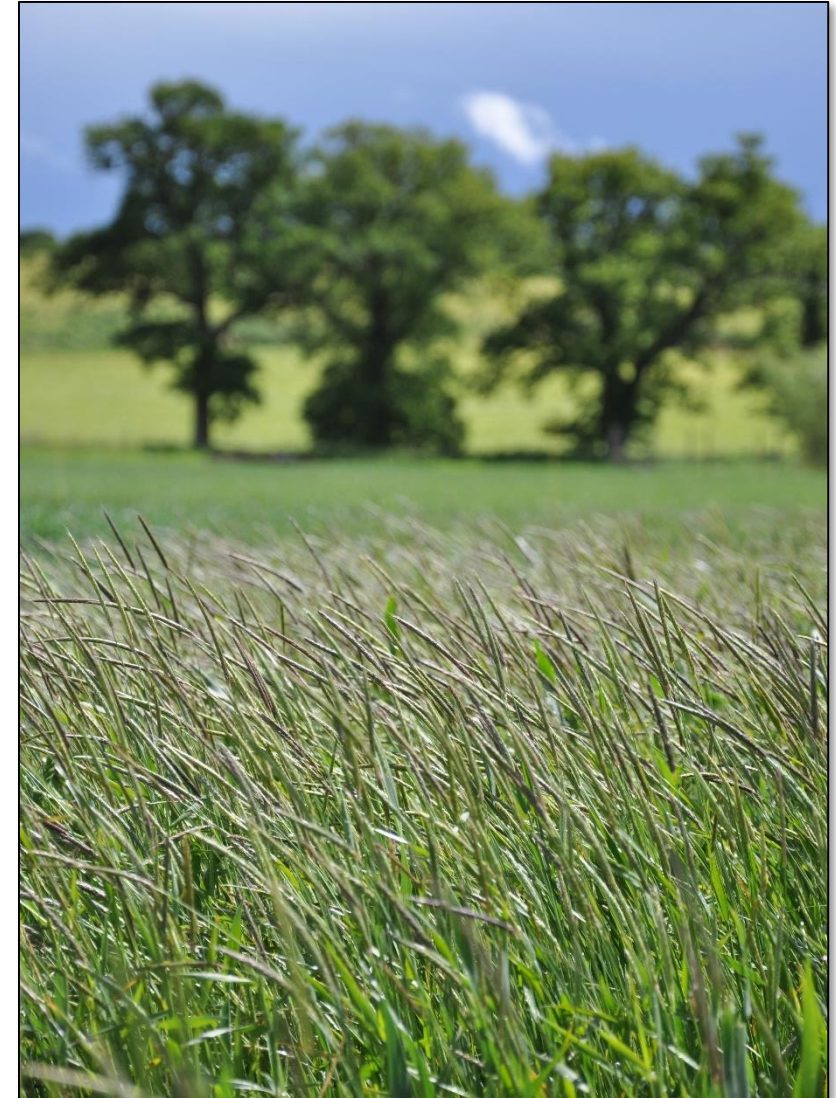


Evolution of glyphosate insensitivity in UK populations of *Alopecurus myosuroides*

Dr. David Comont

Blackgrass: *Alopecurus myosuroides*

- An outcrossing, predominantly autumn germinating annual species.
- Increasing distribution and abundance in the UK and NW Europe.
- Resistance to seven MOAs reported.
- #1 agronomic issue on many UK arable farms.



Aims:

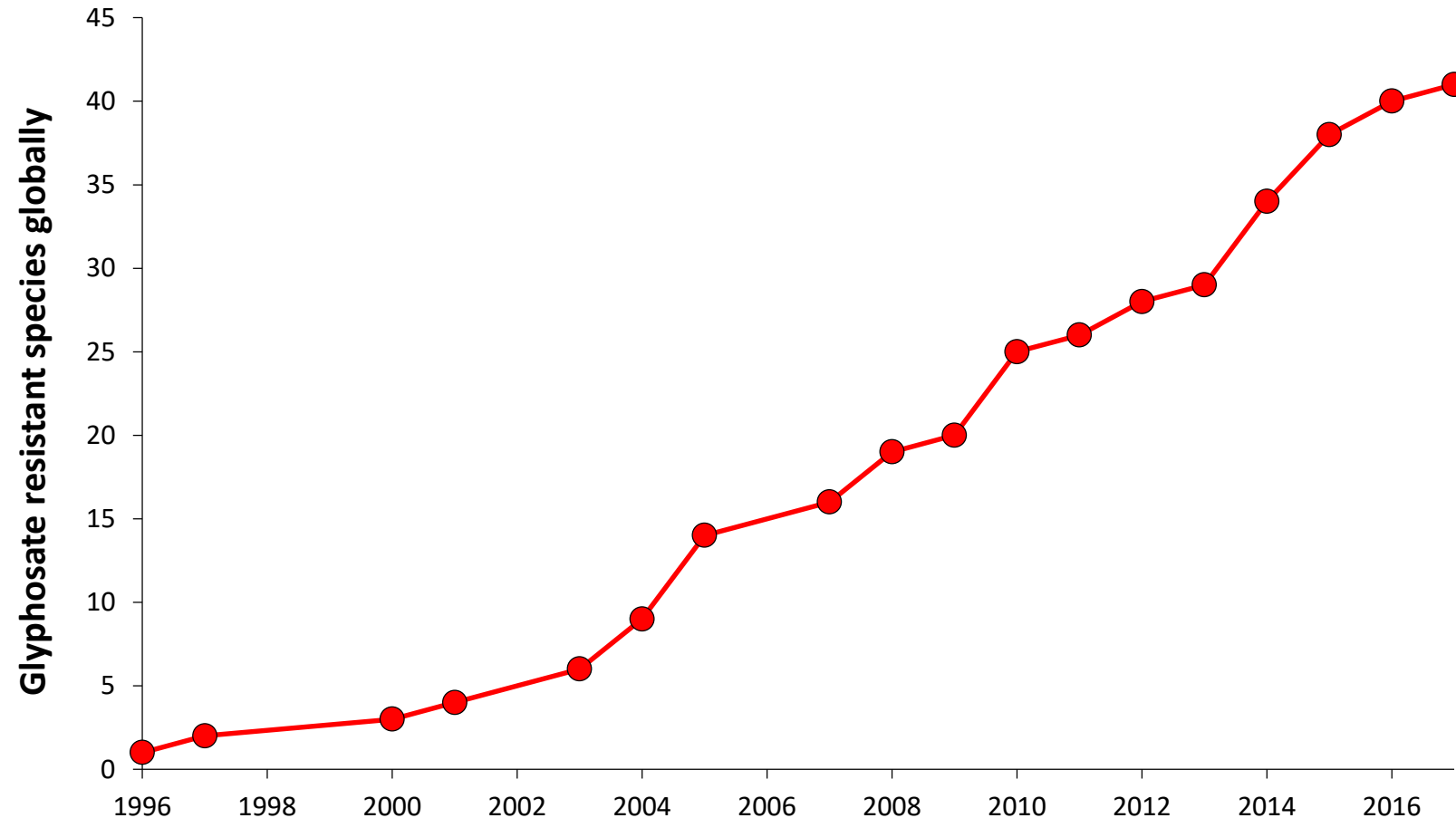
Previous research has demonstrated the extent and key evolutionary drivers of current herbicide resistance in *A. myosuroides*.

