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Managing the resistance risk to retain long-term effectiveness of glyphosate for grass-weed control in UK crop rotations

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This is the final report of a 60-month project (21120023) that started in October 2015. The work was funded by Albaugh, FMC (Headland) Nufarm and Monsanto (Bayer) and a contract for £250,000 from AHDB (with in-kind contributions from Syngenta, Agrii, Frontier, Hutchinsons and Agrovista).

CONTENTS

1. Abstract

This work improved evidence and provided greater precision on the management of glyphosate resistance risks in grass weeds. In particular, the project aimed to quantify the four key management principles: prevent survivors, maximise efficacy, use alternatives and monitor success. The outcomes filled data gaps identified during the development of the Weed Resistance Action Group (WRAG) guidelines (first published in June 2015), with respect to greater precision on how much glyphosate can safely be used (to avoid resistance evolving), at what rates/timings and how that is mitigated by subsequent management.

The experimental work, which included multiple field-based and container-based experiments, used black-grass and Italian rye-grass to inform the key objectives. This five-year research project was required to fully test and verify the objectives. Resistance shifts are less likely to be detected in a shorter timeframe. However, a key aspect of the work was to establish what cost-effective treatments result in no survivors becoming resistant. Treatments were practical and field relevant.

The project investigated the two key risk periods of glyphosate application. Firstly (Objective 1), stubbles/pre-drilling, when multiple applications are applied. Secondly (Objective 2), between crop rows, when application would potentially be to larger plants and not followed by other risk mitigation. Additionally, Objective 3 ensured availability of within-season 'live plant' and seed tests – to determine treatment outcomes and assess whether survivors are due to resistance. Finally, practical management guidelines were agreed and communicated (Objective 4).

The results provided consistent and strong evidence on best application timing, rate and weed growth stage for optimal efficacy, therefore preventing survivors. Seed survivors, collected from experiments, showed a trend towards populations requiring a higher glyphosate rate when they had been exposed to a low rate at a large weed growth stage. In field situations, these survivors are likely to gradually build up the resistance to glyphosate in a population.

The overall key messages were:

- Optimum application timing for black-grass and Italian rye-grass is GS12–13
- Glyphosate rate ≥540g is critical for optimal control
- If target weeds are tillering (from GS21), a higher glyphosate rate (≥720g) is required
- Temperature at application is extremely important (enhancing or reducing control)
- Cultivation of stale seedbed (depth 5 cm) is essential to increase black-grass control
- Maximum of two glyphosate application timings for a stale seedbed

This programme of work provides information to further underpin the WRAG guidelines to manage resistance, in particular quantifying the four key principles: prevent survivors, maximise efficacy, use alternatives and monitor success.

2. Introduction

The herbicide glyphosate has been commercially available for 40 years. It is one of the most frequently used herbicides in the UK in all crop production systems, including annual and perennial crops and non-cropped areas. There are currently no known cases of glyphosate resistance in the UK, however, globally, resistance to glyphosate has evolved as a result of repeated use and over-reliance of this herbicide.

Current changes in usage patterns in the UK have increased the risk of glyphosate resistance development. An over-reliance on a limited group of herbicide modes of action has accelerated the development of herbicide-resistant grass weeds, particularly black-grass (*Alopecurus myosuroides*) and Italian rye-grass (*Lolium multiflorum*). This has been mainly due to a lack of new herbicides, regulatory policy changes, a limited crop rotation and the under-exploitation of cultural control practices. The main threat is in annual arable crops where glyphosate provides a key role in managing grass-weeds which have developed resistance to selective herbicides.

Glyphosate-tolerant crops were introduced in North and South America and Canada in the mid-1990s and this inevitably led to an increase in glyphosate resistant weeds in the years that followed. A clear lesson was that reliance on the use of glyphosate alone was an inappropriate use. Although there are no glyphosate-tolerant crops approved in the UK, patterns of use and reliance of glyphosate have led to a heightened risk. With increasing herbicide resistance, especially in grass-weeds there will be even more use pre-drilling. Additionally it is now possible to use glyphosate within crops. For example, in the UK there are approvals for glyphosate to be applied in a wide variety of vegetable and fruit crops and a new approval of a specific glyphosate product to be applied between wide rows in an oilseed rape crop and more are likely to follow. This has the potential for the target grass-weeds to be large in size and so the dose rate must be optimum to control weeds of that size, otherwise a tolerance to glyphosate may evolve rapidly with frequent exposure. Simulation models developed by Neve *et al*., (2002) investigating the evolution of glyphosate resistance risk in rigid ryegrass showed that the greatest risk factors were sole reliance on glyphosate for pre-drilling control in systems with reduced tillage and in this situation, resistance could evolve in 10-15 years.

The first case of glyphosate resistance in an arable crop in Europe was reported in Italy (Collavo + Sattin, 2014) to ryegrass (*Lolium* spp.), which also showed cross resistance to ACCase and ALS-inhibitor herbicides. Previous to that glyphosate resistant weeds in Europe were located in perennial crops (orchards) where usage patterns differ to arable crops as often no additional actives or cultivations are used. Worldwide there are currently 50 weed species (26 grasses), across 31 different weed families (a total of 324 individual biotypes) with reported glyphosate resistance (Heap, 2020).

Glyphosate is an EPSPS inhibitor (HRAC G) and is a non-selective foliar acting herbicide with no soil residual activity, therefore only used post-emergence. It is fairly slow acting compared to other similar herbicides with symptoms visible after 7 to 10 days and it has very low mammalian toxicity. Glyphosate is one of the most frequently used herbicides across arable, horticultural, ornamental crops and amenity situations and therefore has extremely high national importance. The evolution of weed resistance to glyphosate would therefore have a major impact on the economics of the agriculture, horticulture and amenity industries resulting in a wide scale problem. The cost, socio-economic and environmental impacts of a loss of glyphosate in the UK, which could also equate to future resistance to glyphosate, were discussed by Cook *et al*., (2010) & Wynn *et al*., (2014). There would be a loss of crop production (it was estimated that a 20% yield loss would occur without the use of glyphosate pre-drilling (Clarke *et al*., 2009)) and potential loss of quality due to an increase in resistant weeds hindering all systems. Glyphosate is a relatively cost effective herbicide and there are many products containing this active ingredient available on the market, providing a wide range of choice to the consumer.

Given the increasing amount of active used, the reliance on it for control and other future changes, the risk of glyphosate resistance is now a reality in the UK. It forms an essential component of grass-weed management strategies and ensuring it is effective for many more years is critical to arable production systems.

The programme of work was structured to provide information for the development of more robust guidelines to manage resistance, in particular to quantify the four key principles: prevent survivors, maximise efficacy, use alternatives and monitor success. The outcomes fill data gaps identified by the Weed Resistance Action Group (WRAG) Guidelines (published June 2015) with respect to greater precision on how much glyphosate can safely be used, to avoid resistance evolving, at what rates/timings and how that is mitigated by subsequent management. A key project outcome was to improve evidence and greater precision and detail in future management guidelines.

The experimental work included a combination of both field and container-based methods to determine the individual key objectives. A five year research project was required to fully test and verify the objectives. Resistance shifts are less likely to be detected in a shorter timeframe, however a key aspect of the work was to establish what cost-effective treatments result in no survivors to become resistant. Treatments were practical and field relevant. The main grass-weeds tested were black-grass and Italian rye-grass.

