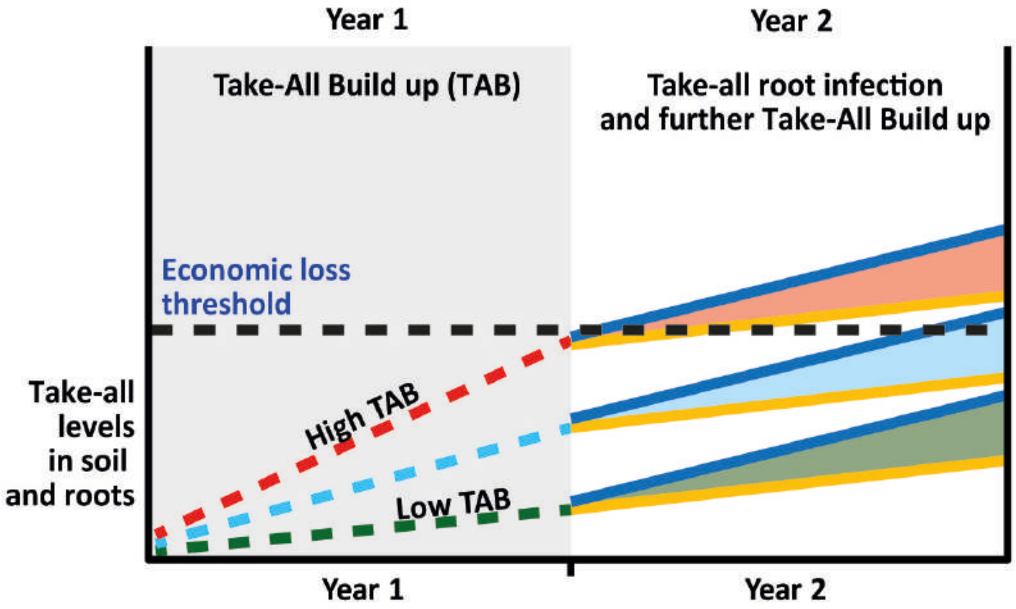
Pseudomonas species affected by the 1st wheat cultivar

ROTHAMSTED
RESEARCH

Sustainable take-all control by 1st wheat choice

Take-all build up influences disease severity and productivity in the second wheat year

(McMillan et al 2018)



Blue= highly susceptible

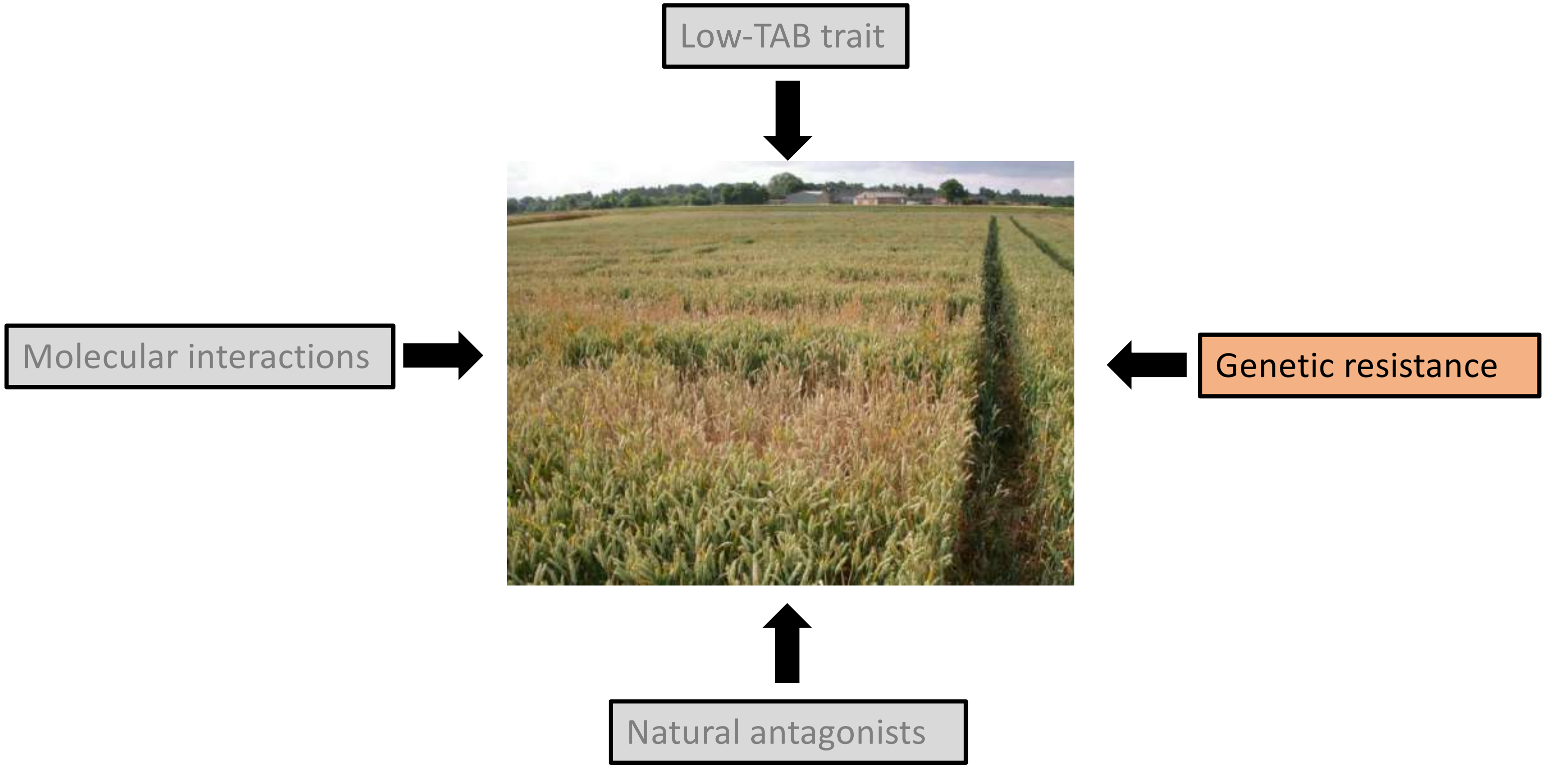
Orange = partially resistant

(Palma-Guerrero *et al*, 2021)

Significantly less disease and higher yields in 2nd wheat after growing a Low-TAB 1st wheat cultivar

ROTHAMSTED
RESEARCH

Sustainable approaches to control Take-all



Low-TAB trait

Molecular interactions

Genetic resistance

Natural antagonists

Genetic resistance to take-all

- Ancestral cultivars and relatives

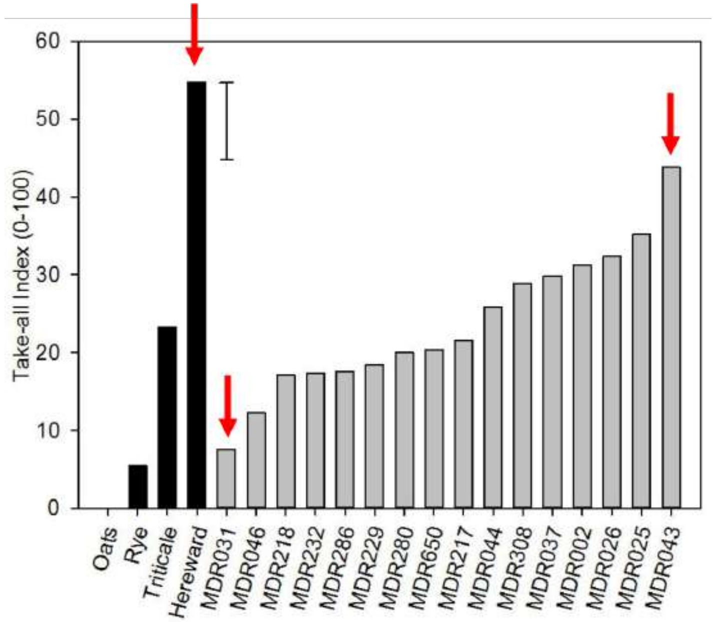
Triticum monococcum, a source of resistance to pathogens

Take-all resistance, unknown mechanisms

Diploid, “A” genome

Field trials screenings identified:

- MDR031 highly resistant
- MDR043 highly susceptible



(McMillan *et al,* 2014)

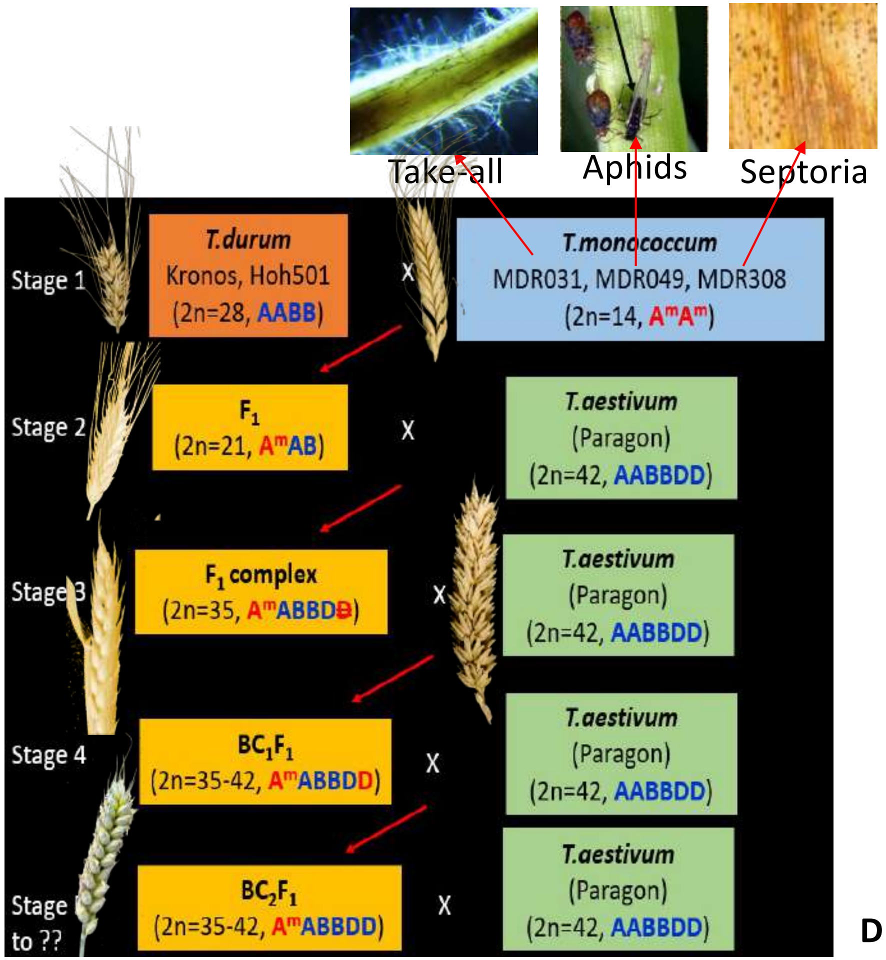
- Mapping population (F6) generated and tested in field experiments

