

WGIN 3 Project kick-off meeting



Kim Hammond-Kosack
Rothamsted Research



4th March 2015
RRes

Background

WGIN 2 extension from Dec 2013 to August 2014 ~ £106,000

Besides the science and networking, three key activities were completed in 2014

- 1. Submitted to Defra in June 2014**
a WGIN Legacy document covering the entire 10 year project
- 2. Submitted to Defra in November 2014**
the final report on the entire WGIN 2 project
- 3. Submitted to Defra in December 2014**
the new two year WGIN 3 project

WGIN phase 3 (WGIN3)

New project title

**Defra Wheat Genetic Improvement Network
- Improving the resilience of the wheat crop
through genetics and targeted traits
analysis**

Wheat Genetic Improvement Network (WGIN3) 2014-2016

Red text -
new to WGIN3

WP1 Management meetings – The Network

WP3 Tools and Resources

Maintain and further develop, mapping popⁿ,
Watkins/Gediflux, *T. monococum* collections (3.1)

Create an A x C NIL TILLING popⁿ (3.2)

T. monococum introgression (3.3)

WPs 2, 3 & 4 Targeted Traits

Aphid resistance (2.2)

Take-all resistance (2.2, 3.4)

Septoria and **yellow rust resistance** (2.2)

Yield and quality resilience (2.2, 3.4)

Yield components (2.2)

Drought tolerance (2.2, 3.4)

Root system function (3.4)

WPs 2 & 4 Genetic and QTL analyses

For each of the targeted traits

Gene-specific marker development (2.4)

Sub-contractors – WP1.3 & 4.2 **NGS genome / exome analyses**

WP1 Enhancing the network and communication of results

Website (1.2)

Annual Stakeholders forum (1.1)

International collaborations (1.4)

Publications + data deposits (1.4)

Electronic Newsletter (1.4)

Focussed workshops (1.1)

Public outreach

Industry-led forum (1.5)

WGIN 3 project partners

Total - £669, 938

John Innes Centre – Simon Griffiths

£252,069

Rothamsted Research - Kim Hammond-Kosack

£417,069

inc admin /ShE

Two sub- contractors £98,821

**Bristol Genomics
Facility
Univ. Bristol, UK**

**MYcroarray
Michigan, USA**

Genotyping

Allelic variation via Exome Capture

Duration - 2 years

Twenty one project milestones

1	(March 15)	First stakeholder meeting
2	(February 15)	Development of new near isogenic lines.
3	(throughout project)	Further maintenance and distribution of Avalon x Cadenza doubled haploid population.
4	(Feb 15)	Genetic characterisation of Paragon mutants.
5	(March 15)	Identification of useful genetic variation in Watkins population.
6	(Apr 15)	Development of new mapping populations.
7	(June 15)	First Interim written report to Defra
8	(Sept 15)	Resistance to cereal aphids, information to establish the likely genetic basis of resistance to cereal aphid (Sept 15)
9	(Aug 15)	Development of new QTL for yield at low and high N input
10	(Sept 15)	Information on stability of yield and nitrogen use efficiency parameters for elite varieties
11	(Oct 15)	Collection of data on variation in canopy longevity and nitrogen remobilisation
12	(Dec 15)	Evaluation of lines with good bread-making properties
13	(Dec 15)	Second stakeholder meeting
14	(Jan 16)	Second Interim written report to Defra/Project evaluation
15	(Feb 16)	Improvement of water use efficiency and drought tolerance traits
16	(Feb 16)	Take-all disease, genetic basis, introgression of lines harbouring resistance
17	(March 16)	Introgression of extreme resistance to Septoria tritici from T. monococcum
18	(Apr 16)	Information on germplasm with new important traits.
19	(Summer 16)	Grain Archiving: from each plot of the annual diversity and Avalon x Cadenza field
20 and 21	(Dec 16)	Third Stakeholder meeting and 21. Report (Interim or final)

Plus a detailed Gantt chart covering all activities linked to these milestone

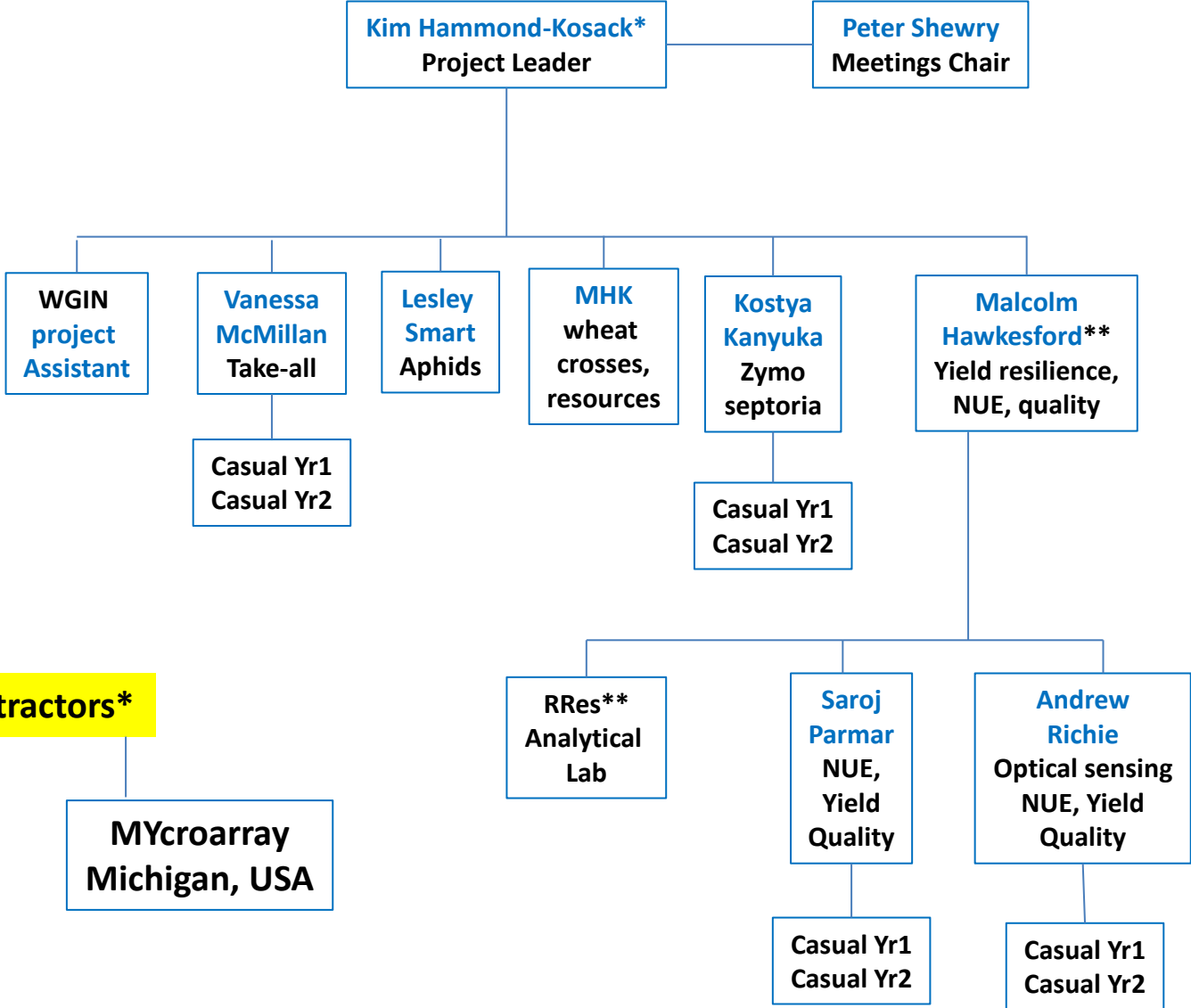
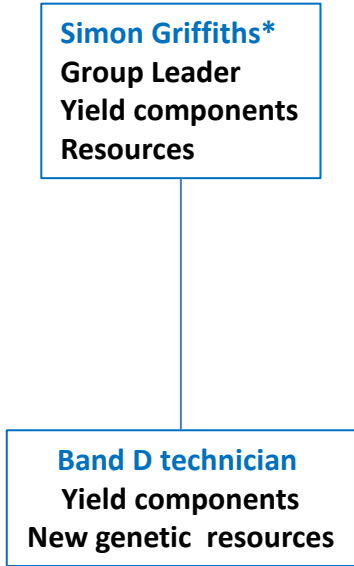
WGIN phase 3

Improving the resilience of the wheat crop through genetics and targeted traits analysis

THE WGIN3 TEAM

John Innes Centre

Rothamsted Research



Sub-contractors*

**Bristol Genomics Facility
Univ. Bristol, UK**

**MYcroarray
Michigan, USA**

John Innes Centre

Director: Dale Saunders Staff: 536

Six Departments

Biological Chemistry

Cell and Developmental Biology

Crop Genetics, Staff: 130
Simon Griffiths + Band D Tech

Metabolic Biology

Molecular Microbiology

Computational and Systems Biology

Farm	IT services
GH & CE Facilities	HR
Contracts	Outreach

Sub-contractors

Bristol Genomics Facility
@Univ. Bristol, UK

MYcroarray
Michigan, USA

Rothamsted Research

Director: Achim Dobermann Staff: 414

Five Departments

AgroEcology

Biological Chemistry and Crop Protection
Lesley Smart

Plant Biology and Crop Science, Staff: 72
Deputy head: **Malcolm Hawkesford**
Peter Shewry,
Kim Hammond-Kosack, Kostya Kanyuka,
Vanessa McMillan, Mike Hammond-Kosack
Andrew Richie, Saroj Parmar, C. Sparks

Computational and Systems Biology
Keywan Hassani-Pak

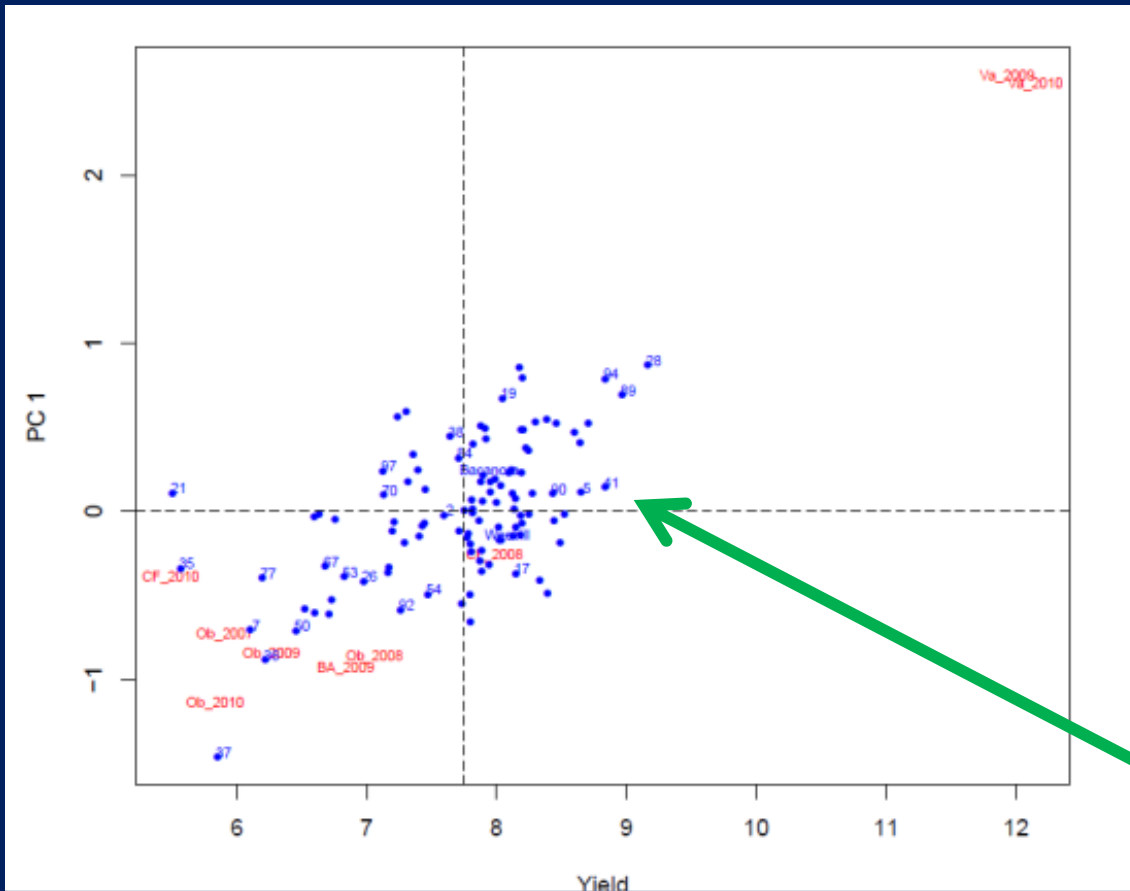
Sustainable Soils and Grassland Systems

Farm, GH & CE Facilities Contracts	Analytical lab HR and IT Outreach
--	---

Genetic Resource Development for UK wheat yield stability



Why has WGIN targeted yield stability?



- Wheat growing conditions are subject to escalating climate volatility
- Acceptably high yield levels, with consistency between locations and years is an increasingly important target.
- It is easy to be stable and low!
- What traits deliver high mean yield?

AMMI plot 2 for Weebill x Bacanora in multiple locations, for stable yield $PC1=0$

Dissection of genetic gain in UK winter wheat

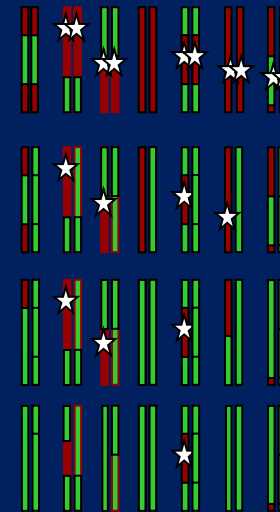
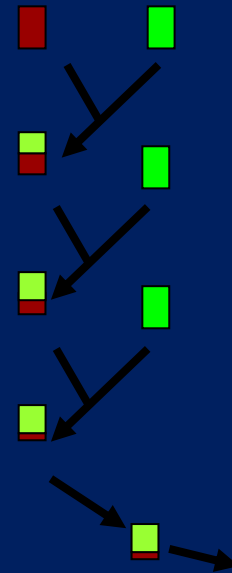
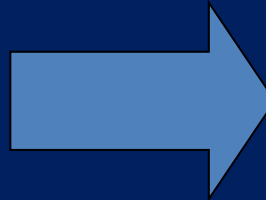
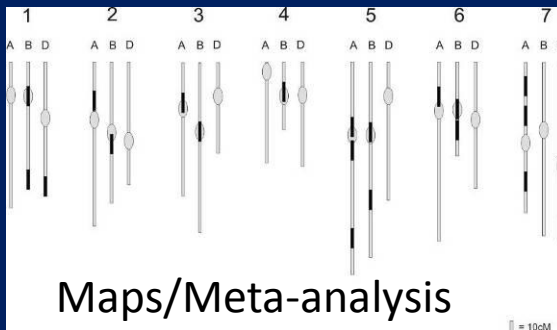
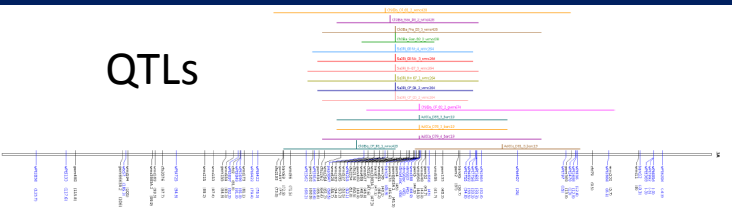
crosses

- Spark x Rialto
- Malacca x Charger
- Avalon x Cadenza
- Savannah x Renesansa
- Buster x Charger
- Lynx x Cadenza
- Charger x Badger
- Beaver x Soissons
- Savannah x Rialto
- Weebil x Bacanora
- Shango x Shamrock
- Milan x Catbird

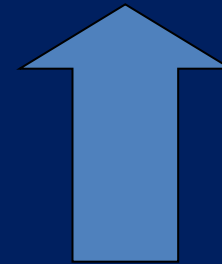
What genes control these traits?
How do alleles
work in combination for genetic gain and
trait stability?



QTLs

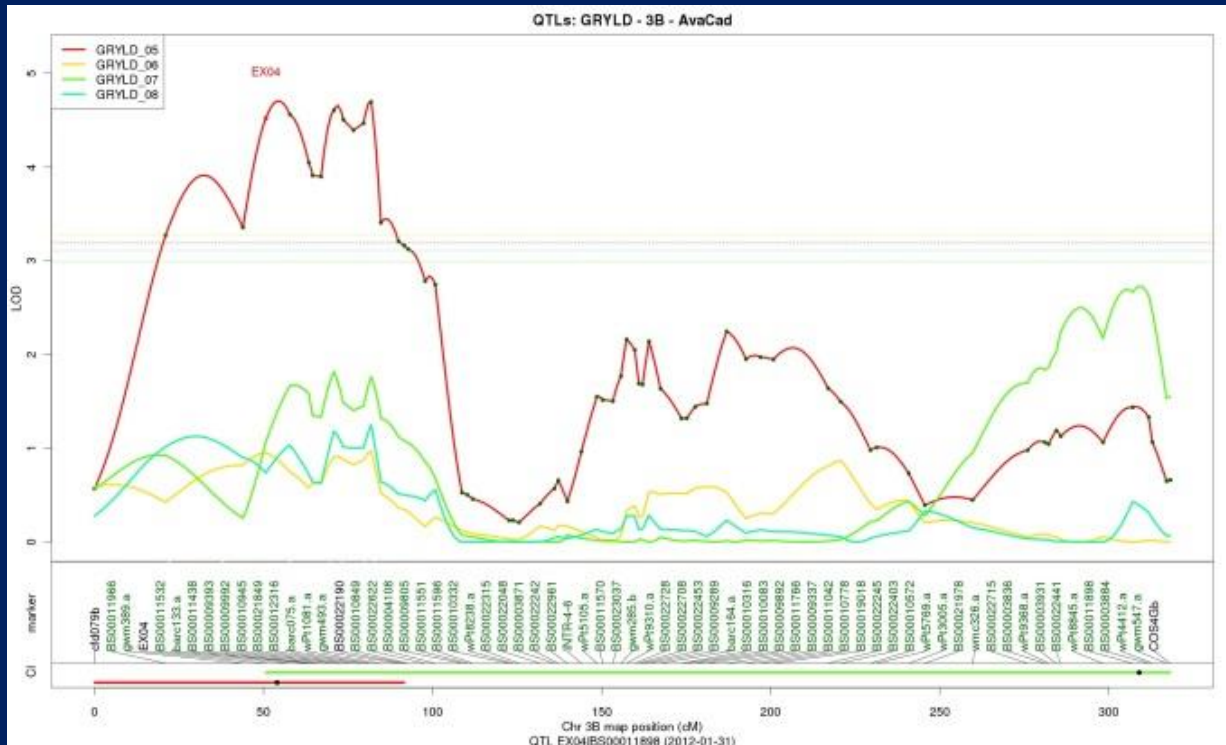


Isogenics



Can we understand the basis of QTL x environment interaction?