- Hicks et al. 2018. The factors driving evolved herbicide resistance at a national scale. *Nature Ecology and Evolution*. 2, 529–536.

Can we adapt this epidemiological approach to be **pro-active**, and screen for potential future resistance risks **before** they become a problem?

- Glyphosate

Glyphosate resistance worldwide



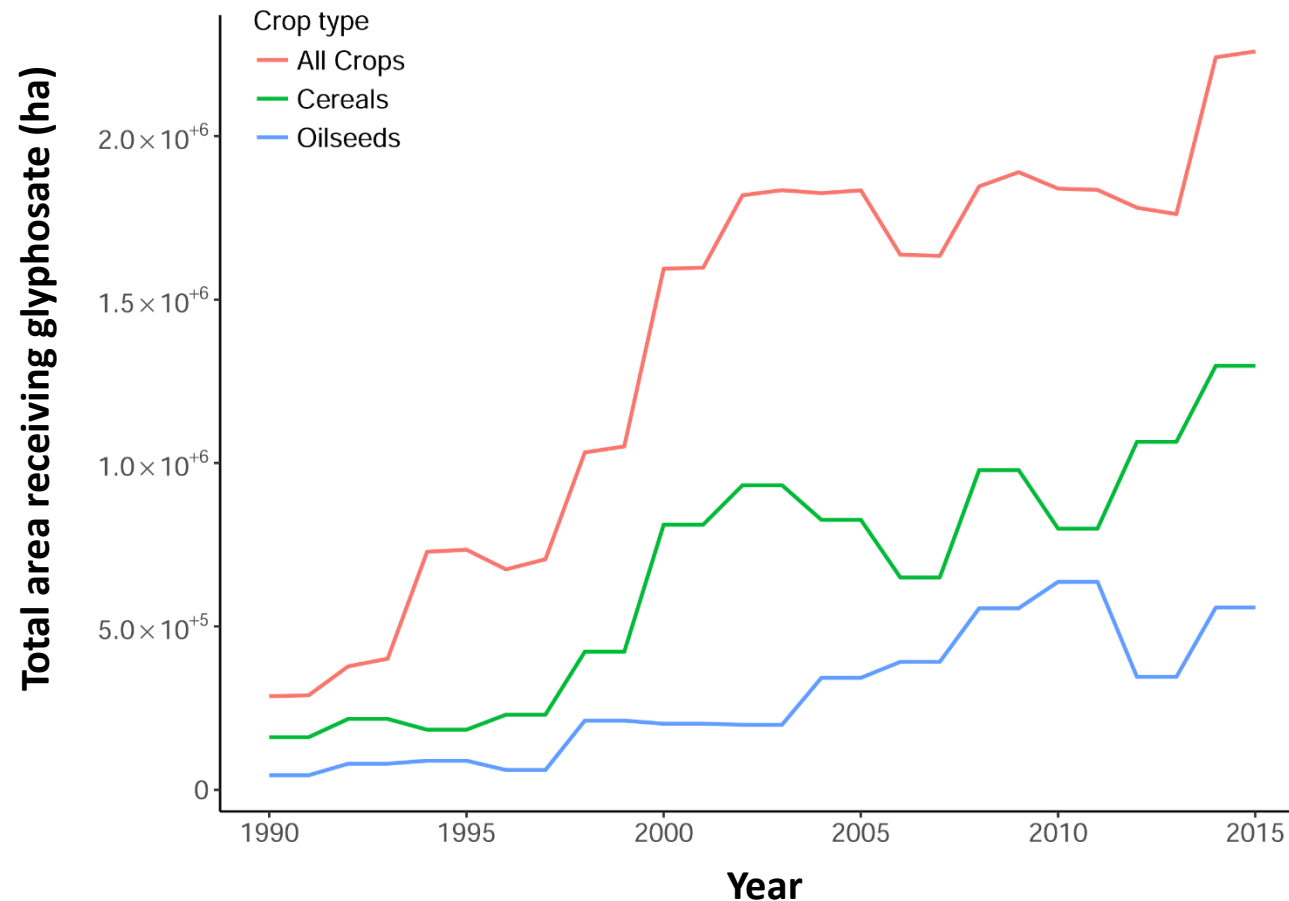
Source: www.weedscience.org

There are no reported cases of glyphosate resistant *A. myosuroides*...

But, global instances of glyphosate resistance are steadily increasing

Resistance reported in regions where glyphosate is intensively used

Glyphosate usage in the UK



Farms in the UK are increasingly reliant on glyphosate

UK glyphosate usage has risen 8-fold since 1990

So, selection pressure for glyphosate resistance is likely increasing

Standing genetic variation for glyphosate resistance?



DOI: 10.1111/wre.12264

Interpopulation variability and adaptive potential for reduced glyphosate sensitivity in *Alopecurus myosuroides*

L R DAVIES* & P NEVE*† 

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Heredity (2009) 103, 318–325

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www.nature.com/hdy

ORIGINAL ARTICLE

Evolution of glyphosate resistance in a *Lolium rigidum* population by glyphosate selection at sublethal doses

R Busi and SB Powles

Western Australian Herbicide Resistance Initiative, School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, Western Australia, Australia