- Currently being genotyped by Kompetitive Allele Specific PCR (KASP)

Dr. Wanxin Chen and Dr. Lawrence Bramham

ROTHAMSTED
RESEARCH

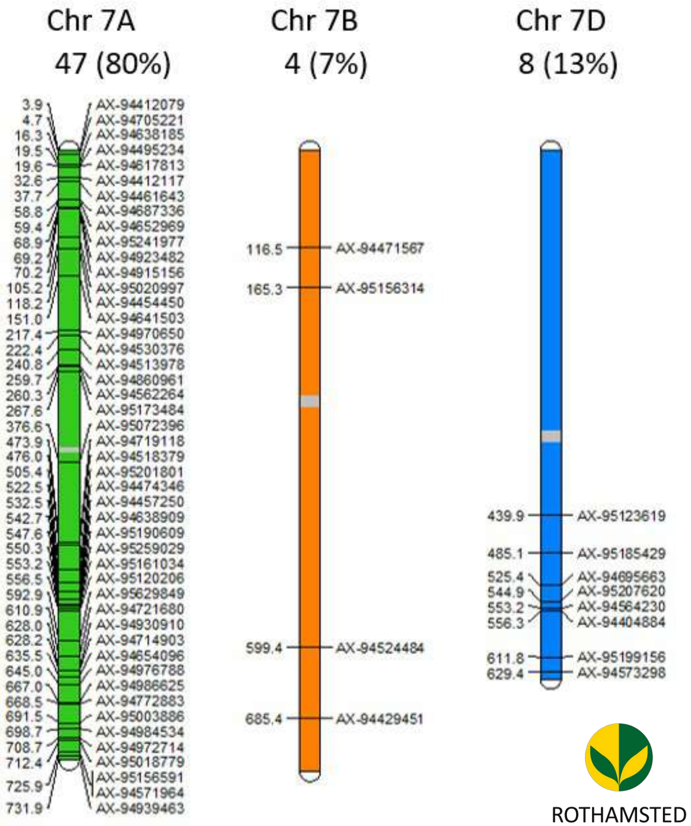
T. monococcum introgression



- **Field trial** with 29 BC1-lines, 960 segregating plants
- **Single Seed Descent** (SSD) of segregating plants to make them homozygous

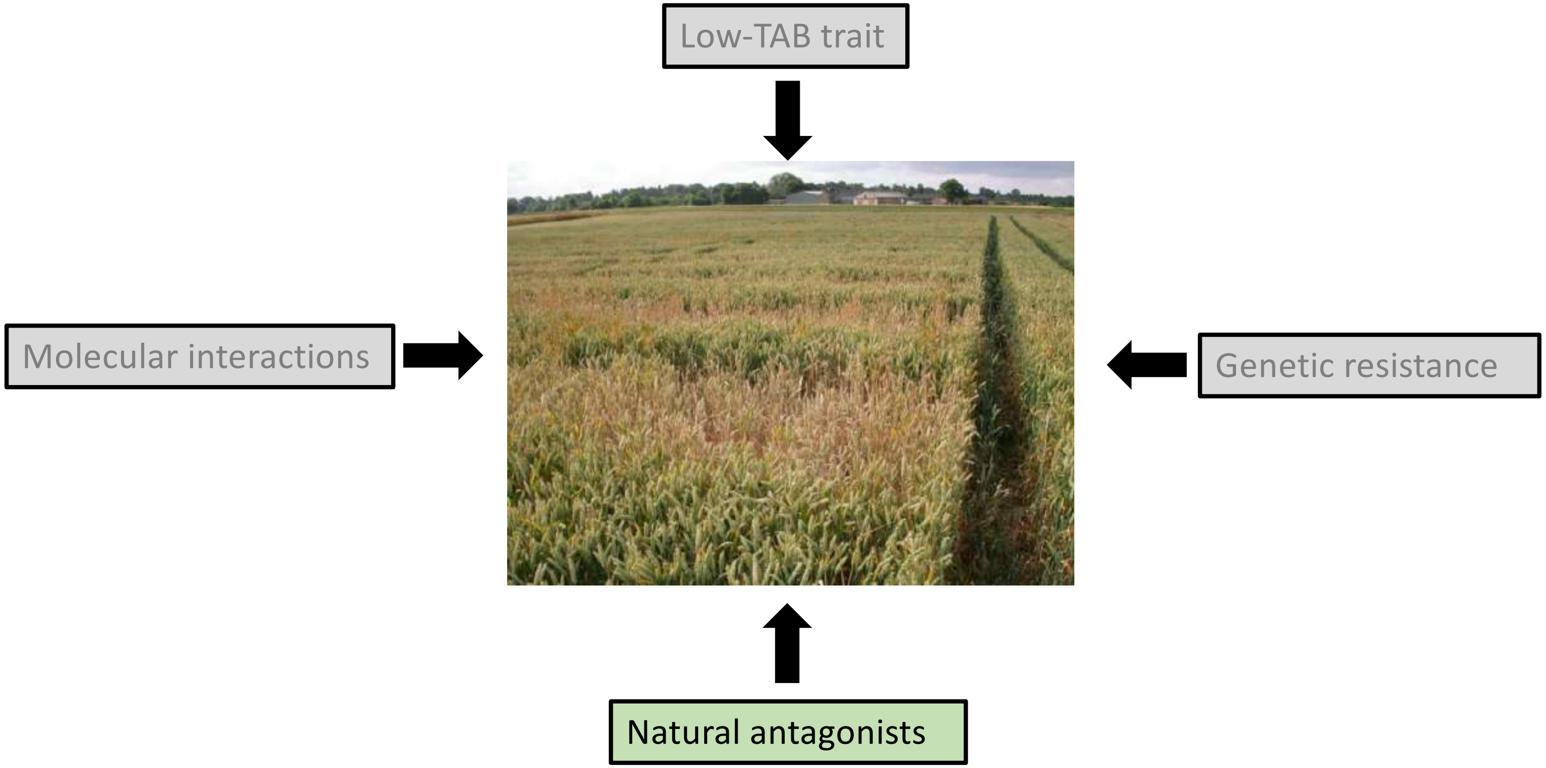
Dr. Mike Hammond-Kosack

hetMarker scoring of F1Complex plants on 35K Axiom Breeders' Array: predominant introgression into the A sub-genome



ROTHAMSTED
RESEARCH

Sustainable approaches to control Take-all



Low-TAB trait

Molecular interactions

Genetic resistance

Natural antagonists

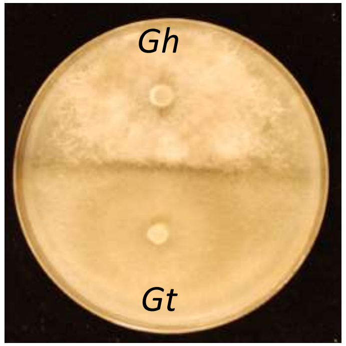
Take-all antagonists in the rhizosphere

Beneficial fungi with different modes of action

Plant defense activation

Gaeumannomyces hyphopodioides (Phialophora)

Suppresses take-all disease (Speakman and Lewis 1978)



Potential biocontrol agent

Direct antifungal activity

Fungi isolated from wheat roots

Beneficial fungus 1

Gt

Beneficial fungus 2

Gt

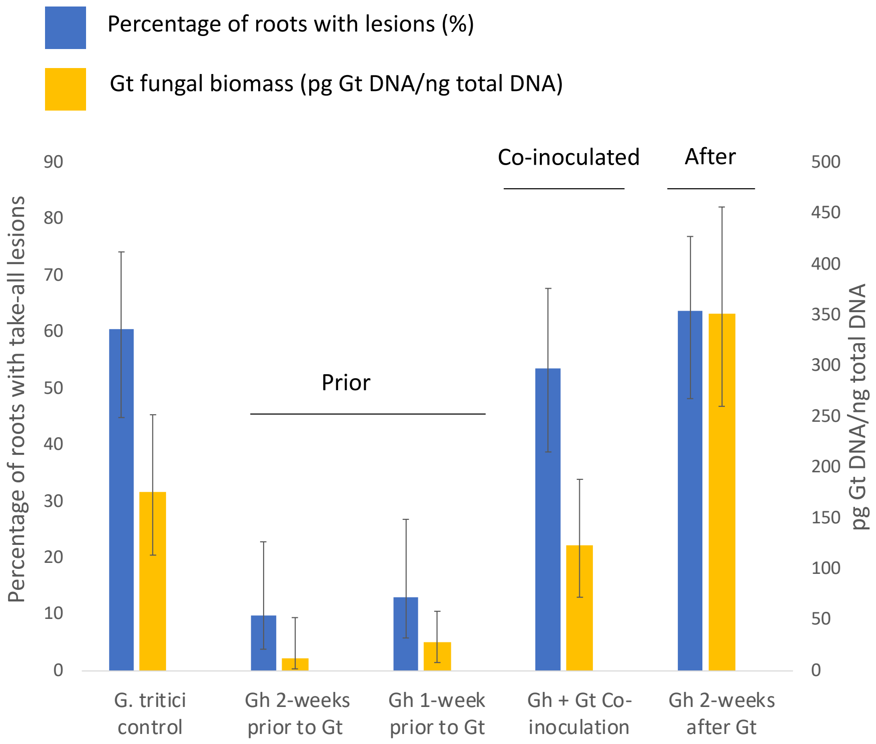
Source of antifungal compounds

ROTHAMSTED
RESEARCH

Take-all antagonists in the rhizosphere

- *Gaeumannomyces hyphopodioides* (Phialophora):

Tania Chancellor (UoN DTP)



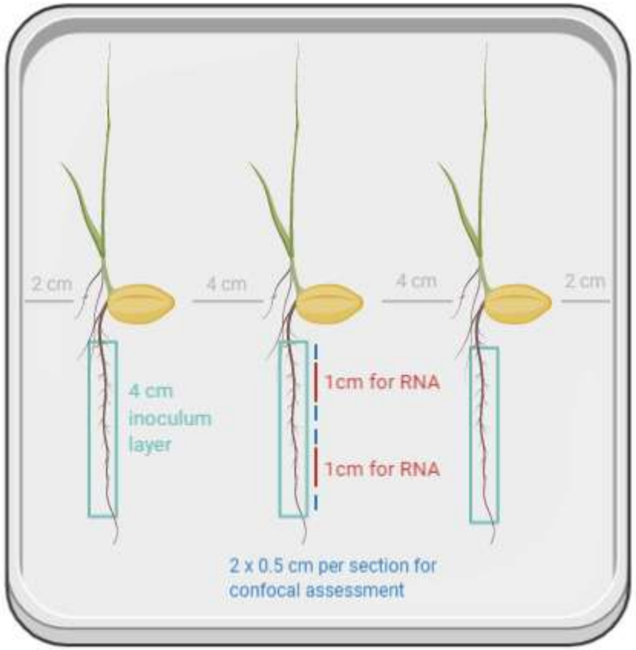
Gh infection protects from take-all disease

ROTHAMSTED
RESEARCH

Plant defence activation by Gh?

