

# WGIN 3

## Update on mineral nutrient interactions

Malcolm J. Hawkesford

5<sup>th</sup> Management Meeting  
7<sup>th</sup> July 2016

# Nitrogen Use Efficiency (NUE), stability of wheat grain yield, wheat grain quality and canopy longevity

## (Work packages 2.3, 2.4, 3.4, 4.3, 4.4 Milestones 9 and 10)

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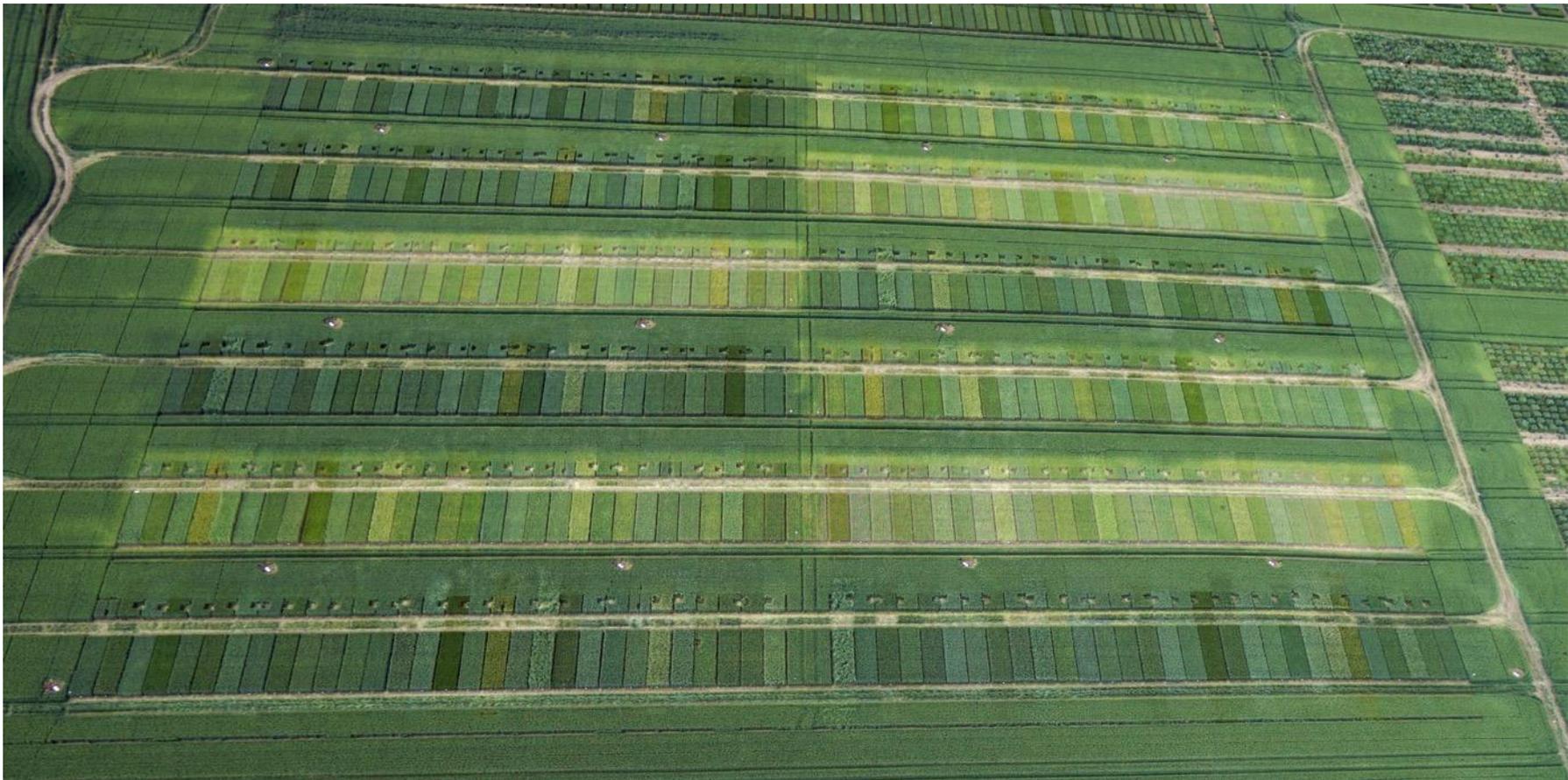
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- The existing dataset includes yield, nitrogen use efficiency (NUE) and associated crop development data. In the next two years this data set will be extended, and the whole dataset analysed finally with respect to **stability and resilience** of these key agronomic traits, drawing on meteorological data from the Rothamsted site
- In 2015 and 2016, **post anthesis uptake of nitrogen and minerals** will be evaluated (mineral composition will be determined by ICP atomic absorption spectroscopic analysis to provide data on a wide range of minerals including P, S, K, Ca, Mg, Fe and Zn).
- **Archive WGIN material** from the previous 3 years (2012-14) (grain and straw) of final harvest grain and straw to give a 5 year dataset on mineral uptake and final partitioning.

# The Rothamsted WGIN Diversity experiment (since 2004; now 13<sup>th</sup> year)



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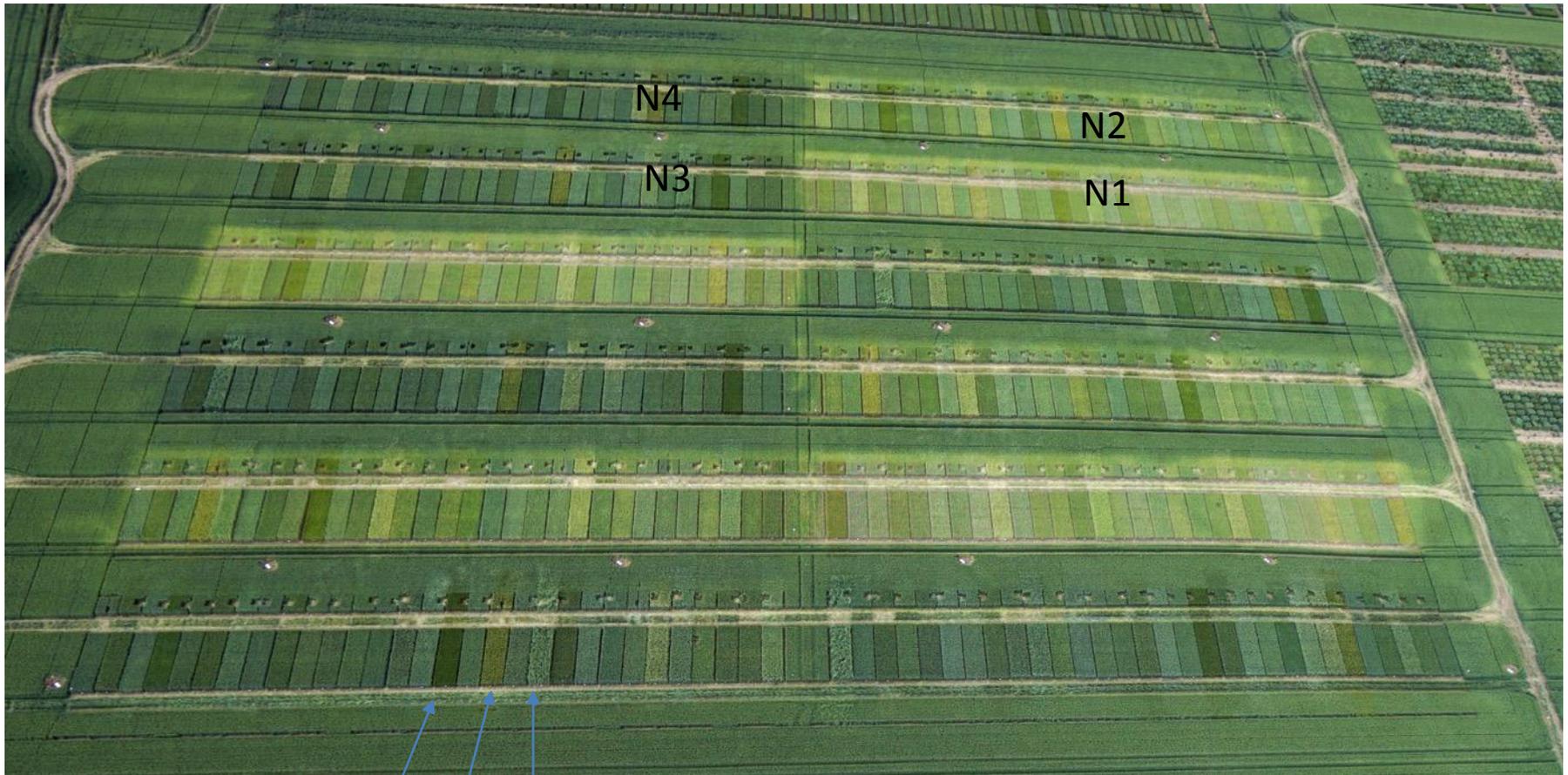


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# The Rothamsted WGIN Diversity experiment (since 2004; now 13<sup>th</sup> year)