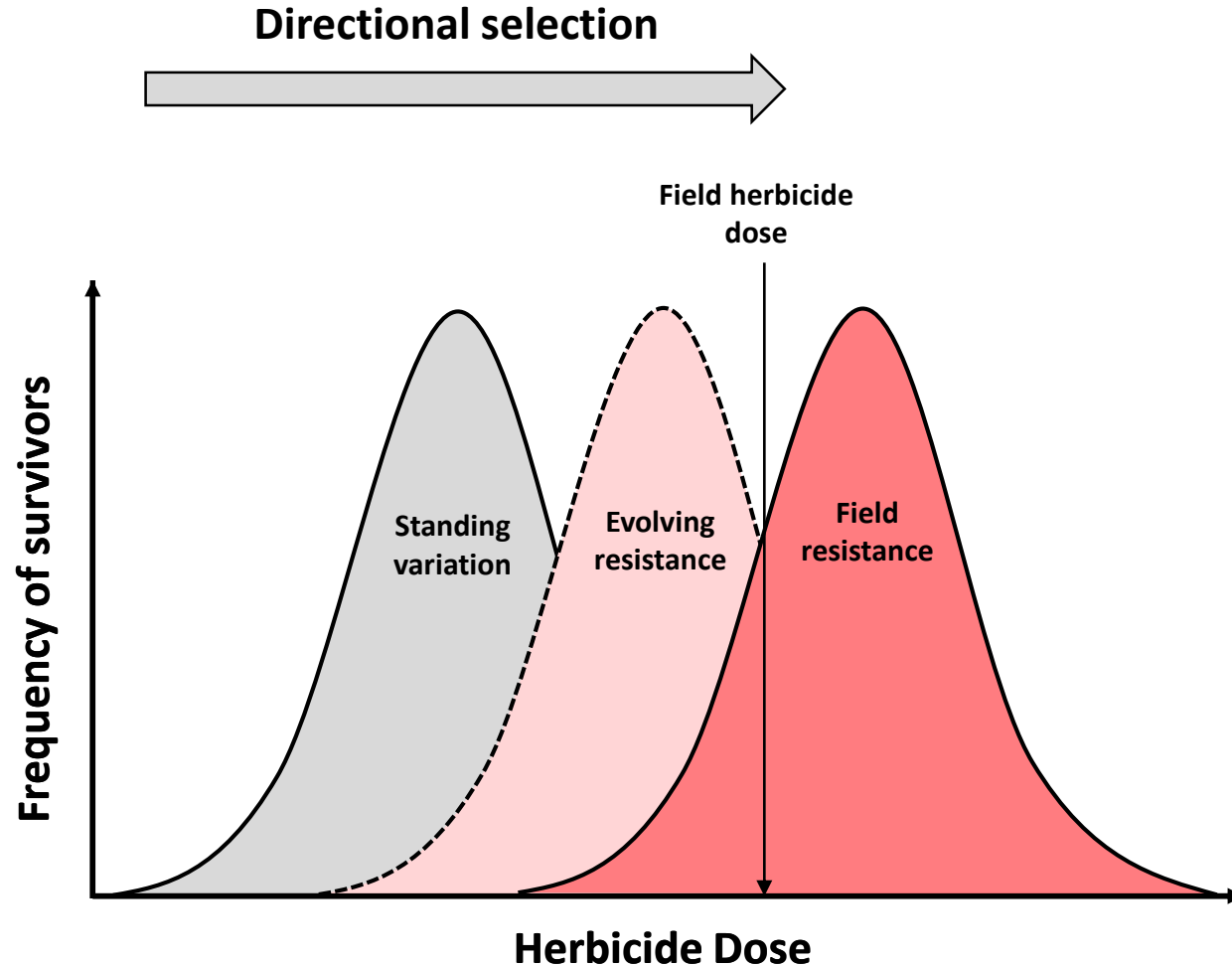
Experimental evidence for heritable variation in glyphosate sensitivity in *A. myosuroides*

Comparable results in *L. rigidum* suggest that selection can lead to a quantitative resistance trait evolving

Is *A. myosuroides* in the early stages of selection for glyphosate resistance?



Pre-emptive detection of resistance evolution?



Can we use epidemiological approaches to detect directional selection for glyphosate resistance in blackgrass?

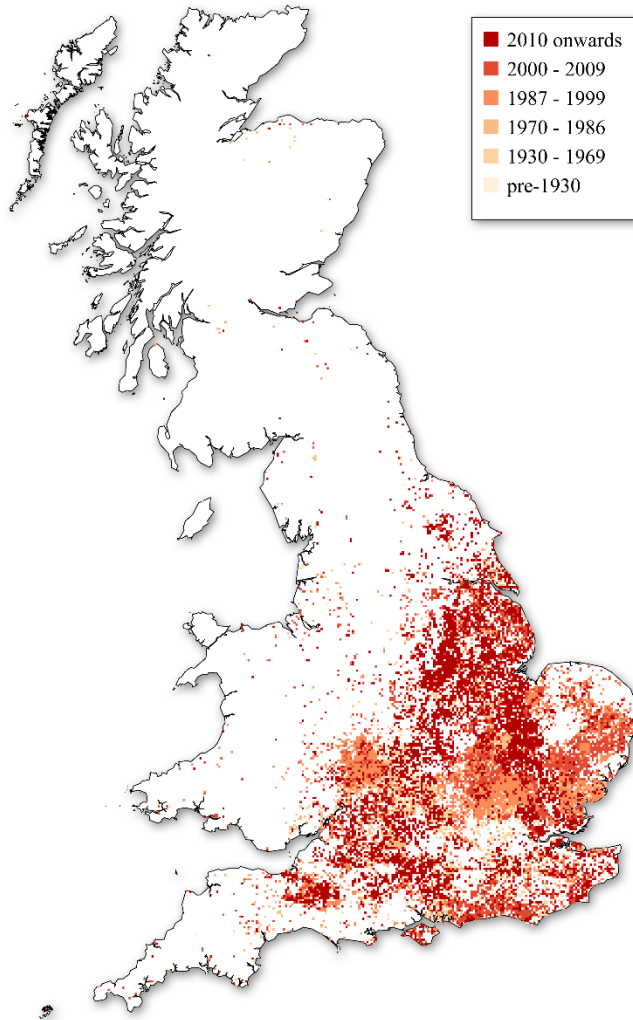
Can we do this pre-emptively, *before* resistance has become a problem?