The project aimed to investigate the two key risk periods of glyphosate application: 1) Stubbles/pre-drilling (Objective 1) when multiple applications are applied and 2) Between crop rows (Objective 2) when application would potentially be to larger plants and not followed by other risk mitigation. Objective 3 ensured availability of within-season ‘live plant’ and seed tests, both to determine treatment outcomes but also to provide a timely assessment of whether survivors are due to resistance. Practical management guidelines were agreed and communicated (Objective 4).

The AHDB project was managed by ADAS with co-funding and collaborative involvement from the Glyphosate Task Force (through Monsanto, now Bayer) with members Bayer (formerly Monsanto), Albaugh, FMC, Nufarm and Syngenta and four distributor companies (Agrii, Agrovista, Frontier and Hutchinsons) providing data and sites. The Black-grass Research Initiative (BGRI) was represented on the steering group and close collaboration was maintained through joint activity. All members of the consortium were involved with knowledge transfer and, in partnership with WRAG, consistent messages have been agreed and widely communicated, especially to agronomists and farmers.

It is important to note that the same formulation of glyphosate was used throughout every experiment within the project for consistency. No adjuvants were used but all experiments used deionised water.

3. Materials and methods

3.1. Application in stubbles (WP1)

To quantify the need for repeat glyphosate applications in stubbles two key aspects needed to be quantified:

1. How important is repeat glyphosate use in increasing the number of weed flushes?

2. What is the optimum rate and timing of glyphosate to minimise the number of applications?

3.1.1. **Data review**

A review was carried out in the first six months of the project building on previous trial data that include comparisons of glyphosate applications pre-drilling with and without cultivations. The distributor project partners and some companies agreed to historic data sharing to inform this review. This helped to build a knowledge base and provide a valuable resource for the production of key messages and practical guidelines for farmers. It also identified gaps in knowledge where new data needed to be gained within the project field sites.

3.1.2. **Field experimentation data (WP1.1)**

Data gaps were identified from the review that then formed the structure of the field experiments. The field trials started in project year two and ran for four years (2016-2020).

Field experiment 1. Glyphosate and weed flushes (winter wheat cropping 2017 & 2018)

A set of three field trials were established in autumn 2017 and 2018 on sites hosted by the partner distributor companies known to have a high black-grass population. The aim of the trials was to evaluate the risk of repeat glyphosate application to stubbles/stale seedbeds prior to crop drilling and to identify the effects of cultivations and glyphosate treatment timing and dose, on black-grass control. The CambsA and CambsH trials were located in Cambridgeshire and the LincsF trials in Lincolnshire in both trial years.

Trial design

Each site received a minimal post-harvest cultivation, but no seed bed established before the trial was marked out. The trial plots measured 24m x 3m and were replicated three times. The plots were split in half with a cultivation treatment just before or at the same time as drilling, reducing the overall plot size to 12m x 3m. There were six glyphosate treatments and timings (Table 1) all were to be applied before drilling.

Table 1 Glyphosate treatment timings and doses for field experiments in winter wheat 2016/17 & 2017/18.

Treatment	Product	Dose rate g a.s./ha	L/ha of product	Application Timing		
				1	2	3
1	NIL	-	-	-	-	-
2	Glyphosate 360g a.s./L	540	1.5	x		
3	Glyphosate 360g a.s./L	540	1.5	x	x	
4	Glyphosate 360g a.s./L	540	1.5		x	
5	Glyphosate 360g a.s./L	540	1.5		x	x
6	Glyphosate 360g a.s./L	1080	3.0			x

The actual timing of each treatment are summarised in the table below (Table 2) for each field site. The final cultivation and drilling dates were site specific as were weather and soil condition dependant. On some occasions the final glyphosate treatment occurred on the day of drilling.

Table 2 Glyphosate application dates for each field site 2016/17 & 2017/18.

Timing	Target date	2016-2017			2017-2018		
		CambsH17	LincsF17	CambsA17	CambsH18	LincsF18	CambsA18
1	Early – Mid Sept	20/09/16	05/09/16	21/09/16	18/09/17	12/09/17	18/09/17
2	Mid – Late Sept	03/10/16	22/09/16	03/10/16	09/10/17	06/10/17	09/10/17
3	Early – Mid Octo	20/10/16	07/10/16	17/10/16	24/10/17	25/10/17	30/10/17

Table 3 Cultivation and drilling dates for each trial site for 2016/17 & 2017/18.

Timing	2016-2017			2017-2018		
	CambsH17	LincsF17	CambsA17	CambsH18	LincsF18	CambsA18
Cultivate	12/10/16	24/10/16	25/10/16	25/10/17	26/10/17	30/10/17
Drill	24/10/16	24/10/16	26/10/16	25/10/17	26/10/17	30/10/17

After drilling the field plots received a robust herbicide programme including a pre-emergence and post-emergence application as decided by the host farm. To enable the trial to have a completely untreated control area, to understand the effect of the pre-drilling glyphosate, plastic sheeting measuring 1m x 1m were placed in each non-glyphosate treated plot x cultivation combination on the day of the application of pre-emergence herbicides. These sheets were removed immediately after application.

There were four assessment periods:

a. Autumn at glyphosate treatments and 2-3 weeks after treatments emergence (assessments 1-7)
b. November plant count (assessment 8)
c. March plant count (assessment 9)

d. June head count (assessment 10)

Emergence, plant and head counts

Black-grass emergence was assessed by placing five fixed quadrats (0.1 m^2) in each treatment plot at the timing of the first glyphosate application and counting emergence at each assessment stage (Table 4).

In November and March/April of each season black-grass plant counts were done by placing five random quadrats (0.1m^2) per plot (Table 4). By now the plots had been split by cultivation so twice as many counts were done compared to the emergence counts. In the non-glyphosate treated plots where pre-emergence herbicide application was prevented using 1 x 1 m plastic covers two plant counts using 0.1m^2 quadrats were done.

The total number of black-grass heads per plot were assessed in late May/June (Table 4) each season using five randomly placed quadrats (0.1m^2). As with the plant counts, in the un-glyphosate treated plots where pre-emergence herbicide application was prevented using 1 x 1 m plastic covers two head counts using 0.1m^2 quadrats were done.

Table 4 Assessment dates for each field site in 2016/17 & 2017/18

	CambsH17		LincsF17		CambsA17		CambsH18		LincsF18		CambsA18	
Pre drilling	Date	GS	Date	GS	Date	GS	2018	GS	Date	GS	Date	GS
1	19/09/16	10-13	08/09/16	10-12	19/9/16	15-25	29/09/17	10-12	15/9/17	10-12	18/09/17	10-11
2	04/10/16	11-12	20/09/16	10-15	5/10/16	11-15	12/10/17	10-21	09/10/17	11-23	09/10/17	11-27
3	04/10/16	11-21	20/09/16	10-25	5/10/16	11-23	12/10/17	10-21	09/10/17	11-23	09/10/17	11-27
4	20/10/16	10-24	06/10/16	10-21	18/10/16	11-13	23/10/17	10-23	24/10/17	11-26	24/10/17	11-30
5	20/10/16	10-24	06/10/16	10-25	18/10/16	11-25	23/10/17	10-23	24/10/17	11-26	24/10/17	11-30
6	27/10/16	10-23	17/10/16	10-26	24/10/16	11-25	-	-	-	-	-	-
7	27/10/16	10-24	17/10/16	10-26	24/10/16	11-25	23/10/17	10-23	24/10/17	11-26	24/10/17	11-30
Post drilling												
8	25/11/16	10-12	17/11/16	10-29	22/11/16	10-24	20/11/17	10	23/11/17	10-29	28/11/17	10
9	29/03/17	10-24	04/04/17	29	21/03/17	13-29	23/03/18	26	23/03/18	29	17/4/18	29
Head count	08/06/17	61-71	23/05/17	61-71	08/06/17	61-71	01/06/18	61-71	31/05/18	61-71	01/06/18	61-71

Field experiment 2. Optimum rate and timing of glyphosate: 2018-19

Objectives

The field trials were still aiming to evaluate the risk of repeat glyphosate application to stubbles/stale seedbeds prior to crop drilling in winter wheat and to identify the effects of glyphosate treatment timing, and cultivations on black-grass control. In 2018 it was decided to split the field trials into winter sown and spring sown crops, so two sites were established for each crop.