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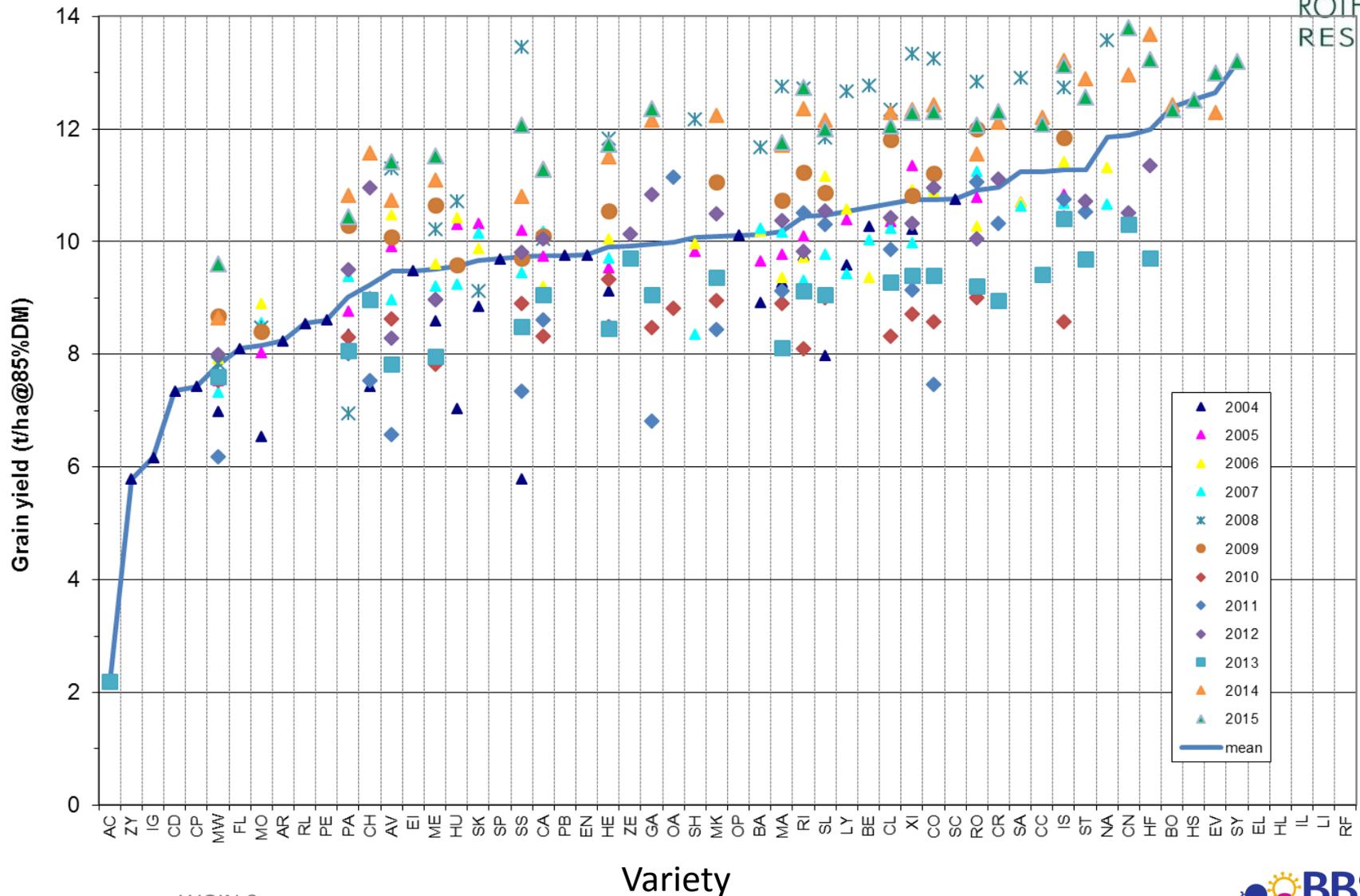
Cr Ss Mw

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# Yield (hence NUE): genetic and year variability



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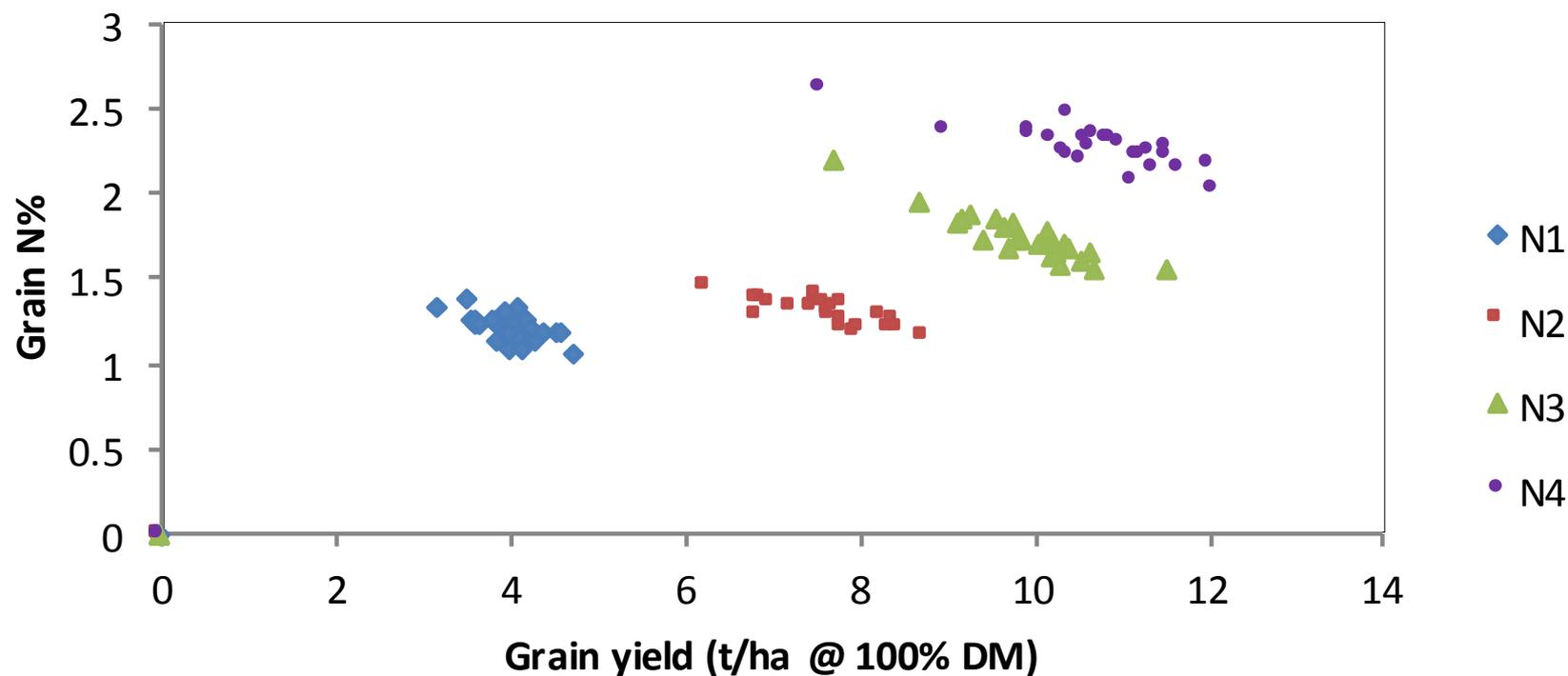
# ICP data sets



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		ICP DATA FOR WIGIN DIVERSITY		
YEAR	FINAL GRAIN	FINAL STRAW	ANTHESIS	NOTES
2012	YES	YES		Archive material
2013	YES	YES		Archive material
2014	YES	YES	some	Archive material
2015	YES	YES	YES	Full data set
2016			YES	In progress