Four key questions and approaches:

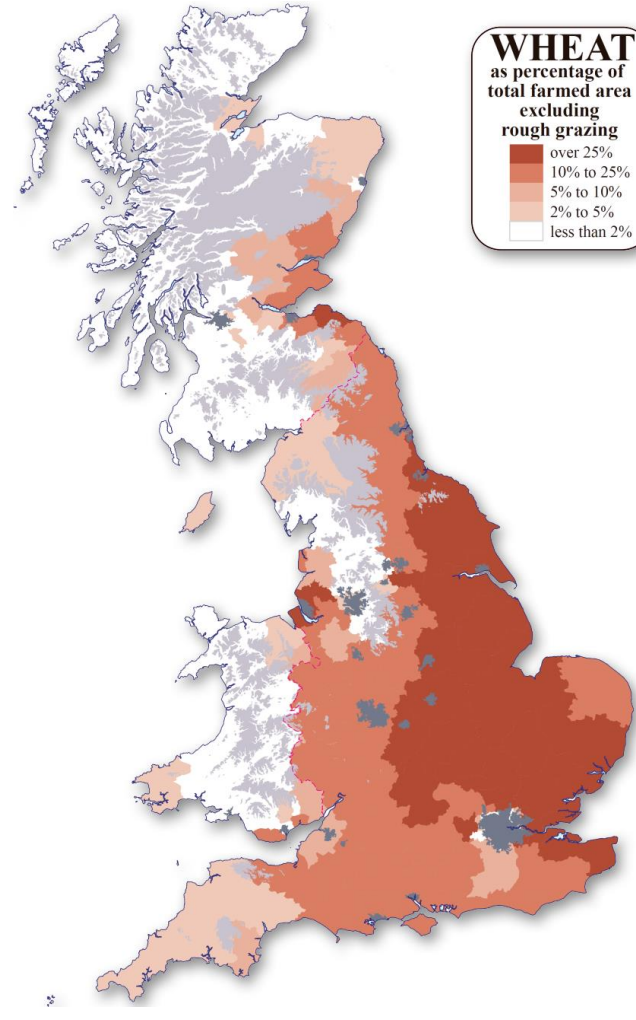
<p>1. Does blackgrass show variability in glyphosate sensitivity? - Glasshouse glyphosate sensitivity assays of UK populations</p>	
<p>2. Does that variability have a heritable genetic basis? - Classical genetics on pedigreed seed families</p>	
<p>3. Can glyphosate selection cause further reduction in sensitivity? - Sensitivity screening in generation following glyphosate selection</p>	
<p>4. Is there evidence for this occurring in the field? - Statistical analysis of UK glyphosate usage</p>	