Wheat transcriptional response to *Gaeumannomyces tritici* and *Gaeumannomyces hyphopodioides* root infection

Confocal microscopy

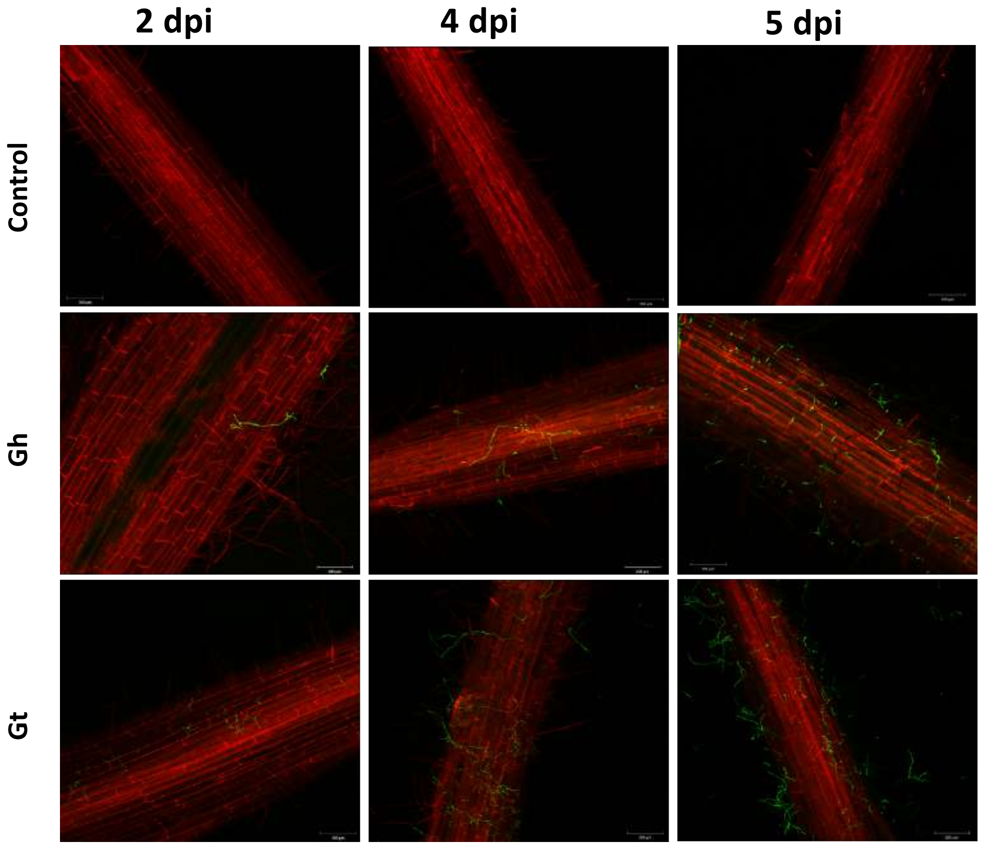


Samples fixed in ethanol and stained

Green = Alexa Fluor – WGA

Red = PI

Tania Chancellor (UoN DTP)



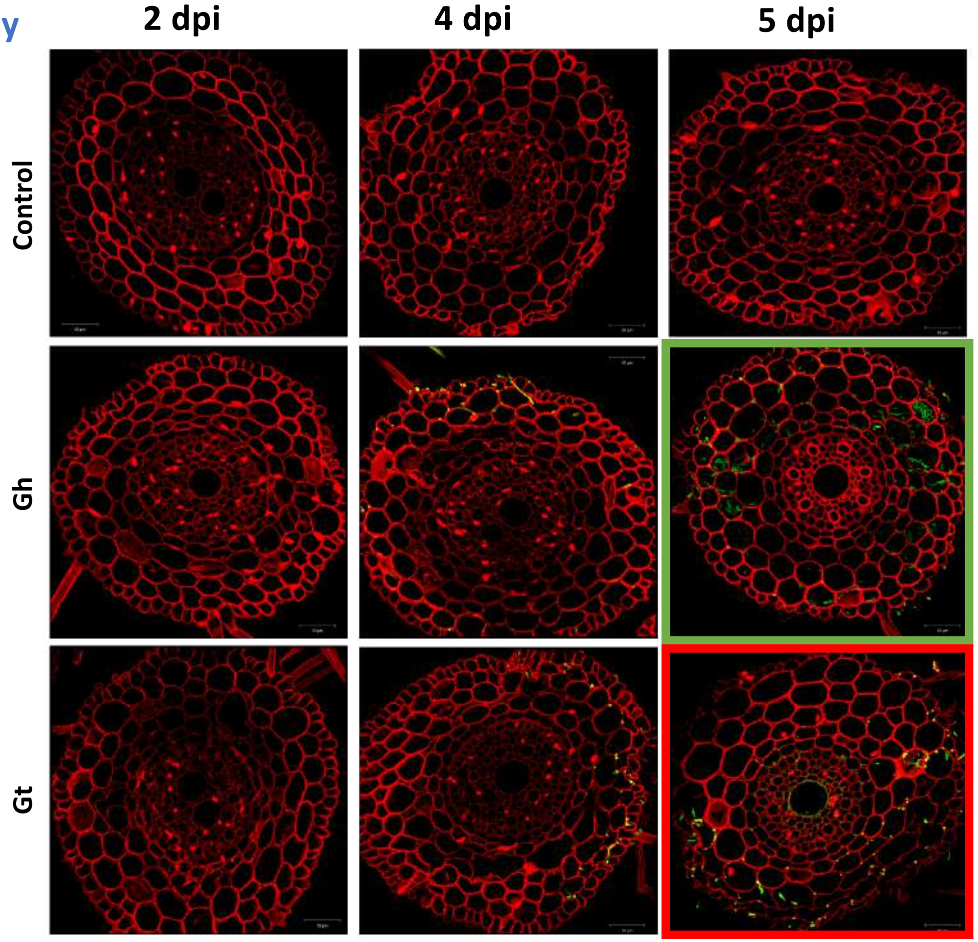
Chinese Spring

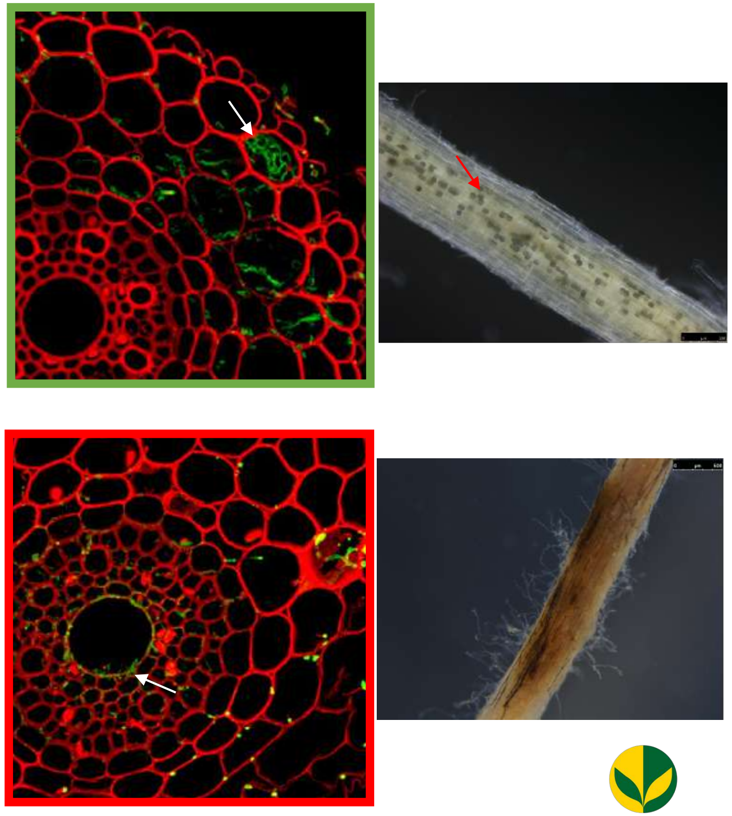
ROTHAMSTED
RESEARCH

Plant defence activation by Gh?