## Grain N% v grain yield

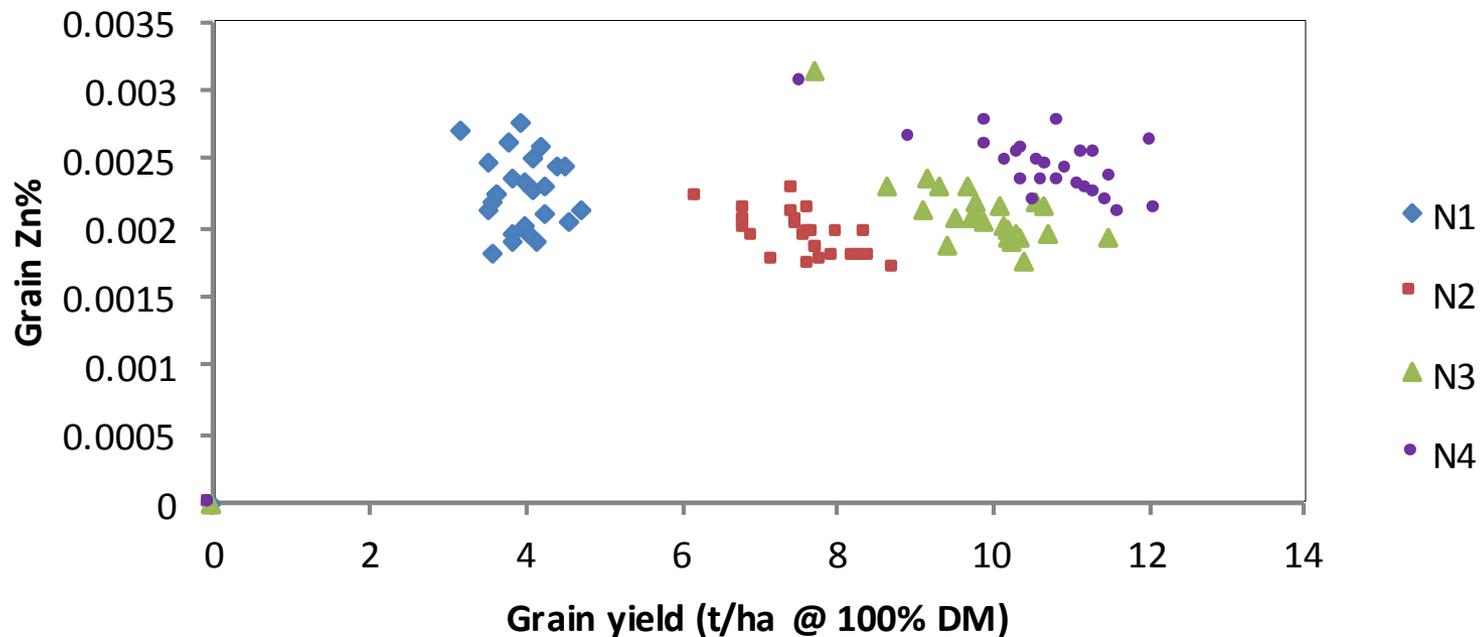


# Mature grain Zn in WGIN varieties in 2015



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## Grain Zn% v grain yield

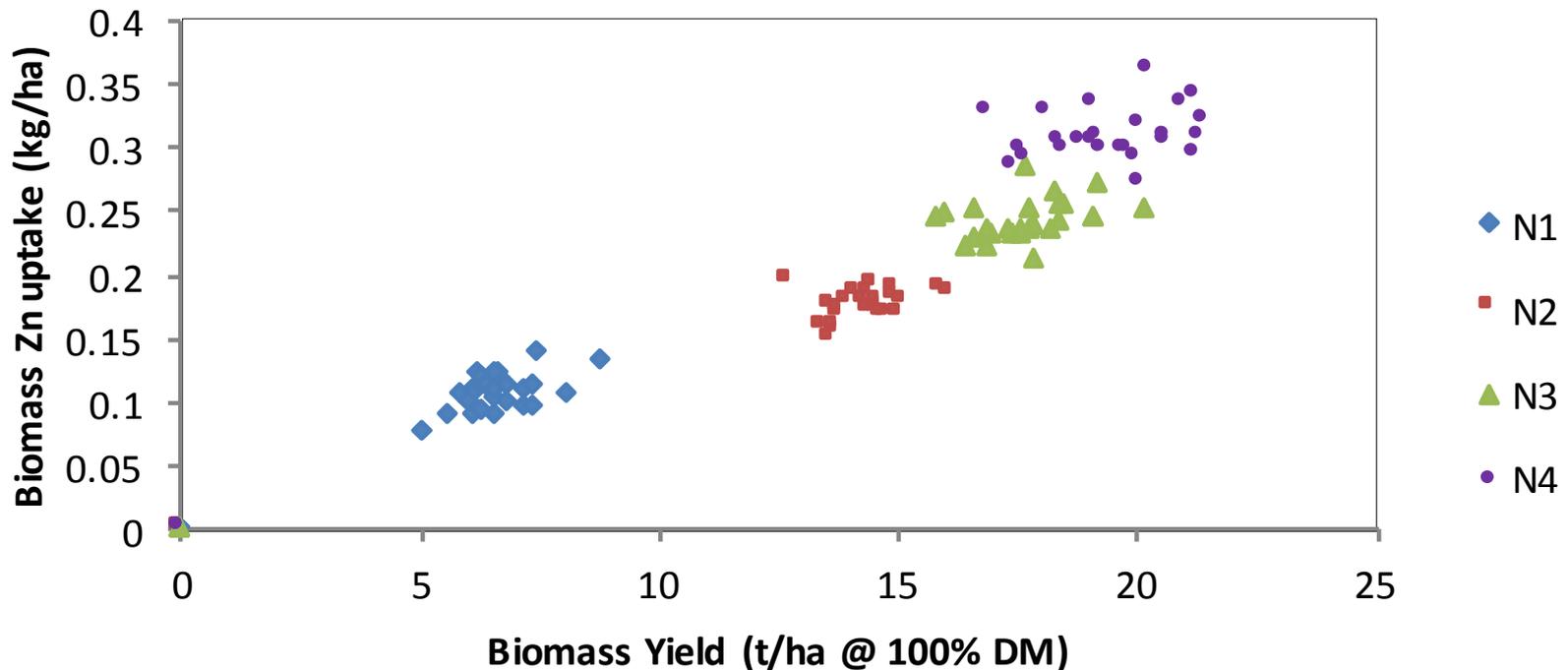


# Total Zn uptake proportional to biomass in WGIN varieties in 2015



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## Biomass Zn uptake v biomass yield

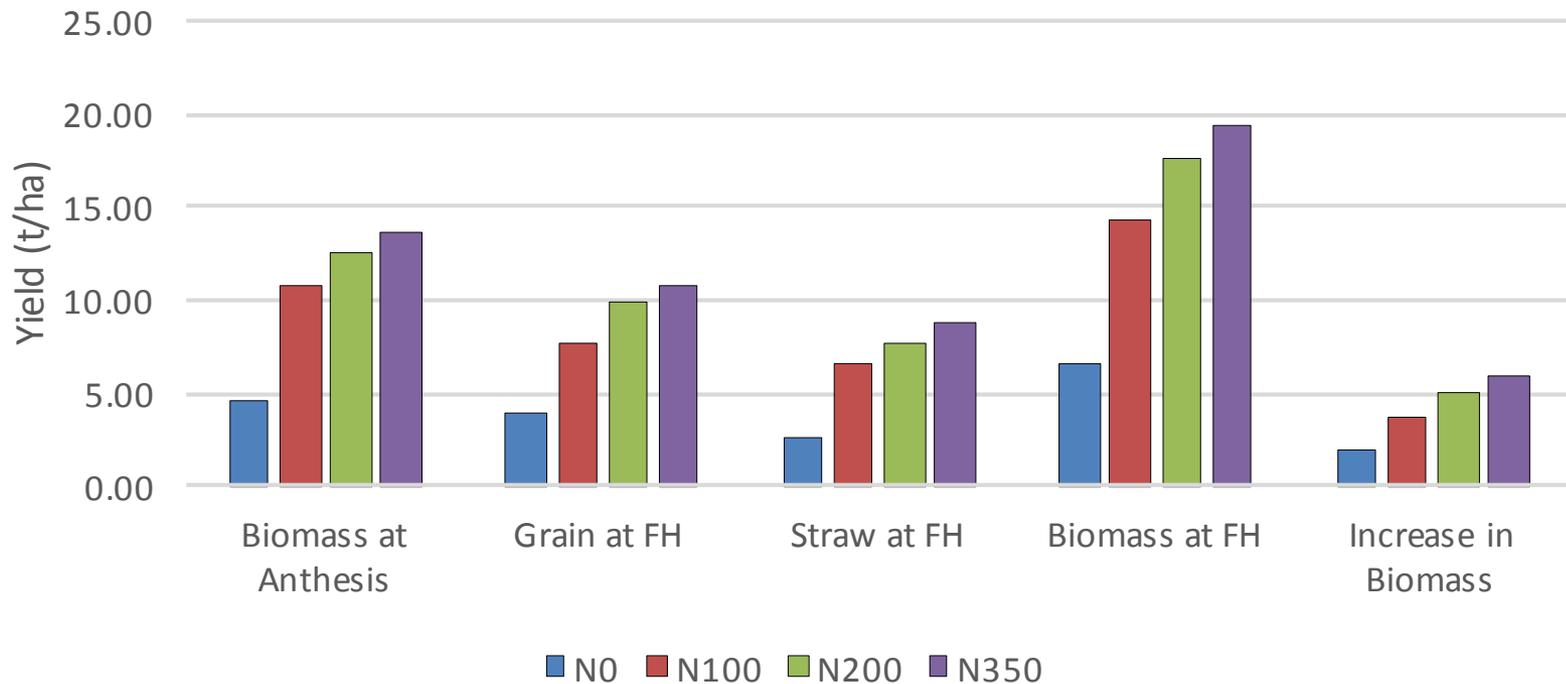


# Summary data, WGIN Diversity, 2015



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## Yields (@ 100% DM)