A blackgrass field network

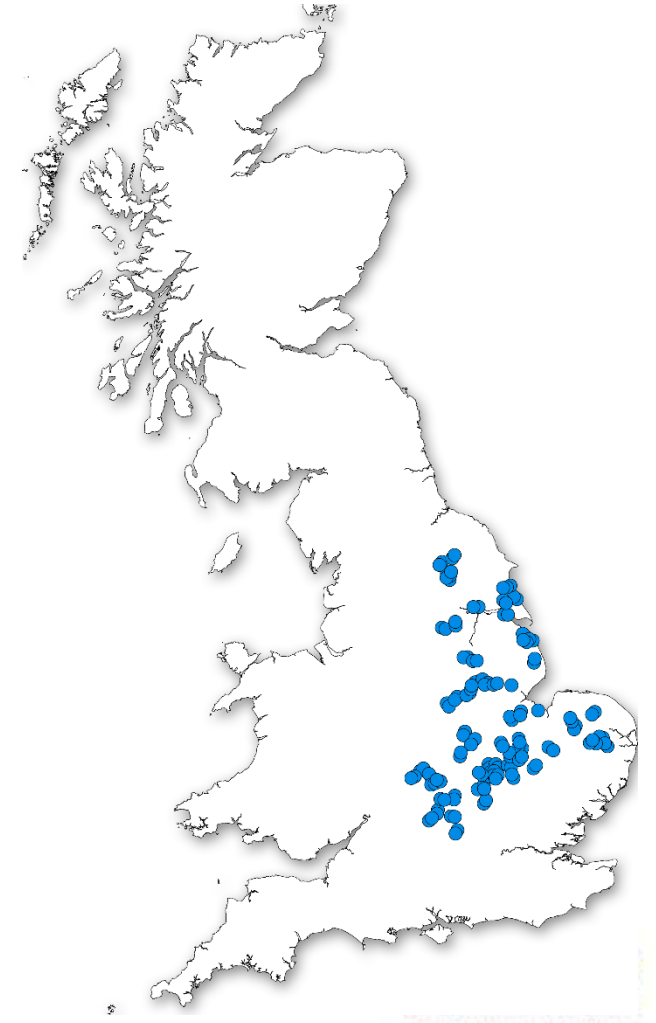
UK Blackgrass distribution



Distribution of wheat cropping



Field populations studied



Glyphosate sensitivity screening



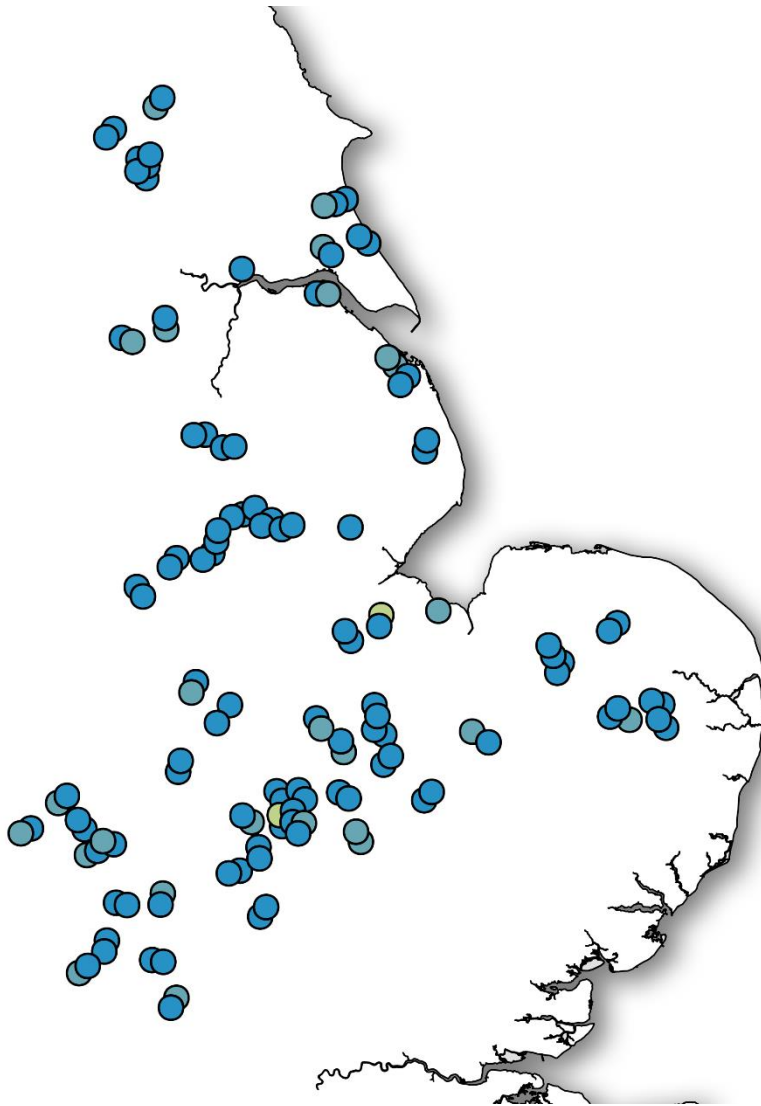
Glyphosate dose-response experiment

- Seven doses
- ~ 16,500 plants

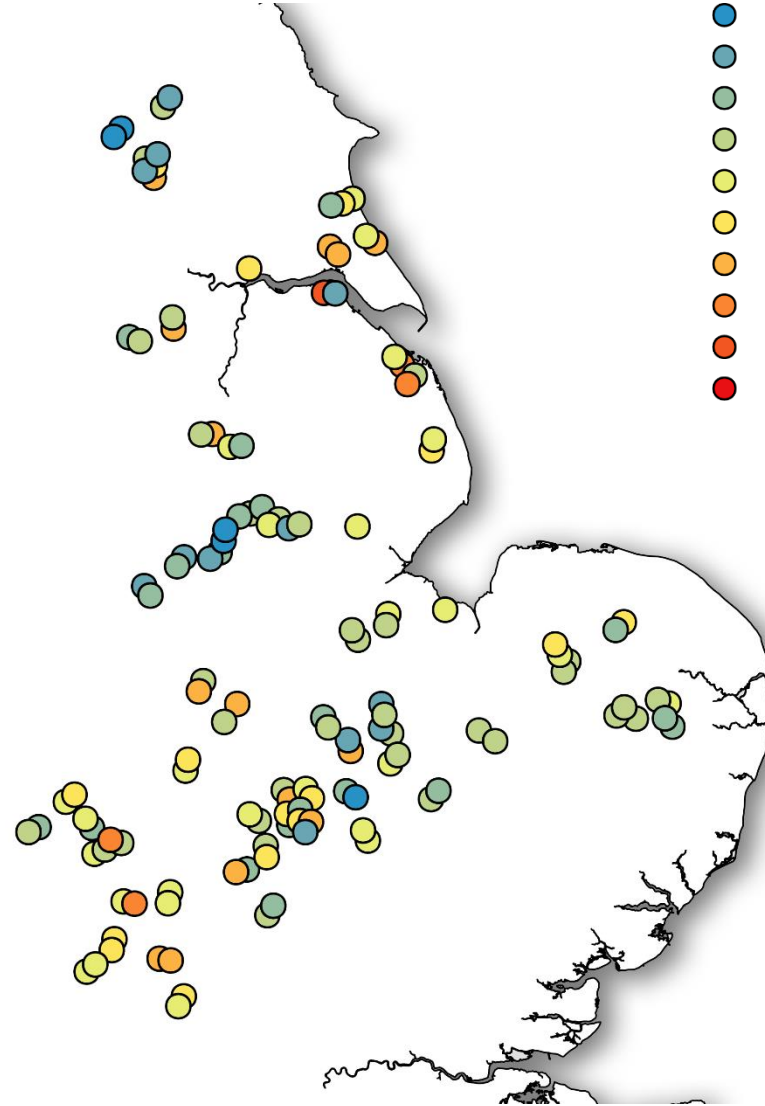
Herbicide rate	Dose (g ha ⁻¹)
0	0
0.125	67.5
0.25	135
0.5	270
0.75	405
1 (field rate)	540
1.25	675

Among-population variability in sensitivity

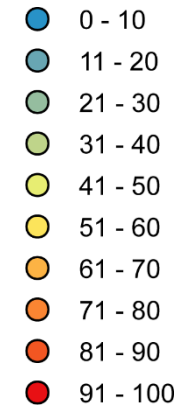
Field rate (540 g ha⁻¹)



$\frac{3}{4}$ rate (405 g ha⁻¹)