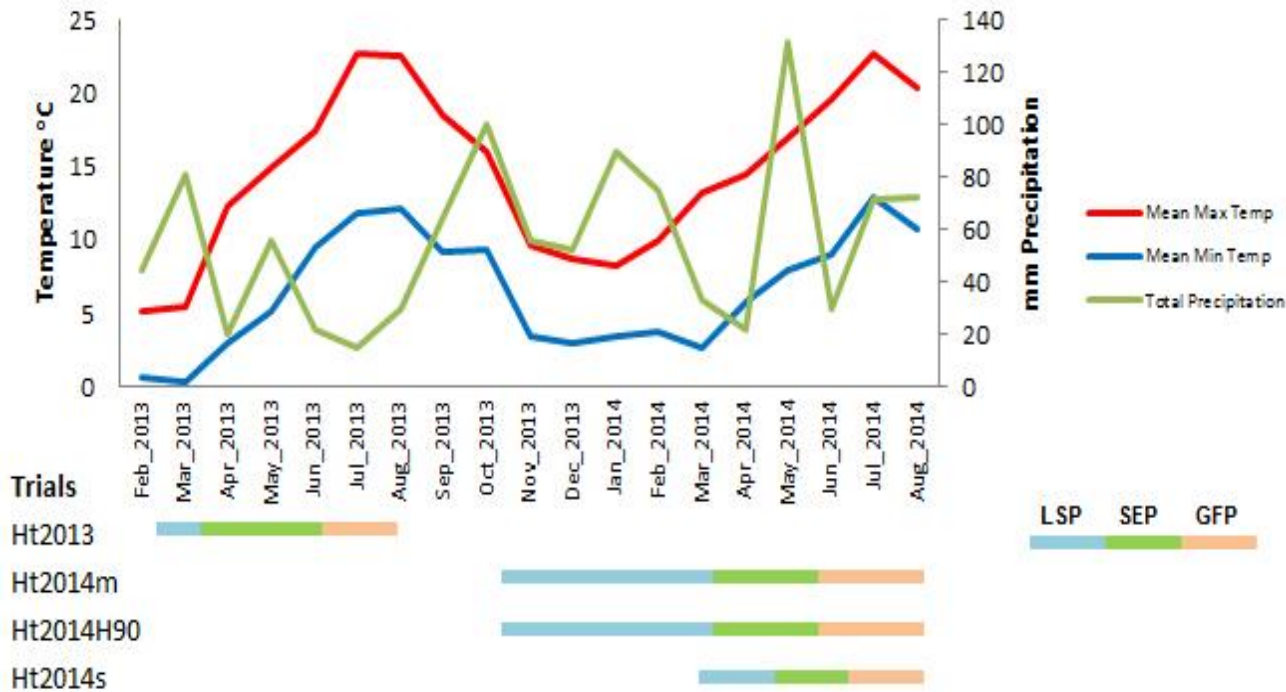
Experiment 2: Understanding genotype x environment interaction in Avalon x Cadenza (WP2.3)



- In this example a grain yield QTL was expressed very strongly at Church Farm in 2006, but not really since!
- What was different?

We will analyse climatic data to look for some QTL x E clues.

Temperature and Rainfall:
Duration of 2013 and 2014 Field Trials, JIC



- Norwich climatic data, together with developmental stage of trial.
- Analysis of this data for AxC DH and NIL trials

GRAIN YIELD

Year: 2013 and 2014

Background: Avalon and Cadenza

Chromosome: 1B, 1D, 2A, 2D, 3A, 3B, 5A, 6A, 7B and 7D

Allele: Avalon and Cadenza

Background	Allele	Chromosome										
		1B	1D	2A	2D	3A	3B	5A	6A	6B	7B	7D
Avalon	a	3	8	9	37	14	4	8	22	25	4	4
	b	7	5	2	37	12	6	8	8	19	2	6
Cadenza	a	8	19	8	27	29	13	-	26	15	-	-
	b	9	22	5	33	22	17	-	22	27	-	-

(nº genotype)

GRAIN YIELD

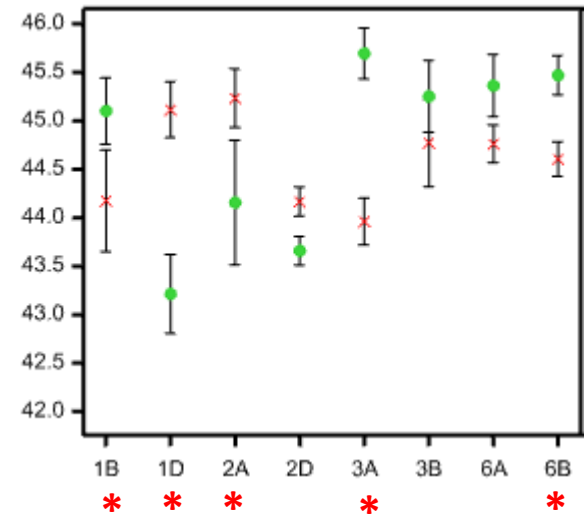
Source of variation	df	Wald-test	p-value
Year (Y)	1	20768.25	<0.001
Background (B)	1	236.54	<0.001
Chromosome (C)	7	51.88	<0.001
Allele (A)	1	37.68	<0.001
Y.B	1	267.16	<0.001
Y.C	7	15.4	0.033
B.C	7	68.32	<0.001
Y.A	1	15.93	<0.001
B.A	1	6.89	0.009
C.A	7	64.5	<0.001
Y.B.C	7	10.22	0.180
Y.B.A	1	2.18	0.140
Y.C.A	7	23.02	0.002
B.C.A	7	13.91	0.055
Y.B.C.A	7	13.27	0.068

DTEM

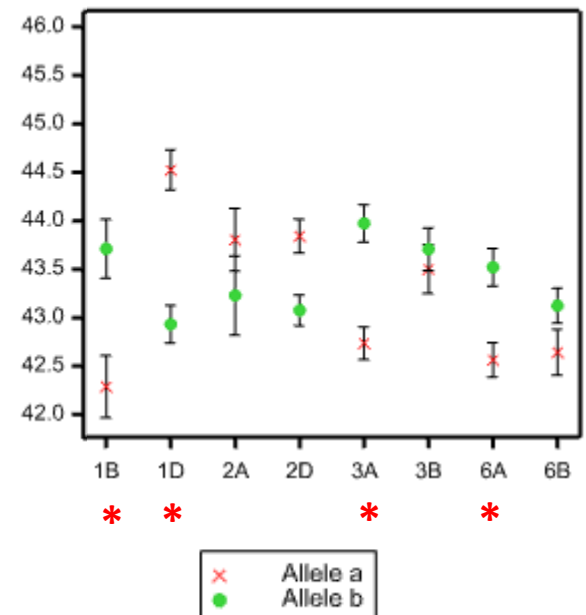
2013

Background	Chromosome	Avalon allele	Cadenza allele	
Avalon	1B	44.17	45.10	
	1D	45.11	43.21	
	2A	45.23	44.16	
	2D	44.17	43.66	
	3A	43.96	45.69	
	3B	44.77	45.25	
	6A	44.76	45.36	
	6B	44.61	45.47	
	Cadenza	1B	42.28	43.7
		1D	44.51	42.92
2A		43.79	43.22	
2D		43.83	43.06	
3A		42.72	43.96	
3B		43.49	43.7	
6A		42.55	43.51	
6B		42.63	43.11	
Average s.e.d		0.40		

Avalon background



Cadenza background

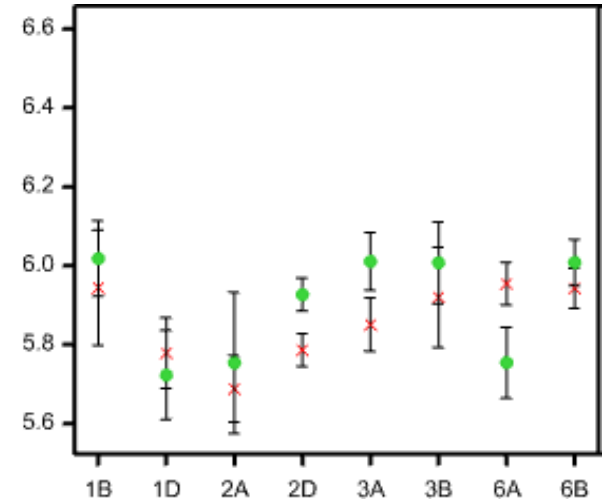


x Allele a
● Allele b

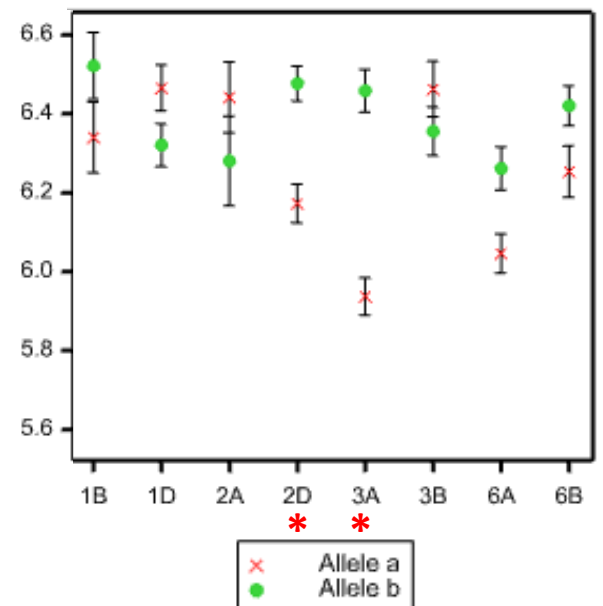
GRAIN YIELD

Background	Chromosome	Avalon allele	Cadenza allele
Avalon	1B	5.231	5.252
	1D	5.117	5.102
	2A	4.943	5.034
	2D	5.053	5.125
	3A	5.143	5.270
	3B	5.143	5.183
	6A	5.210	5.096
	6B	5.195	5.229
Cadenza	1B	5.375	5.507
	1D	5.468	5.376
	2A	5.466	5.368
	2D	5.273	5.487 *
	3A	5.098	5.457 *
	3B	5.455	5.420
	6A	5.212	5.276
	6B	5.328	5.436
Average s.e.d		0.137	

Avalon background

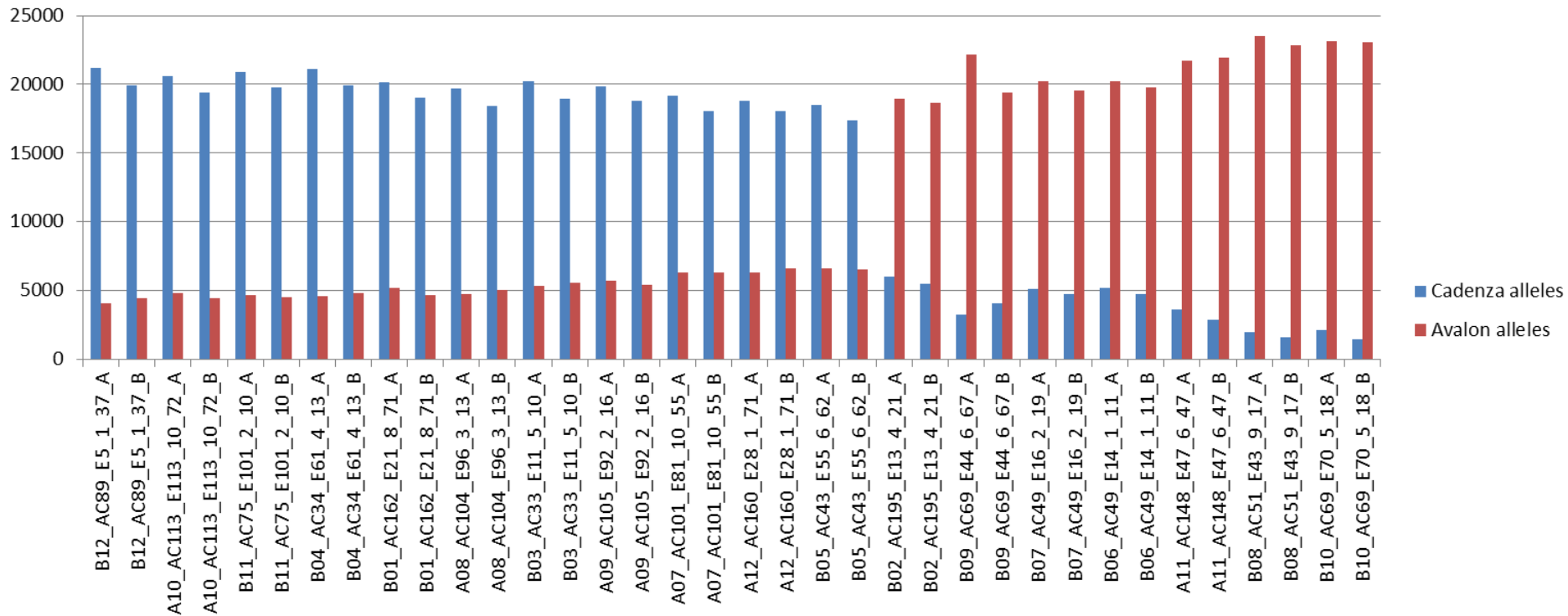


Cadenza background



Genotypic composition of back/foreground

Axiom 817 K analysis of Avalon x Cadenza Near Isogenic Line sub set
Experiment 1: A chromosome segment substitution library for Avalon x Cadenza (AxC) (WP3.2)



Can we tile the whole genome to make recombinant substitution lines for whole genome?

250 BC₂ NILs in Avalon background
302 in Cadenza

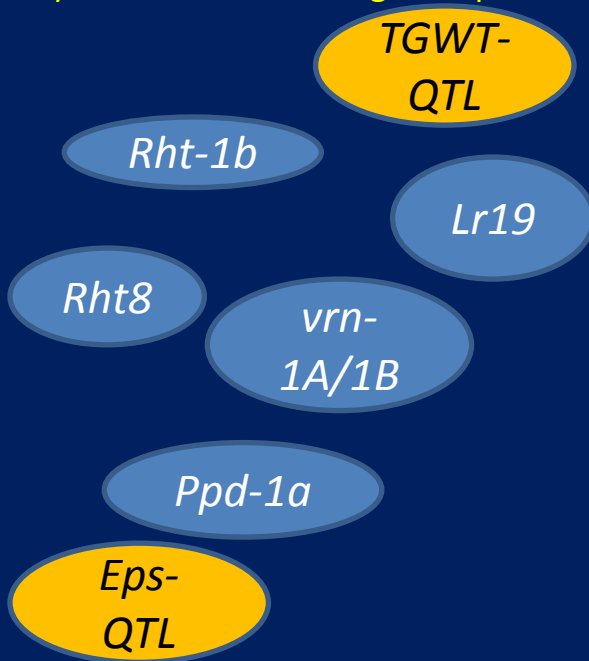


Etc!

Combining alleles in a uniform genetic background is a powerful way to assess genetic interactions and test a hypothesis for breeding

Experiment 4: Quantifying agronomic impact of WGIN target genes using the Paragon NIL library (WP2.3, WP3.2)

Experiment 5: Informing multiple marker assisted selection for yield stability using Paragon library (WP3.2)



Combining alleles in a uniform genetic background is a powerful way to assess genetic interactions and test a hypothesis for breeding

Experiment 4: Quantifying agronomic impact of WGIN target genes using the Paragon NIL library (WP2.3, WP3.2)
Experiment 5: Informing multiple marker assisted selection for yield stability using Paragon library (WP3.2)



Combining alleles in a uniform genetic background is a powerful way to assess genetic interactions and test a hypothesis for breeding

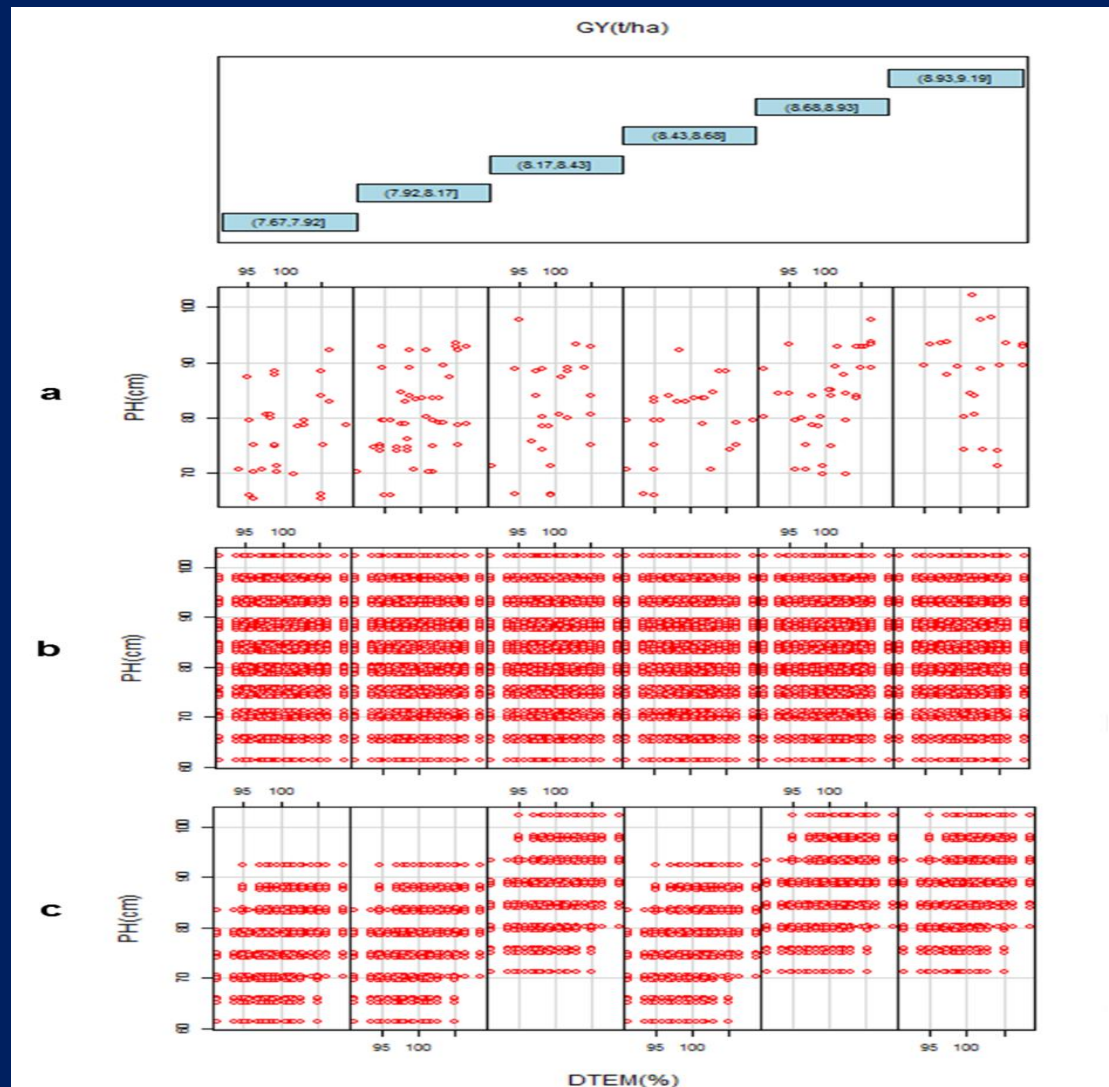
Experiment 4: Quantifying agronomic impact of WGIN target genes using the Paragon NIL library (WP2.3, WP3.2)
Experiment 5: Informing multiple marker assisted selection for yield stability using Paragon library (WP3.2)



The UK reference population Avalon×Cadenza as a platform for simulated breeding strategies for grain yield in elite Western European bread wheat

Experiment 4: Applying WGIN data to breeding by design for UK yield stability (WP3.2)

- Based on the work described, can we develop tools for breeding stability by design
- ‘Best’ simulated genotypes will be by crossing closest DHs and then MAS



Is improved drought tolerance one route to increased stability for UK wheat?