Confocal microscopy

Tania Chancellor (UoN DTP)

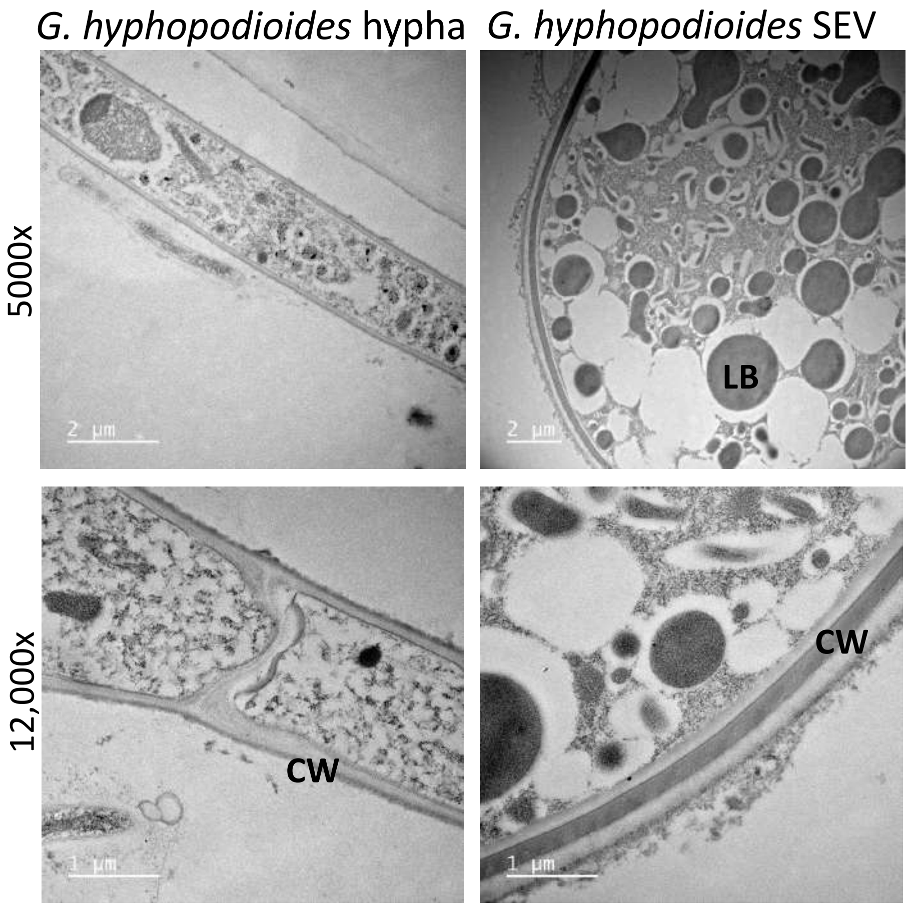




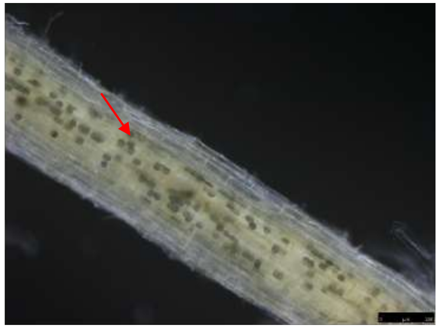
Green = Alexa Fluor – WGA   Red = PI

ROTHAMSTED
RESEARCH

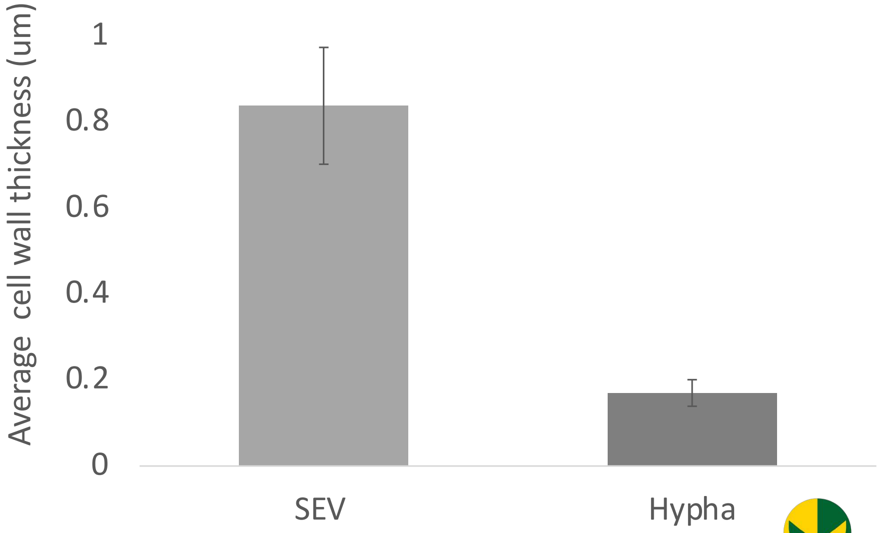
Are the subepidermal vesicles resistance structures?


G. hyphopodioides hypha *G. hyphopodioides* SEV

CW= Cell Wall, LB= Lipid Body.



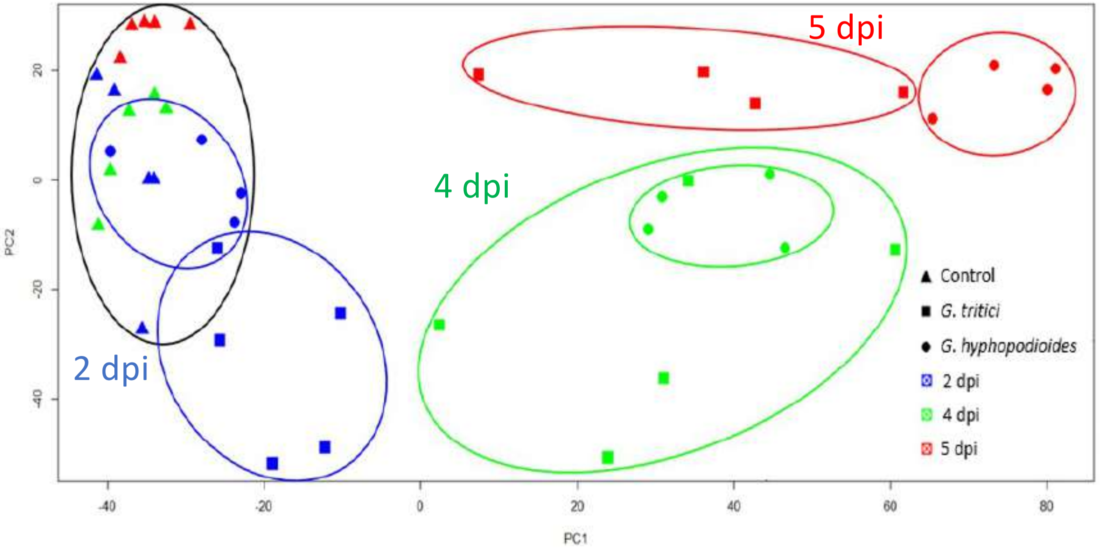
Tania Chancellor (UoN DTP)