% survival

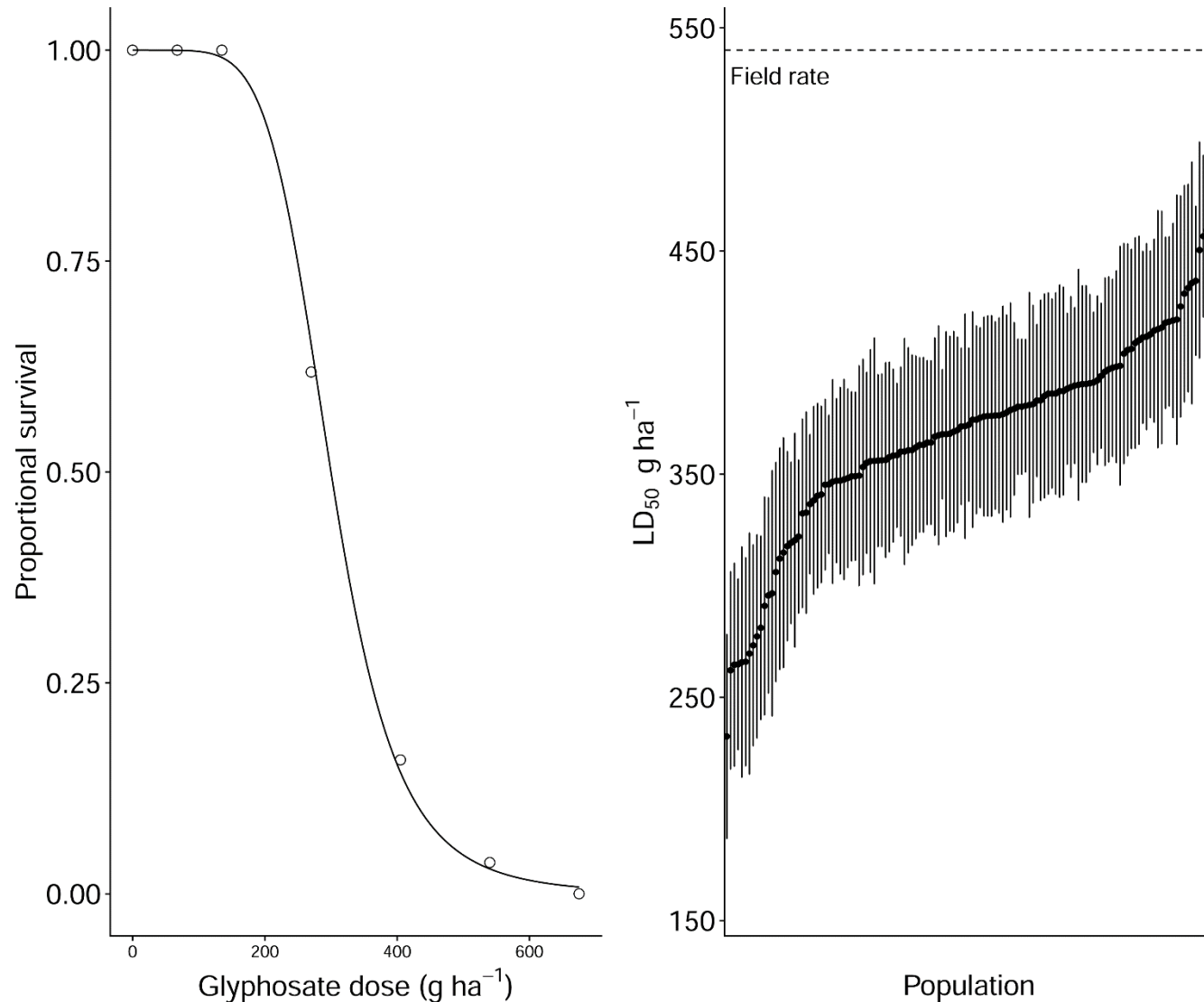


Little survival at field rate

Variable % survival below field rate

High survival is not clustered in any one geographic location

Among-population variability in sensitivity



Herbicide sensitivity:

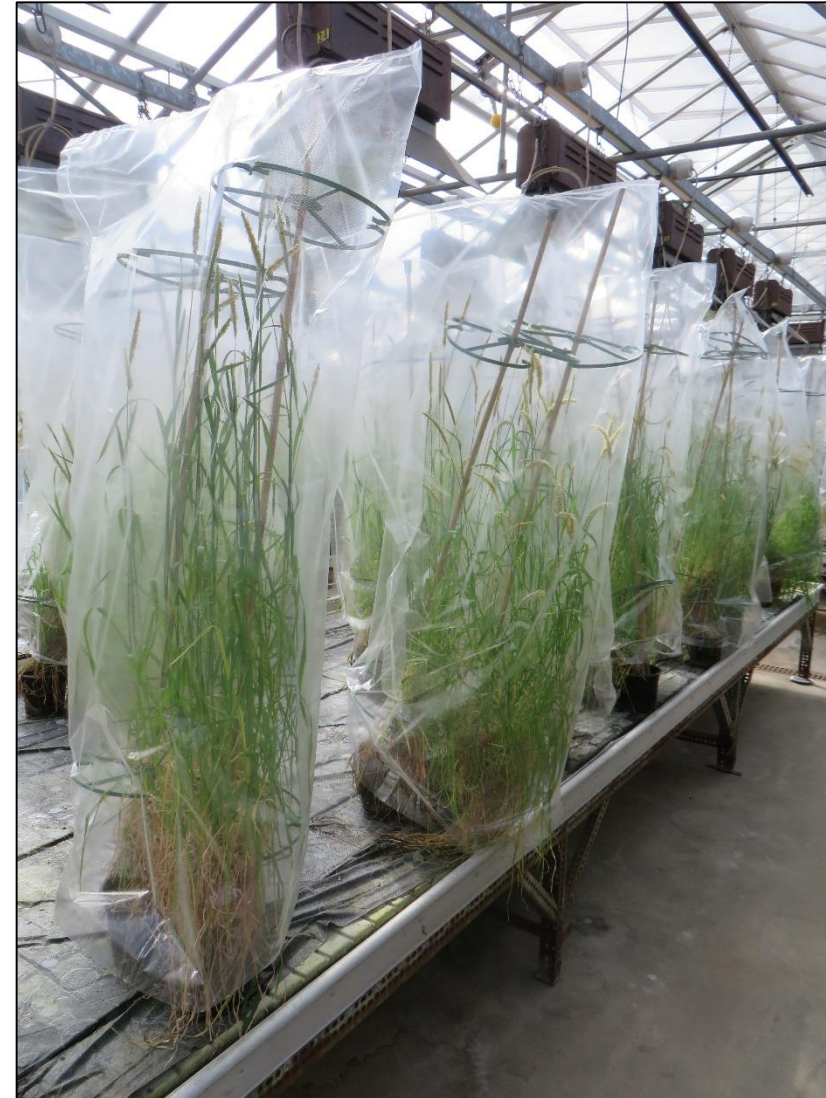
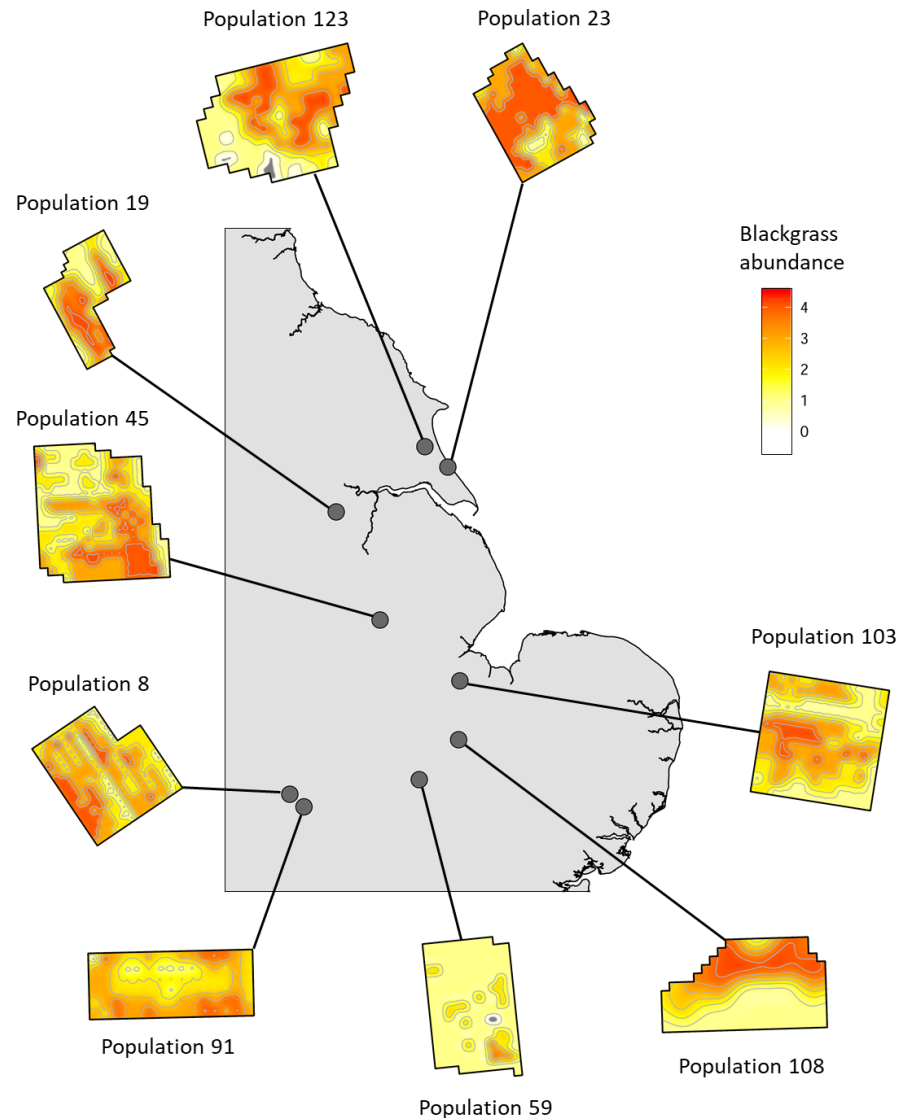
LD₅₀ = The herbicide dose needed to kill 50% of individual plants within a population

