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
INTRODUCTION

The results were of immediate importance depending on fertiliser or manure applications, N, P and K accumulated or diminished in soil plot. Analyses of soil samples showed how details of the quantity and composition of each fertiliser applied, enabled a balance sheet for crop nutrient requirements. Lawes and Gilbert realised that much useful information could be gained by continuing them over many growing seasons. Nine experiments were continued of which still continue. Although they were not intended to be long-term, Lawes and Gilbert reasoned that it was the best way to learn about individual effects of abandoning arable land. When Lawes died in 1900, the eight remaining experiments were established in the 1880s to examine the results. In particular, two Wilderness studies gained by continuing them over many growing years and, later, further changes were made which they abandoned only one, in 1878. Some treatments were changed during the first few seasons. Nine experiments were planned. Modifications have been made to the experiments in the world. They are the oldest, continuous agronomic experiments in the world. The Classical experiments is the name now given to those experiments started by Lawes and Gilbert between 1843 and 1856, and but whose separate actions as plant nutrients amounts in crops and farmyard manure (FYM), sodium and magnesium (N, P , K, Na and Mg), containing nitrogen, phosphorus, potassium, their main objectives were to measure the effects on crop yields of inorganic compounds made at Rothamsted by treating bones with sulphuric acid), the sulphates of K, Na and Mg (often referred to then, and in this Guide, as nitrate (as alternative sources of nitrogen). The effects of these inorganic fertilisers were compared with those of FYM and rape cake minerals), and ammonium salts and sodium sulphuric acid), the sulphates of K, Na and Mg (often referred to then, and in this Guide, as nitrate (as alternative sources of nitrogen). The effects of these inorganic fertilisers were compared with those of FYM and rape cake minerals), and ammonium salts and sodium sulphuric acid), the sulphates of K, Na and Mg (often referred to then, and in this Guide, as nitrate (as alternative sources of nitrogen). The effects of these inorganic fertilisers were compared with those of FYM and rape cake.
Interactions in the rhizosphere

A complex ecosystem

Up to $10^6 \text{--} 10^9$ bacteria, $10^5 \text{--} 10^6$ fungi and $10^1 \text{--} 10^2$ nematodes per gram of soil

(Chuberre et al 2018)

Beneficials

Pathogens

Impact on yield and quality

(Lopez Raez et al 2017)
Take-all disease

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

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

It also infects barley and triticale, but not oats $\rightarrow$ Avenacin (Osborn \textit{et al} 1994)

No completely effective fungicide treatment or resistant cultivar $\rightarrow$ crop rotations

Wheat is an important staple crop produced and consumed globally

\textbf{Urgent need for new control methods}
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)

Controlling the disease is important for food security and for the environment
Figure 3. Gaeumannomyces tritici Life Cycle.

(A) Life cycle within a crop season. The cycle begins when seeds are drilled into soil containing infected crop debris and/or fungal mycelium, the seedlings roots are infected, and runner hyphae can be observed on roots. As the infection progresses disease lesions appear on wheat roots and crown tissue. Perithecia, containing asci and ascospores, can be observed on stem bases and stubble. Secondarily infections occur by runner hyphae growing through root bridges, which can happen with in a plant or between roots from different plants. Diseased plants with stunted growth and whiteheads appear in patches in the field. After the harvest the fungus survives saprophytically in the crop debris.

(B) Take-all level when successive years of wheat crops are grown in the field. The first wheat crop after a rotation break (i.e., non-cereal crop) has low levels of take-all inoculum build-up (TAB) in the rhizosphere and has low take-all disease levels. In subsequent years, take-all disease in the wheat roots increases and peaks during years 2–4 (depending on local conditions) and then declines.

(C) The 2-year, synergistic genetic traits concept to reduce take-all root disease in wheat crops. In year 1, the growing of low, intermediate or high take-all inoculum build-up (TAB) cultivars (respectively, green, blue, and red broken lines) leads to different levels post-harvest of take-all fungal mycelium left in the rhizosphere, even though the roots of the first wheat crop remain take-all disease free. In year 2, when a partially resistant wheat cultivar is grown (orange solid line), the disease incidence and severity is lower than when a fully susceptible wheat cultivar is grown (blue solid line). In fields where a high TAB situation has developed in year 1, the economic threshold for severe yield losses is likely to be reached irrespective of second wheat choice.