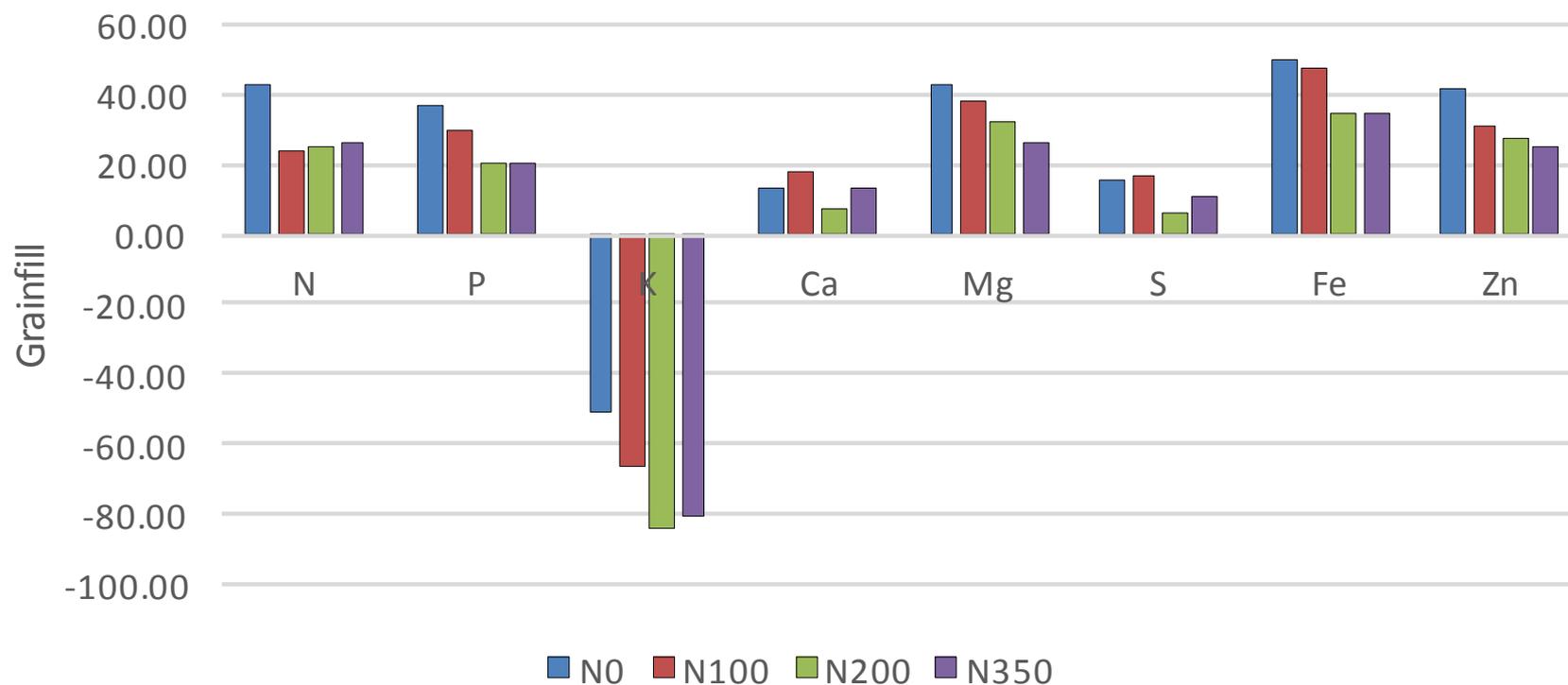


# Elemental (total biomass) acquisition after anthesis



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## Uptake during grainfill as a % of total uptake

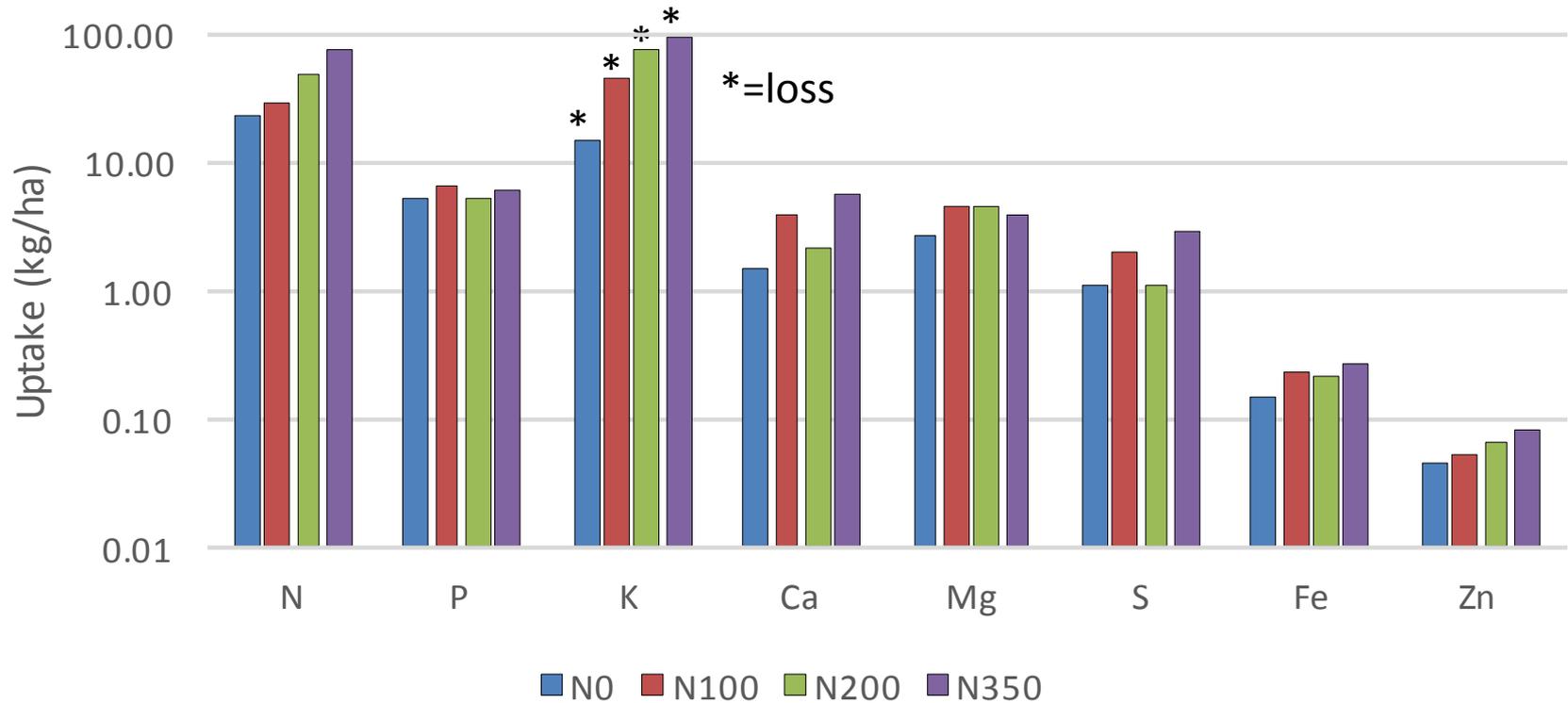


# Elemental (total biomass) acquisition after anthesis



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## Uptake during grainfill



# Rothamsted field platform includes mini-WGIN Diversity experiment



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WGIN 3

# Thanks

- WGIN team
- Rothamsted Farm staff
- Andrew Riche, Saroj Parmar, March Castle, Adam Michaelski, Grzegorz Kulczycki
- Gantry: Pouria Sadeghi, Kasra Sabermanesh, Nicolas Virlet



# WGIN 3

## Resistance to take-all and foliar diseases

Vanessa McMillan  
Kim Hammond-Kosack



# Resistance to take-all and foliar diseases

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Characterisation of hexaploid wheat germplasm previously shown to exhibit a high level of resistance to multiple foliar diseases

## 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?



# 10 Watkins accessions with high degree of resistance to all 4 foliar pathogens



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Accession	Growth habit	Country of Origin	2008 Disease assessments			
			Yellow rust	Brown rust	Septoria	Mildew
18	Spring	India	0	0	T	T
137	Spring	Australia	T	T	0	T
203	Winter	India	0	0	0	T
231	Spring	Hungary	0	0	T	0
262	Spring	Canary Islands	0	0	0	0
399	Spring	China	T	0	T	0
495	Spring	Morocco	0	0	T	0
610	Spring	Yugoslavia	0	0	T	T
733	Spring	Iran	T	T	T	T
786	Spring	USSR	0	T	T	0

0 – no disease , T = trace



# WGIN 3 Watkins foliar disease experiment 2015

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- 10 Watkins lines + controls sown in both **1<sup>st</sup> wheat (no take-all)** and **3<sup>rd</sup> wheat (high take-all)** field trials in autumn 2014 (1 or 2 replicates per line in each trial)
- No fungicides applied to allow natural disease to develop
- Score for foliar diseases + take-all



# Watkins foliar disease field trial 2015



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26<sup>th</sup> June 2015      1<sup>st</sup> wheat Long Hoos 4