LD₅₀ values ranging from 230 – 470 g ha⁻¹

Significant variability *is* present

1. Does blackgrass show variability in glyphosate sensitivity? - Glasshouse glyphosate sensitivity assays of UK populations	✓
2. Does that variability have a heritable genetic basis? - Classical genetics on pedigreed seed families	
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4. Is there evidence for this occurring in the field? - Statistical analysis of UK glyphosate usage	

Quantitative genetics for glyphosate sensitivity



Nine blackgrass populations chosen

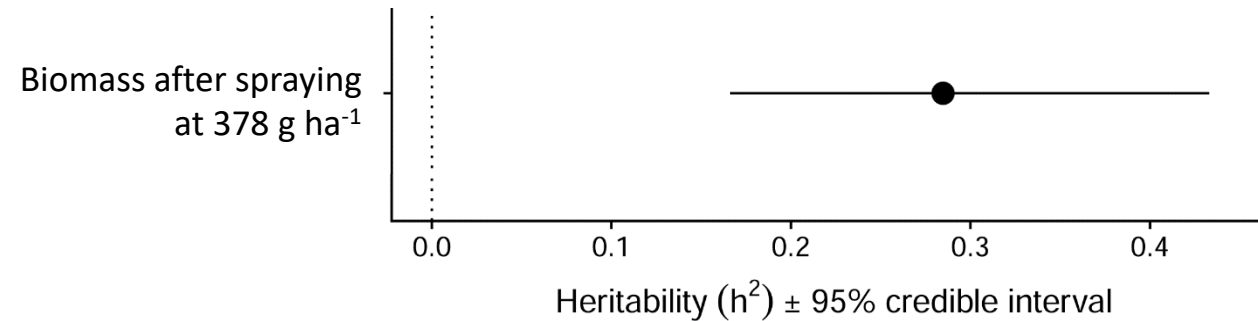
Individual pairs of plants cross pollinated to produce seeds

400 seed families produced

Quantitative genetics for glyphosate sensitivity

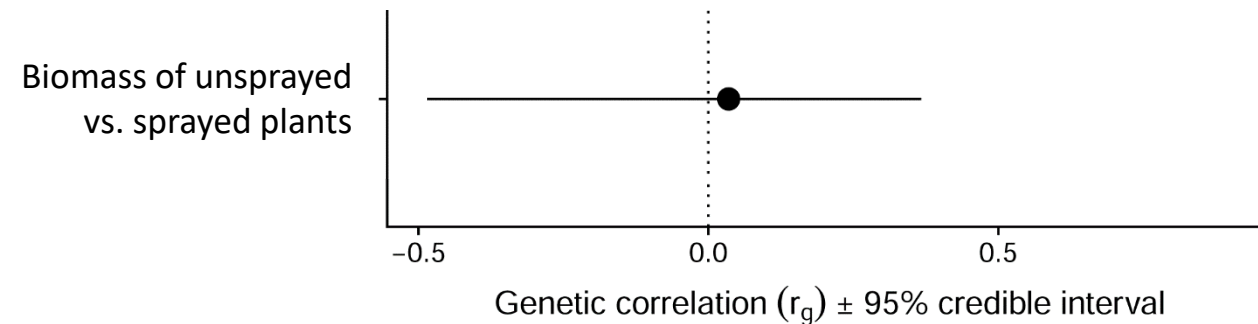
Narrow-sense heritability:

(How much of the phenotype is due to additive genetic effects)



Genetic correlation:

(Do the same genes determine unsprayed and sprayed biomass)



Glyphosate sensitivity *is* heritable

1. Does blackgrass show variability in glyphosate sensitivity? - Glasshouse glyphosate sensitivity assays of UK populations	✓
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Response to further glyphosate selection