(Disease cycle (Palma-Guerrero et al., 2021))
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
Sustainable approaches to control Take-all

- Low-TAB trait
- Genetic resistance
- Natural antagonists
- Molecular interactions
Sustainable approaches to control Take-all

- Low-TAB trait
- Molecular interactions
- Natural antagonists
- Genetic resistance
Take-all Build-Up

1st wheat
Yield average 12.69t/ha

3rd wheat
Yield average 7.64t/ha

1st and 3rd wheat field trials; 45 elite winter wheat cultivars
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
Wheat cultivars differ in TAB levels

1st wheat crop take-all build-up

P = 0.009

44% difference between Hereward and Cadenza

Associated to changes in soil microbiome
Rhizosphere microbiome changes

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

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

(Mauchline et al 2015)
Sustainable take-all control by 1\textsuperscript{st} wheat choice

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

(McMillan et al 2018)

Orange = partially resistant
Blue = highly susceptible

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

(Palma-Guerrero \textit{et al}, 2021)
Sustainable approaches to control Take-all

Low-TAB trait

Molecular interactions

Natural antagonists

Genetic resistance
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

- 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
**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
Sustainable approaches to control Take-all

Low-TAB trait

Molecular interactions

Natural antagonists

Genetic resistance
Take-all antagonists in the rhizosphere

Beneficial fungi with different modes of action

**Plant defense activation**

*Gaeummannomyces hyphopodioides* (Phialophora)

Suppresses take-all disease  (Speakman and Lewis 1978)

Potential biocontrol agent

**Direct antifungal activity**

Fungi isolated from wheat roots

Source of antifungal compounds
Take-all antagonists in the rhizosphere

- *Gaeumannomyces hyphopodioides* (Phialophora):

Gh infection protects from take-all disease

Tania Chancellor (UoN DTP)
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)

2 dpi  |  4 dpi  |  5 dpi
---|---|---
Control

Gh

Chinese Spring
# Plant defence activation by Gh?

<table>
<thead>
<tr>
<th>Confocal microscopy</th>
<th>2 dpi</th>
<th>4 dpi</th>
<th>5 dpi</th>
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</table>

Green = Alexa Fluor – WGA  \[Red = PI\]
Are the subepidermal vesicles resistance structures?

G. hyphopodioides hypha  G. hyphopodioides SEV

SEVs resemble chlamydospores

Tania Chancellor (UoN DTP)

CW = Cell Wall, LB = Lipid Body.
PCA of RNAseq reads

Different wheat response to Gt and Gh

Percentage of reads mapped to the wheat genome

Number of differentially expressed genes (DEGs)

Tania Chancellor UoN DTP
Dr. Dan Smith
Different wheat responses to Gt and Gh

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

- aromatic amino acid family metabolic process
- biological process
- carbohydrate derivative transport
- cell communication
- cell cycle
- cell division
- cell recognition
- cell wall organization or biogenesis
- cellular component organization or biogenesis
- establishment or maintenance of cell polarity
- hydrogen peroxide catabolism
- immune system process
- isoprenoid biosynthesis
- localization
- localization of signal transduction molecules
- mannose metabolism
- metabolic process
- microtubule-based movement
- mitotic cell cycle
- movement of cell or subcellular component
- multi-molecular complex process
- multi-organism process
- negative regulation of endocytosis
- nitrate transport
- organic compound metabolic process
- photosynthesis
- protein metabolic process
- regulation of jasmonic acid-mediated signaling pathway
- response to abiotic stimulus
- response to chemical
- response to nitrate
- response to stimulus
- steroid biosynthetic process
- steroid metabolic process
- starch metabolic process
- U9P-glucose transport regulator protein translation
- water transport

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Dr. Dan Smith
Different wheat responses to Gt and Gh

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

<table>
<thead>
<tr>
<th></th>
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<th>Gt ↓</th>
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**Abstract**

**Background:** Cell walls (CWs) are protein-rich polysaccharide matrices essential for plant growth and environmental acclimation. The CW constitutes the first physical barrier as well as a primary source of nutrients for microbes interacting with plants, such as the vascular pathogen *Fusarium oxysporum* (Fo). Fo colonizes roots, advancing through the plant primary CWs towards the vasculature, where it grows causing devastation in many crops. The pathogenicity of Fo and other vascular microbes relies on their capacity to reach and colonize the xylem. However, little is known about the root-microbe interaction before the pathogen reaches the vasculature and the role of the plant CW during this process.

**Results:** Using the pathosystem Arabidopsis-Fo5176, we show dynamic transcriptional changes in both fungus and root during their interaction. One of the earliest plant responses to Fo5176 was the downregulation of primary CW synthesis genes. We observed enhanced resistance to Fo5176 in Arabidopsis mutants impaired in primary CW cellulose synthesis. We confirmed that Arabidopsis roots deposit lignin in response to Fo5176 infection, but we show that lignin-deficient mutants were as susceptible as wildtype plants to Fo5176. Genetic impairment of jasmonic acid biosynthesis and signaling did not alter Arabidopsis response to Fo5176, whereas impairment of ethylene signaling did increase vasculature colonization by Fo5176. Abolishing ethylene signaling attenuated the observed resistance while maintaining the dwarfism observed in primary CW cellulose-deficient mutants.

**Conclusions:** Our study provides significant insights on the dynamic root-vascular pathogen interaction at the transcriptome level and the vital role of primary CW cellulose during defense response to these pathogens. These findings represent an essential resource for the generation of plant resistance to Fo that can be transferred to other vascular pathosystems.