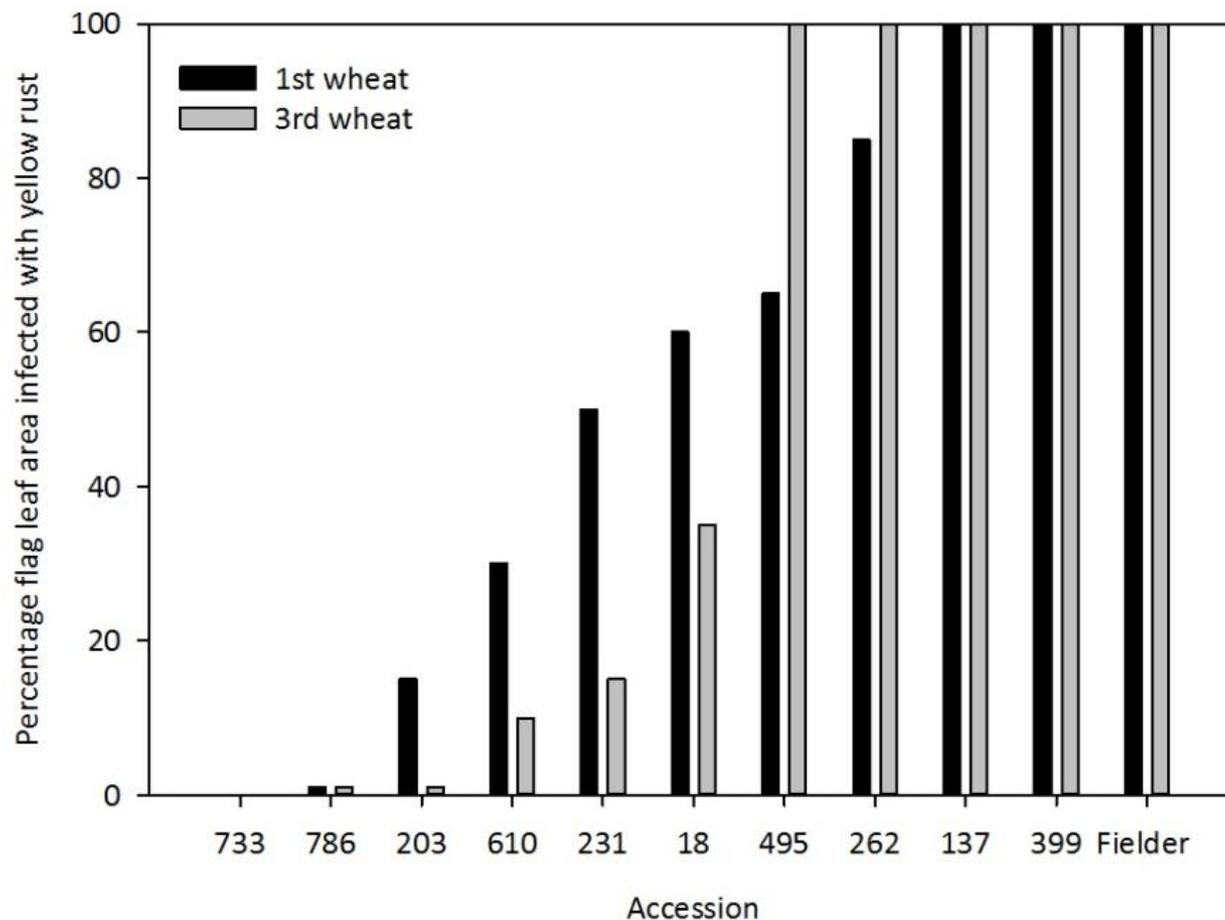
Yellow rust dominant disease that developed across 2015 field trials



# Evidence of resistance to yellow rust



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- Evidence of induced response due to take-all?
- Take-all disease assessments still to be completed



# Evidence of resistance to yellow rust



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Watkins 203

Low levels of yellow rust sporulation



Watkins 733

No sporulation



# Watkins foliar disease trial 2016

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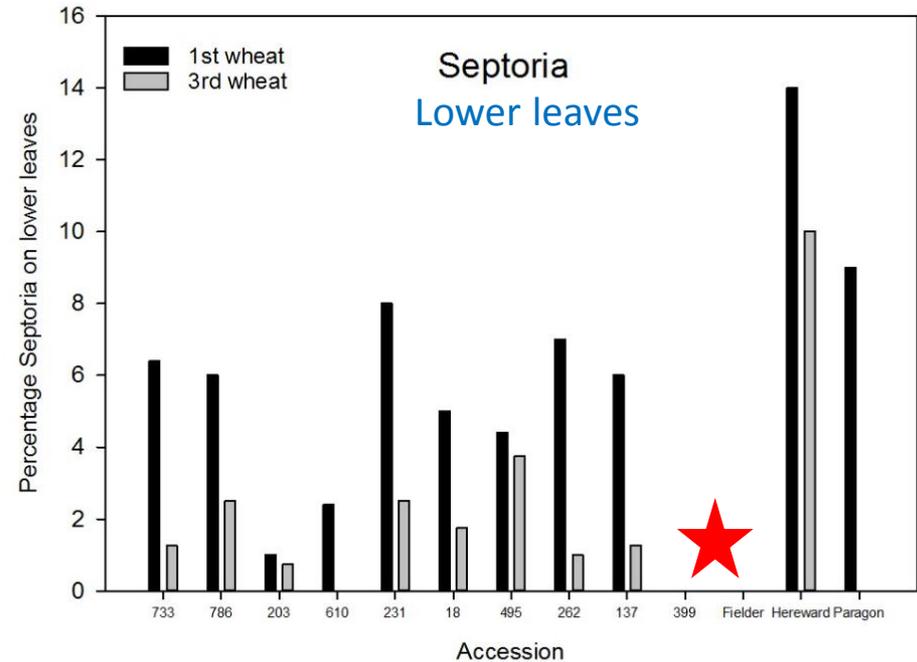
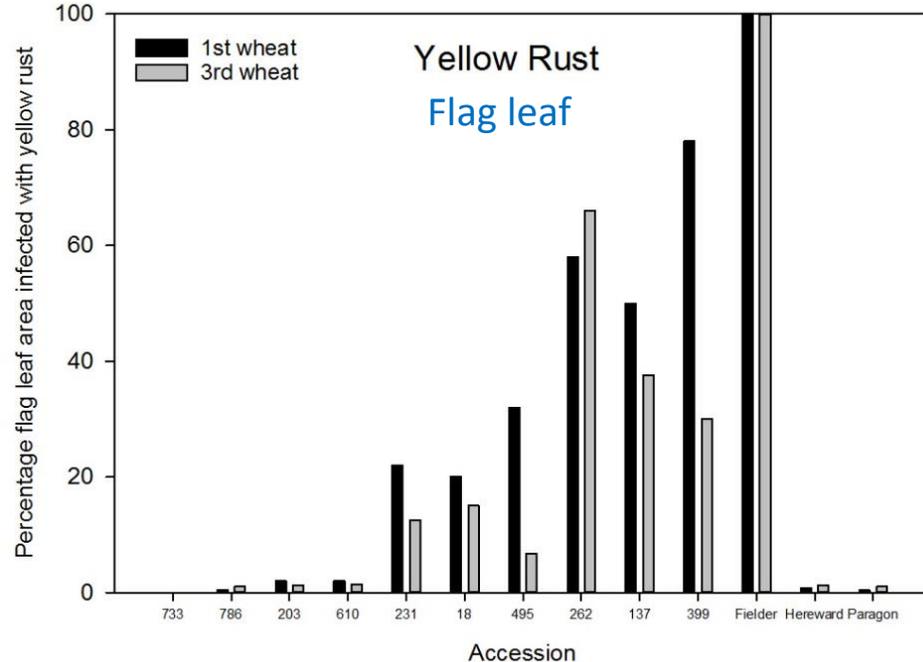
- Repeat field trial with 5 replicates of each genotype sown in autumn 2015 in Long Hoos 5 (1<sup>st</sup> wheat) and Long Hoos 6/7 (3<sup>rd</sup> wheat)



# 6<sup>th</sup> June 2016 – Yellow rust and Septoria



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- Yellow Rust shows similar pattern to 2015

★ Low levels of Septoria on all genotypes except 399 and Fielder where high levels of yellow rust are found, therefore impossible to assess Septoria infections