Winter wheat sites 2018-19

Site locations

There were two field sites in winter wheat drilled in 2018 and harvested in 2019, CambsA19 in Cambridgeshire and LincsF19 in Lincolnshire.

Treatments and cultivations

The trials included three glyphosate application timings (Table 5) at a rate of 720g a.s./ha. It was considered that this rate reflected true field practice. The application timings were applied a minimum of 10 days apart pre-drilling of the crop (Table 6). The herbicide treatments were fully randomised within each cultivation block. There were three cultivations (Table 7) that included no soil disturbance until drilling (C1), a shallow cultivation between glyphosate applications (C2) and a 'flexible' treatment (C3) to be decided by the host site to suit their field conditions and establishment equipment. The cultivation blocks were not randomised for practical reasons.

Table 5 Herbicide treatments for both field sites 2018-19

Treatment	Product	Dose rate g a.s./ha	L/ha of product	Number of applications	Application Timing	
					1	2
T1	NIL	-	-	-	-	-
T2	Glyphosate 360g a.s./L	720	2.0	1	T1	-
T3	Glyphosate 360g a.s./L	720	2.0	1	-	T2
T4	Glyphosate 360g a.s./L	720	2.0	2	T1	T2

Table 6 Actual herbicide application timings for both trial sites in 2018

		Field site	
Application timing	Target date	LincsF19	CambsA19
T1	Mid-September	24/09/18	13/09/18
T2	Mid-October	19/10/18	11/10/18

Table 7 Cultivation dates and timings for both winter wheat sites in 2018-19

Cultivation no.	Cultivation type	Timing	Cultivation and drilling date at each site	
			LincsF19	CambsA19
C1	Direct drilling	Action of drilling	28/10/18	12/10/18
C2	Shallow cultivation	Between glyphosate applications	26/09/18 straw rake	14/09/18
C3	'Flexible' cultivation to suit the site/conditions	Flexible depending on each site	08/10/18 Power harrow	12/10/18 Combi drill

After drilling the field plots received a robust herbicide programme including a pre-emergence and post-emergence application as decided by the host farm. To enable the trial to have a completely untreated control area, to understand the effect of the pre-drilling glyphosate, plastic sheeting measuring 1m x 1m were placed in each non-glyphosate treated plot x cultivation combination on the day of the application of pre-emergence herbicides. These sheets were removed immediately after application.

Assessments

There were three assessment periods:

a. Autumn at glyphosate treatments and 2-3 weeks after treatments, emergence counts (assessments 1-2)
b. November, plant count (assessment 3)
c. June, head count (assessment 4)

Black-grass emergence was assessed by placing ten random quadrats (0.1 m^2) in each treatment plot at the timing of the first glyphosate application and counting emergence at each assessment stage (Table 8).

In November of each season black-grass plant counts were done by placing five random quadrats (0.1m^2) per plot (Table 8). By now the plots had been split by cultivation so twice as many counts were done compared to the emergence counts. In the non-glyphosate treated plots where pre-emergence herbicide application was prevented using 1 x 1 m plastic covers two plant counts using 0.1m^2 quadrats were done.

The total number of black-grass heads per plot were assessed in late May/June (Table 8) each season using five randomly placed quadrats (0.1m^2). As with the plant counts, in the un-glyphosate treated plots where pre-emergence herbicide application was prevented using 1 x 1 m plastic covers two head counts using 0.1m^2 quadrats were done.

Table 8 Assessment dates for both field sites 2018/19

Assessment	Field site LincsF19	CambsA19
1. Emergence count	27/09/18	14/09/18
2. Emergence count	25/10/18	04/10/18
3. Late autumn count	*Not done*	19/11/18
4. Head count	11/06/19	10/06/19

Spring wheat sites 2018-19

Site locations

There were two field sites in spring wheat crop drilled in 2019 in Cambridgeshire, CambsBx19 and CambsH19. Plots measured 3m x 12m at CambsBx19 and 6m x 8m at CambsH19, both with two replicate blocks.

Treatments and cultivations

There were two glyphosate treatments at a higher dose of 720g a.s./ha to be consistent with the winter wheat trials. The application timings were required in the autumn and at pre-drilling of the crop in the spring (Table 9, Table 10). The herbicide treatments were fully randomised within each cultivation block. There were two cultivations (Table 11) that included minimal soil disturbance until drilling (C1), and a shallow cultivation approximately 10 days after the autumn glyphosate applications (C2). The cultivation blocks were not randomised for practical reasons.

Table 9 Herbicide treatments

Treatment	Product	Active ingredient	Dose rate g a.s./ha	L/ha of product	No. times applied	Application Timing	
						1 (autumn)	2 (spring)
T1	NIL	-	-	-	-	-	-
T2	Glyphosate 360g a.s./L	Glyphosate	720	2.0	1	x	-
T3	Glyphosate 360g a.s./L	Glyphosate	720	2.0	1	-	x

The growth stage of the black-grass for the autumn glyphosate application was GS11 and for the spring application it varied between GS11-29, with the majority of plants over GS21.

Table 10 Application timings for both field sites in spring wheat 2018/19

Treatment	Product	L/ha of product	Application Timing			
			Autumn		spring	
			CambsBx19	CambsH19	CambsBx19	CambsH19
T1	NIL	-	-	-	-	-
T2	Glyphosate 360g a.s./L	2.0	19/10/18	17/10/18	-	-
T3	Glyphosate 360g a.s./L	2.0	-	-	22/02/19	15/02/19

The whole trial area was lightly cultivated post-harvest. The following cultivations were then part of the trial treatments.

Table 11 Cultivations and drilling dates at both spring sown field sites 2018-19

Cultivation no.	Cultivation	Timing	Cultivation and drilling date at each site	
			CambsBx19	CambsH19
C1	None	Act of drilling only	02/03/19	16/02/19
C2	Shallow cultivation	After glyphosate application T2 (10 days minimum) in autumn	09/11/18	25/11/18

A pre-emergence herbicide was applied to the CambsBx19 site on 23/03/19 and the same method of covering the ground with 1m x1m plastic sheets as described above was repeated. No post-emergence herbicides were applied.

Assessments

There were three assessment periods with five assessments:

a) Autumn at glyphosate treatments, and 2-3 weeks after treatments, emergence counts (assessments 1-2)
b) Spring glyphosate treatments and 2-3 weeks after treatment/or post cultivation (Assessment 3-4)
c) June, head count (assessment 5)

Black-grass emergence was assessed by placing 10 random quadrats (0.1 m^2) in each treatment plot at the timing of the first glyphosate application in the autumn and counting emergence at each assessment stage (Table 12).

In spring 2019 black-grass plant counts were done by placing 10 random quadrats (0.1m^2) per plot (Table 12). In the non-glyphosate treated plots where pre-emergence herbicide application was prevented using 1 x 1 m plastic covers two plant counts using 0.1m^2 quadrats were done.