Experiment 3: Dissecting UK drought tolerance in Paragon x Garcia (WP2.3, WP4.3, Milestone 19)

- Paragon x Garcia, a RIL population of 351 lines was developed by WGIN
- Designed for UK drought study
- Genetic map developed in CiRC
- Will be grown +/- irrigation at Church Farm
- Measured traits will be height, heading, yield, and components

Does this analysis raise some new questions for the genetic dissection of certain traits for stability?

Experiment 3: Foundations for a new generation segregating populations for studying yield stability in the UK (WP2.1)

	RL Group1	RL Group2	RL Group3	RL Group4
RL Group1				
RL Group2	X			
RL Group3	X	X		
RL Group4	X	X	X	

Production of all possible F1s (or provision of them) provides all options for the production of a new 'stability population'.

WGIN team at JIC

- Clare Lister
- Alba Farre Martinez

WGIN 3

Malcolm J. Hawkesford

1st Management Meeting
25th February 2015

3/7/14



ROTHAMSTED
RESEARCH



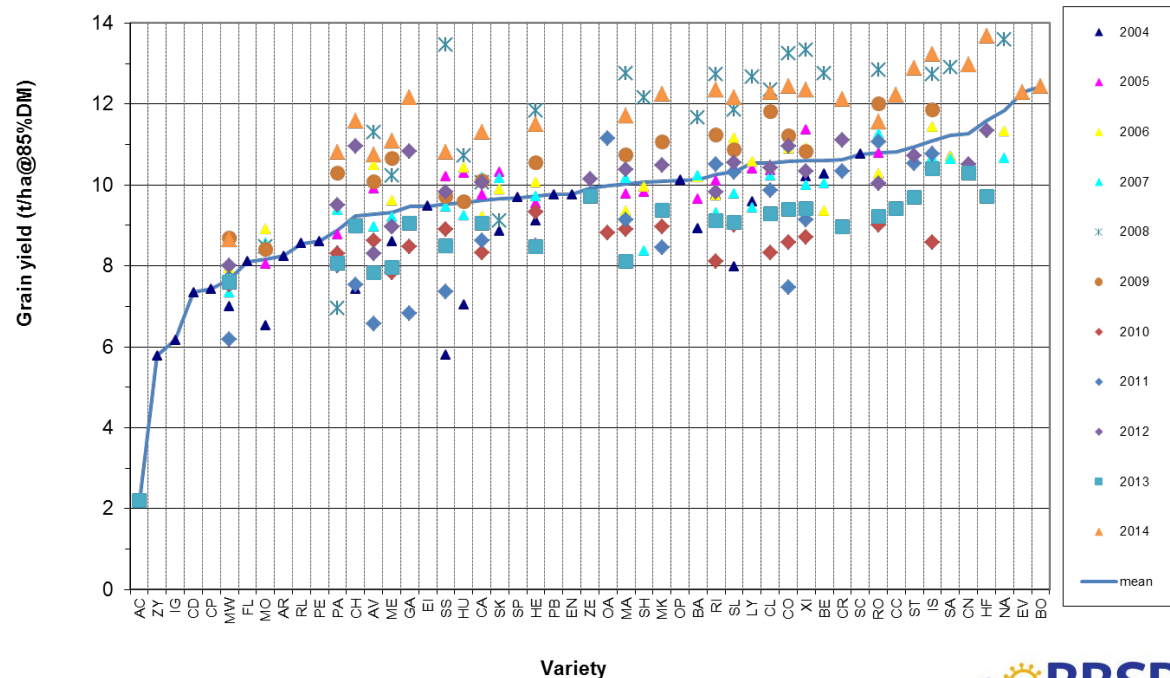
WGIN 3

Stability/resilience



ROTHAMSTED
RESEARCH

- Add to data since 2004 (13 year total)
- Some variety adjustments
- Analysis referencing meteorology
- Include long term data (Broadbalk)



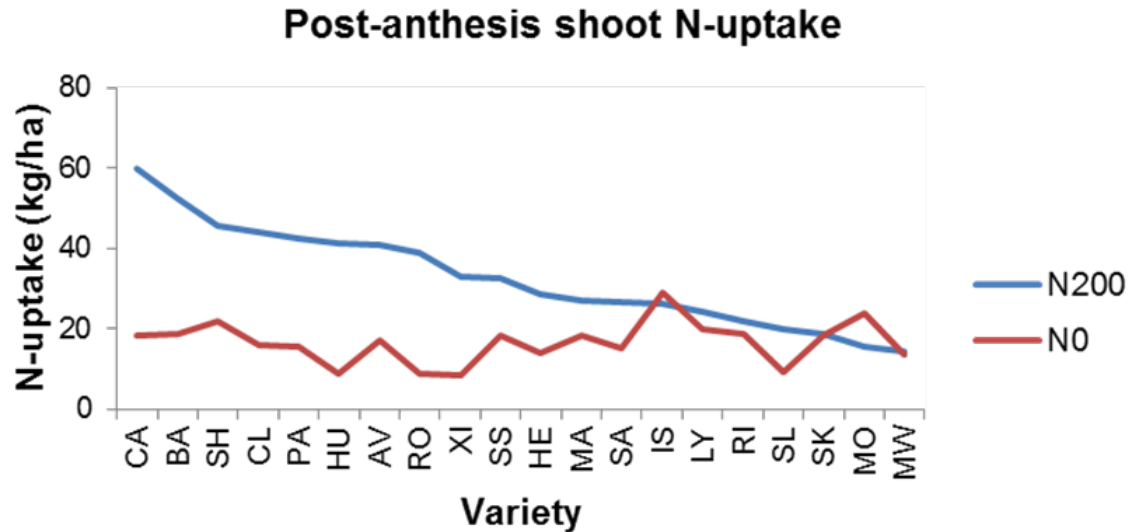
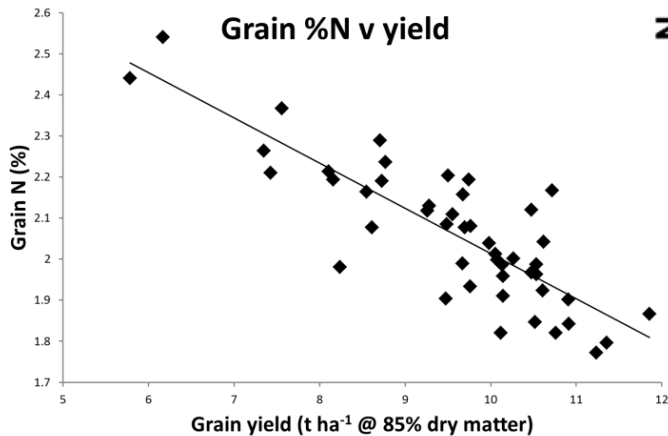
WGIN 3

Post harvest mineral uptake



ROTHAMSTED
RESEARCH

- Link to GPD?
- N and other minerals



Field Crops Research xxx (2013) xxx-xxx

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Genotypic variation in the uptake, partitioning and remobilisation of nitrogen during grain-filling in wheat[☆]

Peter B. Barraclough^{*}, Rafael Lopez-Bellido¹, Malcolm J. Hawkesford

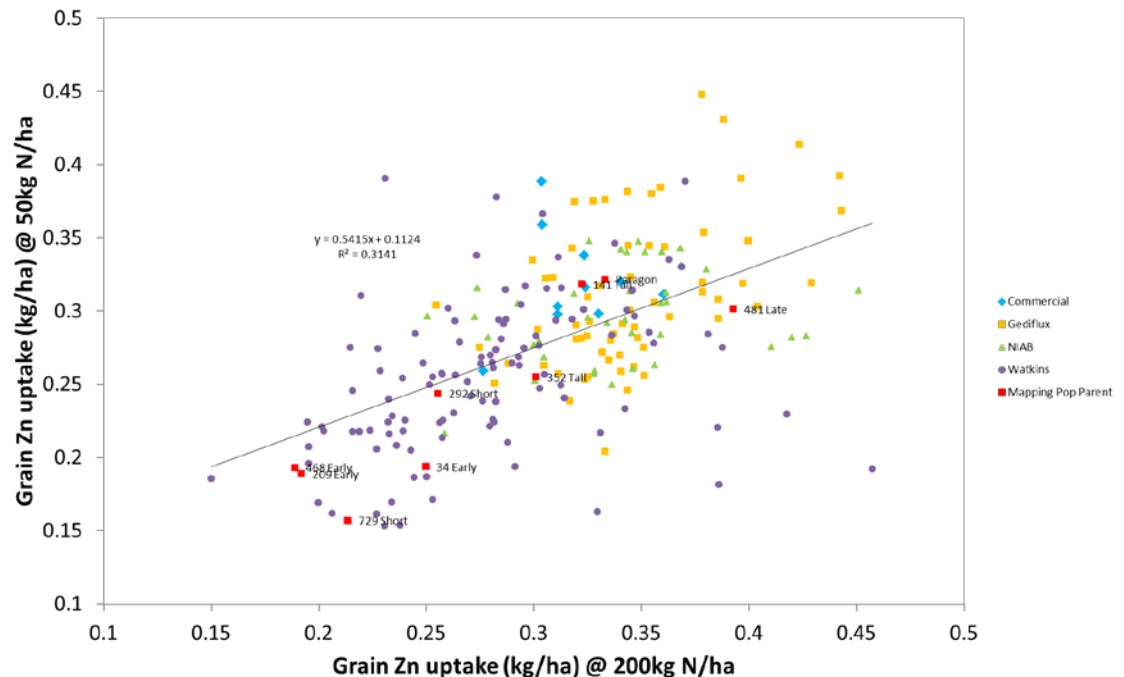
Plant Biology and Crop Science Department, Rothamsted Research, West Common, Harpenden, Hertfordshire AL5 2JQ, UK

Interactions between nutrients



ROTHAMSTED
RESEARCH

- Diversity/N panel
- ICP AES for P, S, K, Ca, Mg, Fe and Zn
- 2015 and 2016, anthesis and final
- Partitioning
- 3 past years



Canopy longevity

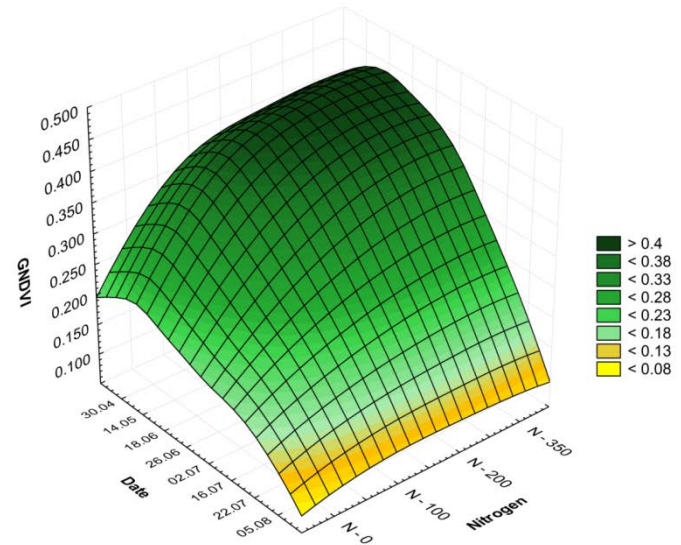


ROTHAMSTED
RESEARCH

- Diversity/N-panel
- Some existing data (2014)
- 2015 and 2016



The change GNDVI during the growing season of winter wheat depending on the level of nitrogen fertilization



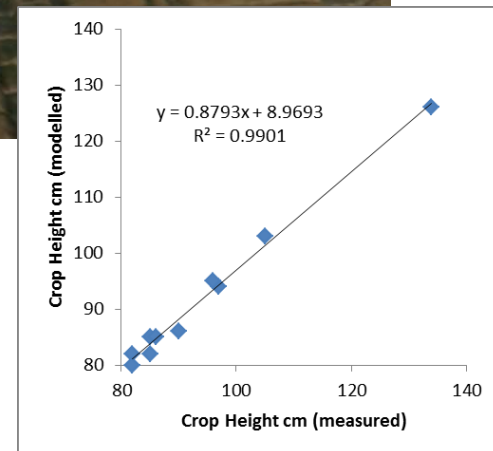
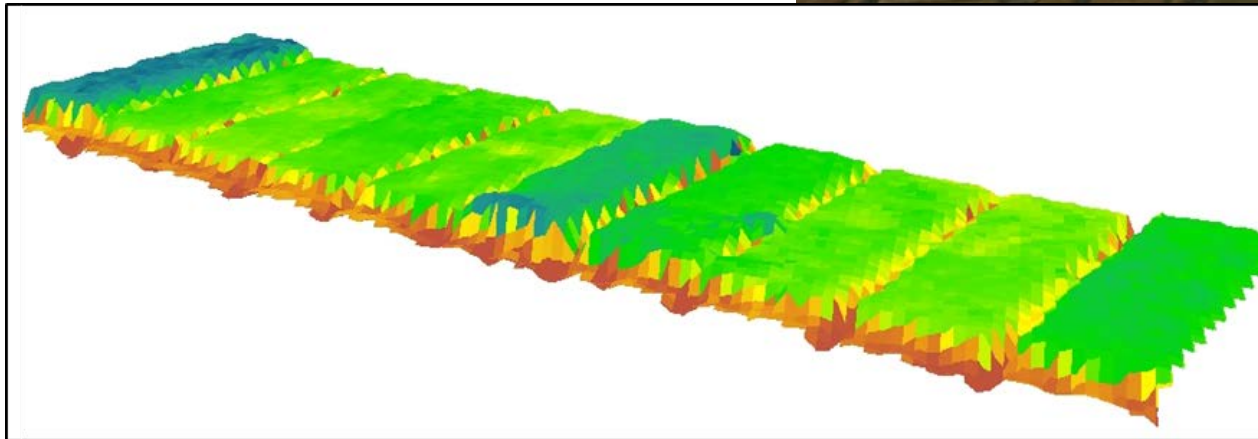
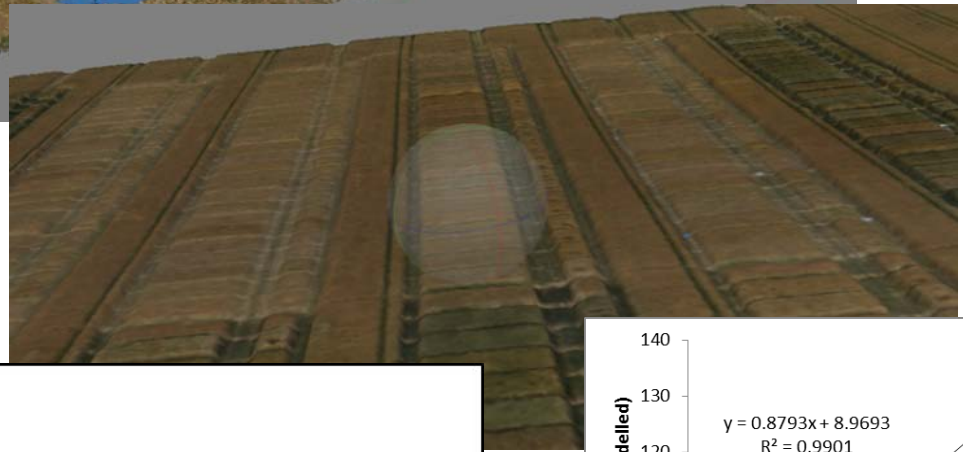
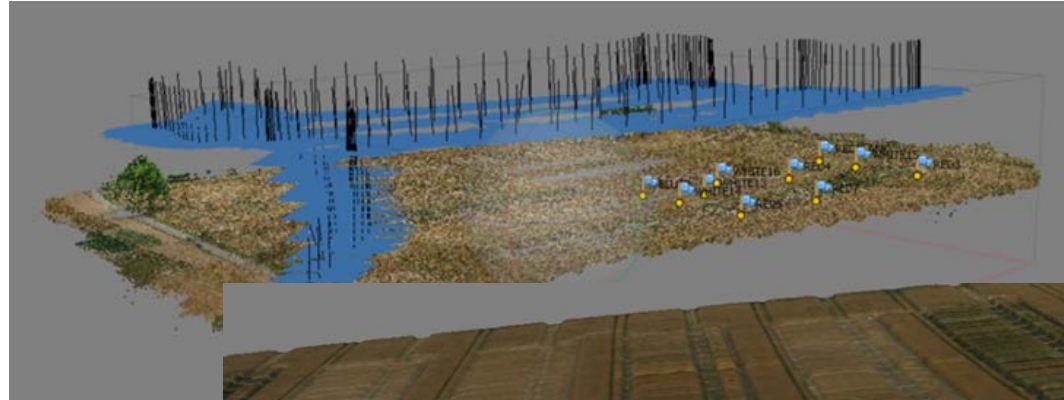
Data analysis – height



ROTHAMSTED
RESEARCH

Estimating height from DEMs (Digital Elevation Models)

- 10 Ground Control Points
- 40m flight altitude
- Imaged every second
- Only 10 plots estimated



30/4/14



ROTHAMSTED
RESEARCH



WGIN 3



3/7/14



ROTHAMSTED
RESEARCH



WGIN 3



16/7/14



ROTHAMSTED
RESEARCH



WGIN 3



WGIN3

Screening germplasm for resilience to aphids (WP2.3)

Lesley Smart

Background of aphid work in Lola and WISP

Part of Phenotyping theme

Screen wheat lines for evidence of resistance to the main cereal aphid pests in the UK – the bird cherry-oat aphid *Rhopalosiphum padi* and the grain aphid *Sitobion avenae*.