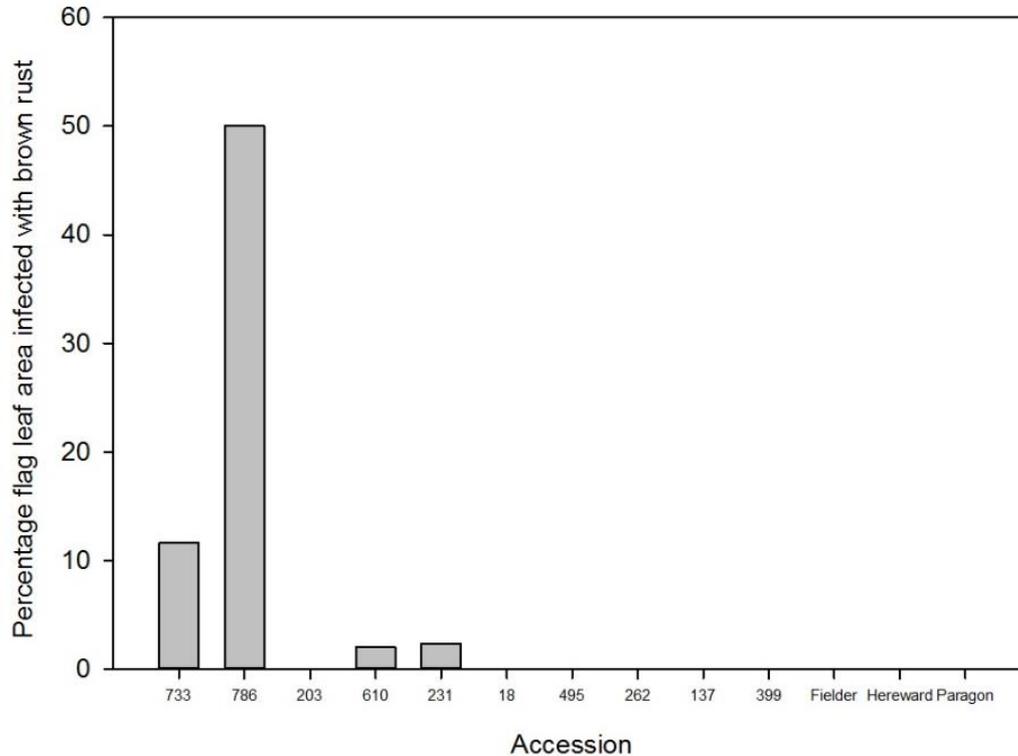
- All genotypes at GS 57-61, except 610 at GS 47



# 22<sup>nd</sup> June 2016 – Brown rust developed across the 1<sup>st</sup> wheat trial site



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- Watkins 733 and 786 were most resistant to yellow rust but are very susceptible to brown rust – do not possess multi disease resistance
- Watkins 203 most promising for showing high levels of resistance against both yellow and brown rust



# Watkins mapping population development



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## Summer 2015

Field crossing with cv. Fielder

Accession Number	Growth habit	Country of Origin	Ears crossed	F <sub>1</sub> grains
203	Winter	India	8	31
231	Spring	Hungary	8	54
610	Spring	Yugoslavia	6	33
733	Spring	Iran	6	49
786	Spring	USSR	N/A	N/A

## Summer 2016

- F<sub>1</sub> grain sown in glasshouse to generate F<sub>2</sub> and for backcrossing to cv. Fielder – grain soon to be harvested
- Watkins 786 x Fielder crossing carried out in glasshouse – grain soon to be harvested
- F<sub>1</sub> grain included in a spring field trial 2016 to study inheritance



# F<sub>1</sub> plants – spring field trial



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- Six F<sub>1</sub> grain from each of the 4 crosses sown
- Two replicate plots of parent genotypes (40 seeds per plot)
- Yellow Rust assessments on 30<sup>th</sup> June 2016

Parents		Fielder x 203	
Fielder	203	Plant 1	Plant 2
100%	2.5%	50%	50%

Parents		Fielder x 231
Fielder	231	Plant 1
100%	5%	30%

Parents		Fielder x 610	
Fielder	610	Plant 1	Plant 2
100%	7.5%	50%	70%

Parents		Fielder x 733				
Fielder	733	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
100%	0%	0%	trace	0%	0%	0%



## OVERALL SUMMARY

- 2015 trials only yellow rust (6 accessions evidence of moderate to high resistance)
- 2016 trials – yellow rust (YR), brown rust, septoria present
- Watkins 203 most promising accession high level of resistance to all three diseases.
- Watkins 733 extreme resistance to YR dominant
- Watkins 203, 231 and 610 reduced susceptibility appears to be recessively inherited.
- UK yellow rust races have undergone at least three complete ‘genetic sweeps’ since 2008, i.e the older races are no longer present in the current pan UK-European population
- Trend towards less overall disease in 3<sup>rd</sup> wheat crops evident in both seasons

# Many thanks to



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Kim Hammond-Kosack  
Vanessa McMillan  
Gail Canning

## PhD students

Sarah-Jane Osborne  
Joseph Moughan

## Undergraduate summer students

Erin Baggs  
Eleanor Leane  
Tessa Reid

Mike Hammond-Kosack – crossing and introgression  
Lucy Nevard and Leanne Freeman – seed preparation

Rodger White - statistics

RRes farm and glasshouse staff

Sarah Holdgate (NIAB)  
Simon Orford (JIC)



# Exome Capture

Kim Hammond-Kosack



**MY**croarray

Ann Harbor, Michigan, USA

7<sup>th</sup> July 2016

# Exome Capture

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

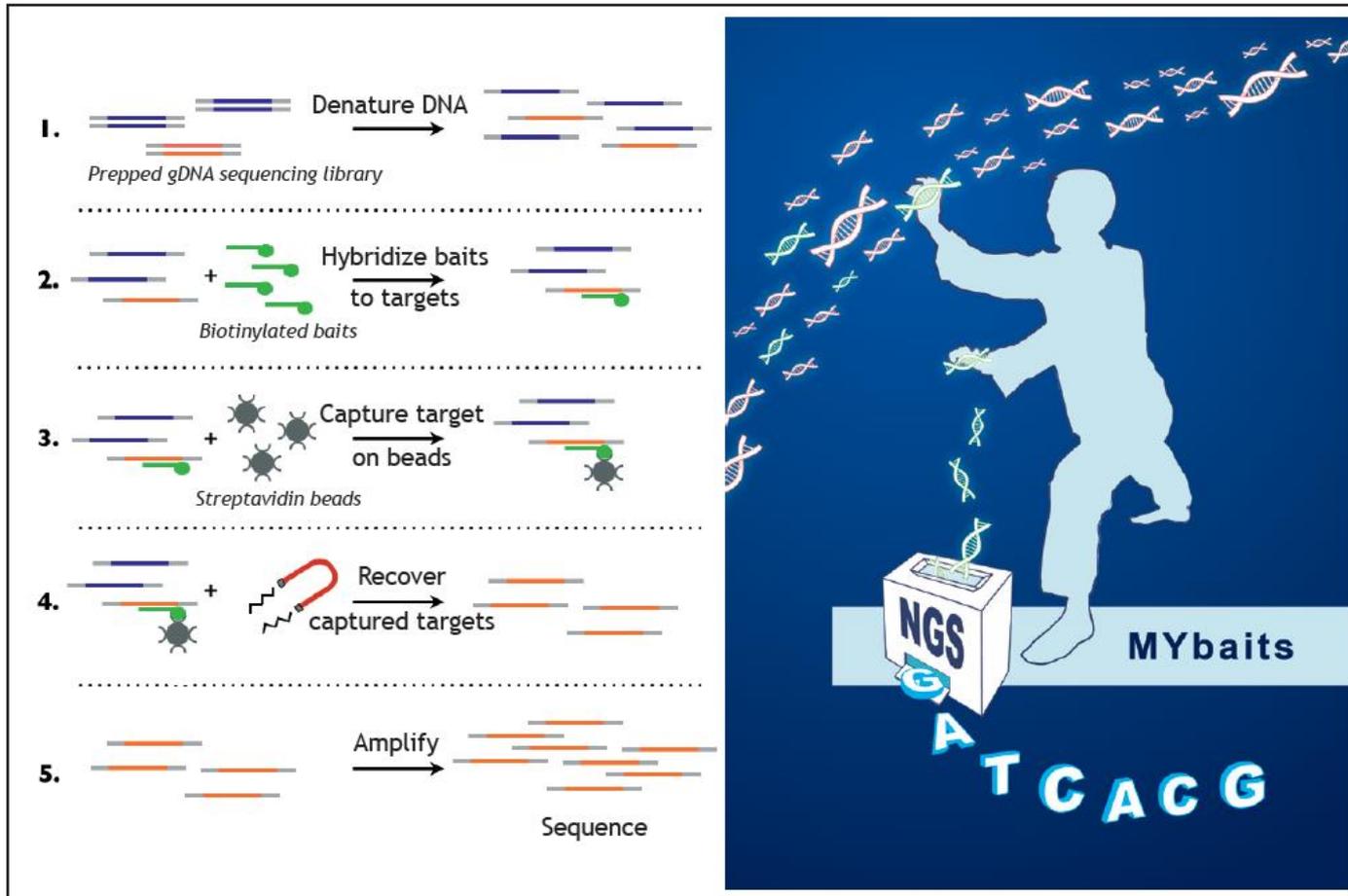
## **Exome capture (WP 4.2, 4.4 and Milestones 18)**

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**.