The total number of black-grass heads per plot were assessed in late June 2019 (Table 12) each season using 10 randomly placed quadrats ($0.1m^2$). As with the plant counts, in the un-glyphosate treated plots where pre-emergence herbicide application was prevented using 1 x 1 m plastic covers two head counts using $0.1m^2$ quadrats were done.

Table 12 Assessment dates at both spring sown sites 2018-19

	CambsBx19		CambsH19	
Assessment	Date	Growth stage	Date	Growth stage
1. Emergence	24/10/18	10-13	24/10/18	10-12
2. Emergence	27/11/18	10-23	13/12/18	12-23
3. Spring plant count	25/02/19	11-29	-	-
4. Spring plant count	25/03/19	21-29	06/03/19	10-29
5. Black-grass heads	19/06/19	61-71	14/06/19	61-71

Spring cropping 2019-2020

One field trial was located at ADAS Boxworth (CambsBx20), plot size was 6m x 8m with four replicates. The field crop was spring barley.

Treatments and cultivations

There were two glyphosate treatments at a higher dose of 720g a.s./ha. The application timings were required in the autumn and at pre-drilling of the crop in the spring (Table 13). The herbicide treatments were fully randomised within each cultivation block. There were two cultivations (Table 14) that included minimal soil disturbance in October 2019 (C1), and a shallow cultivation on the same day as the spring glyphosate application in March 2020 (C2). The cultivation blocks were not randomised for practical reasons and the trial was laid out as a matrix design with cultivation in one direction and glyphosate treatment in the other (randomised by replicate).

Table 13 Herbicide application timings for spring barley trial 2019-20

Treatment	Product	Dose rate g a.s./ha	L/ha of product	No. times applied	Application Timing 1 (autumn)	Application Timing 2 (spring)
T1	Glyphosate 360g a.s./L	720	2.0	1	06/11/19	-
T2	Glyphosate 360g a.s./L	720	2.0	1	-	19/03/20

Table 14 Cultivations for spring barley trial 2019-20

Cultivation no.	Cultivation	Timing	Date
C1	Shallow	October	23/10/19
C2	Shallow	Spring (pre-glyphosate application)	19/03/20

The spring barley crop was drilled on 22/03/20 and rolled 30/03/20.

Assessments

There were three assessment periods with five assessments:

a) Autumn at glyphosate treatments, and 2-3 weeks after treatments, emergence counts (assessments 1-2)
b) Spring glyphosate treatments and 2-3 weeks after treatment/or post cultivation (assessment 3-4)
c) June, head count (assessment 5)

Black-grass emergence was assessed by placing 10 random quadrats (0.1 m^2) in each treatment plot at the timing of the first glyphosate application in the autumn and counting emergence at each assessment stage (Table 15).

In spring 2020 black-grass plant counts were done by placing 10 random quadrats (0.1m^2) per plot (Table 15).

The total number of black-grass heads per plot were assessed in late June 2019 (Table 15) each season using 10 randomly placed quadrats (0.1m^2).

Table 15 Assessments in spring barley trial 2019-20

Assessment	Date	Black-grass growth stage
1. Emergence count	06/11/19	12
2. Emergence count	02/12/19	11-24
3. Spring plant count	18/03/20	21-29
4. Late spring plant count	12/05/20	10-61
5. Head count	31/07/20	61-71

3.2. Container experiments (WP1.2 and WP3)

The aim of the container based experiments (1, 2 & 3) was to determine the optimum weed growth stage and glyphosate dose for full herbicide efficacy. In addition, how any risks are mitigated by cultivation or subsequent herbicide applications was also investigated.

3.2.1. Container Experiment 1. The effect of glyphosate dose against weed growth stage

The aim of this experiment was to determine and validate the most effective glyphosate dose for a specific weed size. The experiment began in autumn 2015 and was repeated in autumn 2016 with seed collected from the survivors of the first year. Three black-grass and three Italian rye-grass populations (one susceptible and two resistant (to other herbicide modes of action (Table 71)) were selected (Table 16).

Table 16 Grass weed populations selected for the glyphosate dose and weed size container experiment

Sample no.	ADAS Sample name	Source of seed	Population reference
1	BG01	40 acres field Boxworth 2015	SD 0172
2	BG Susceptible	Susceptible standard seed	SD 0043
3	Peldon resistant	Peldon resistant standard seed	SD 0032
4	IRG01	Syngenta seed lot PS 6757	SD 0173
5	IRG Susceptible	Susceptible standard seed	SD 0041
6	IRG Resistant	Resistant standard seed	SD 0174

There were 12 herbicide treatments, including three glyphosate doses and three weed growth stages (Table 17), replicated three times.

Table 17 Herbicide treatments and application growth stage for the glyphosate dose & weed size container experiment 1.

Treatment Number	Product	Active	Growth Stage for application	Dose (g a.s./L)	Dose (L/ha)
1	Untreated	-	1 leaf (GS 10)	-	-
2	Untreated	-	2-3 lvs (GS 12-13)	-	-
3	Untreated	-	Tillering (GS 21-22)	-	-
4	MON79376	Glyphosate	1 true leaf (GS 10)	360	1.0
5		360g a.s./L	1 true leaf (GS 10)	540	1.5
6			1 true leaf (GS 10)	720	2.0
7	MON79376	Glyphosate	2-3 true leaves (GS 12-13)	360	1.0
8		360g a.s./L	2-3 true leaves (GS 12-13)	540	1.5
9			2-3 true leaves (GS 12-13)	720	2.0
10	MON79376	Glyphosate	Tillering (GS 21-22)	360	1.0
11		360g a.s./L	Tillering (GS 21-22)	540	1.5
12			Tillering (GS 21-22)	720	2.0

A total of 216 plastic containers (measuring 310mm x 210 mm x 145 mm) were filled with sterilised Kettering loam mix (Rothamsted ‘weed mix’, 4:1 loam: lime free 3-6mm grit plus 2kg/m^3 Osmacote mini) to a depth of 3cm below the rim, with the soil level and even. Containers were laid out in the fruit cage at ADAS Boxworth and watered well using an overhead watering system. Seed were weighed out (0.5g seed/container for black-grass and Italian rye grass) from each population for each individual container into small plastic vials with lids. Seed were mixed a very small amount of soil and sprinkled evenly over the soil surface of the container (Table 18) and covered with a very shallow layer of soil (no more than 1cm). All containers were watered lightly after sowing with a watering can with a fine rose attachment. The trial was arranged in a randomised block design.

Table 18. Glyphosate dose and weed growth stage experiments summary of application and assessment dates.

Harvest Year	Sowing date	Herbicide application date			Plant count	Head count
		GS10	GS12-13	GS21-22		
2016	10/11/15	10/12/15	06/01/16	22/03/16	06/05/16	05/07/16
2017	19/10/16	22/11/16 – IRG* 28/11/16 – BG*	16/01/17	09/03/17	03/05/17	17/07/17

**IRG= Italian rye-grass, BG=Black-grass*

At the correct weed growth stages containers were grouped into treatments and moved to the spray area. Herbicides were applied to containers using a hand-held 2m boom and knapsack sprayer at 2 bar, F110 02 nozzles at a water volume of 80 L/ha. De-ionised water was used. The treatment was allowed to dry on the foliage before placing the containers back in to the fruit cage and were not watered for at least six hours post-herbicide application. The number of plants and heads per container were counted (Table 18) each year. Containers were isolated into mesh cages in their herbicide treatments (three replicates per cage) in April each year to ensure there was no cross-pollination. Seeds were collected from all survivors in July 2016. The treatment and population replicates were bulked together and seed were weighed and re-sown in autumn 2016. As the quantity of seed from every treatment varied there was not enough seed available to re-sow every treatment for experiment year two as some treatments had been very effective. For those particular treatments the baseline seed was used (Table 19).