SEVs ressemble chlamydospores

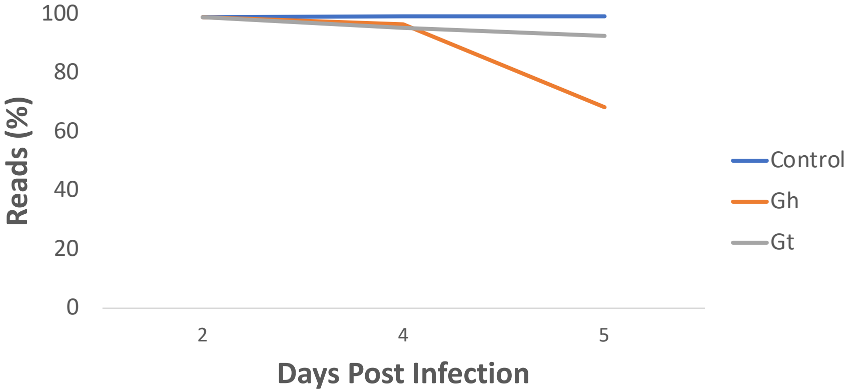
Different wheat response to Gt and Gh

PCA of RNAseq reads

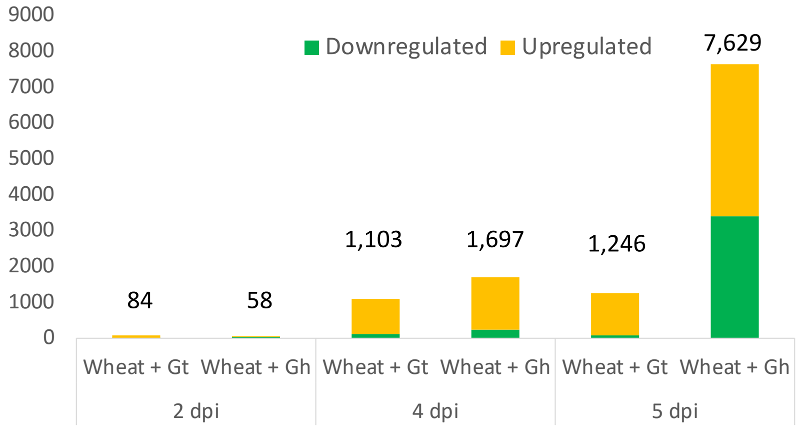


Tania Chancellor UoN DTP
Dr. Dan Smith

Percentage of reads mapped to the wheat genome

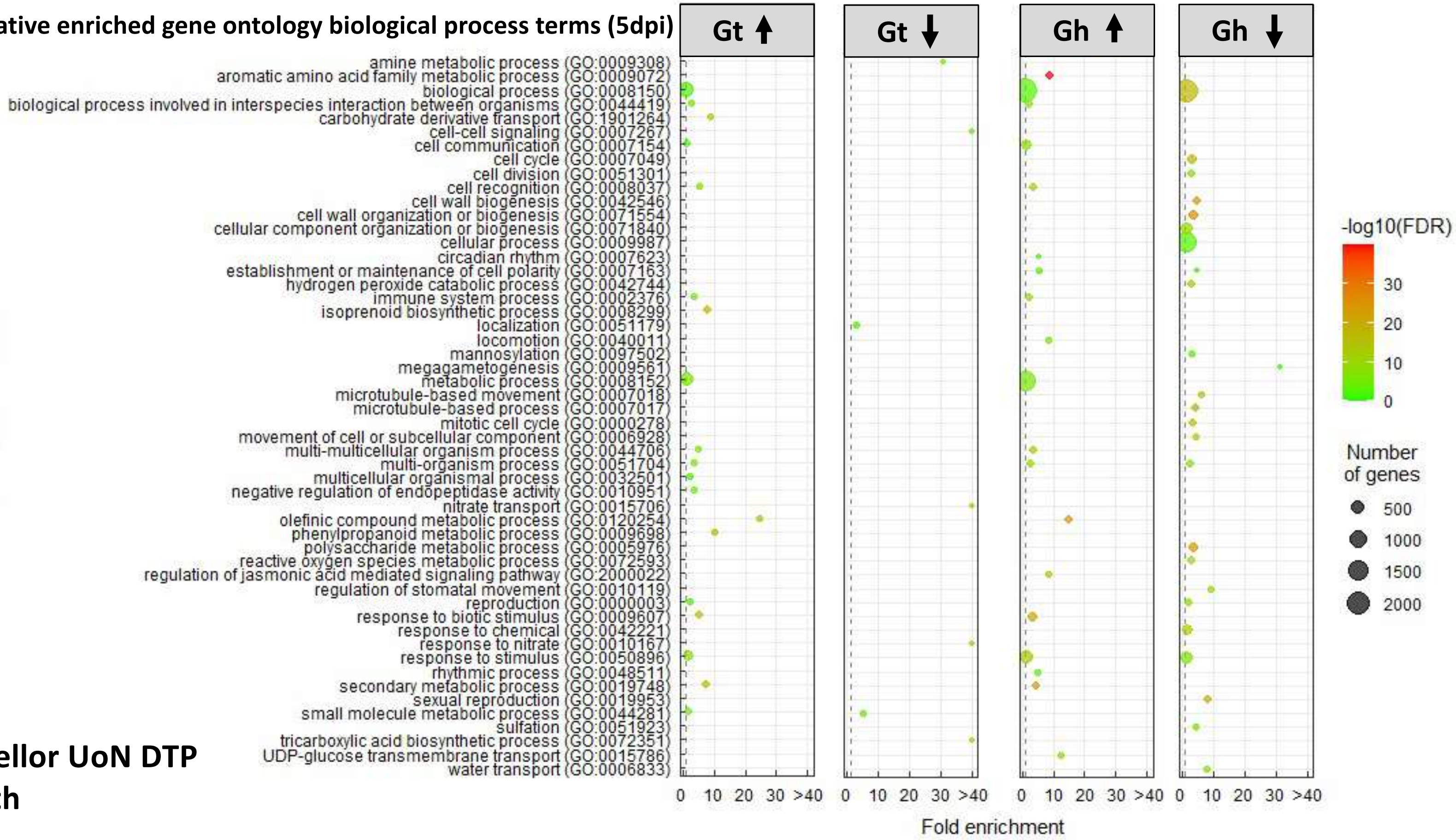


Number of differentially expressed genes (DEGs)



Different wheat responses to Gt and Gh

Most representative enriched gene ontology biological process terms (5dpi)

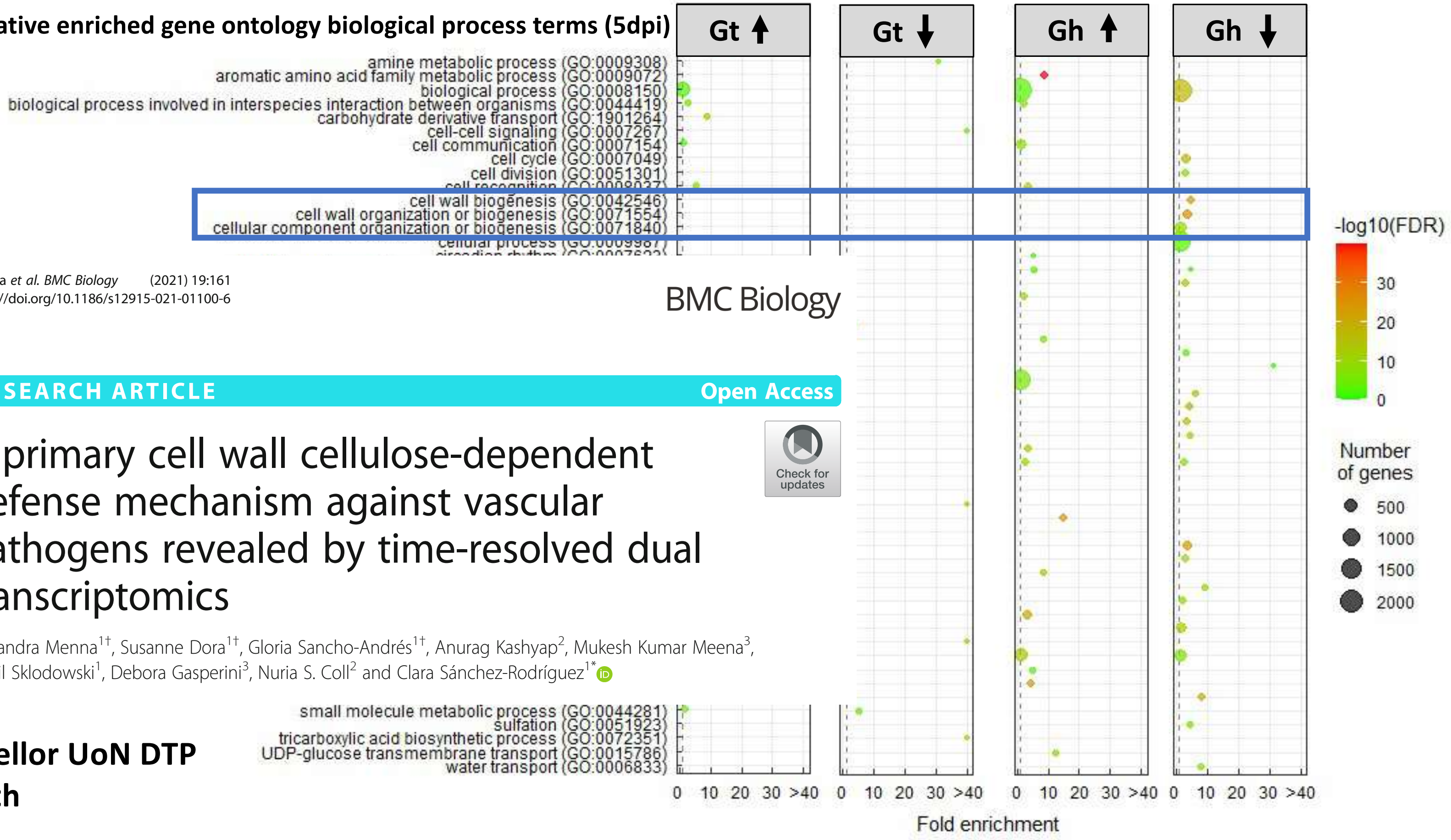


Tania Chancellor UoN DTP
Dr. Dan Smith

ROTHAMSTED
RESEARCH

Different wheat responses to Gt and Gh

Most representative enriched gene ontology biological process terms (5dpi)



Menna *et al. BMC Biology* (2021) 19:161
https://doi.org/10.1186/s12915-021-01100-6

BMC Biology

RESEARCH ARTICLE **Open Access**

Check for updates

A primary cell wall cellulose-dependent defense mechanism against vascular pathogens revealed by time-resolved dual transcriptomics

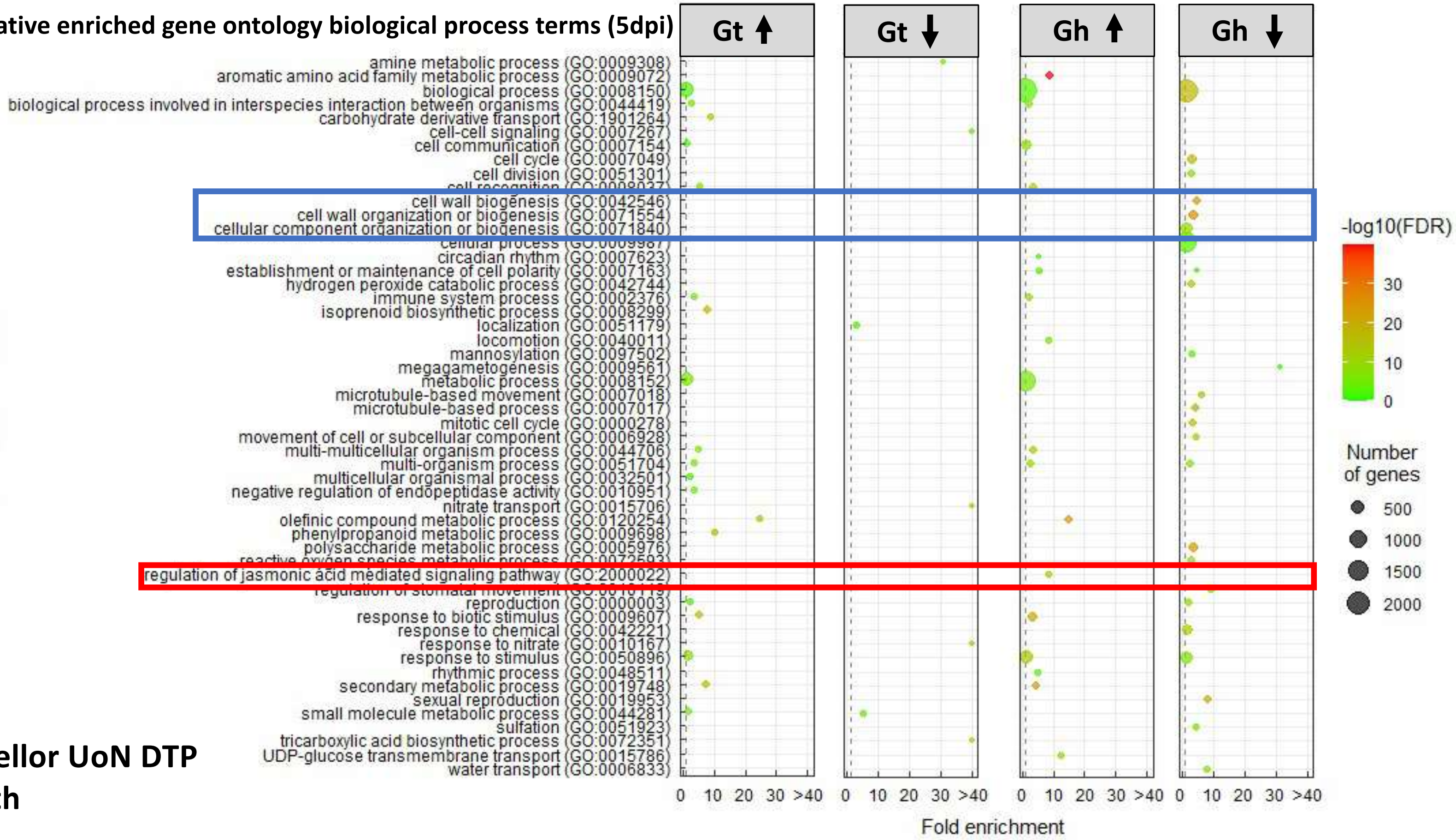
Alexandra Menna[1†], Susanne Dora[1†], Gloria Sancho-Andrés[1†], Anurag Kashyap[2], Mukesh Kumar Meena[3], Kamil Sklodowski[1], Debora Gasperini[3], Nuria S. Coll[2] and Clara Sánchez-Rodríguez[1*]