**Focus : promoter analysis**

# 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.

# **Summary of exome capture discussions @ WGIN Stakeholder meeting 16<sup>th</sup> April 2015**

**Focus : promoter sequences (~1kb) – NOVELTY**

**A, B and D genome sequences to be  
individually captured**

- Bioinformatics will be quite challenging to  
ID the 3 homoeologous promoters**

# Summary of exome capture discussions @ WGIN Stakeholder meeting 16<sup>th</sup> April 2015

Design: 120-mers across each promoter,  
each overlapping by 60 bp (i.e. 2-fold coverage)  
16 probes per promoter (960 bp)  
48 probes to cover A, B and D promoters / gene  
416 x 3 promoters  
Or some only evaluated for 1 homoeologue

Need to include published **positive controls**  
to validate the technology  
For example - *ppd1, vrn1A*

Need to remove

**MITEs** - miniature inverted-*repeat* transposable elements  
from the probe sets developed

# Summary of exome capture discussions @ WGIN Stakeholder meeting 16<sup>th</sup> April 2015

## Developing the list of 96 cultivars – with reasons

Need to relate to ongoing / previous wheat projects  
(WGIN and beyond)

### Generic Resources

Avalon

Cadenza

Paragon

Chinese Spring

Kronos (tetraploid)

Diploids

Alchemy, Hereward, **Rialto**, Robigus, Savannah and Xi19

- Wingfield et al (2012) PBJ study

### Specific traits

Garcia

Watkins 777

# **Summary of exome capture discussions @ WGIN Stakeholder meeting 16<sup>th</sup> April 2015**

**Developing the promoter – gene list – 416 x 3 genomes**

## **Traits**

- 1. Yield resilience**
- 2. Grain quality**
- 3. Biotic stress – fungi and insects**
- 4. Abiotic stress – drought, high temp**
- 5. Nutrient use efficiency**
- 6. Canopy development**
- 7. Flower biology**
- 8. Root architecture**

**50 nominated promoters per trait category**

# Who to be involved ?

Yield resilience

Grain quality

Biotic stress – fungi and insects

Abiotic stress – drought, high temp

Nutrient use efficiency

Canopy development / whole plant architecture) Simon Griffiths,

Flower biology

Root architecture

Cristobal Uauy\*, **need a 2<sup>nd</sup>**

Peter Shewry, Kay Trafford (NIAB),  
Rowan Mitchell (RRes)

Kim H-K (RRes), Brandt Wulff (JIC),  
Matthew Moscow (SL), **insects ??**

John Foulkes, **need a 2<sup>nd</sup>**

Malcolm Hawkesford **and Nottingham**

Allison Bentley (NIAB), Andy Phillips\*

Zoe Wilson, **need a 2<sup>nd</sup>**

Malcolm Bennett, **need a 2<sup>nd</sup>**

**Breeding community - nominations?**

\* BBSRC BBR wheat tilling project and exome capture

# Gene nomination - NEW

- Suggestions directly into an Excel spread sheet
- Gene ID numbers to come from just one source
  - ENSEMBL plants
- Need to specify separately the ID numbers for the A, B and D homoeologues – sheet 1
  - or could just nominate 1 homoeologue (with reason)
- Larger gene families where homoeologues cannot yet be resolved enter ENSEMBL Gene ID numbers – sheet 2
- Aim to use the ‘comments column’ for participants to nominate candidate positive controls –
  - where transcriptomics data already suggests one or more copies are silent / over expressed

# Exome capture – next steps

– summer / autumn 2016

4-6 individuals interested in taking this WP forward

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

**Series of  
Skype calls  
/ WORKSHOP**

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

## *Wheat, Barley and Maize Target Enrichment Designs for Exome Sequencing Available from Roche NimbleGen*

November 14, 2013

Roche (SIX: RO, ROG; OTCQX: RHHBY) announced the release of SeqCap EZ Exome Designs for target enrichment of the wheat, barley and maize genomes. These agriculture exome designs were developed with key opinion leaders in crop genome research. The goal is to provide researchers a cost-effective and easy-to-use alternative sequencing method beyond whole genome sequencing.

The Wheat Barley Exome Consortium (WBEC) worked closely with Roche NimbleGen to develop both the Wheat and Barley Exome Designs for public use. The WBEC is a collaboration of researchers from the University of Liverpool, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), James Hutton Institute, Kansas State University, University of Minnesota, University of Saskatchewan, and BIOGEMMA.

The Maize Exome design resulted from the collaboration between Roche NimbleGen and researchers at Iowa State University and the University of Minnesota. It is based on a comprehensive collection of the exon content from a range of North American lines of maize and maize relatives from the *Zea* genus.

"Using NimbleGen's target enrichment design in a maize GWAS study allowed us to focus our sequencing resources on the exome, which proved to be a more rapid and cost-effective method to identify trait associated loci over traditional detection methods," said Dr. Patrick Schnable, Distinguished Professor and Director, Center for Plant Genomics at Iowa State University.

# 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.

# 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**).

## **Taken a pause - Why ?**

- **Considerably more Chinese Spring sequencing data to be released into the public domain in Nov 2015**
- **To be uploaded into ENSEMBL (EBI)**
- **Transfer annotations from any public source onto this new genome release once a quarter**
- **EBI contacts are Paul Kersey and Dan Bolser**

# WGIN Promoter capture experiment

Focus : promoter sequences (~1kb) – **NOVELTY**

A, B and D genome sequences to be individually captured

- Bioinformatics will be quite challenging to ID the 3 homoeologous promoters

**Advantages going forward will be**

- Nominators just need to identify the correct locus ID in ENSEMBL wheat and enter this into the Excel sheet and also view the promoter sequence.
- Homoeologous promoter identification should be easier, because of the wheat chromosome maps in ENSEMBL
- The RRes bioinformatician can directly use the ENSEMBL software to pull back the correct 1kb promoter sequences using the locus ID number.

# Tools, resources, genotyping and phenotyping

Clare Lister  
07/07/16

# WGIN3 Projects: Griffiths' Lab

1. Dissecting UK drought tolerance in Paragon x Garcia
2. Quantifying agronomic impact of WGIN target genes using the Paragon NIL library
3. Informing multiple marker assisted selection for yield stability using Paragon library
4. A chromosome segment substitution library for Avalon x Cadenza
5. Understanding genotype x environment interaction in Avalon x Cadenza
6. Foundations for a new generation segregating populations for studying yield stability in the UK
7. Applying WGIN data to breeding by design for UK yield stability
8. Curation and distribution of WGIN germplasm

# WGIN3 Projects: Griffiths' Lab

1. Dissecting UK drought tolerance in Paragon x Garcia
2. Quantifying agronomic impact of WGIN target genes using the Paragon NIL library **IN DROUGHT TRIAL AND BELOW PHENOSPEX (CL/JZ)**
3. Informing multiple marker assisted selection for yield stability using Paragon library (Rht stacking / winter Paragon x Rht's) **(SEO/AFM/CL)**
4. A chromosome segment substitution library for Avalon x Cadenza
5. Understanding genotype x environment interaction in Avalon x Cadenza **(AFM)**
6. Foundations for a new generation segregating populations for studying yield stability in the UK **CROSSES FROM RL UNDERWAY (SEO)**
7. Applying WGIN data to breeding by design for UK yield stability **CROSSES & SEED BULKING UNDERWAY (SEO/CL)**
8. Curation and distribution of WGIN germplasm **ONGOING (CL)**

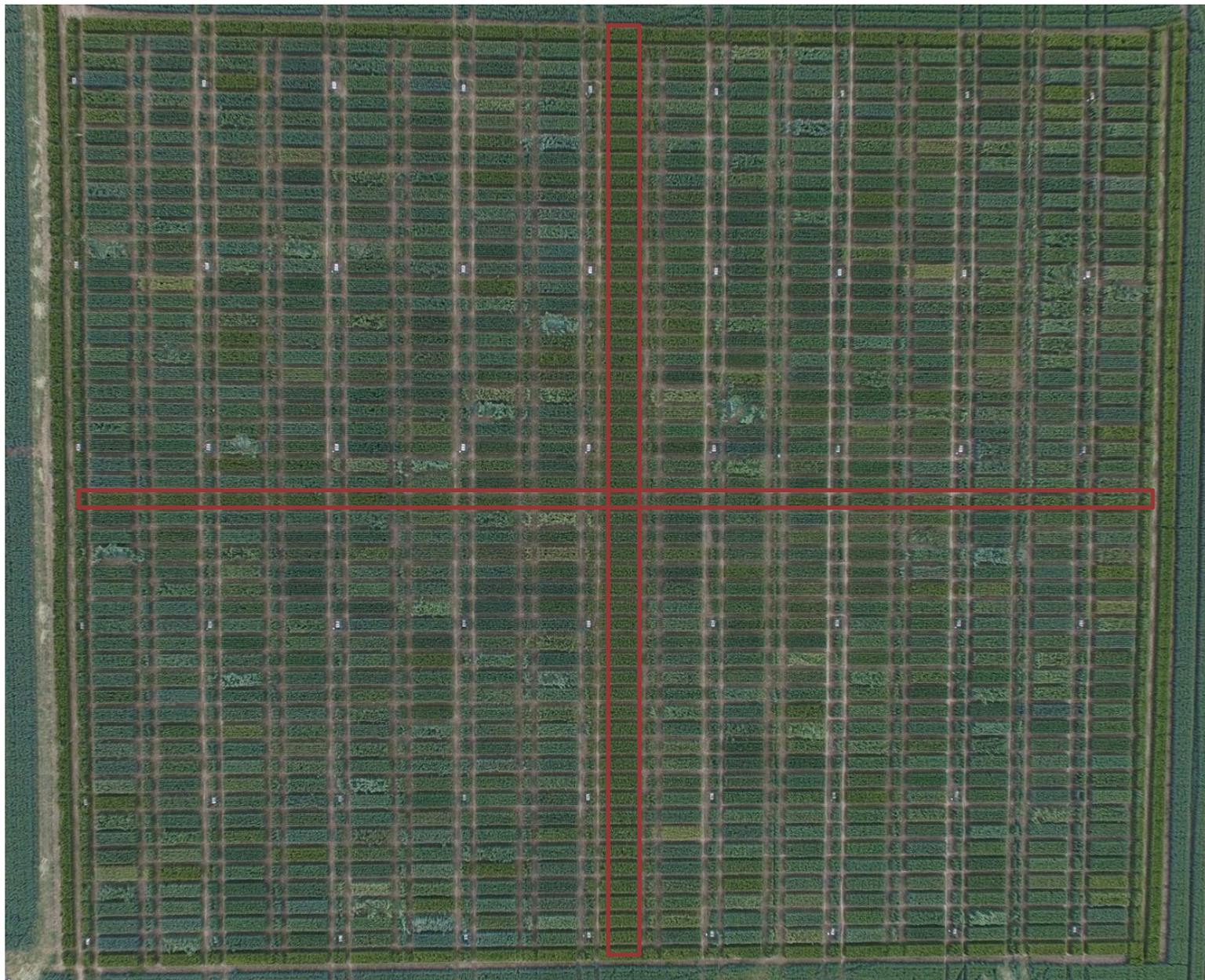
# 1. UK drought tolerance in Paragon x Garcia