- Initial study included Hexaploids from the Gediflux and Watkins collections
- And more recently Diploid wheat species including lines of *Aegilops speltoides* (from JIC), *Ae. tauschii* (from NIAB) and *Triticum monococcum* (from RRes WGIN).
Synthetic wheat lines (from NIAB)

The Target Pests



Rhopalosiphum padi

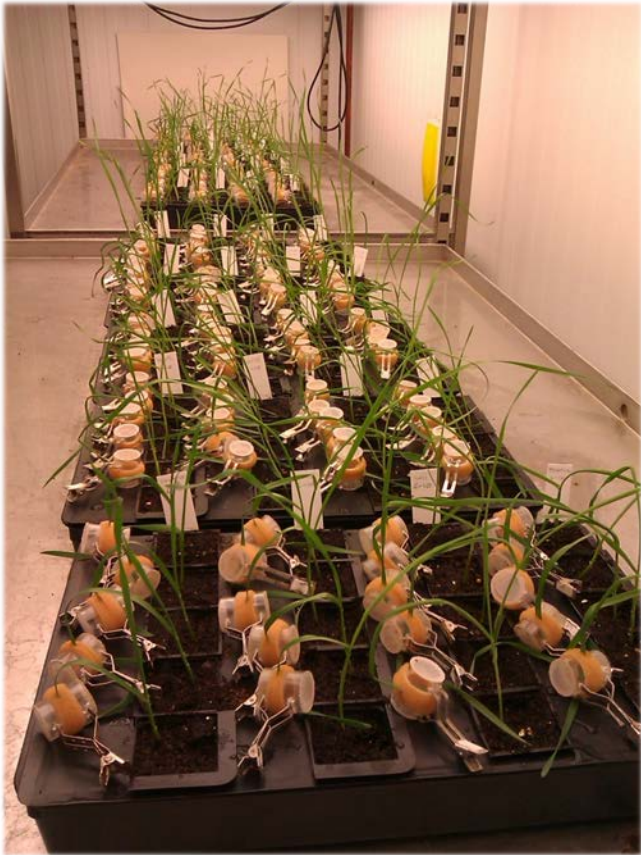


Sitobion avenae

Phenotyping Screen



Replicate 1



Replicate 2

WV1				WV17	WV1				WV17
WV2				WV18	WV2				WV18
		...		C1			...		C1
				C2					C2

Tray 1 Tray 2 Tray 3 Tray 4 Tray 5 Tray 1 Tray 2 Tray 3 Tray 4 Tray 5

Row 1
Row 2
Row 3
Row 4

Phenotyping Screen

18 lines/week plus a duplicated control (Solstice)

Alates caged on first leaf of seedlings 7 days after sowing.

R. padi left for 24h, *S. avenae* left for 48h.

Alates then removed, nymphs counted and re-caged for 6-7 days.

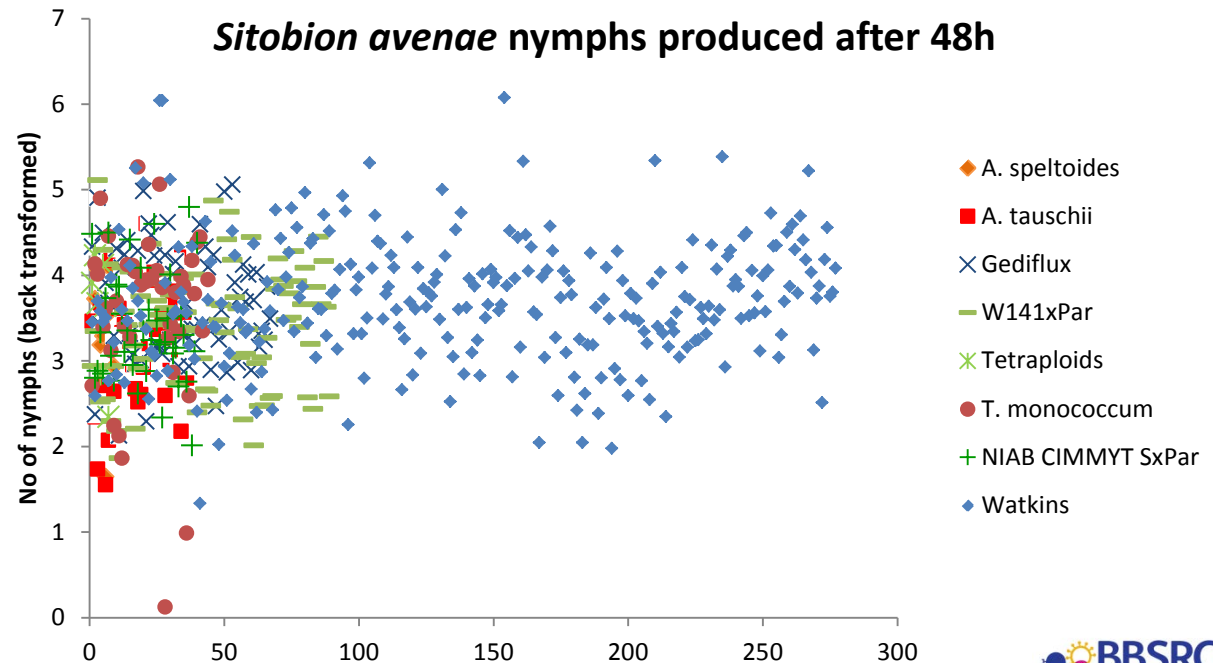
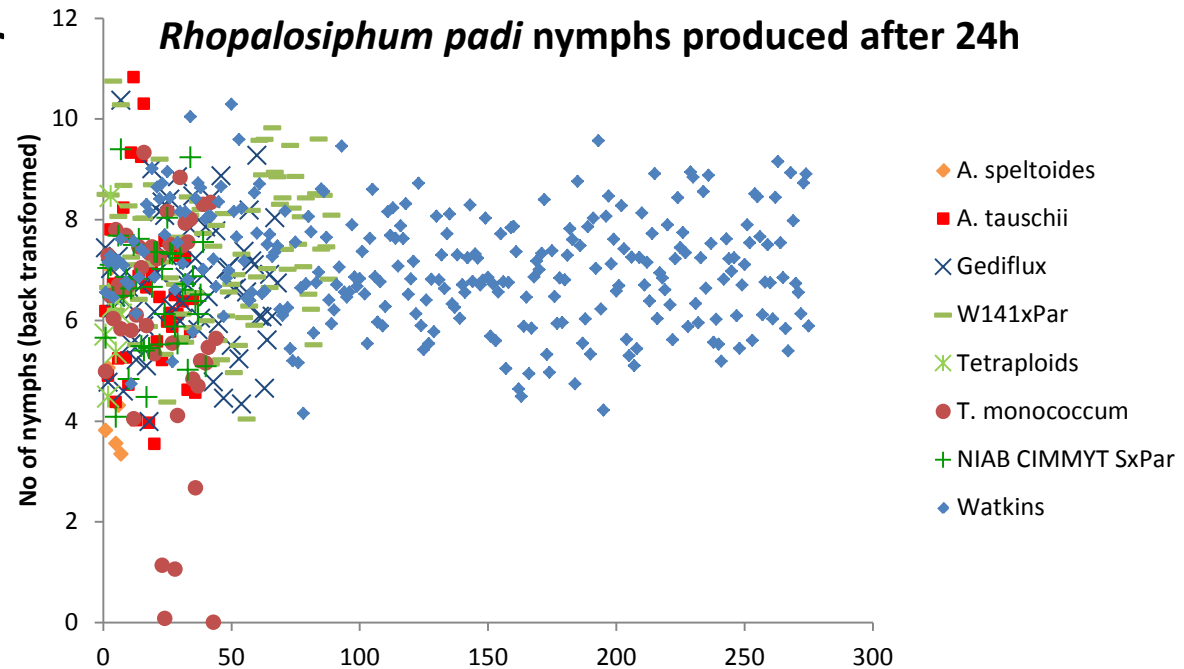
Nymphs recounted and weighed in their batches.

Statistical analyses of data to compare nymph number, survival and weight to the control.

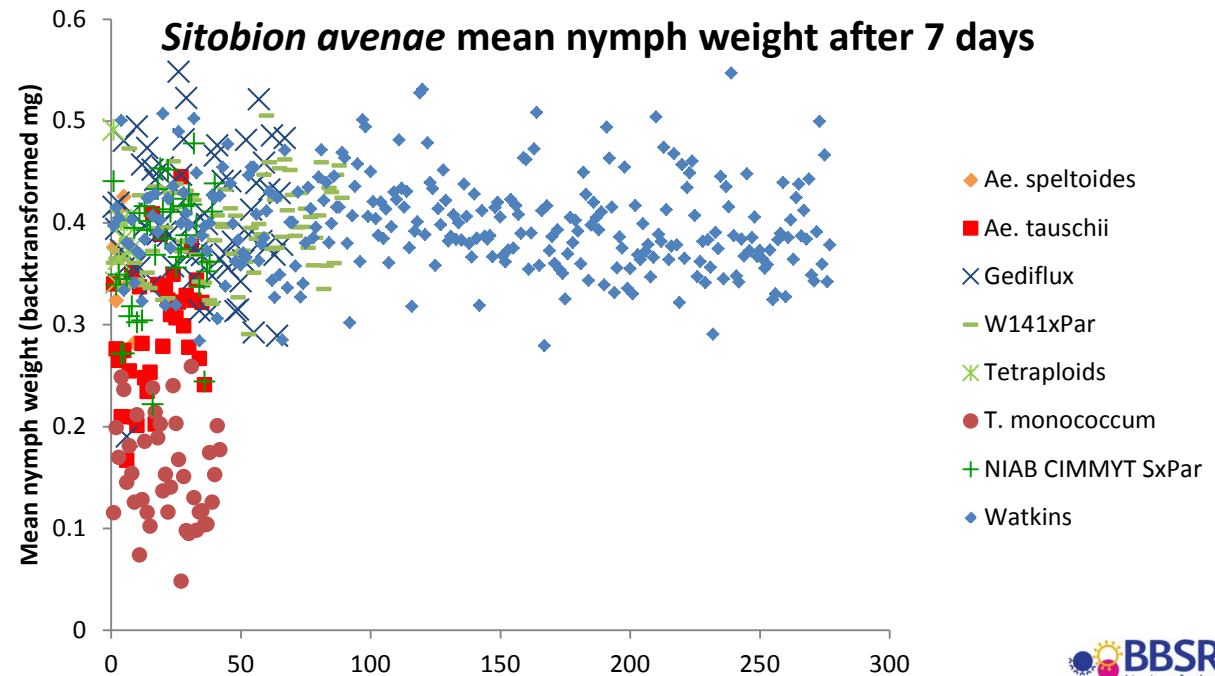
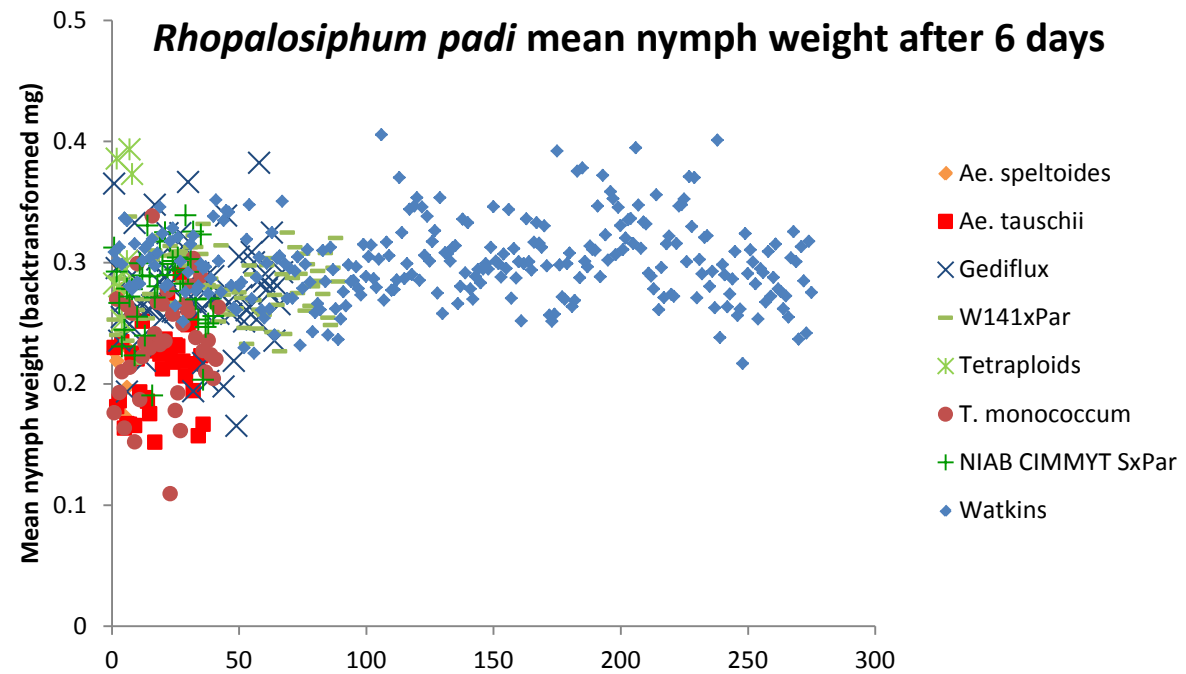
Assay provides

- an assessment of antixenotic effects on alate settlement and commitment to nymph production
- an assessment of antibiotic effects on nymph development
- An illustration of the difference in response of the two aphid species to different lines

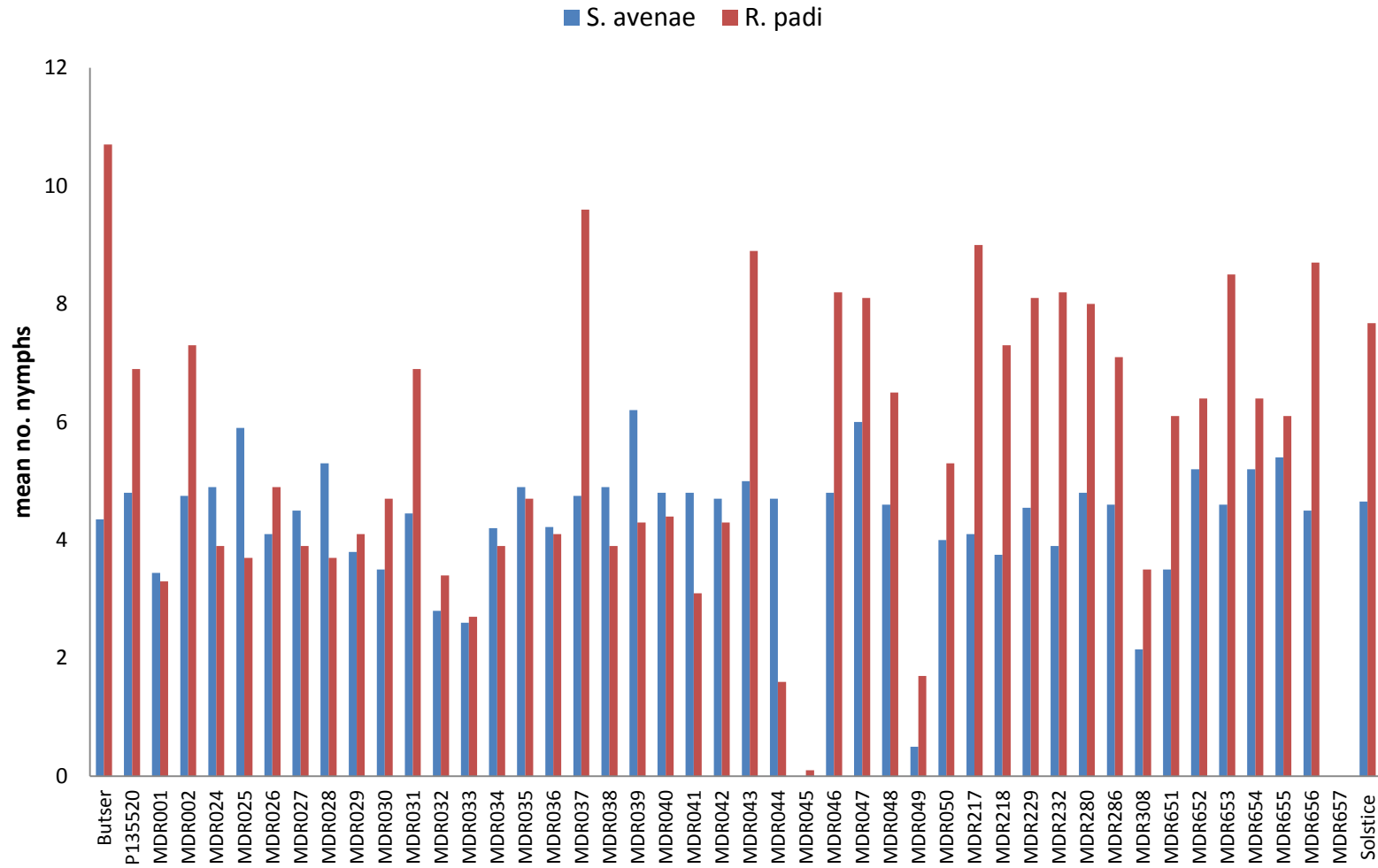
Nymph number



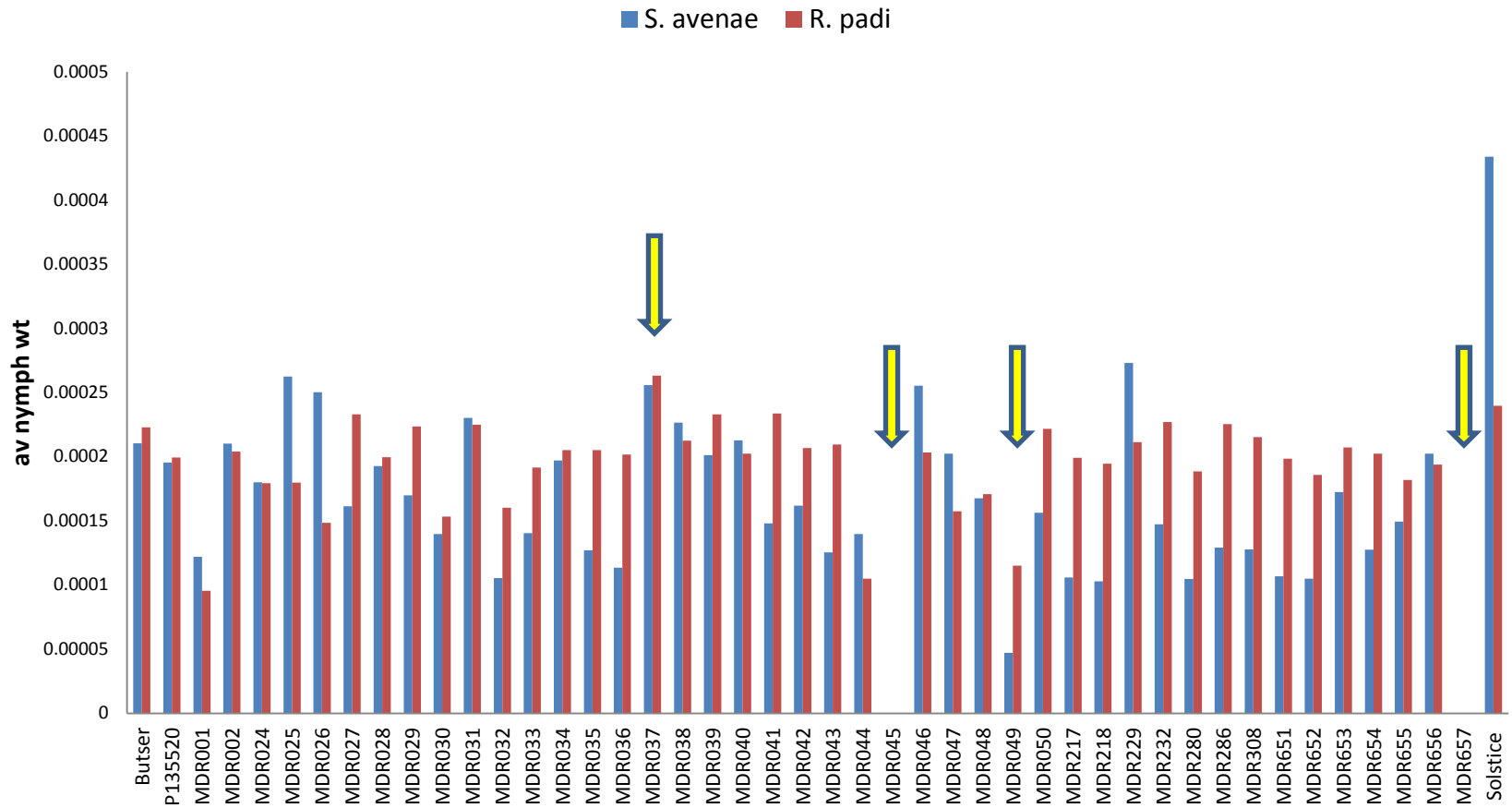
Nymph weight



Nymphs on *Triticum monococcum* lines



Nymph weight on *Triticum monococcum* lines

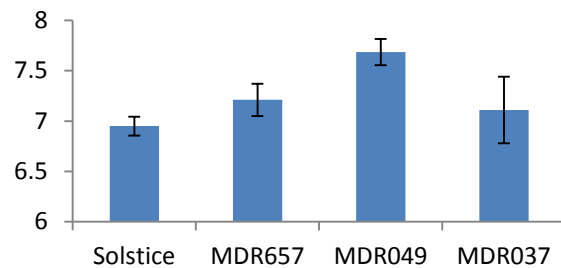


Fecundity assays – Intrinsic rate of increase (r_m)