Tania Chancellor UoN DTP
Dr. Dan Smith

ROTHAMSTED
RESEARCH

Different wheat responses to Gt and Gh

Most representative enriched gene ontology biological process terms (5dpi)



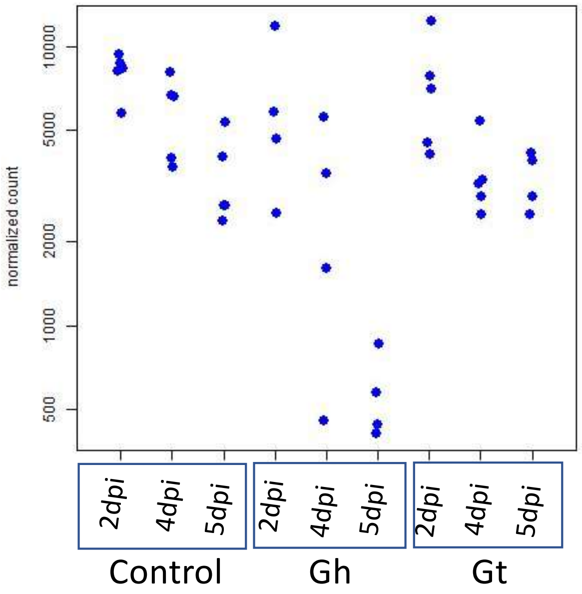
Tania Chancellor UoN DTP
Dr. Dan Smith

Downregulation of cell wall biogenesis and upregulation of JA signaling

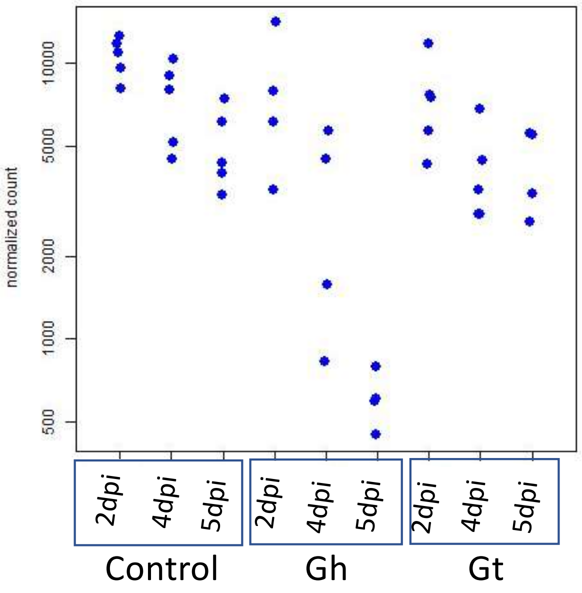
ROTHAMSTED
RESEARCH

Cellulase synthesis downregulation

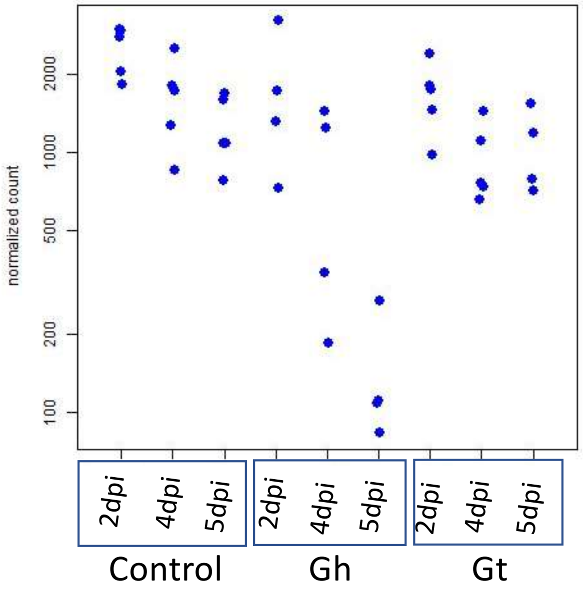
CESA4



CESA7



COBL-5

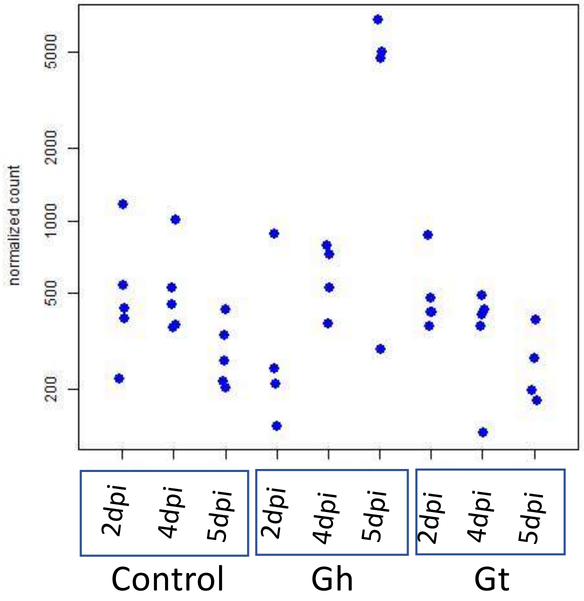


Defense activation?

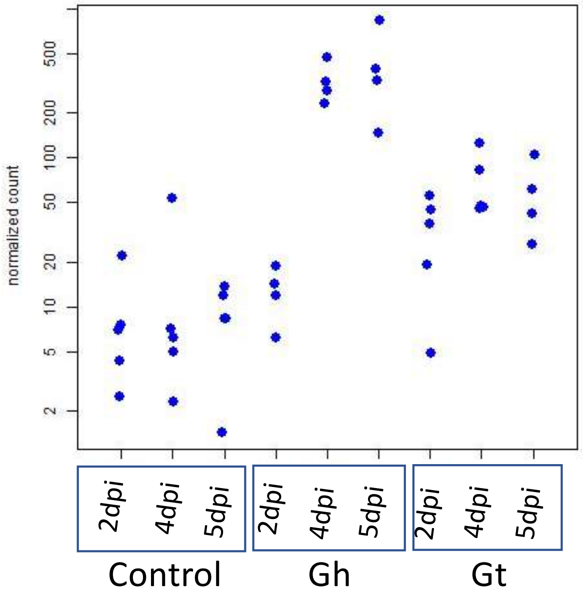
Tania Chancellor UoN DTP
Dr. Dan Smith

Plant defense activation by Gh

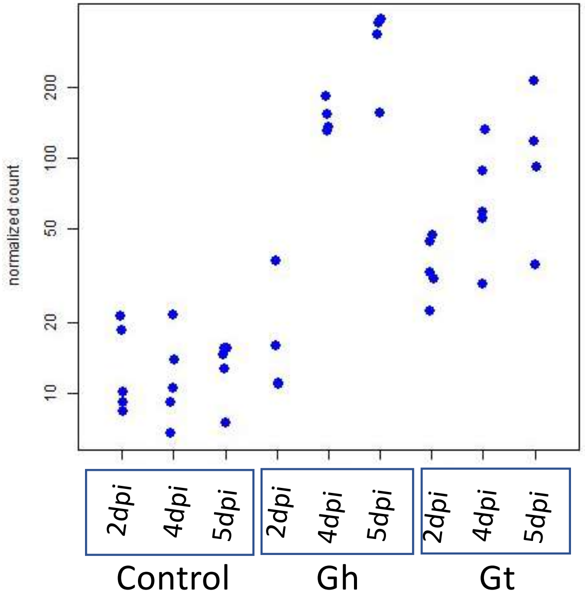
OPR1-like



WRKY28-like



WRKY75-like



Role in JA biosynthesis

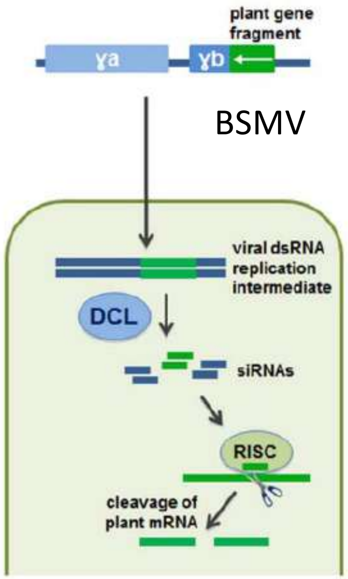
Transcription factors with role in resistance to fungal pathogens

Defense activation?

Tania Chancellor
Dr. Dan Smith

Virus Induced Gene Silencing

VIGS Mechanism



(Lee *et al*, 2012)

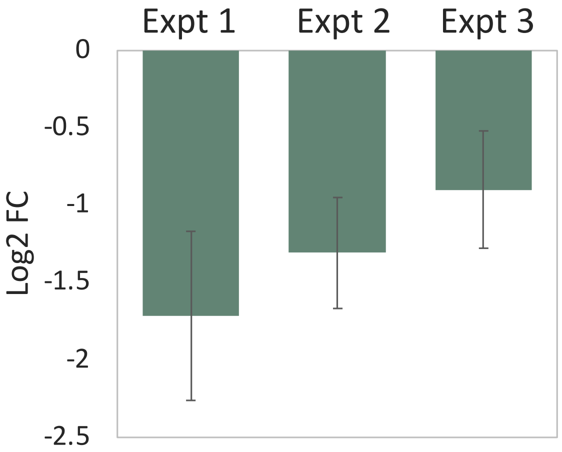
Gene	Characteristics	Silencing phenotype
Root hairless 3 (TaRTH3)	COBRA-like protein involved in cell expansion and cell-wall biosynthesis	Short roots and root hairs in maize mutants

Tania Chancellor UoN DTP
Dr. Wanxin Chen
Dr. Kostya Kanyuka

BSMV:Mcs4 (-)