Table 19 Treatment where baseline seed was required for trial year two as there were no survivors in year one.

Treatment number	BG01	BG Sus	BG Peldon resistant	IRG01	IRG Sus	IRG Res
1		Baseline		Baseline	Baseline	
3			Baseline			
5	Baseline	Baseline				
6		Baseline			Baseline	
7	Baseline	Baseline		Baseline	Baseline	
8	Baseline	Baseline	Baseline	Baseline	Baseline	
9	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
10			Baseline			
11						Baseline
12	Baseline		Baseline	Baseline		

In July 2017 seed were collected from survivors. These seed were used for a glasshouse dose response experiment presented in Section 3.4.4.

3.2.2. Containers Experiment 2. The effect of glyphosate dose, weed size and cultivations

The aim of this experiment was to determine and validate the most effective glyphosate dose for a specific weed size, with the addition of a simulated cultivation. The experiment began in autumn 2015 and was repeated in autumn 2016. Three black-grass and three Italian rye-grass populations (one susceptible and two resistant (to other herbicide modes of action (Table 71)) were selected (Table 16) as in experiment 1.

There were 12 herbicide treatments, including two glyphosate doses, two weed growth stages, two simulated cultivations (Table 20), replicated three times.

Table 20 Herbicide treatments, cultivation type and application growth stage for the glyphosate dose, weed size and cultivation container experiment 2.

Treatment Number	Product (active)	Cultivation Type	Cultivation depth (cm)	Growth stage	Dose L/ha
1		Deep (C1)	10		
2	Untreated	Shallow (C2)	5	GS 12-13	-
3		None (C3)	none		
4		Deep (C1)	10		
5	Untreated	Shallow (C2)	5	GS21-22	-
6		None (C3)	none		
7	MON79376	Deep (C1)	10		
8	(Glyphosate	Shallow (C2)	5	GS 12-13	1.0
9	360g a.s./L)	None (C3)	none		
10	MON79376	Deep (C1)	10		
11	(Glyphosate	Shallow (C2)	5	GS21-22	1.0
12	360g a.s. /L)	None (C3)	none		

The simulated cultivations were to mimic different types of inversion in the field. A deep cultivation (C1) was to a depth of 10cm and a shallow cultivation (C2) was to a depth of 5cm.

A total of 216 plastic containers (measuring 310mm x 210 mm x 145 mm) were filled with sterilised Kettering loam mix (Rothamsted ‘weed mix’, 4:1 loam: lime free 3-6mm grit plus 2kg/m^3 Osmacote mini) to a depth of 3cm below the rim, with the soil level and even. Containers were laid out in the hard standing area at ADAS Boxworth and watered well using an overhead watering system. Seed were weighed out (0.5g seed/container for black-grass and Italian rye grass) from each population for each individual container into small plastic vials with lids. Seed were mixed a very small amount of soil and sprinkled evenly over the soil surface of the container (Table 21) and covered with a very shallow layer of soil (no more than 1cm). All containers were watered lightly after sowing with a watering can with a fine rose attachment. The trial was arranged in a randomised block design.

Table 21 Glyphosate dose, weed growth stage & cultivations experiment summary of application and assessment dates.

Harvest Year	Sowing date	Herbicide application and cultivation date				Plant count	Head count
		GS 12-13		GS 21-22			
		Spray	Cultivation	Spray	Cultivation		
2016	13/11/16	11/01/16	25/01/16	22/03/16	01/04/16	06/05/16	05/07/16
2017	13/10/17	22/11/16* IRG 28/11/16* BG	02/12/16* IRG 09/12/16* BG	19/01/17	30/01/17	n/a	28/06/17

**IRG = Italian ryegrass, BG = black-grass*

At the correct weed growth stages containers were grouped into treatments and moved to the spray area (Table 21). Herbicides were applied to containers using a hand-held 2m boom and knapsack sprayer at 2 bar, F110 02 nozzles at a water volume of 80 L/ha. De-ionised water was used. The treatment was allowed to dry on the foliage before placing the containers back in to the hard standing and were not watered for at least six hours post-herbicide application. The cultivation treatments (Table 20) were carried out between 10-14 days after herbicide applications to simulate a cultivation in the field after a stale seedbed technique. The cultivation treatments were disturbed on the soil surface using a small gardening hand fork to mimic a blade passing through. A small amount of soil was turned on top of the plants and then additional soil was placed over the plants to cover the required depth (either 10cm (C1) or 5cm (C2)). One treatment had no disturbance ('none' C3). The number of heads per container were counted (Table 21) each year. In 2016 the number of plants per container were also counted, however in 2017 this assessment was not carried out due to the large number of plants making the assessment very difficult to do. It was considered that the head count data would be a more accurate assessment.

3.2.3. Container Experiment 3. The effect of glyphosate dose, weed size and subsequent pre- and post-emergence selective herbicide programmes.

The aim of this experiment was to determine whether the glyphosate resistance risk is reduced through getting effective control when a sub-optimal glyphosate dose for a specific weed size has been used on a population in a stubble but is then followed by a robust herbicide programme with other modes of action. A pre-emergence and post-emergence herbicide were chosen by the project steering group to represent these herbicide application timings. They were not applied as a sequence as it was considered that in the container conditions they would be more active than field conditions. The treatments selected were to enable the building blocks of a sequence to be understood where the risk of plants exposed to sub-optimal glyphosate were present. This was only tested on three black-grass populations (Table 22) and not Italian rye-grass as the trial size was large. The black-grass populations included one susceptible standard and two populations with known resistance to other modes of action (Table 71). This experiment started in autumn 2017 and was repeated in 2018. A total of 162 containers (three replicates) were filled with the same loam mix and used the same sowing methodology as described in section 3.1.3.1.

Table 22 Black-grass populations used for container Experiment 3.

Sample no.	ADAS Sample name	Source of seed	Population reference
1	BG01	Boxworth 40 acres field (known resistant)	SD 0026
2	BGSus	Susceptible standard seed	SD 0043
3	BGPeldon resistant	Peldon resistant standard seed	SD 0032

Herbicide treatments were applied at the timings stated in Table 23 and actual dates in Table 24. Treatments 7 to 18 all received an application of glyphosate at the required black-grass growth stage and were then either followed by no herbicides, a pre-emergence alone (14 days after glyphosate) or a post-emergence alone. This is obviously not normal practice, however the reason that the pre-emergence herbicide is being applied at that timing was to prove the level of kill from the glyphosate (as if it were a stale seedbed in the field) versus the pre-emergence.

Table 23 Herbicide treatments and application timings for container Experiment 3.