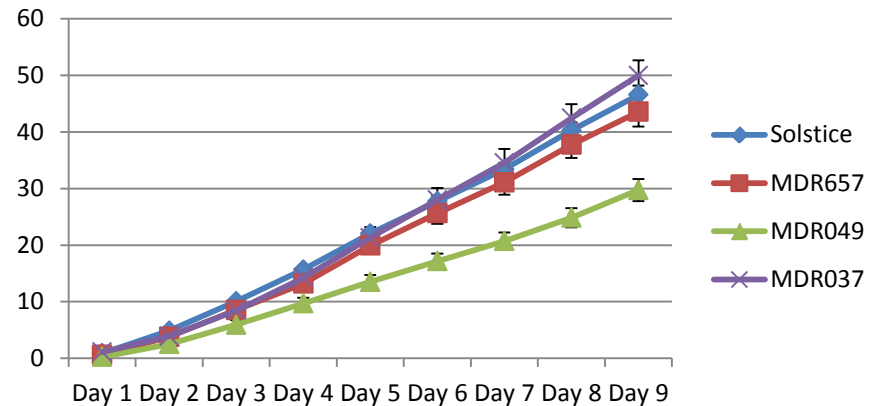
$$r_m = (\ln(FD)/D) \times C (0.74) \text{ (Wyatt and White, 1977)}$$

Rhopalosiphum padi – no nymphs on MDR045

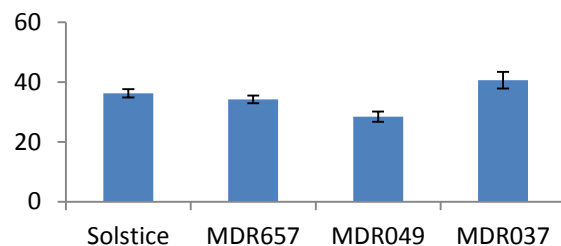
D (days from birth to 1st nymph production)



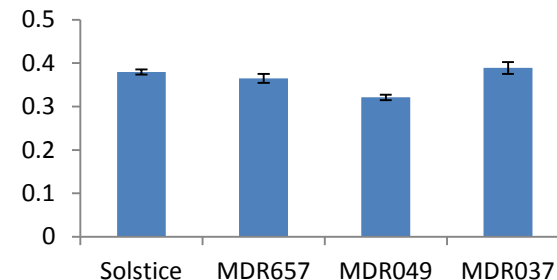
Cumulative nymph production



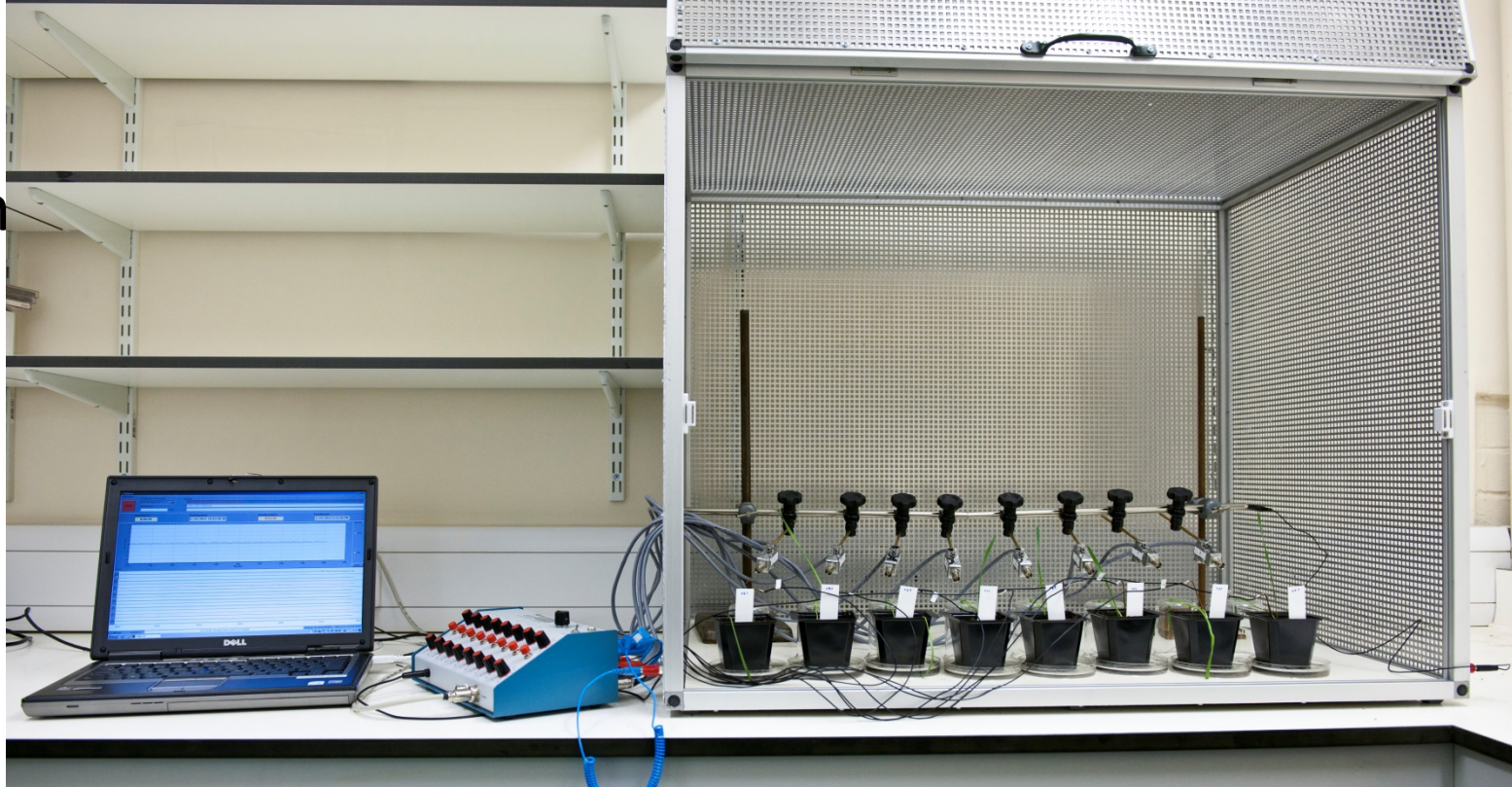
FD (nymphs produced over time D)



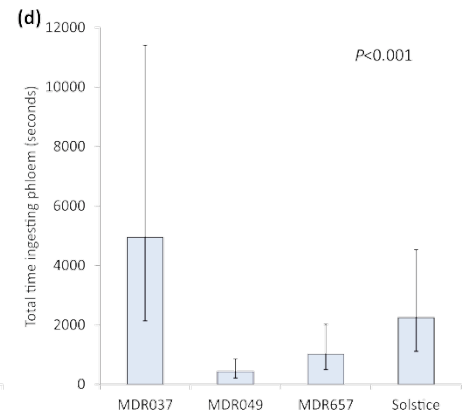
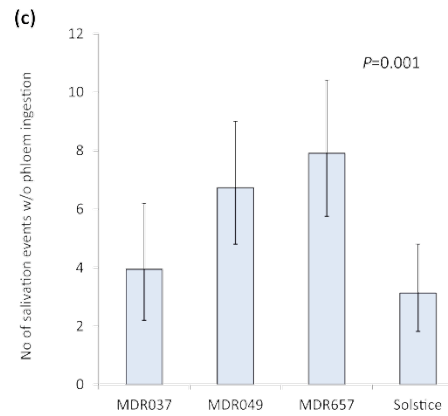
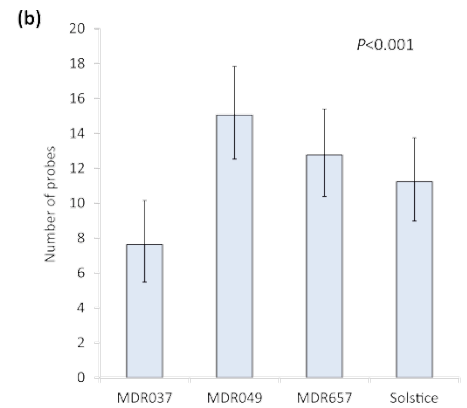
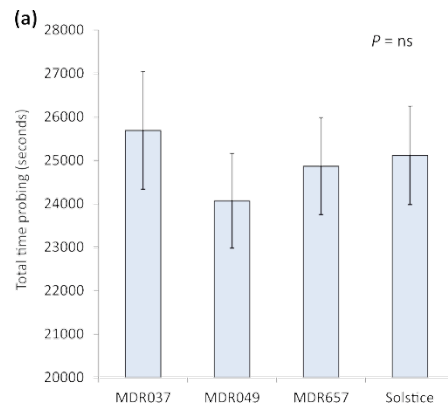
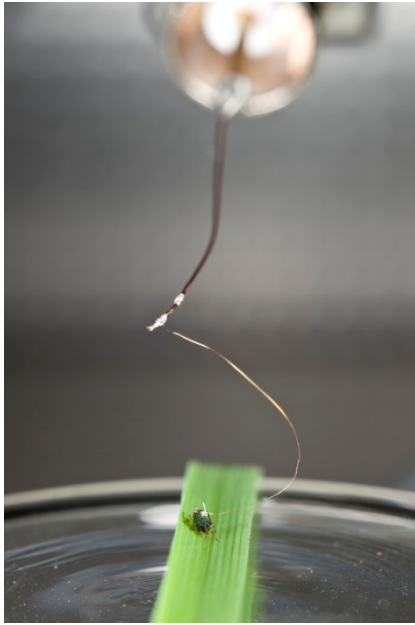
r_m



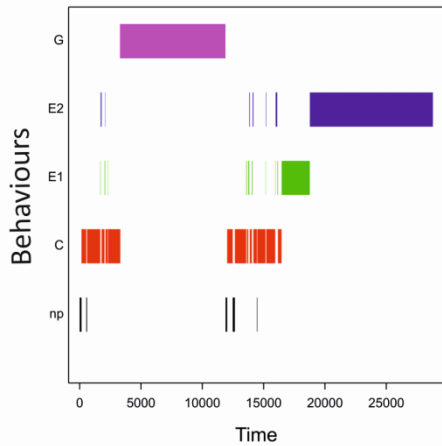
Electrical Penetration Graph for aphid feeding behaviour



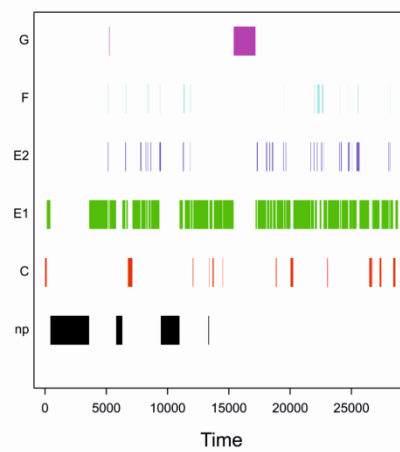
Feeding behaviour *R. padi* recorded by EPG



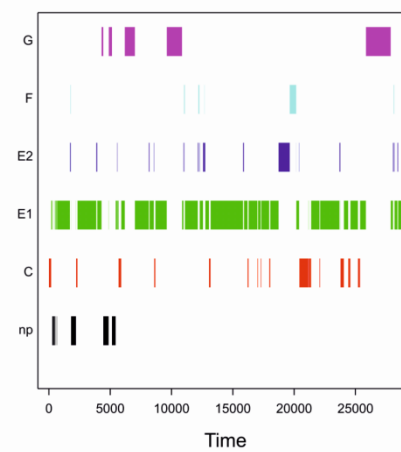
MDR037



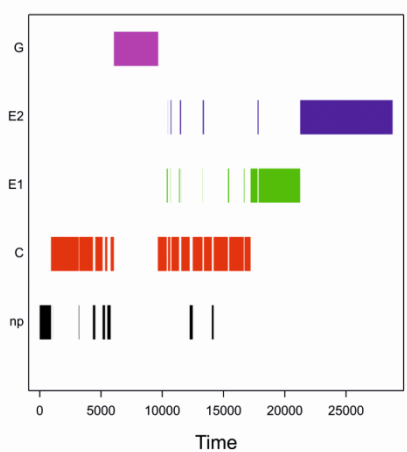
MDR049



MDR657



Solstice



Work in WGIN 3

Triticum monococcum crosses already made by Mike Hammond-Kosack

MDR037 x MDR045, MDR049 and MDR657

Test F1 generations of these crosses in phenotyping screen for both aphid species

Take lines to F2 and beyond with further assays and backcrosses as necessary

Acknowledgements

The entomology team



Dr Gia Aradottir



Alex Greenslade



Janet Martin

WGIN 3

Resistance to take-all and foliar diseases

Vanessa McMillan
Kim Hammond-Kosack



Resistance to take-all and foliar diseases



ROTHAMSTED
RESEARCH

Objectives:

1. Complete development of *Triticum monococcum* mapping populations for genetic analysis of resistance to take-all
2. Continue the introgression of resistance to take-all from *T. monococcum* to the BC1 stage
3. Examine the resistance of *Triticum monococcum* to yellow rust
4. Characterise hexaploid wheat germplasm previously shown to exhibit a high level of resistance to multiple foliar diseases

Take-all disease



ROTHAMSTED
RESEARCH

Major root disease of wheat

Ascomycete soil-borne fungal pathogen

Gaeumannomyces graminis var. *tritici* (Ggt)



Take-all infected wheat seedling



Take-all patch showing stunting and premature ripening of the crop

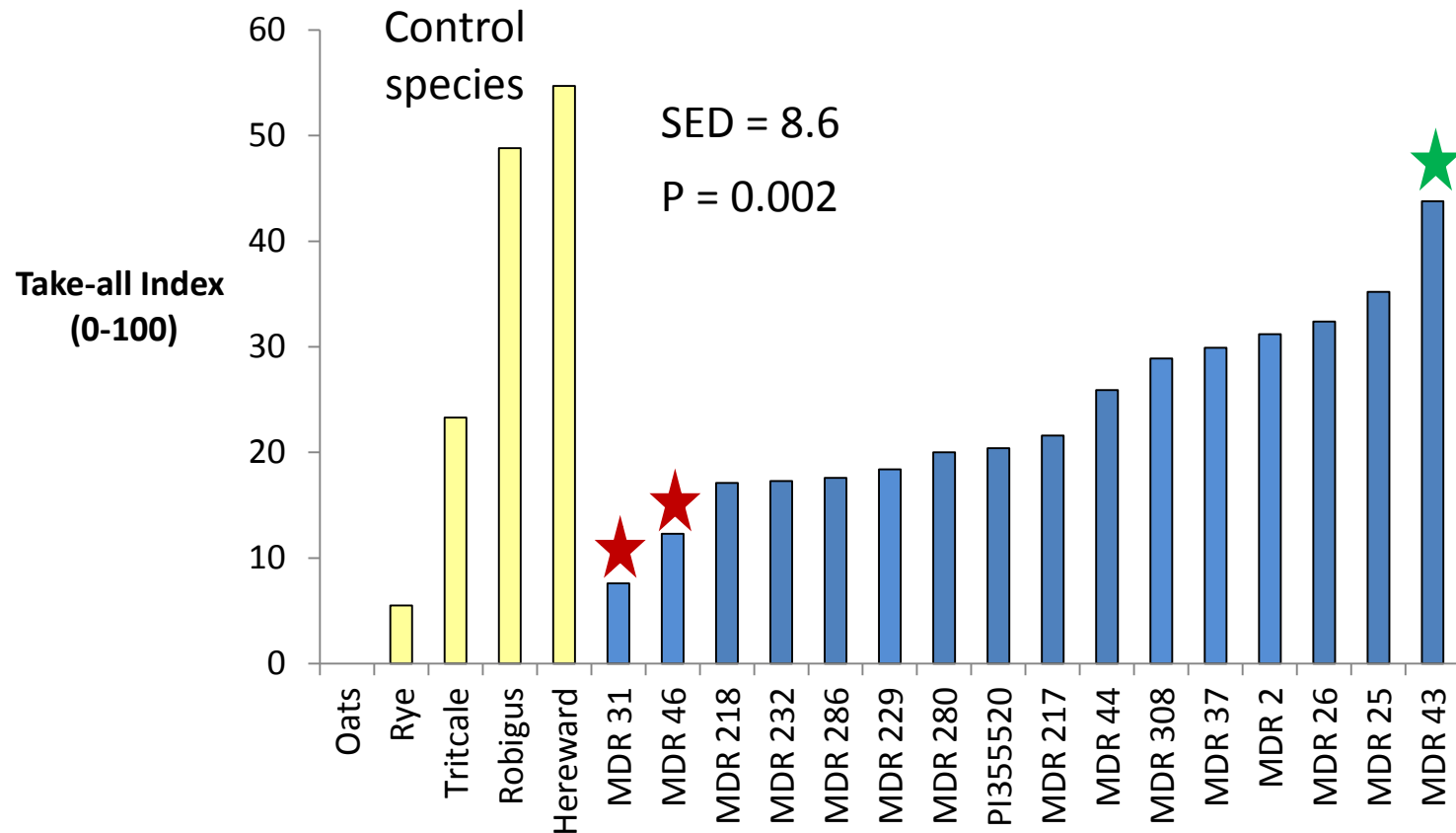
Limited control options

Resistance to take-all in *Triticum monococcum*



ROTHAMSTED
RESEARCH

- 3rd wheat field trials 2006-2011 (WGIN 1 and 2)
- 34 *T. monococcum* accessions tested over 5 years