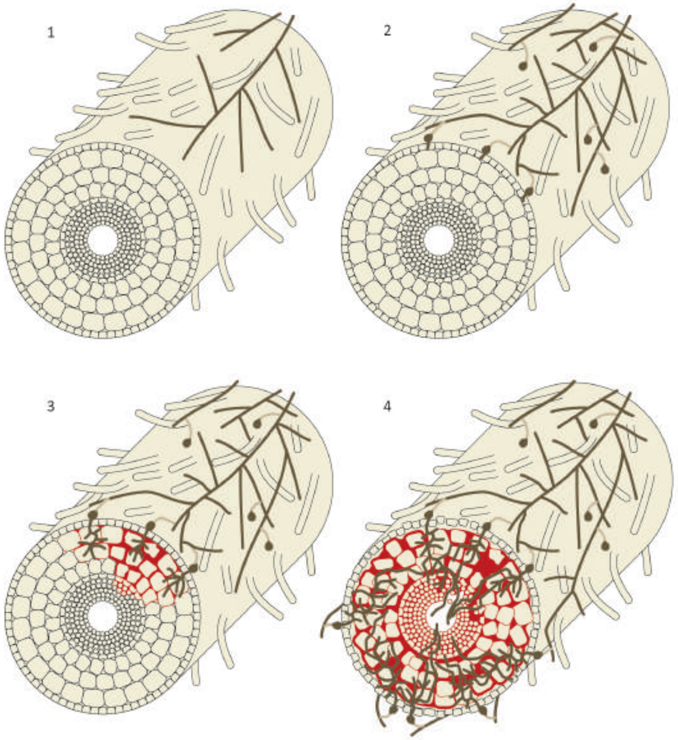
BSMV:TaRTH3

TaRTH3 relative expression levels

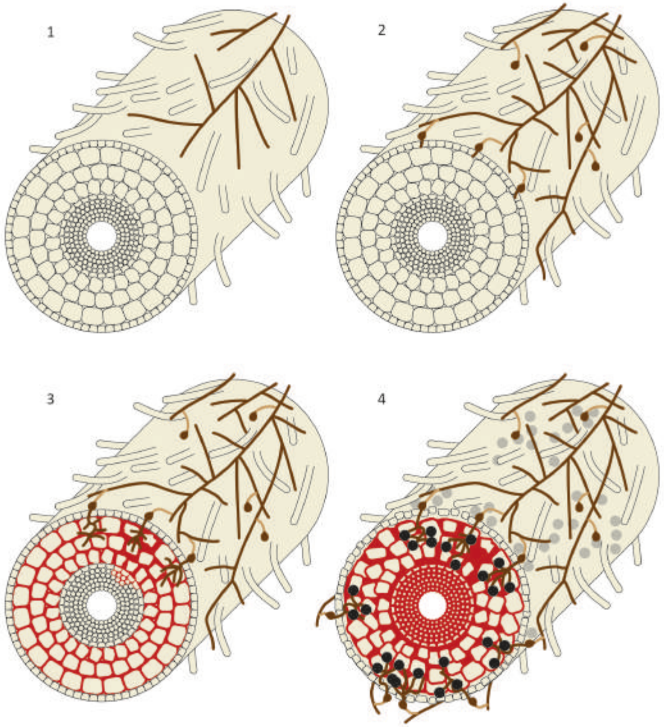


The infection process by Gt and Gh

Gt infection process

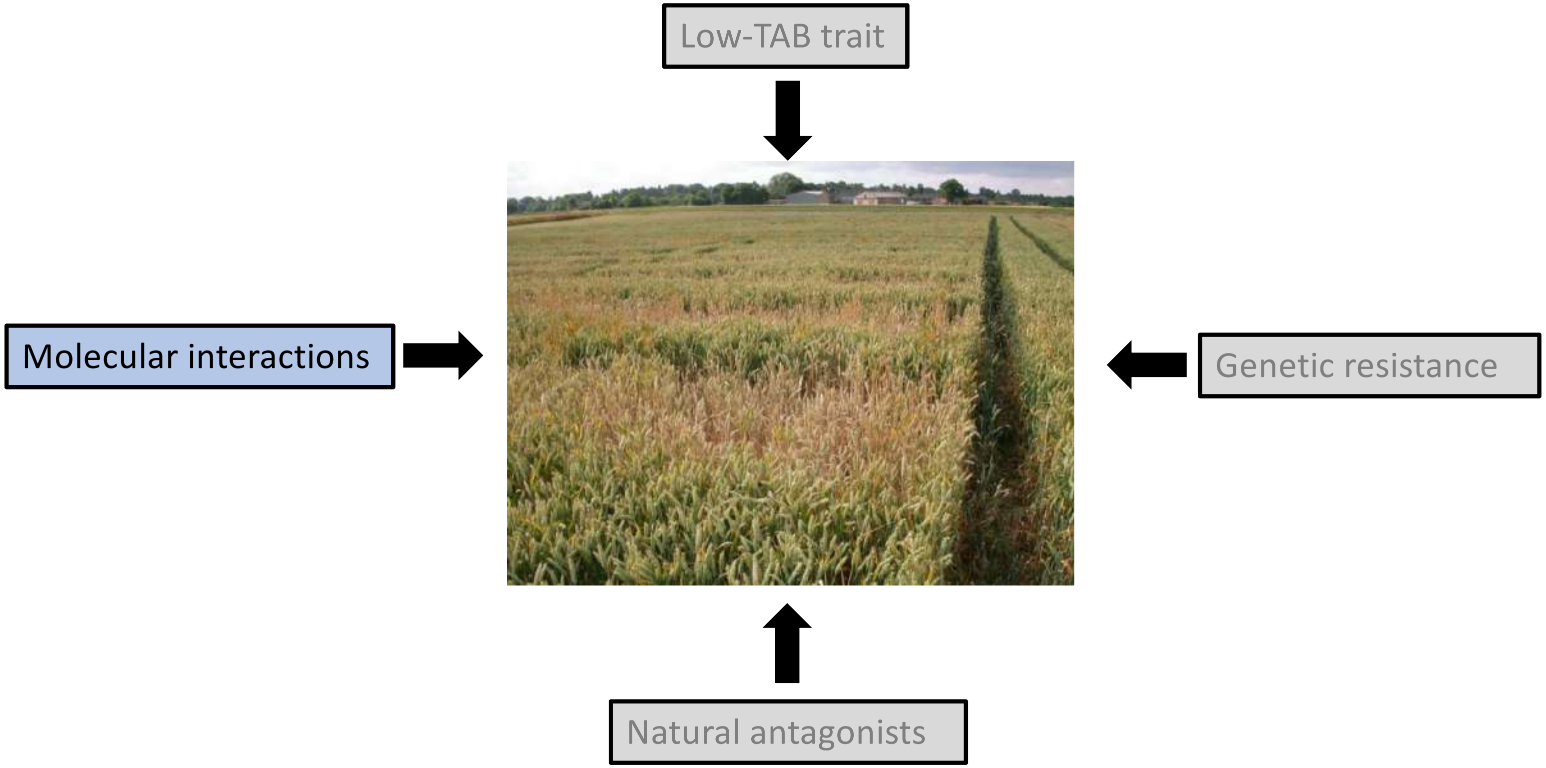


Gh infection process



What makes *G. tritici* successful?

Sustainable approaches to control Take-all



Low-TAB trait

Molecular interactions

Genetic resistance

Natural antagonists

Molecular interactions between *G. tritici* and wheat

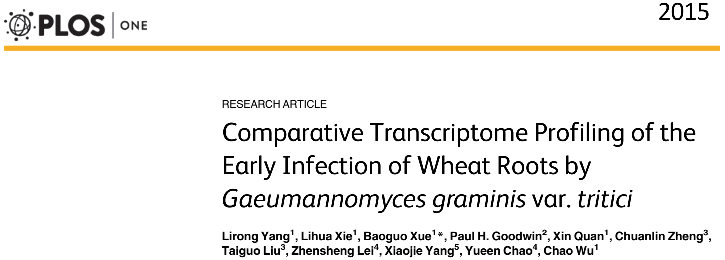
Molecular basis of virulence in *G. tritici* are mainly unknown

No mutants available

Genome sequenced in 2015 = 43.6 Mb (Okagaki *et al* 2015)

Transcriptomic studies:

2015



PLOS | ONE

RESEARCH ARTICLE

Comparative Transcriptome Profiling of the Early Infection of Wheat Roots by *Gaeumannomyces graminis* var. *tritici*

Lirong Yang[1], Lihua Xie[1], Baoguo Xue[1]*, Paul H. Goodwin[2], Xin Quan[1], Chuanlin Zheng[3], Taiguo Liu[3], Zhensheng Lei[4], Xiaojie Yang[5], Yueen Chao[4], Chao Wu[1]

Signal transduction pathways
CWDEs
Response to plant defense compounds: laccasses

MPMI Vol. 32, No. 10, 2019, pp. 1336–1347. https://doi.org/10.1094/MPMI-03-19-0066-R *e*-Xtra*

A Comparative Transcriptomic and Proteomic Analysis of Hexaploid Wheat's Responses to Colonization by *Bacillus velezensis* and *Gaeumannomyces graminis*, Both Separately and Combined

Xingxing Kang,[1] Lanhua Wang,[1] Yu Guo,[1] Muhammad Zain ul Arifeen,[1] Xunchao Cai,[1] Yarong Xue,[1,†] Yuanqin Bu,[2] Gang Wang,[3] and Changhong Liu[1,†]

SA-mediated response to take-all infection

frontiers in Microbiology

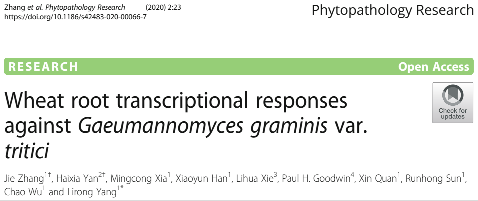
ORIGINAL RESEARCH
published: 09 July 2019
doi: 10.3389/fmicb.2019.01474

Check for updates

Comparative Transcriptome Profiling of *Gaeumannomyces graminis* var. *tritici* in Wheat Roots in the Absence and Presence of Biocontrol *Bacillus velezensis* CC09

Xingxing Kang[1], Yu Guo[1], Shuang Leng[1], Lei Xiao[2], Lanhua Wang[1], Yarong Xue[1] and Changhong Liu[1]**

Inhibitors of Papain-like cysteine protease
Catalase peroxidases
Enzyme involved in ABA biosynthesis



Zhang *et al. Phytopathology Research* (2020) 2:23
https://doi.org/10.1186/s42483-020-00066-7