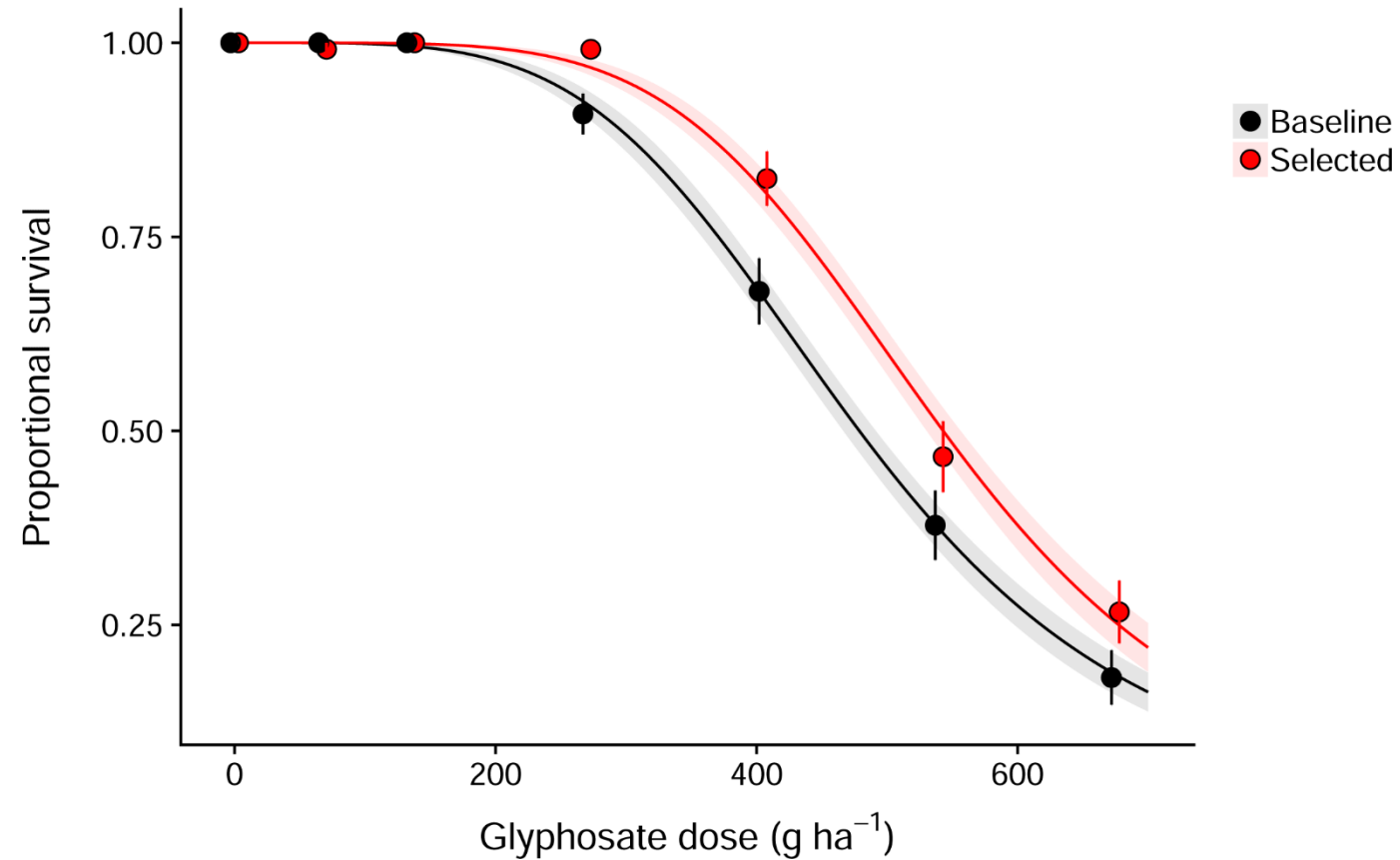


**Glyphosate survivors
kept to produce
seeds**

**Next generation
tested for glyphosate
sensitivity**



Response to further glyphosate selection



**Reduced sensitivity to
glyphosate *does* respond to
further selection**

See also: Davies and Neve (2017).
Weed Research. 57, 323–332

Glyphosate sensitivity *does* respond to selection

1. Does blackgrass show variability in glyphosate sensitivity? - Glasshouse glyphosate sensitivity assays of UK populations	✓
2. Does that variability have a heritable genetic basis? - Classical genetics on pedigreed seed families	✓
3. Can glyphosate selection cause further reduction in sensitivity? - Sensitivity screening in generation following glyphosate selection	✓
4. Is there evidence for this occurring in the field? - Statistical analysis of UK glyphosate usage	

Selection histories collected



Received management data from over 80 fields on:

- Herbicide use (products, rates, application dates)
- Fertiliser use (organic/inorganic, rates, dates)
- Crop rotation (species, seed rates, planting dates)
- Soil cultivation (methods, dates)
- Soil type (broad category)
- Harvest (crop yield, harvest date)

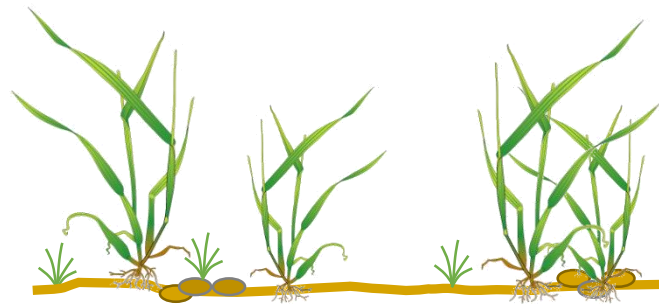
Is there an association between glyphosate usage and current levels of glyphosate sensitivity?

An informative measure of glyphosate usage?

Autumn

Spring

Summer



Herbicidal glyphosate: Mean number of glyphosate applications over Autumn and Spring

Desiccant glyphosate: Mean number of glyphosate applications over Summer (pre-harvest)

Association between glyphosate use and sensitivity?

LMM for the glyphosate LD₅₀ values:

Fixed effect	Df	Estimate	SE	Sum Sq	P value
Desiccant Glyphosate use	1	0.041	0.119	0.074	0.729 ns
Herbicidal Glyphosate use	1	0.261	0.117	3.157	0.030 *

Glyphosate use as a desiccant is not associated with glyphosate sensitivity (LD₅₀)

But, herbicidal glyphosate use *is*

Glyphosate selection in the field?

Management factor	Sums of squares	P value
<u>Population size and cultivation</u>		
Black-grass abundance	-0.007	0.217 ns
Proportion autumn sown	0.408	0.517 ns
Black-grass emergence	0.270	0.026 *
Cultivation intensity	0.150	0.661 ns
<u>Herbicide usage</u>		
Herbicidal Glyphosate	0.452	0.008 **
MOA turnover	0.164	0.142 ns
MOA diversity	-0.126	0.447 ns
MOA mixing	-0.092	0.763 ns
<u>Herbicide resistance</u>		
Mesosulfuron resistance	0.277	0.081 ns
Cycloxydim resistance	-0.330	0.096 ns
Fenoxaprop resistance	0.170	0.238 ns

R² marginal: 0.240

R² conditional: 0.565

Same approach as the Hicks et al.
(2018) Nature Ecology Evolution paper

**Glyphosate use is the strongest
predictor of current glyphosate
sensitivity (LD₅₀)**

**Fields with higher glyphosate usage
have higher survival of glyphosate**

Also see: Evans et al. (2016). *Pest
management science*. 72, 74-80.

Directional selection *is* seen in the field

<p>1. Does blackgrass show variability in glyphosate sensitivity? - Glasshouse glyphosate sensitivity assays of UK populations</p>	✓
<p>2. Does that variability have a heritable genetic basis? - Classical genetics on pedigreed seed families</p>	✓
<p>3. Can glyphosate selection cause further reduction in sensitivity? - Sensitivity screening in generation following glyphosate selection</p>	✓
<p>4. Is there evidence for this occurring in the field? - Statistical analysis of UK glyphosate usage</p>	✓

Conclusions:

- Significant among-population variation in glyphosate susceptibility
- Heritable basis for this sensitivity trait
- Responds to further glyphosate selection
- Strong (and increasing) selection pressure from glyphosate use
- Evidence for directional response to selection occurring in the field
- BUT, no widespread field-resistance yet

A pro-active epidemiological approach *can* reveal signatures of directional selection, *before* field resistance evolves

Acknowledgements



Rob Edwards Alina Goldberg-Cavalleri Nawaporn Onkokesung

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- Land use change, impact assessments



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- Ecology, population genetics, population dynamics, modelling, management

UNIVERSITY of York



Louise Jones

- Epigenetics



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- Quantitative genetics



Thanks for listening