Objective 1: Complete development of *Triticum monococcum* mapping populations



ROTHAMSTED
RESEARCH

F₆ populations:

MDR037 (S) x **MDR046 (R)** – 79 F6 lines (started with ~180 F3 plants)

MDR037 x MDR229 – 85 F6 lines

F₂ Tm cross progeny numbers:

Parentage		Estimated F ₃ progeny number
MDR031 (R) x MDR043 (vS)	31 ears from 3 plants	450 ←
MDR031 x MDR229	16 ears from 1 plant	320
MDR031 x MDR650	48 ears from 3 plants	900
MDR043 (vS) x MDR031 (R)	48 ears from 3 plants	960
MDR043 (vS) x MDR046 (R)	36 ears from 3 plants	750 ←
MDR229 x MDR031	94 ears from 6 plants	2000

Now at F₄, taking forward to F₆



Objective 2: Introgression of resistance to take-all from *T. monococcum* to the BC1 stage (MH-K)



ROTHAMSTED
RESEARCH

5 lines selected for crossing

Line	(datasets)	Variety	Origin	Type
MDR031	(3 field years of data)	<i>monococcum</i> ; <i>macedonicum</i>	Turkey	Spring
MDR046	(4 field years of data)	<i>atriaristatum</i> ; <i>macedonicum</i>	Romania	Spring
MDR232	(3 field years of data)	<i>nigricultum</i>	Yugoslavia	Winter
MDR286	(4 field years of data)	84TK154-034	Turkey	Winter
MDR229	(4 field years of data)	3962	Spain	Spring

Objective 2: Introgression of resistance to take-all from *T. monococcum* to the BC1 stage (MH-K)



ROTHAMSTED
RESEARCH

Introgression method

First cross *Tm* to Paragon *ph-1* mutant (Paragon as female parent)

Backcross the F₁ plants into Paragon (wild-type)

Objective 2: Introgression of resistance to take-all from *T. monococcum* to the BC1 stage (MH-K)



ROTHAMSTED
RESEARCH

Tm x *Ta* Paragon *ph-1* mutant - Crossing outcome

MDR	TOTAL F ₁ grain	F ₁ grain set per ear	Total number of crossed ears
031	79	5, 11, 8, 6, 3, 3, 18, 14, 11	9
046	36*	3, 11, 4, 18	4
229	45	8, 0, 0, 0, 13, 14, 10	7
232	49	4, 7, 3, 16, 13, 6	6
286	81	4, 0, 8, 18, 16, 10, 5, 6, 11, 3	10
Totals	290		36

* crossing stopped after 4 ears

F1 ear images



Ta (ph-1) x Tm



Ta (ph-1) x Ta (ph-1)



Control



Ta (ph-1)
self



Next steps – the backcross



ROTHAMSTED
RESEARCH

Method for generating the F₁ plants : University of Nottingham (Julie King)

- Surface sterilise the F₁ grain, then germinate *in vitro*
- After 72hr when no germination is evident then embryo rescue and culture *in vitro*

Paragon

F₁ Ta x Tm

F₁ Ta x Tm

Anthers

Anther extrusion

Anthers opening

But no viable pollen



6 months from embryo rescue
to anthesis



Objective 3: Examine the resistance of *Triticum monococcum* to yellow rust



ROTHAMSTED
RESEARCH

Background: *T. monococcum* grown at RRes since 2004, but never any obvious yellow rust infections

- Total *T. monococcum* collection (263 accessions) sown in field trial autumn 2014 (one replicate per accession)
- Spreader rows of the highly susceptible hexaploid cultivar Robigus sown in between *T. monococcum* plots
- 3 yellow rust isolates obtained from NIAB – Solstice isolate 08/21, KWS Sterling isolate 11/140 and Warrior isolate 11/08
- Yellow rust inoculated seedlings to be planted out in mid-March
- Entire *T. monococcum* collection to be genotyped by University of Bristol – association analysis approach

Objective 4: Characterise hexaploid wheat germplasm previously shown to exhibit a high level of resistance to multiple foliar diseases



ROTHAMSTED
RESEARCH

Background

- WGIN 2: 3rd wheat, Take-all field experiment in 2008 – Watkins collection (740 lines) – Richard Gutteridge
- Single replicate of each Watkins line
- 5 blocks of 8 controls (Oats, Triticale, Rye, and 5 currently grown wheat varieties including Hereward)
- No fungicides
- Trial assessed for yellow rust, brown rust, septoria and powdery mildew infection and plant samples taken for take-all assessments on the root systems

Watkins field trial – June 2008

WGIN 2





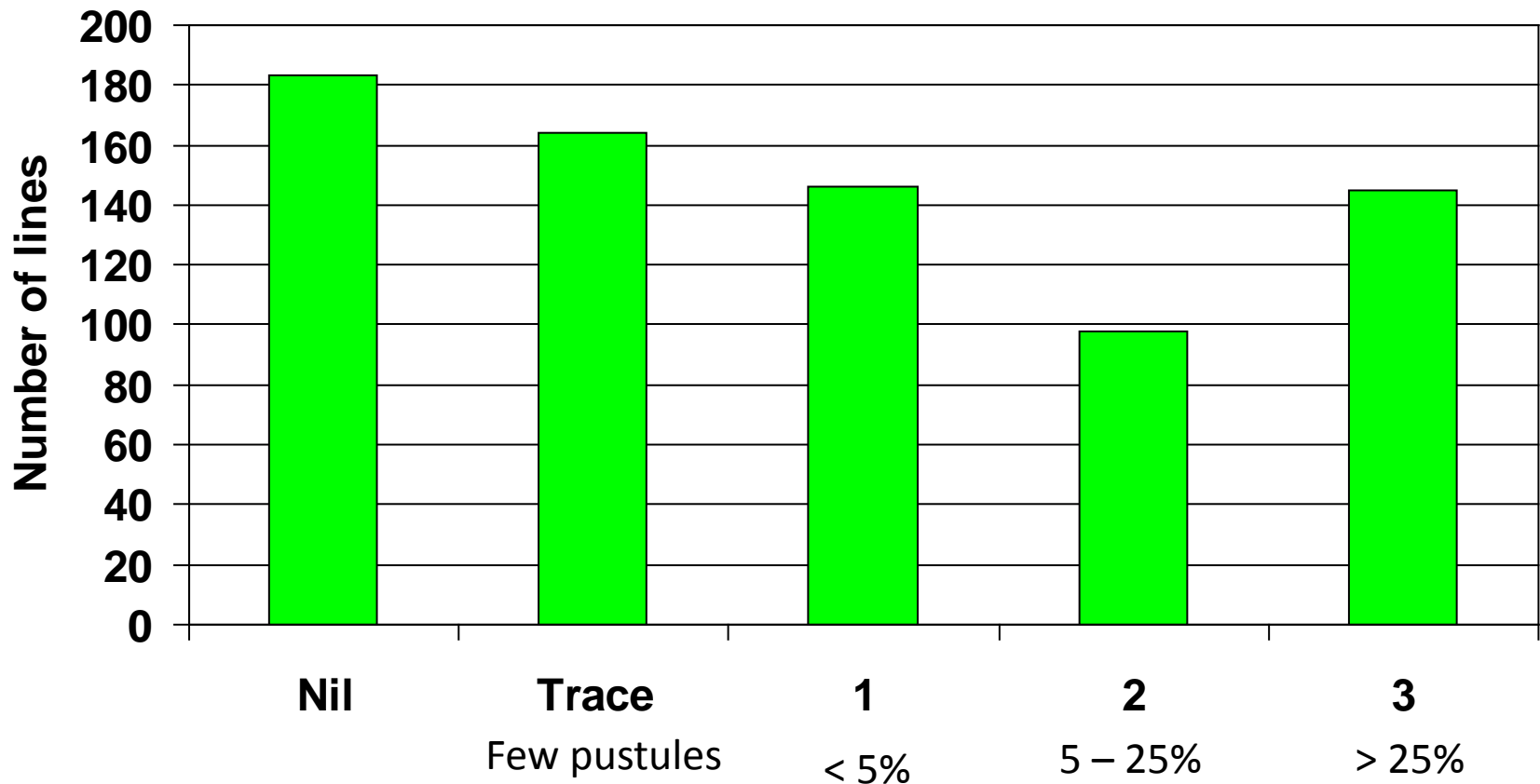




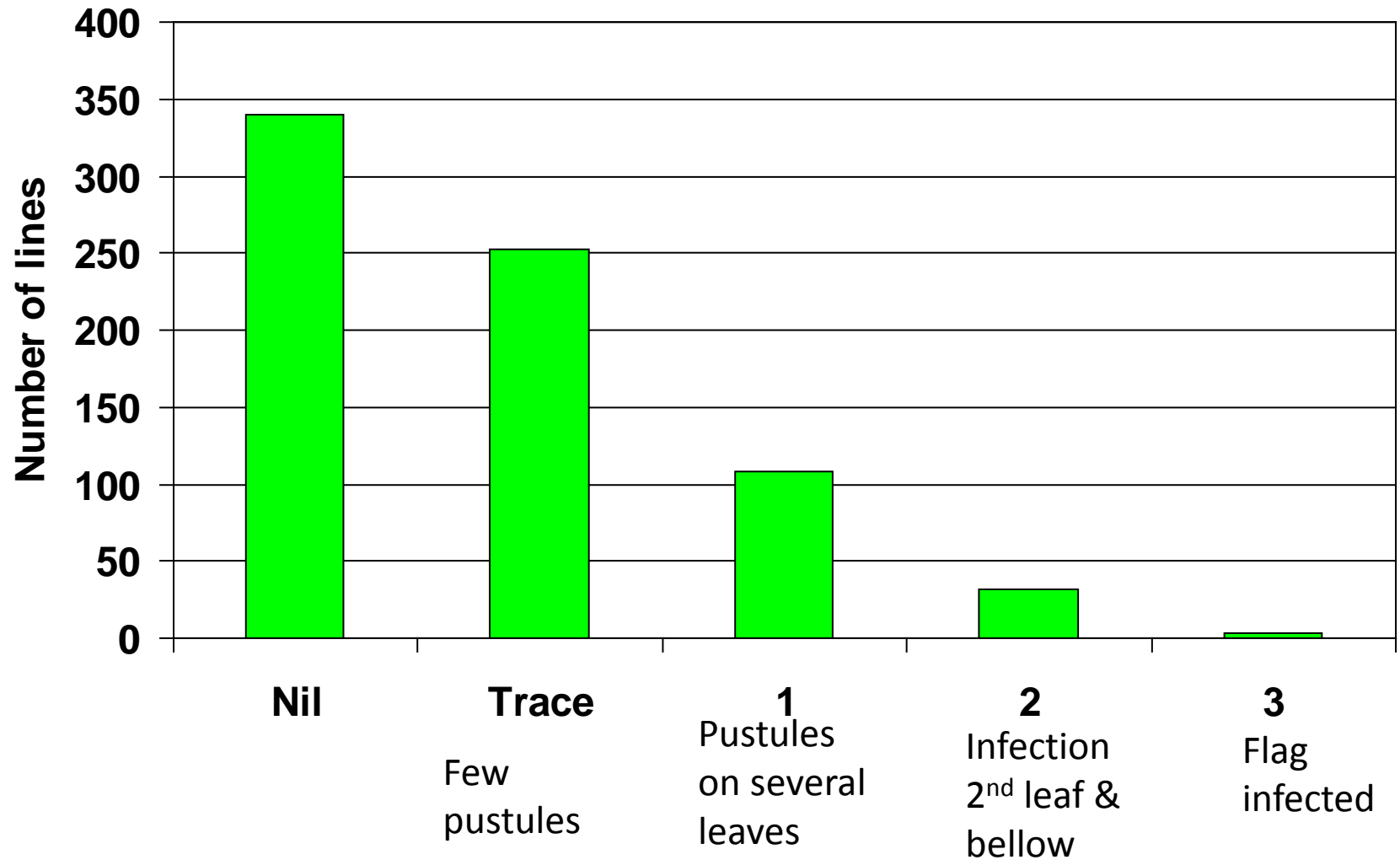




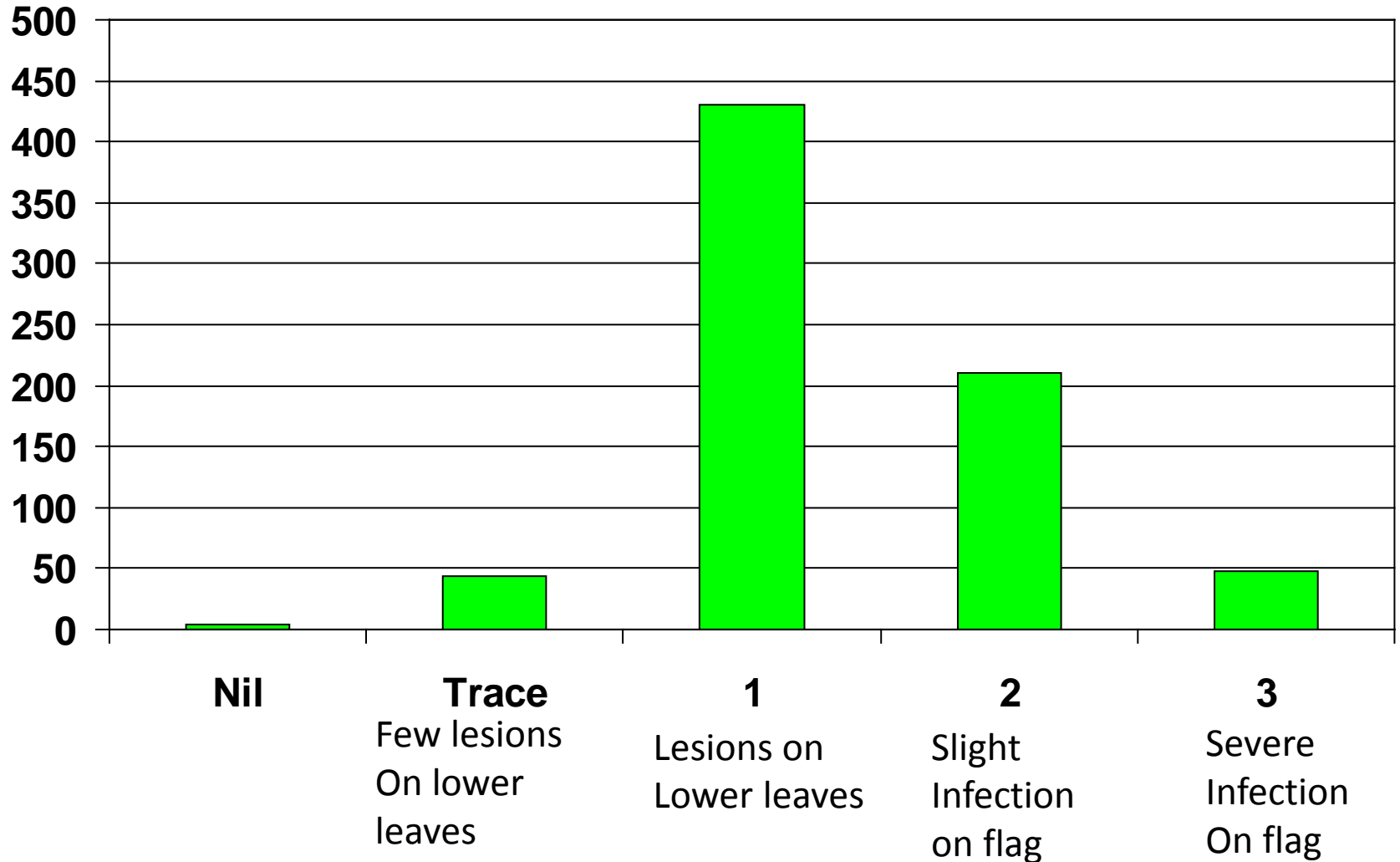
Yellow Rust on flag leaf – percentage area of leaf affected



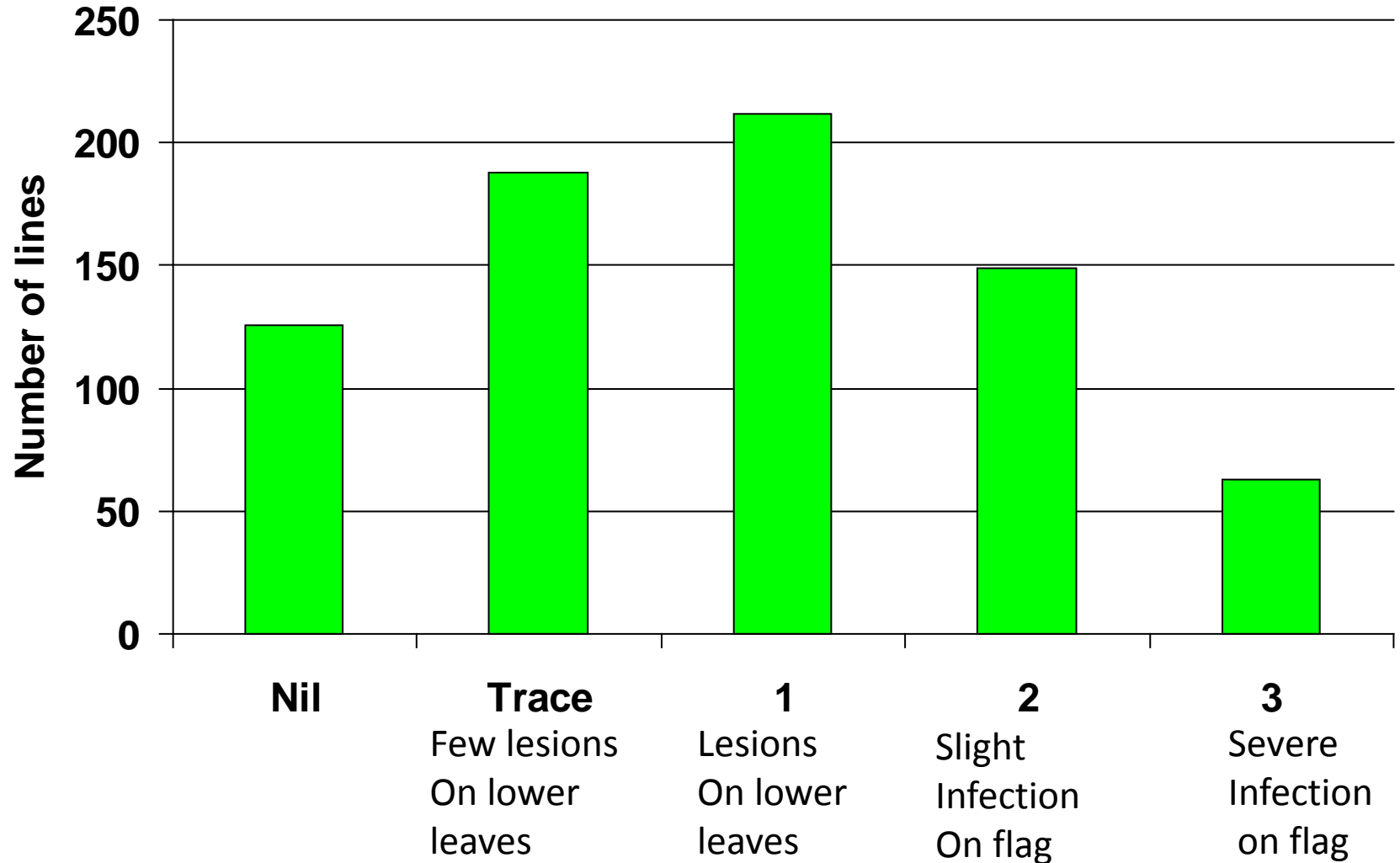
Brown Rust Infection score



Septoria Infection score



Mildew Score



Objective 4: Characterise hexaploid wheat germplasm previously shown to exhibit a high level of resistance to multiple foliar diseases



ROTHAMSTED
RESEARCH

Background

- Watkins 2008 field trial – **10 Watkins accessions** with a high degree of resistance to all 4 foliar pathogens
- Also a high take-all disease year with root infection early in the season
- Was the foliar disease resistance an induced plant response?