Phytopathology Research

RESEARCH Open Access

Check for updates

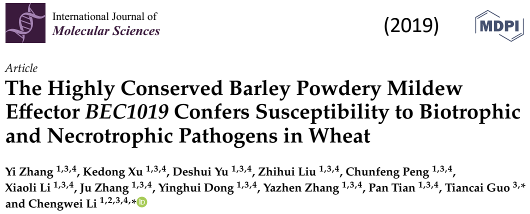
Wheat root transcriptional responses against *Gaeumannomyces graminis* var. *tritici*

Jie Zhang[1†], Haixia Yan[2†], Mingcong Xia[1], Xiaoyun Han[1], Lihua Xie[3], Paul H. Goodwin[4], Xin Quan[1], Runhong Sun[1], Chao Wu[1] and Lirong Yang[1*]

Defense and stress responses
WRKY, MAPKs, PR genes, SA genes

None of the genes have been functionally validated

One effector gene characterized by HIGS



International Journal of *Molecular Sciences*

(2019)

MDPI

Article

The Highly Conserved Barley Powdery Mildew Effector *BEC1019* Confers Susceptibility to Biotrophic and Necrotrophic Pathogens in Wheat

Yi Zhang [1,3,4], Kedong Xu [1,3,4], Deshui Yu [1,3,4], Zhihui Liu [1,3,4], Chunfeng Peng [1,3,4], Xiaoli Li [1,3,4], Ju Zhang [1,3,4], Yinghui Dong [1,3,4], Yazhen Zhang [1,3,4], Pan Tian [1,3,4], Tiancai Guo [3,*] and Chengwei Li [1,2,3,4,*]

Molecular basis of virulence in *G. tritici*

- Genome sequences being generated by Mark McMullan (Earlham Institute) as part of DFW

DFW

20 strains used for Pac Bio sequencing. Among them 4 Gt, 2 Gh, and beneficial fungi

G. tritici reference strains

Year	Cultivar	Soil core	TAB trait	Type A/B	Silthiofam Sensitivity
2017	Cadenza	19d1	Low	A	Insensitive
2017	Cadenza	23d	Low	B	Sensitive
2014	Hereward	14LH10	High	B	Insensitive
2017	Hereward	8d	High	A	Sensitive

+ 60 strains used for Illumina sequencing. Including 48 Gt strains and 4 Gh strains

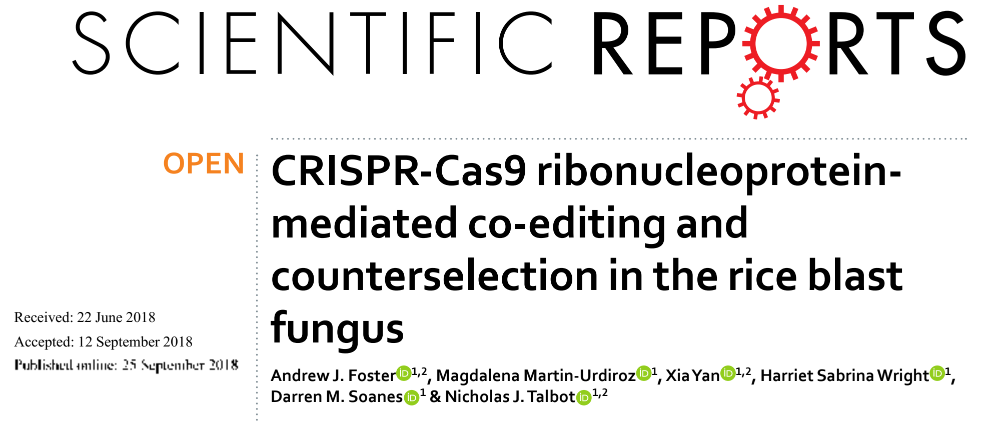
Species pangenome and

Identifying core genome and sp

Fungal transcriptional repr

Identifying genes differential

- Establishing CRISPR/CAS9 for genome editing in *G. tritici*



SCIENTIFIC **REPORTS**

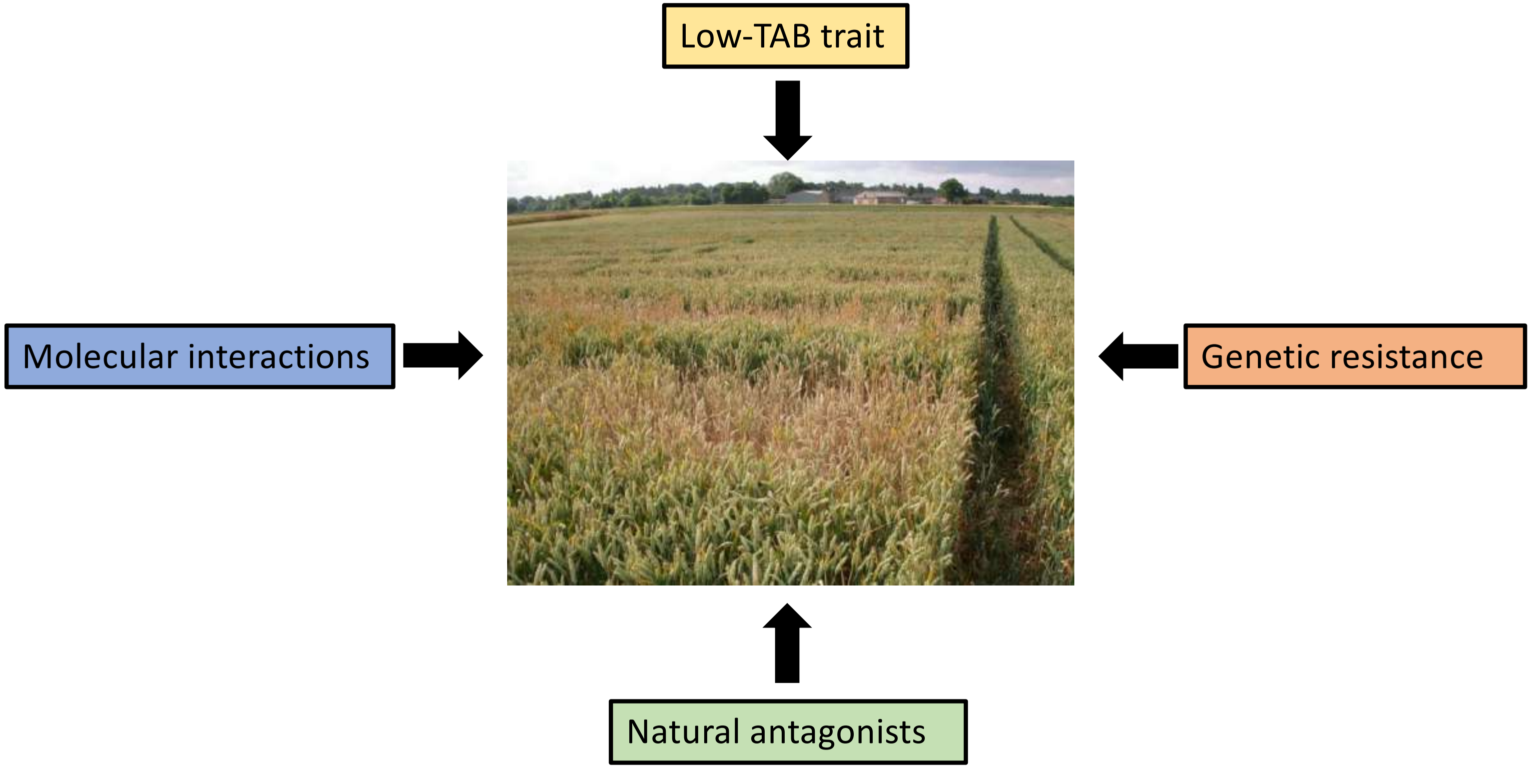
OPEN

CRISPR-Cas9 ribonucleoprotein-mediated co-editing and counterselection in the rice blast fungus

Received: 22 June 2018
Accepted: 12 September 2018
Published online: 25 September 2018

Andrew J. Foster[1,2], Magdalena Martin-Urdiroz[1], Xia Yan[1,2], Harriet Sabrina Wright[1], Darren M. Soanes[1] & Nicholas J. Talbot[1,2]

Sustainable approaches to control Take-all



Low-TAB trait

Molecular interactions

Genetic resistance

Natural antagonists

Increase wheat yield and protect the environment

ROTHAMSTED
RESEARCH

Thanks!

Take-all team
Gail Canning
Tania Chancellor
Dr. Shuang Gu
Dr. Wanxin Chen
Danni Zou

Previous members
Dr. Richard Gutteridge
Dr. Vanessa McMillan
Dr. Sarah-Jane Osborne
Dr. Joseph Moughan
Jess Spong
Dr. Marie Laborde
Jess Hammond

Wheat Pathogenomics
Prof. Kim Hammond-Kosack
Dr. Kostya Kanyuka
Dr. Martin Urban
Dr. Jason Rudd
Dr. Dan Smith
Dr. Mike Hammond-Kosack
Dr. Lawrence Bramham
Dr. Martin Darino

Biointeractions and Crop Protection Department

Collaborators:
Dr. Tim Mauchline and Dr. Ian Clark – **Molecular Microbial Ecology Group**
Dr. David Withall – **Chemical Ecology Group**
Dr. Mark McMullan, Dr. Sabrina Ward, Dr. Michelle Grey – **Earlham Institute**
Dr. Smita Kurup, Kirstie Halsey, Dr. Eudri Venter, and Hannah Walpole – **Bioimaging**

DFW

Wheat
Genetic
Improvement
Network

defra
Department for Environment Food and Rural Affairs

UKRI
Biotechnology and
Biological Sciences
Research Council

ROTHAMSTED
RESEARCH

If you want to know more about take-all disease:

CellPress
OPEN ACCESS

2021

Trends in
Plant Science

Review

Take-All Disease: New Insights into an Important Wheat Root Pathogen

Javier Palma-Guerrero[1,*,@], Tania Chancellor[1], Jess Spong[1], Gail Canning[1], Jess Hammond[1], Vanessa E. McMillan[1] and Kim E. Hammond-Kosack[1,*,@]

https://www.youtube.com/watch?v=JcVlx7R5QZI https://en.wikipedia.org/wiki/Take-all

Javier.palma-guerrero@rothamsted.ac.uk

ROTHAMSTED
RESEARCH