Treatment Number	Glyphosate Treatment	Growth stage	Glyphosate product dose (L/ha)	Pre-emergence herbicide product & dose 14 days post-glyphosate*	Post-emergence herbicide product & dose *(pre-tillering black-grass)*
1		2-3 lvs (GS 12-13)	-	-	-
2	UTC	2-3 lvs (GS 12-13)	-	Liberator @ 0.6 L/ha[a]	-
3		2-3 lvs (GS 12-13)	-	-	Hamlet @1.5 L/ha[b]
4		Tillering (GS21-22)	-	-	-
5	UTC	Tillering (GS21-22)	-	Liberator @ 0.6 L/ha[a]	-
6		Tillering (GS21-22)	-	-	Hamlet @1.5 L/ha[b]
7		2-3 lvs (GS 12-13)	0.75	-	-
8		2-3 lvs (GS 12-13)	0.75	Liberator @ 0.6 L/ha[a]	-
9	MON79376 (Glyphosate 360g a.s./L)	2-3 lvs (GS 12-13)	0.75	-	Hamlet @1.5 L/ha[b]
10		Tillering (GS21-22)	0.75	-	-
11		Tillering (GS21-22)	0.75	Liberator @ 0.6 L/ha[a]	-
12		Tillering (GS21-22)	0.75	-	Hamlet @1.5 L/ha[b]
13		2-3 lvs (GS 12-13)	1.125	-	-
14		2-3 lvs (GS 12-13)	1.125	Liberator @ 0.6 L/ha[a]	-
15	MON79376 (Glyphosate 360g a.s./L)	2-3 lvs (GS 12-13)	1.125	-	Hamlet @1.5 L/ha[b]
16		Tillering (GS21-22)	1.125	-	-
17		Tillering (GS21-22)	1.125	Liberator @ 0.6 L/ha[a]	-
18		Tillering (GS21-22)	1.125	-	Hamlet @1.5 L/ha[b]

[a]*Liberator contains flufenacet + diflufenican*

[b]*Hamlet contains mesosulfuron-methyl + iodosulfuron-methyl-sodium + diflufenican*

Treatments were applied using the same method and equipment as in section 3.1.3.1.

The number of plants and heads per container were assessed in spring and summer of each testing year (Table 24).

Table 24 Summary of sowing, application and assessment dates for container Experiment 3.

Harvest Year	Sowing date	Herbicide application date			Plant count	Head count
		Glyphosate	Pre-em	Post-em		
2018	02/11/17	(GS12-13) - 16/01/18	30/01/18	13/02/18	10/05/18	01/06/18
		(GS 21-22) - 12/03/18	30/03/18	10/04/18		
2019	30/10/18	(GS12-13) – 10/01/19	23/01/19	06/02/19	02/04/19	03/06/19
		(GS 21-22) – 12/02/19	26/02/19	13/03/19		

3.3. Applications between crop rows (WP2)

To determine the implications on resistance risk from use of glyphosate post-emergence, such as between crop rows.

3.3.1. To investigate the resistance risk of glyphosate applications to larger weed growth stages (Container experiment)

The aim of this experiment was to determine whether a larger weed growth stage (as would be expected with a post-emergence application such as in-row shielded treatments) was more susceptible to glyphosate resistance evolution and therefore dependent on the correct rate and timing. This experiment started in autumn 2017 and was repeated for two years to validate the data. Two black-grass populations were selected (Susceptible standard and Peldon resistant standard, with known resistance to other herbicide modes of action (Table 71) (Table 25).

Table 25 Black-grass populations used in the large growth stage container experiment.

Sample no.	ADAS Sample Reference	Source of seed	Population reference
1	BGSus	Susceptible standard seed	SD 0043
2	BGPeldon resistant	Peldon resistant standard seed	SD 0032

A total of 42 containers were filled with the same loam mix and used the same sowing methodology as described in section 3.2.1. Black-grass plants were treated at three different large growth stages GS 23, GS 25-28 and GS 32, with three glyphosate doses, including an untreated control, and replicated three times (Table 26 and Table 27). Treatments were applied using the same method and equipment as in section 3.2.1.

Table 26 Application rates and timings for the large growth stage container experiment.

Treatment Number	Product	Active	Dose (g a.s./L)	Dose (product) L/ha	Application timing (Growth stage)
1	Untreated	-	-	-	
2	MON79376	Glyphosate 360g a.s./L	360	1.0	T1 (GS 23)
3			180	0.5	
4			360	1.0	T2 (GS 25-28)
5			180	0.5	
6			360	1.0	T3 (GS 32)
7			180	0.5	

In April of each experiment year the containers were isolated into their different treatments to prevent cross-pollination. The total number of heads per container were counted (Table 27) and seed were collected from survivors in the July. Seed were tested for resistance status in a glasshouse pot test in January 2020 (see section 3.4.3).

Table 27 Sowing, application and assessment dates for large growth stage container experiment.

Harvest Year	Sowing date	Herbicide application date			Head count
		GS23	GS25-28	GS32	
2018	27/10/17	14/03/18	12/04/18	26/04/18	06/06/18
2019	25/10/18	20/02/19	06/03/19	02/04/19	27/06/19

3.4. Resistance testing (Glasshouse and container experiments) (WP3)

To determine resistance status (testing) of seeds and rapid within-season whole plant assays.

3.4.1. A glyphosate dose response experiment testing black-grass populations from a long-term field trial (Glasshouse Experiment 1).

Black-grass seed were collected in July 2015 from selected plots within a large long-term (five year) field trial in Cambridgeshire (CambsA15). Plots were selected based on previous cultivation, glyphosate use and other herbicide history. The range of cultivations included continuous plough, continuous minimal tillage or a rotational combination of both. Field blocks were sub-divided with a range of glyphosate applications pre-drilling and a full pre- and post-emergence herbicide programme. Seven field populations and two standard reference population were tested (Table 28).

Table 28 Seed populations collected from a long-term glyphosate field experiment and tested in a glasshouse dose response experiment to glyphosate.

Sample no.	Population reference	Seed source
1	2015AG01	
2	2015AG02	
3	2015AG03	
4	2015AG04	CambsA15
5	2015AG05	
6	2015AG06	
7	2015AG07	
8	Standard susceptible	Herbiseed purchased 2015
9	Standard resistant	Peldon resistant

Pre-germination and transplanting of seed

For each of the seed populations, plastic Petri dishes (10 x 90mm) were filled with three Whatmans no. 1 filter papers size 85 mm, and 1 Whatmans GR/A glass microfibre filter paper size 90mm. A total of four Petri dishes were required per population. Approximately 75 black-grass seeds were sprinkled into the prepared Petri dishes and labelled. A 0.2% potassium nitrate solution was made by dissolving 2 g KN03 in 1.0 litre of distilled water and 7mls of solution were syringed into each dish.

Petri-dishes were stacked in groups of 10 with a blank dish (containing filter paper) at the top and bottom giving 12 Petri-dishes altogether. The stacks were placed in a clear polythene bag sealed with sellotape and placed into an incubator set at 17ºC, 14 hour day with neon lights and 11ºC, 10 hour night with no lights. After six to seven days the chitted seed were transferred to the glasshouse ready to be transplanted into plant pots filled with soil.

A total of 270 pots (9cm diameter) were filled with sterilised loam mix (Rothamsted 'weed mix' - Sterilised Kettering loam and Lime free grit 3-6mm in a 4:1 ratio plus 2kg/m^3 Osmacote mini) to a depth of 2 cm below the pot rim. Pots were laid out in the glasshouse at Boxworth in trays and watered well using an overhead watering system. Ten pre-germinated seeds were transplanted into each pot ensuring that they were evenly spaced and at least 15 mm from the edge of the pot. The seeds were covered with fine soil to an even depth of 1 cm of soil.

Spraying

At GS 10 pots were carefully thinned to six plants per pot. Herbicides (Table 29) were applied to pots at GS12-13 using a hand-held 2m boom and knapsack sprayer at 2 bar, F110 02 nozzles at a water volume of 80 L/ha. De-ionised water was used for mixing up the spray. The foliage was left to dry before placing the pots back into the glasshouse.