Objective 4: Characterise hexaploid wheat germplasm previously shown to exhibit a high level of resistance to multiple foliar diseases



ROTHAMSTED
RESEARCH

WGIN 3 experiments

- 10 Watkins lines + controls sown in both 1st wheat (no take-all) and 3rd wheat (high take-all) field trials in autumn 2014 (1 or 2 replicates per line in each trial)
- No fungicides will be applied to allow natural disease to develop
- Score for foliar diseases + take-all
- F₁ crossing between the Watkins accessions and Paragon/Bobwhite will be carried out to develop mapping populations

Many thanks to



ROTHAMSTED
RESEARCH

RRes Farm staff

Mike Hammond-Kosack

Richard Gutteridge

Kim Hammond-Kosack

Gail Canning

Lucy Nevard

Rodger White (Stats)

Sarah Holdgate (NIAB)

Simon Orford (JIC)



Septoria resistance from *T. monococcum*



ROTHAMSTED
RESEARCH

Kostya Kanyuka

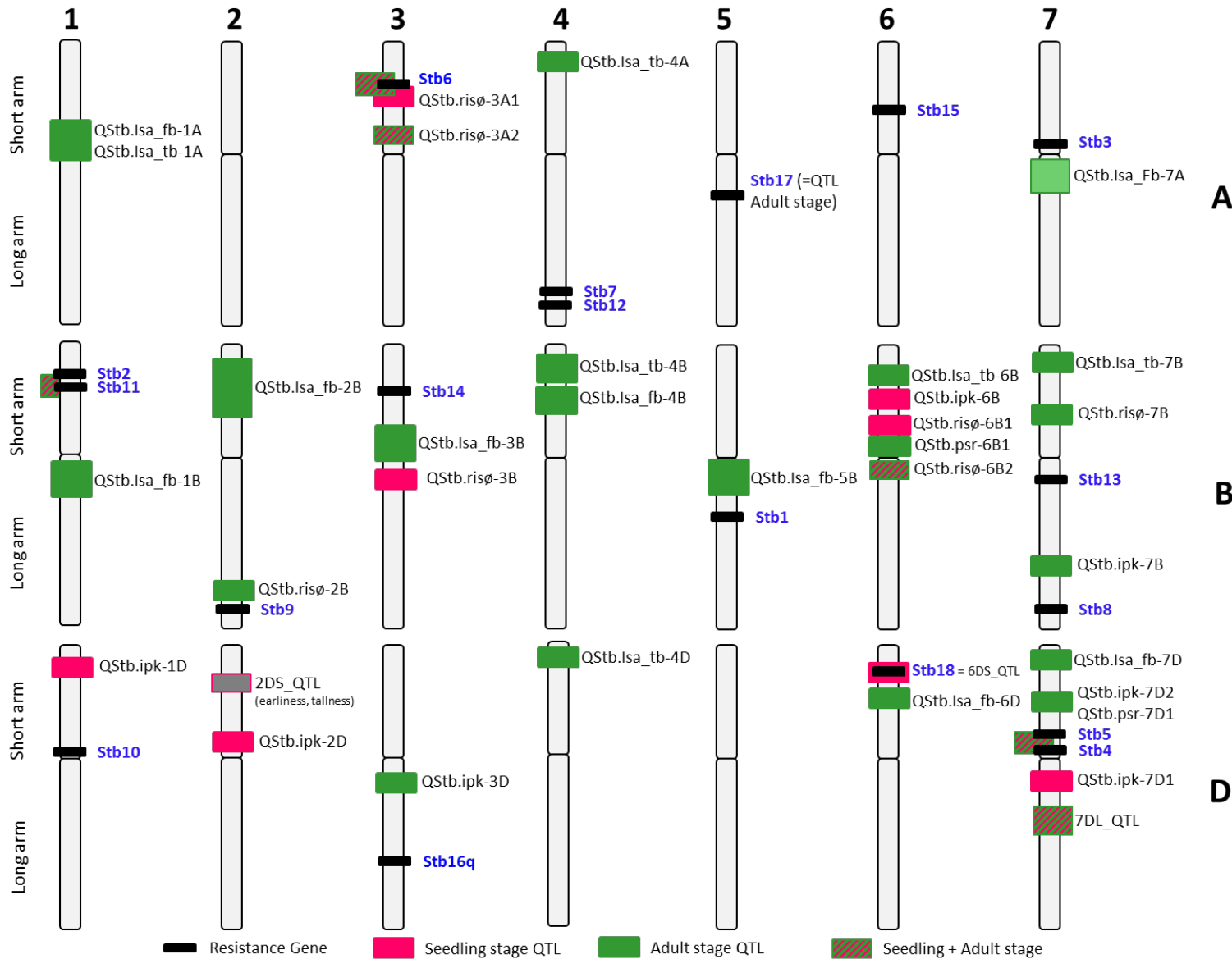
Rothamsted Research

WGIN3 Meeting, 4th March 2015

18 major genes (*Stb*) for resistance to *Z. tritici* and a number of resistance QTLs are known



ROTHAMSTED
RESEARCH



Resistance to STB in cereal species related to wheat



ROTHAMSTED
RESEARCH

Hexaploid wheat cv. Hereward



Diploid wheat *T. monococcum*



no necroses or pycnidia !

Field assessment of 30 *T. monococcum* lines over 4 years

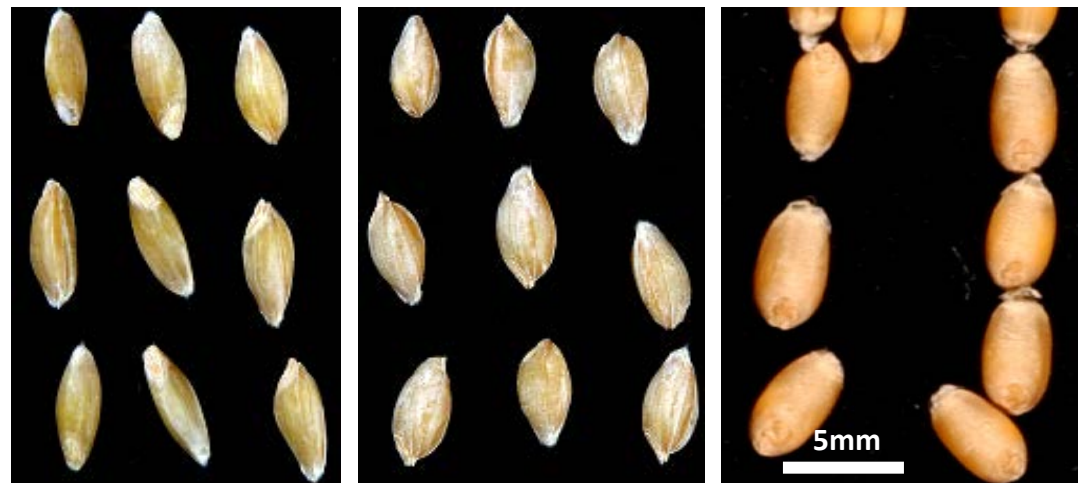
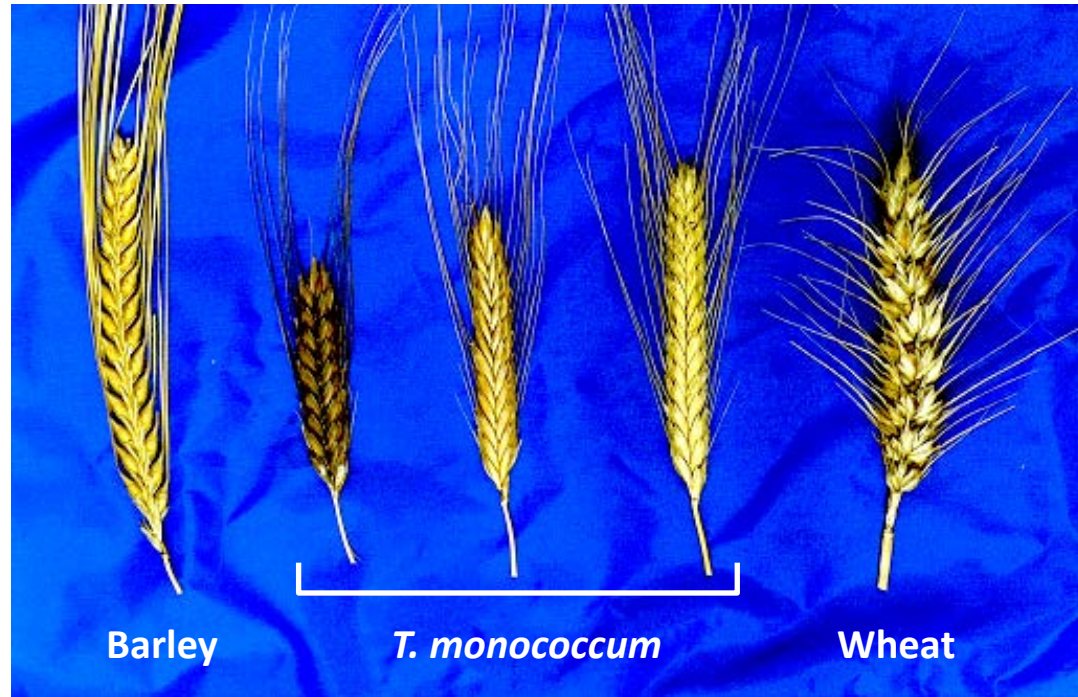
Jing et al. (2007) J Exp Bot

Diploid wheat *Triticum monococcum*



ROTHAMSTED
RESEARCH

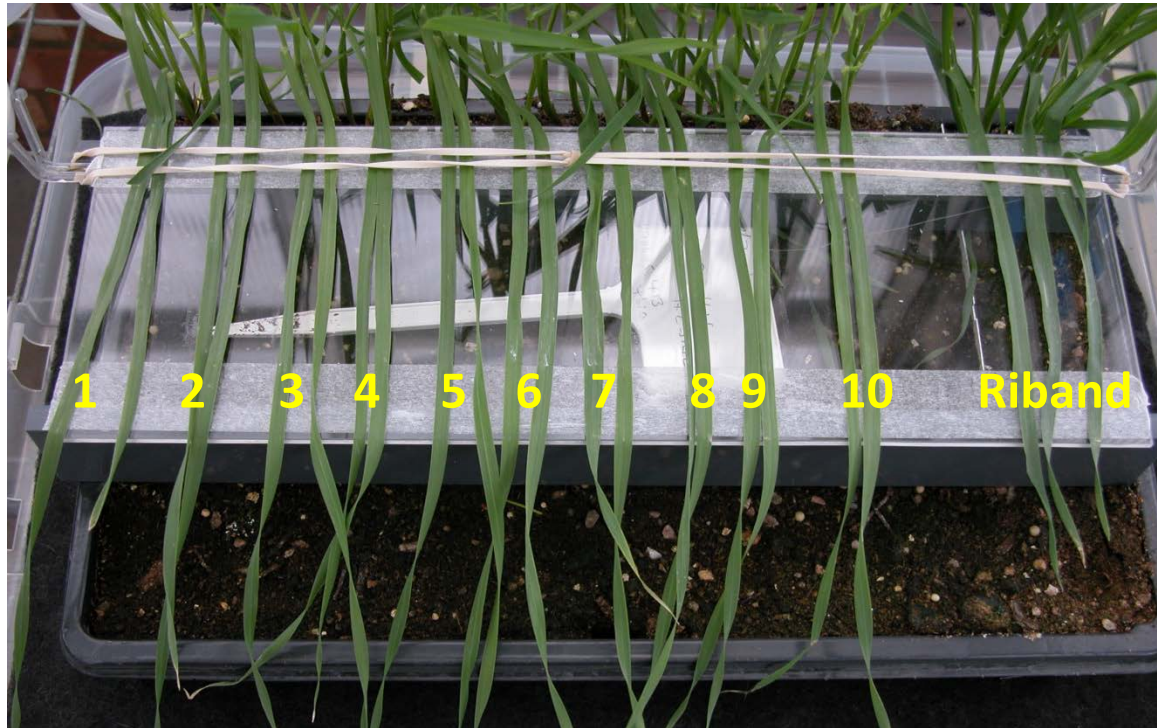
T. monococcum



Responses of 120 *T. monococcum* genotypes to nine diverse *Zymoseptoria tritici* isolates



ROTHAMSTED
RESEARCH



<u>Isolate</u>	<u>Origin</u>
IPO87019	Uruguay
IPO88004	Ethiopia
IPO89011	Netherlands
IPO94269	Netherlands
IPO92006	Portugal
IPO001	Netherlands
IPO90012	Mexico
IPO323***	Netherlands
IPO95052	Durum wheat

*****Sequenced**

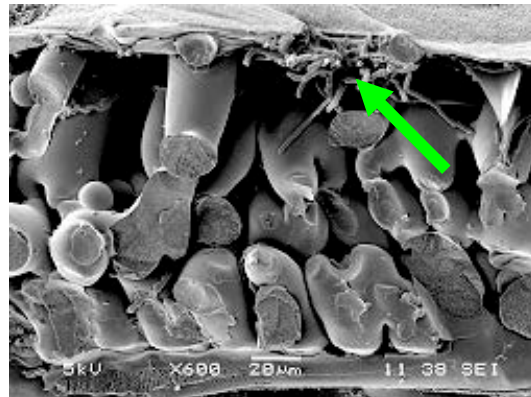
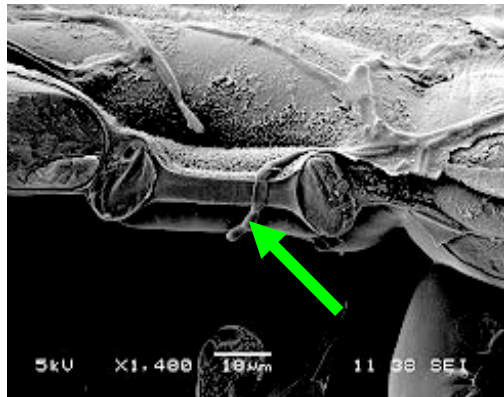
Resistance to *Zymoseptoria tritici* isolate IPO323



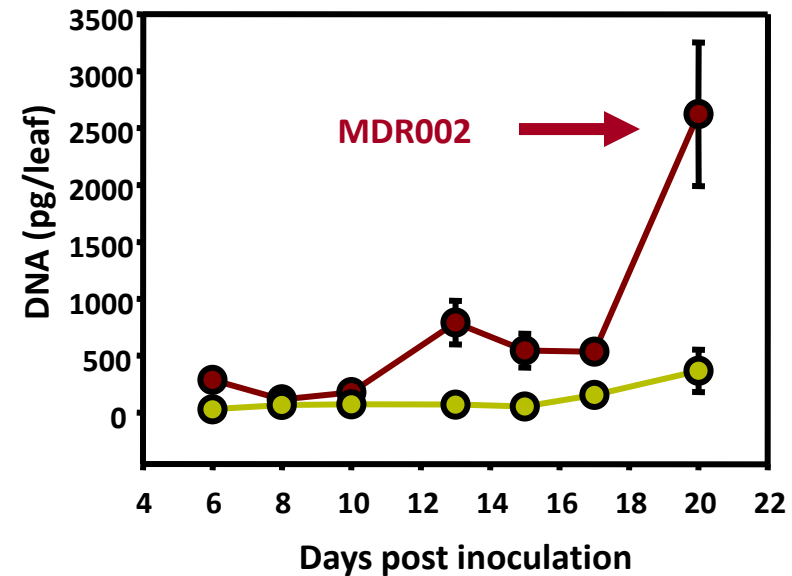
ROTHAMSTED
RESEARCH

DV92/MDR308
(resistant)

MDR2
(susceptible)



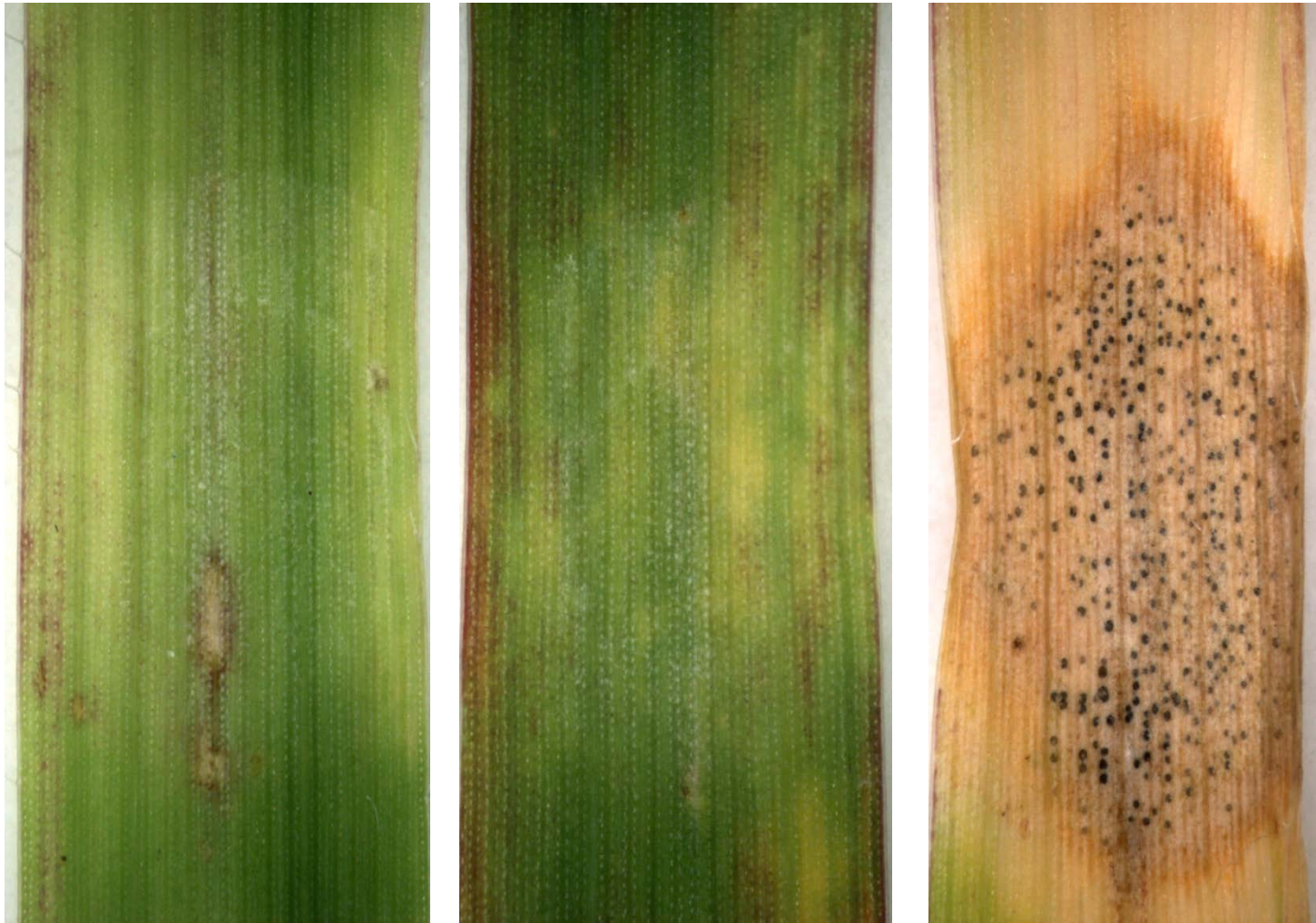
Fungal biomass qPCR



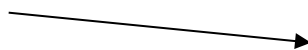
Resistance to *Zymoseptoria tritici* isolate IPO323