- Measured stage 31 (P x Gar) – not yet analysed
- Stem sections around stage 31 – **1 rep only** – not yet analysed
- Measured DTEM, Booting and Height + QTL mapping
- Will measure Yield, TGWT and calculate grains/m<sup>2</sup>
- Monitor/record drought-recovery strategies
- Photos of each plot – **1 rep only**
- Periodic imaging of plots during growth to track senescence, using UAV

# 1. UK drought tolerance in Paragon x Garcia

NOT IRRIGATED REPS



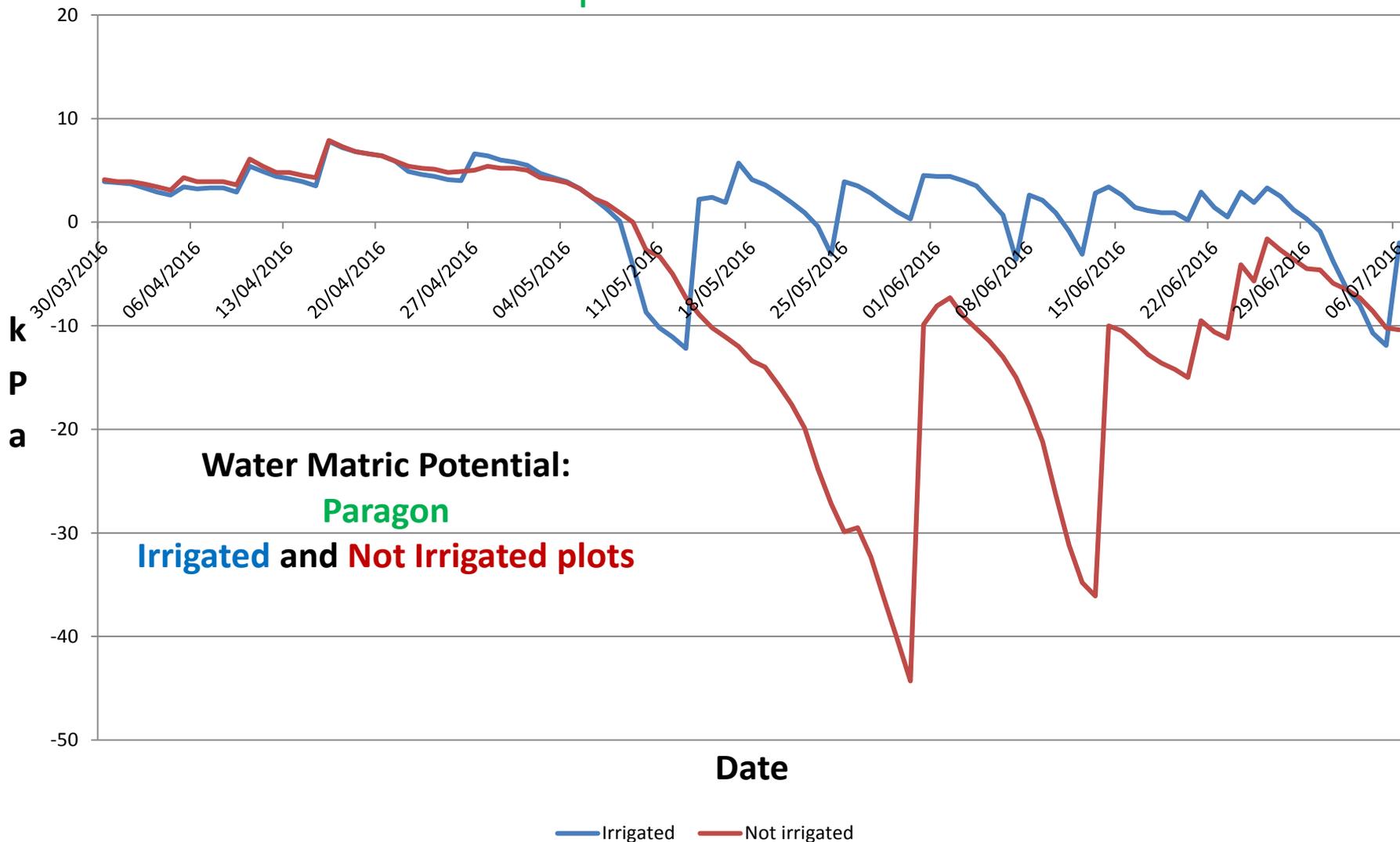
IRRIGATED REPS

# 1. UK drought tolerance in Paragon x Garcia

- To correlate the response of the plants to conditions during the growing season data collected from one **irrigated Paragon** plot and one **Paragon plot not irrigated**.
- Measured soil temperature, soil water content and water potential – ongoing from.. Date
- Using monitoring equipment from **DeltaT**
- Weather data for Church Farm plots will also be available
- Collaborating with John Foulkes (Uni Nott.)
- 2D root screening to characterise variation in key root architectural traits (including root length, angle, and distribution)

# 1. UK drought tolerance in Paragon x Garcia

- Using irrigation to maintain a water matric potential difference between the plots



# 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

- Genotyping of 94 lines representing the QTLs in the AxC NILs (DTEM, Ht, YLD)

Background	Chromosome	Trait	QTL Allele	# of lines
Avalon	1B	EM	Cadenza	5
Avalon	1D	EM	Cadenza	3
Avalon	2A	Ht	Cadenza	2
Avalon	2D	Ht	Cadenza	5
Avalon	2D	YLD	Cadenza	3
Avalon	3A	Ht	Cadenza	5
Avalon	3B	Ht	Cadenza	5
Avalon	5A	YLD	Cadenza	5
Avalon	6A	Ht	Cadenza	5
Avalon	6B	Ht	Cadenza	5
Avalon	7B	YLD	Cadenza	1
Avalon	7D	YLD	Cadenza	3

Background	Chromosome	Trait	QTL Allele	# of lines
Cadenza	1B	EM	Avalon	5
Cadenza	1D	EM	Avalon	5
Cadenza	2A	Ht	Avalon	5
Cadenza	2D	Ht	Avalon	6
Cadenza	3A	Ht	Avalon	6
Cadenza	3B	Ht	Avalon	5
Cadenza	3B	YLD	Avalon	5
Cadenza	6A	Ht	Avalon	5
Cadenza	6B	EM & Ht	Avalon	5

## 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

- NILs being backcrossed to the recurrent parent twice to generate lines where QTL region and random segments have been separated
- Backcrosses to recurrent parents (Winter 15-16)

	Avalon Background	Cadenza Background
Crosses done	23	28
Crosses successful	8	12
Previous crosses (CJM)	7	3

- Backcrosses to recurrent parents (Summer 16)

	Avalon Background	Cadenza Background
Crosses done	38	46
Crosses successful	35	46

- Will genotype BC1 progeny, with parent line, to confirm crosses before proceeding

# 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

- Genotyping carried out on 94 NILs (+ Avalon and Cadenza) using Breeders 35K array
- Data still being analysed
- **Guestimate** of % coverage of background (+ foreground) for each chromosome
- Using AxC map positions of markers
- But may need to remap as order not correct
- Will give accurate % coverage

GUESSTIMATE!		
	Avalon	Cadenza
	43 lines	46 lines
1A	75%	95%
1B	90%	70%
1D	30%	80%
2A	80%	95%
2B	80%	80%
2D	95%	70%
3A	95%	95%
3B	80%	95%
3D	80%	60%?
4A	70%	70%
4B	70%?	80%
4D	80%?	95%
5A	90%	80%
5B	80%	75%
5D	95%	80%
6A	95%	85%
6B	95%	95%
6D	95%	85%
7A	60%	80%
7A or 7D	50%?	95%
7B	40%	85%
7D	95%	30%
INCLUDES FOREGROUND		