**Keywords:** Arabidopsis, *Fusarium oxysporum*, *Ralstonia solanacearum*, plant-pathogen interaction, dual-time course transcriptomics, cellulose, ethylene, defense response

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† Alexandra Menna, Susanne Dora and Gloria Sancho-Andrés contributed equally to this work.

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

RESEARCH ARTICLE

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

Alexandra Menna†, Susanne Dora†, Gloria Sancho-Andrés†, Anurag Kashyap‡, Mukesh Kumar Meena‡, Kamil Sklodowski‡, Debora Gasperini‡, Nuria S. Col‡ and Clara Sánchez-Rodríguez†

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)

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<td>regulation of cell wall organization or biogenesis</td>
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Downregulation of cell wall biogenesis and upregulation of JA signaling
Cellulase synthesis downregulation

Defense activation?

Tania Chancellor UoN DTP
Dr. Dan Smith
Plant defense activation by Gh

ROLE IN JA BIOSYNTHESIS

Transcription factors with role in resistance to fungal pathogens

Defense activation?

Tania Chancellor
Dr. Dan Smith
Virus Induced Gene Silencing

**Gene Characteristics Silencing phenotype**

<table>
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<tr>
<th>Gene</th>
<th>Characteristics</th>
<th>Silencing phenotype</th>
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<tr>
<td>Root hairless 3 (TaRTH3)</td>
<td>COBRA-like protein involved in cell expansion and cell-wall biosynthesis</td>
<td>Short roots and root hairs in maize mutants</td>
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**TaRTH3 relative expression levels**

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<td>-1.5</td>
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**BSMV Mechanism**

(Lee *et al*, 2012)

Tania Chancellor UoN DTP
Dr. Wanxin Chen
Dr. Kostya Kanyuka
The infection process by Gt and Gh

Gt infection process

1

2

3

4

Gh infection process

1

2

3

4

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:

**Signal transduction pathways**

**Response to plant defense compounds: laccases**

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

Xiong Kong, Liuhan Wang, Yu Guo, Muhammad Zain ul Arifeen, Xunchao Cai, Yarong Xue, Yuqin Liu, Gang Wang, and Changhong Liu

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

**Defense and stress responses**

WRKY, MAPKs, PR genes, SA genes

None of the genes have been functionally validated

One effector gene characterized by HIGS

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

Xiong Kong, Yu Guo, Shuang Long, Lei Xie, Liuhan Wang, Yarong Xue* and Changhong Liu*

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

Lixia Li, Lihua Xie, Baoguo Xue* Paul H. Goodwin, Xin Quan, Chuanlin Zheng, Tonglu Liu, Zhenheng Lei, Xiaojie Yang, Yuwen Zhao, and Chao Wu

**Molecular interactions between microorganism, although the gene expression pattern was transcriptional or translational program than for each single gene, and a plant have been analyzed predominantly from the genome for RNA ex-**

**The research is financially supported by the National Natural**

**SA–JA interactions resulted in antagonistic**

**Moreover, the logo stands for “electronic extra” and indicates that five sup-**

**In this study, transcriptome se-**

**Additionally,**

**The highly conserved barley powdery mildew effector BEC1019 confers susceptibility to biotrophic and necrotrophic pathogens in wheat**

**Endophytic bacteria, pathogenic fungi, phytopathology, RNA sequencing, wheat disease**

**Keywords: endophytic bacteria, pathogenic fungi, phytopathology, RNA sequencing, wheat disease**

**©**
Molecular basis of virulence in *G. tritici*

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

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

**G. tritici reference strains**

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<th>Year</th>
<th>Cultivar</th>
<th>Soil core</th>
<th>TAB trait</th>
<th>Type A/B</th>
<th>Silthiofam Sensitivity</th>
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</table>

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

- Establishing CRISPR/CAS9 for genome editing in *G. tritici*
Sustainable approaches to control Take-all

- Low-TAB trait
- Molecular interactions
- Genetic resistance
- Natural antagonists

Increase wheat yield and protect the environment
Thanks!

<table>
<thead>
<tr>
<th>Take-all team</th>
<th>Previous members</th>
<th>Wheat Pathogenomics</th>
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<tbody>
<tr>
<td>Gail Canning</td>
<td>Dr. Richard Gutteridge</td>
<td>Prof. Kim Hammond-Kosack</td>
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<tr>
<td>Tania Chancellor</td>
<td>Dr. Vanessa McMillan</td>
<td>Dr. Kostya Kanyuka</td>
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<td>Dr. Shuang Gu</td>
<td>Dr. Sarah-Jane Osborne</td>
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<td>Dr. Joseph Moughan</td>
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<td>Danni Zou</td>
<td>Jess Spong</td>
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<td>Dr. Marie Laborde</td>
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<td>Dr. Martin Darino</td>
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**Collaborators:**

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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**
Review

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


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

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