ROTHAMSTED
RESEARCH



DV92 (R)



F₁



MDR2 (S)

Genetics of resistance to *Z. tritici* IPO323 in *Triticum monococcum*



ROTHAMSTED
RESEARCH

♀	♂	F ₁	F ₂	F ₃
DV92 (R)	MDR2 (S)	18	400	94*

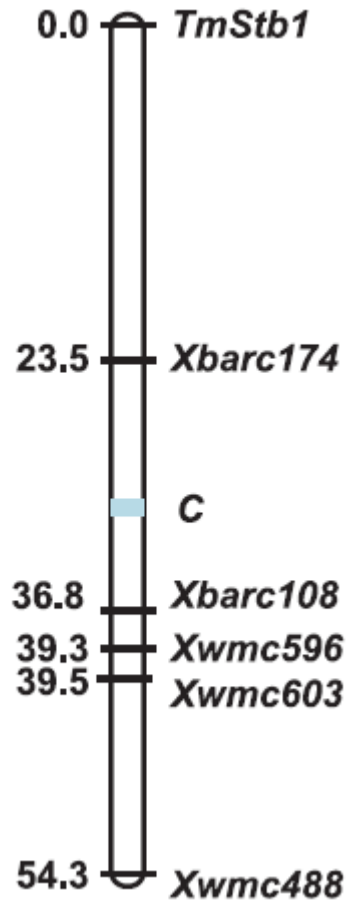
* 94 F₃ families were screened for segregation of resistance / susceptibility to Zt **IPO323**



Resistance to *Z. tritici* IPO323 in *T. monococcum* DV92 appears to be monogenically inherited



ROTHAMSTED
RESEARCH

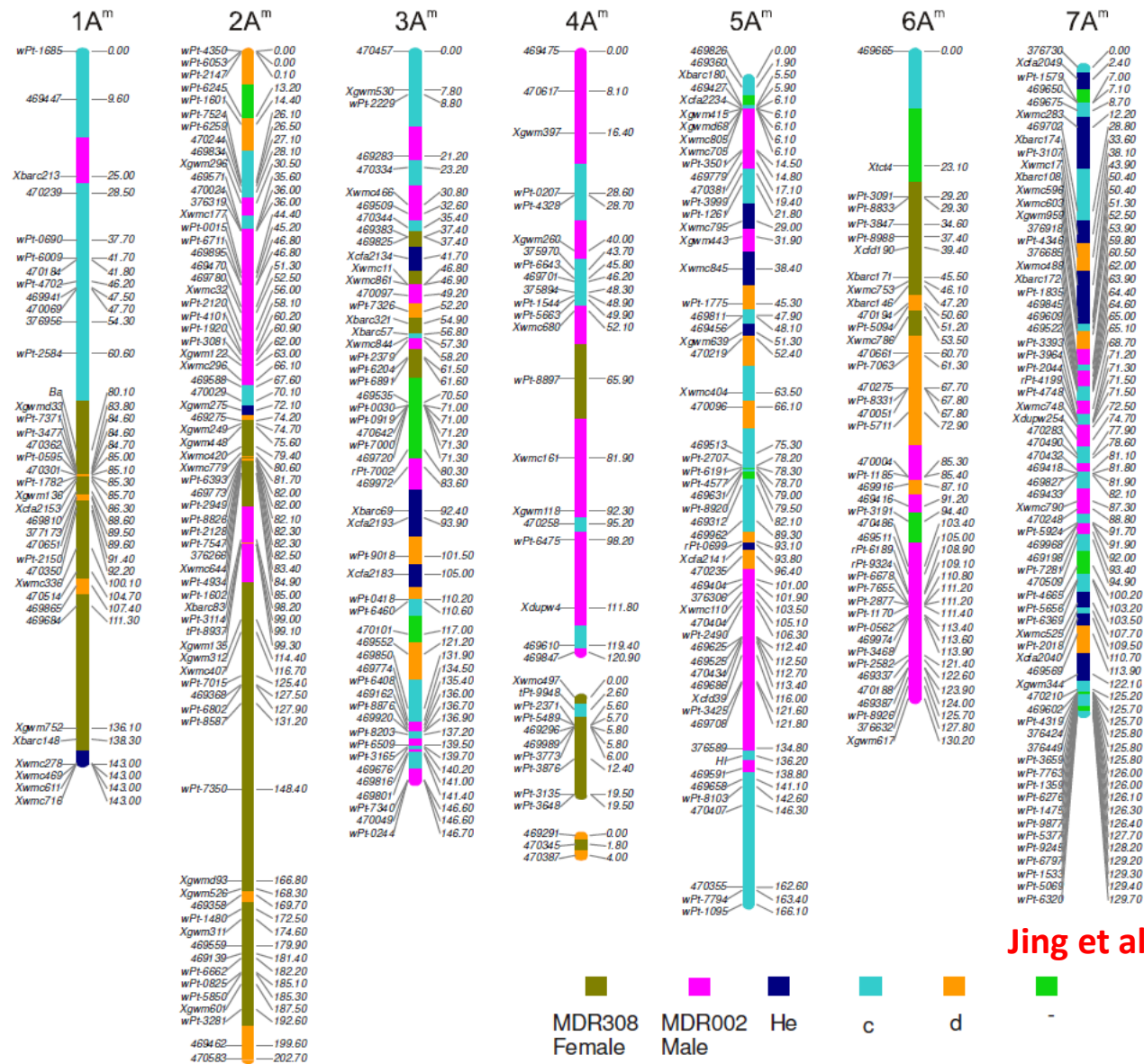


Jing et al. (2008) *New Phytologist*

Refined and higher resolution genetic map around *TmStb1*



ROTHAMSTED
RESEARCH



TmStb1

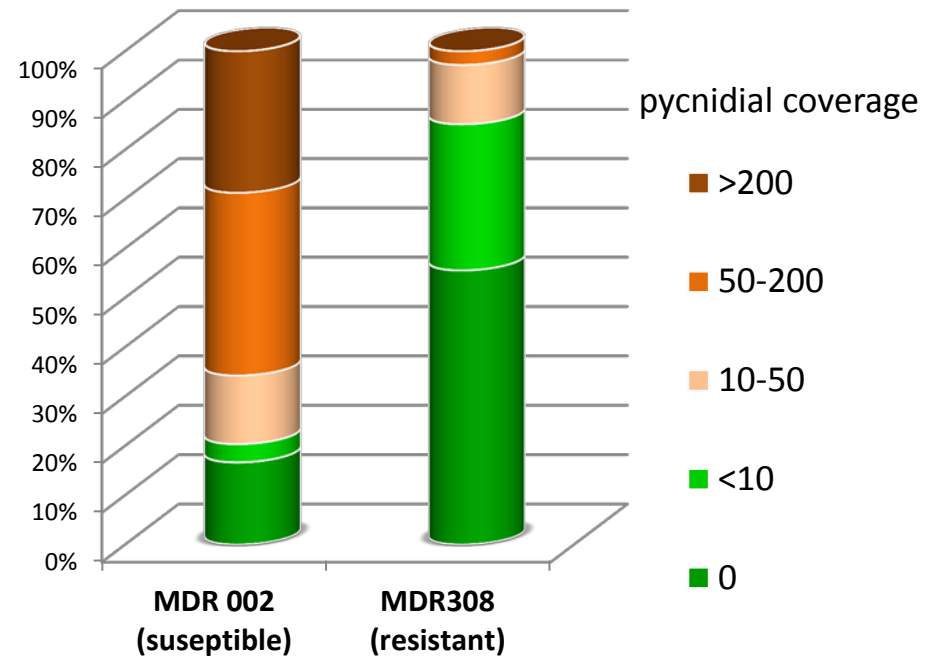
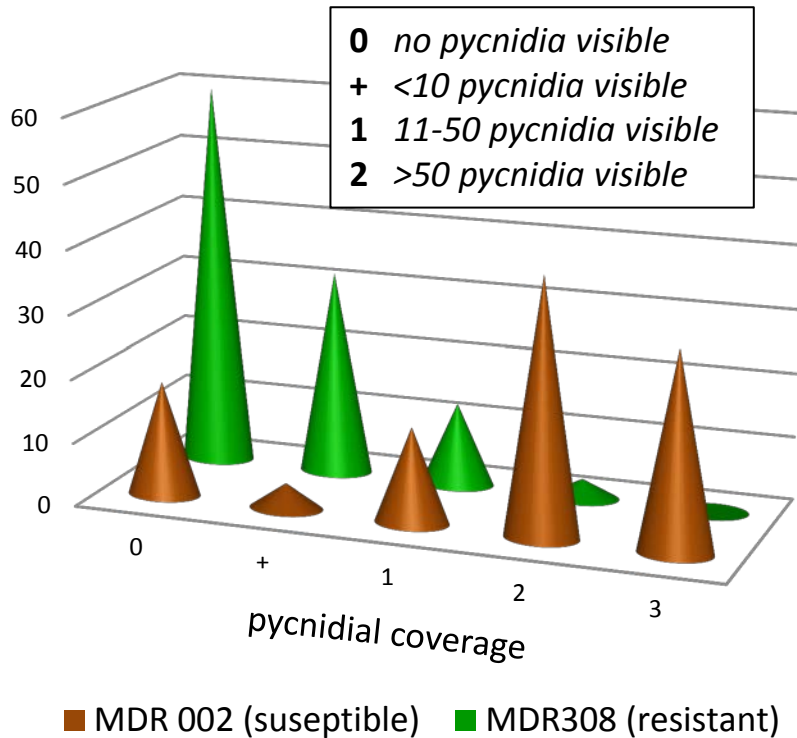
Jing et al. 2009. *BMC Genomics*



Difficulties encountered during phenotyping



ROTHAMSTED
RESEARCH



Based on testing of >100 plants

Phenotyping of a new *T. monococcum* MDR002 x MDR308 pop for resistance to *Z. tritici* IPO323



ROTHAMSTED
RESEARCH

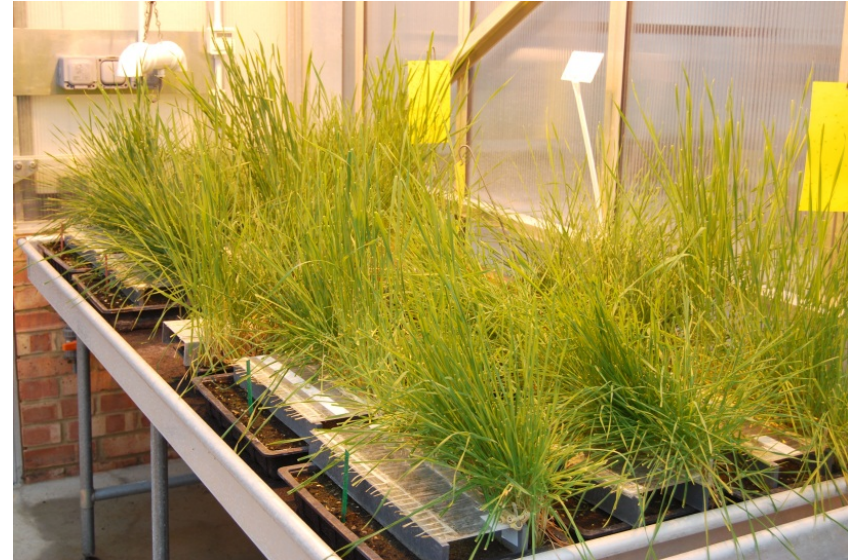
Phenotyped 411 F₂ plants (2 leaves per plant)

Selected 214 F₂ plants for further study, and taken these to F₃

Selected 106 F₃ families for re-screening

Screened 79 F₃ families (12 individuals per family)

30 F₃s were fully resistant, 25 F₃s were fully susceptible, and 19 F₃s segregated for resistance



Acknowledgements



ROTHAMSTED
RESEARCH

Hai-Chun Jing*

Steve Freeman*

Carlos Bayon

Daniel Jenk

Michael Hammond-Kosack

Kim Hammond-Kosack



Exome Capture

Kim Hammond-Kosack



MYcroarray

Ann Harbor, Michigan, USA

WGIN3 project

The overall goal is to exome capture to identify genetic variation in candidate or known genes that are responsible for the desired trait (s)

Exome capture (WP 4.2, 4.4 and Milestones 18)

A designated group of WGIN scientists will interact with the company MYcroarray to decide on the best way to represent wheat genes on the **20,000 bit array**.

This will be done via a series of Skype meetings held during months 1-3.

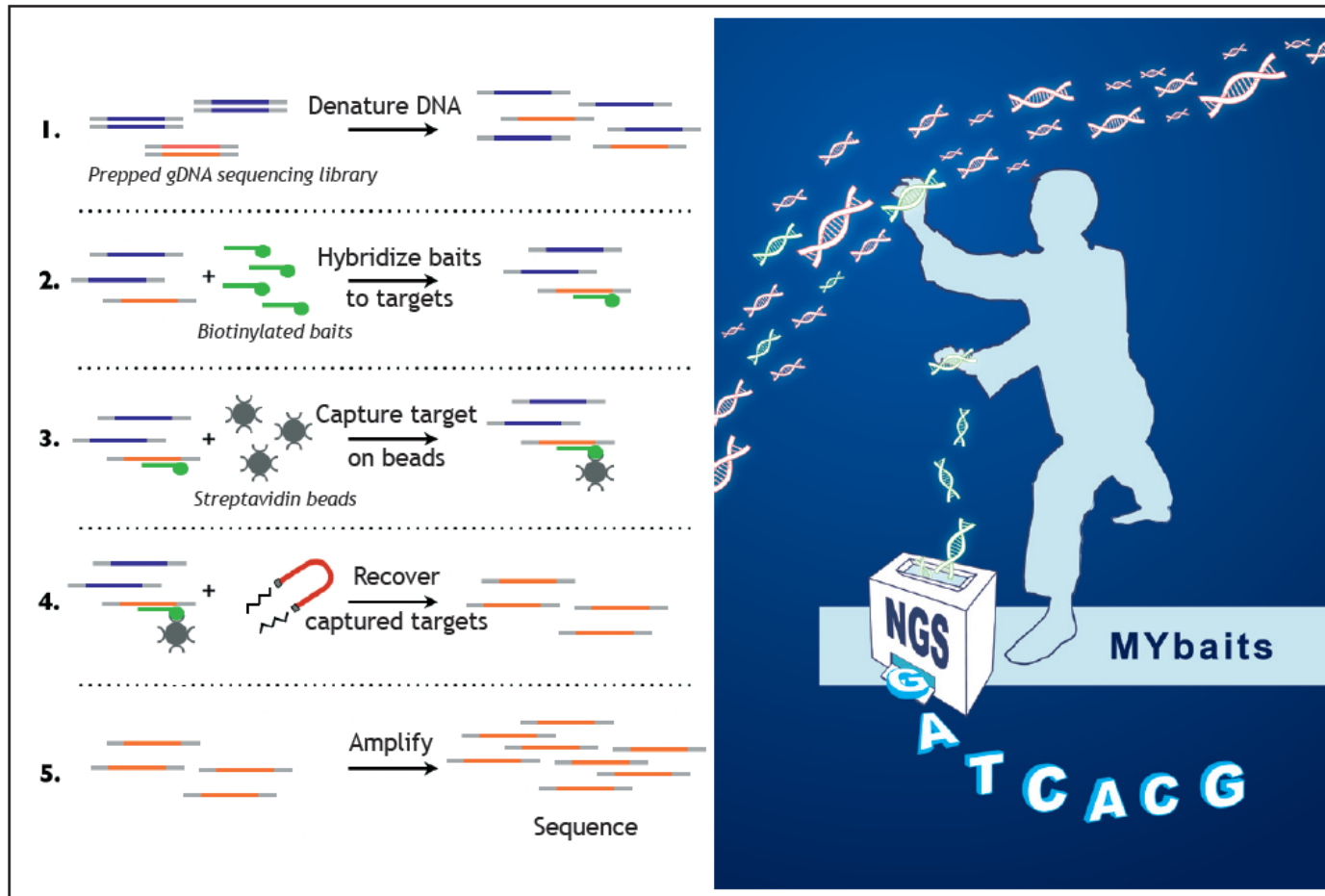
A workshop will be held to **priorities the gene list and the 96 wheat genotypes** to be tested.

A pilot experiment will be done to ensure the DNA is of the correct quality to ensure success.

The full sample set will be sent for the capture using the most appropriate secure carrier.

Custom bait libraries for target sequencing

Mybaits is a fully customisable liquid-phase DNA capture system for targeted sequencing



High percentage of reads on target.

A wheat example from Andy Phillips@RRes

MYcoarray helped design the oligo array for ~1700 wheat genes and made the oligos,

The array “design” was very simple – 120-mers across the whole of each CDS, each overlapping by 60 bp (ie 2-fold coverage). But this naïve design resulted in some variation in capture efficiency .

Used a single set of oligos for each gene, based on a single homoeologue. The on-target homoeologue represented ~50% of all reads, with the other two homoeologues having ~25% each, on average.

Additional comments

A minimum of 20,000 baits – corresponding to ~1200 coding sequences of average length 1kb.

You will achieve a **more comprehensive capture by using genomic sequence not CDS** for oligo design (we lost small exons in our captures) so that you can add some flanking intron sequence (**and promoter, probably important for surveying natural variation**).

Exome capture

Looking for 4-6 individuals interested in taking this WP forward

- select the oligo design method
- select the wheat gene list
- select the 96 wheat genotypes

WORKSHOP

Interact with the BBSRC funded BBR project which include some exome capture for wheat (Uauy and Philips)