Table 29 Treatment list for glasshouse dose response experiment on seed collected from a long-term field experiment.

Treatment Number	Product Name	Active	Dose (g a.s./L)	Dose (product) L/ha
1	Untreated	-		-
2	MON79376	Glyphosate 360g a.s./L	180	0.5
3			360	1.0
4			540	1.5
5			720	2.0

Assessments

Plants were assessed 3-4 weeks post-spraying. A record of the total number of plants in the pot and total number of alive plants in the pot was taken. The fresh weight of all plants per pot (g) was assessed by carefully cutting the plant at the base and recording the total plants per pot weight.

3.4.2. **Annual seed testing for glyphosate resistance (Glasshouse Experiment 2)**

Black-grass seed were sent into ADAS Boxworth from any farmers or agronomists who had concerns with the level of control from glyphosate in the field. Seed were only submitted for testing in 2015 (four populations), 2016 (two populations) and 2018 (three populations).

Pre-germination and transplanting of seed

Seed were pre-germinated as described in section 3.4.1 above.

A total of 180 pots (9cm diameter) were filled with sterilised loam mix (Rothamsted 'weed mix' - Sterilised Kettering loam and Lime free grit 3-6mm in a 4:1 ratio plus 2kg/m^3 Osmacote mini) to a depth of 2 cm below the pot rim. Pots were laid out in the glasshouse at Boxworth in trays and watered well using an overhead watering system. Ten pre-germinated seeds were transplanted into each pot ensuring that they were evenly spaced and at least 15 mm from the edge of the pot. The seeds were covered with fine soil to an even depth of 1 cm of soil.

Spraying

At GS 10 pots were carefully thinned to six plants per pot. Herbicides (Table 30 & Table 31) were applied to pots at GS12-13 using a hand-held 2m boom and knapsack sprayer at 2 bar, F110 02 nozzles at a water volume of 80 L/ha. De-ionised water was used for mixing up the spray. The foliage was left to dry before placing the pots back into the glasshouse.

Table 30 Herbicide treatments for 2015 and 2016 commercial resistance testing samples

Treatment	Product	Active	Rate g a.s./ha	L/ha of product
1	NIL	-	-	-
2	MON79376	Glyphosate 360g a.s./ha	405	1.125
3	MON79376	Glyphosate 360g a.s./ha	540	1.5

Table 31 Herbicide treatments for 2018 commercial resistance testing samples

Treatment	Product	Active	Rate g a.s./ha	of product
1	NIL	-	-	-
2	MON79376	Glyphosate 360g a.s./ha	360	1.0
3	MON79376	Glyphosate 360g a.s./ha	540	1.5
4	MON79376	Glyphosate 360g a.s./ha	720	2.0

Assessments

Plants were assessed 3-4 weeks post-spraying. A record of the total number of plants in the pot and total number of alive plants in the pot was taken. The fresh weight of all plants per pot (g) was assessed by carefully cutting the plant at the base and recording the total plants per pot weight.

3.4.3. Container experiment seed survivors: Glyphosate dose response testing

Testing seed survivors from first two years of container trials (2018)

Black-grass and Italian rye-grass seed were collected from the container experiment (3.2.1) in July 2017 that had had two years exposure to different glyphosate treatments (see section 3.2.1 for treatment and population details). There were a total of 21 populations tested, 10 black-grass and 11 Italian rye-grass including standard populations for comparison (Table 32).

Table 32 Seed survivor populations collected from container experiment 3.2.1 and tested in a glasshouse dose response to glyphosate.

Species	Population reference	*Original treatment reference (see Experiment 3.2.1)*
Black-grass	SD 0548	*BG01, UTC*
	SD 0549	*BG01, Glyphosate 720 (GS12-13)*
	SD 0550	*BG01, Glyphosate 360 (GS21-22)*
	SD 0551	*BG susceptible, UTC*
	SD 0552	*BG susceptible, Glyphosate 360 (GS21-22)*
	SD 0553	*BG Peldon, UTC*
	SD 0554	*BG Peldon, Glyphosate 720 (GS10)*
	SD 0555	*BG Peldon, Glyphosate 360 (GS21-22)*
Italian rye-grass	SD 0556	*IRG01, UTC*
	SD 0557	*IRG01, Glyphosate 360 (GS21-22)*
	SD 0558	*IRG susceptible, UTC*
	SD 0559	*IRG susceptible, Glyphosate 360 (GS21-22)*
	SD 0560	*IRG susceptible, Glyphosate 720 (GS21-22)*
	SD 0561	*IRG resistant, UTC*
	SD 0562	*IRG resistant, Glyphosate 720 (GS10)*
	SD 0563	*IRG resistant, Glyphosate 360 (GS21-22)*
	SD 0564	*IRG resistant, Glyphosate 540 (GS21-22)*
	SD 0565	*IRG resistant, Glyphosate 720 (GS21-22)*
Black-grass	SD 0200	*Peldon resistant population*
	SD 0525	*Standard susceptible*
Italian rye-grass	SD 0215	*Standard susceptible*

The dose response experiment was a randomised block design with five glyphosate treatments, plus an untreated control, replicated six times (Table 33).

Pre-germination and transplanting

Seed were cleaned using the air-column separator and pre-germinated in the laboratory using the method described in section 3.4.1. For black-grass and Italian rye-grass six Petri dishes per population were used for pre-germination (total of 126 dishes). A total of 756 pots (9cm diameter) were filled with sterilised loam mix (Rothamsted ‘weed mix’ - Sterilised Kettering loam and Lime free grit 3-6mm in a 4:1 ratio plus 2kg/m^3 Osmacote mini) to a depth of 2 cm below the rim. Pots were laid out in the glasshouse at Boxworth in trays and watered well using an overhead watering system.

Spraying

The same method as described in section 3.4.1 was used for transplanting, thinning seeds and for the spray application. The glyphosate treatment was applied at GS12-13 (Table 33).

Table 33 Glyphosate dose response treatments

Treatment	Product	Active	Dose a.s./L	L/ha of product
1	NIL	-	-	-
2			180	0.5
3			360	1.0
4	MON79376	Glyphosate 360g a.s./L	540	1.5
5			720	2.0
6			1080	3.0

Assessments

Plants were assessed 3-4 weeks post-spraying. A record of the total number of plants in the pot and total number of alive plants in the pot was taken. The fresh weight of all plants per pot (g) was assessed by carefully cutting the plant at the base and recording the total plants per pot weight.

Testing seed survivors from the large growth stage container experiment

Black-grass seed were collected from container experiment (3.2.1) in June 2019 that had two years exposure to different glyphosate treatments (see section 3.2.1 for treatment and population details). There were a total of 16 populations tested including standard populations for comparison (Table 34). In 2019 some of the populations did not have enough seed for a pot test, so seed from 2018 (only one year of selection) was used and is shown in bold in Table 34.

Table 34 Seed survivor populations collected from large growth stage container experiment 3.2.1 and tested in a glasshouse dose response to glyphosate.

Population reference	*Original treatment reference (see Experiment 3.2.1)*
SD 0795	*Susceptible UTC, 2019 seed*
SD 0796	*Susceptible, 360g glyphosate, GS23, 2019 seed*
SD 0683	***Susceptible, 180g glyphosate, GS23, 2018 seed***
SD 0684	***Susceptible, 360g glyphosate, GS25-28, 2018 seed***
SD 0685	***Susceptible, 180g glyphosate, GS25-28, 2018 seed***
SD 0798	*Susceptible, 360g glyphosate, GS32, 2019 seed*
SD 0797	*Susceptible, 180g glyphosate, GS32, 2019 seed*
SD 0805	*Rothamsted 19 susceptible*
SD 0800	*Peldon resistant UTC, 2019 seed*
SD 0799	*Peldon resistant, 360g glyphosate, GS23, 2019 seed*
SD 0690	***Peldon resistant, 180g glyphosate, GS23, 2018 seed***
SD 0691	***Peldon resistant, 360g glyphosate, GS25-28, 2018 seed***
SD 0692	***Peldon resistant, 180g glyphosate, GS25-28, 2018 seed***
SD 0801	*Peldon resistant, 360g glyphosate, GS32, 2019 seed*
SD 0802	*Peldon resistant, 180g glyphosate, GS32, 2019 seed*
SD 0032	*Peldon resistant baseline seed, SD 0032*

The method of testing and assessing was exactly the same as described in the first glyphosate dose response experiment (3.4.1). The same treatments were applied (Table 33).

Testing seed survivors from selection containers

Black-grass seed were collected from container experiment (3.4.5) in June 2019 that had four years exposure to different glyphosate treatments (see section 3.4.5 for treatment and population details). There were a total of 12 populations tested including standard populations for comparison (Table 35). In 2019 some of the populations did not have enough seed for a pot test, so seed from 2018 (only three years of selection) was used and is shown in bold in Table 35.

Table 35 Seed survivor populations collected from selection container experiment 3.3.5 and tested in a glasshouse dose response to glyphosate.

Population reference	*Original treatment reference (see Experiment 3.3.5)*
SD 0790	*Susceptible, UTC (2019 seed)*
SD 0676	***Susceptible, 180g Glyphosate (2018 seed)***
SD 0791	*Susceptible, 90g Glyphosate (2019 seed)*
SD 0805	*Rothamsted 2019 susceptible*
SD 0792	*BG01, UTC (2019 seed)*
SD 0673	***BG01, 180g Glyphosate (2018 seed)***
SD 0793	*BG01, 90g Glyphosate (2019 seed)*
SD 0172	*BG01 baseline seed (SD 0172)*
SD 0794	*Peldon resistant, UTC (2019 seed)*
SD 0679	***Peldon resistant, 180g Glyphosate (2018 seed)***
SD 0680	***Peldon resistant, 90g Glyphosate (2018 seed)***
SD 0032	*Peldon resistant baseline seed (SD 0032)*

The method of testing and assessing was exactly the same as described in the first glyphosate dose response experiment (3.4.1). The same treatments were applied (Table 33).

3.4.4. Whole plant testing of survivors: RISQ test method

One of the key challenges for understanding resistance risk is to know whether plants will, or are surviving, due to evolving resistance. It is difficult with glyphosate to know if plants will die prior to needing to re-spray. It is therefore important to determine if a rapid/instant test could prevent further selection within-season. The Syngenta RISQ (Resistance In-Season Quick) test method (Kaundun *et al*., 2011) was used to test whole plants that have 'survived' a glyphosate application. This followed on from work by Syngenta (Kaundun *et al*., 2014) where Italian rye-grass was tested but not black-grass. Plants either came from the trials sets within the project or via farmer contacts through the project partners. The RISQ test was carried at ADAS Boxworth and Rosemaund with support and assessment validation from the resistance team at Syngenta's International Research Station at Jealott's Hill, UK.

Methodology

The standard RISQ test method was used with glyphosate with two aims; Firstly to determine the optimum glyphosate dose to use in future testing, and secondly to establish whether bigger tillered plants could be used, and not just small GS10-12 plants.

A series of six different experiments were carried out between 2016-2019 using black-grass and Italian rye-grass populations to validate the optimal glyphosate dose and plant size methods. Tests included four rates of glyphosate when plants were GS10-12 or GS12-14, (Table 36), however for tillering plants only one dose was used (50µM) as early tests on smaller plants showed this rate to be robust and this was the rate suggested by Syngenta. Three replicates were used in each experiment.

Table 36 Treatment list for the RISQ test method with glyphosate during the testing phase.

Treatment number	Treatment	Dose	Plant growth stage
1	Untreated	0µM	Tillered GS 21-24
2	Glyphosate 360 g a.s. /L	50µM	
3	Untreated	0 µM	GS 12-14
4		35 µM	
5	Glyphosate 360 g a.s. /L	50 µM	
6		75 µM	
7	Untreated	0 µM	GS 10-12
8		35 µM	
9	Glyphosate 360 g a.s. /L	50 µM	
10		75 µM	

Assessments

Agar plates can be assessed between 14-21 days after inserting the plants. This requires scoring the root growth by observing the underneath of the dish and not appearance of leaves. Roots are assessed on Syngenta scale of 1-3: 1 Alive, lots of new growth, 2 Yellow, some new growth, 3 Dead, no new growth.

3.4.5. **Selection experiments: To determine how quickly black-grass populations shift their glyphosate tolerance status**

The aim of this experiment was to determine how quickly different populations of black-grass shift their tolerance to glyphosate after being repeatedly sprayed with selected low doses of glyphosate over a three year period. The black-grass populations included a susceptible standard and two populations resistant to other herbicide modes of action (Table 37).

Table 37 Details of black-grass seed populations used in selection experiments

ADAS Sample Reference	Source of seed	ADAS seed code
Boxworth	Boxworth 40 acres field 2015	SD 0172
Susceptible	Susceptible standard seed	SD 0043
Peldon resistant	Resistant standard seed	SD 0032

A total of 36 containers were filled with the same loam mix and used the same sowing methodology as described in section 3.2.1. Black-grass plants were treated with three glyphosate doses (Table 38), including an untreated control, using the same application method as described in 3.2.1, at a growth stage of GS 14-21 and replicated three times.

Table 38 Herbicide treatments and dose used in the selection experiments

Treatment Number	Product	Active	Dose (g a.s./L)	Dose (product) L/ha
1	Untreated	-	-	-
2	MON79376	Glyphosate 360g a.s./L	360	1.0
3	MON79376	Glyphosate 360g a.s./L	180	0.5
4	MON79376	Glyphosate 360g a.s./L	90	0.25

In April every year the containers were isolated into mesh cages into their respective treatments to prevent cross-pollination. The total number of heads per container were counted in June or July (Table 39).

Table 39 Sowing, application and assessment timings for selection experiments

Harvest Year	Sowing date	Application date	Head count
2016	20/11/15	03/05/16	05/07/16
2017	07/10/16	15/12/16	06/07/17
2018	15/11/17	20/03/18	06/06/18
2019	23/10/18	07/02/19	26/06/19

After the head count assessment was complete seed from any survivors were collected when ripened and re-sown each autumn and re-sprayed with the same treatment. Seed collected in summer 2019 were tested in a glasshouse dose response experiment to determine any shifts (Section 4.4.5).

4. Results

4.1. Application in stubbles (WP1)

4.1.1. Data review

A summary of the collation of the historic glyphosate trials data provided by distributors and companies were grouped into rate, timing and number of applications. The data held by the companies was very variable, with experiments undertaken in different fields with varying levels of black-grass infestation, over different years. The summary of the data provided is therefore based on a limited number of very varied trials which should be considered when interpreting the results.

Glyphosate rate

Higher glyphosate rates generally increased black-grass control. The optimum rate being approximately 1080g a.s./ha (Figure 1). Application rates above 1080g provided no increase in control.

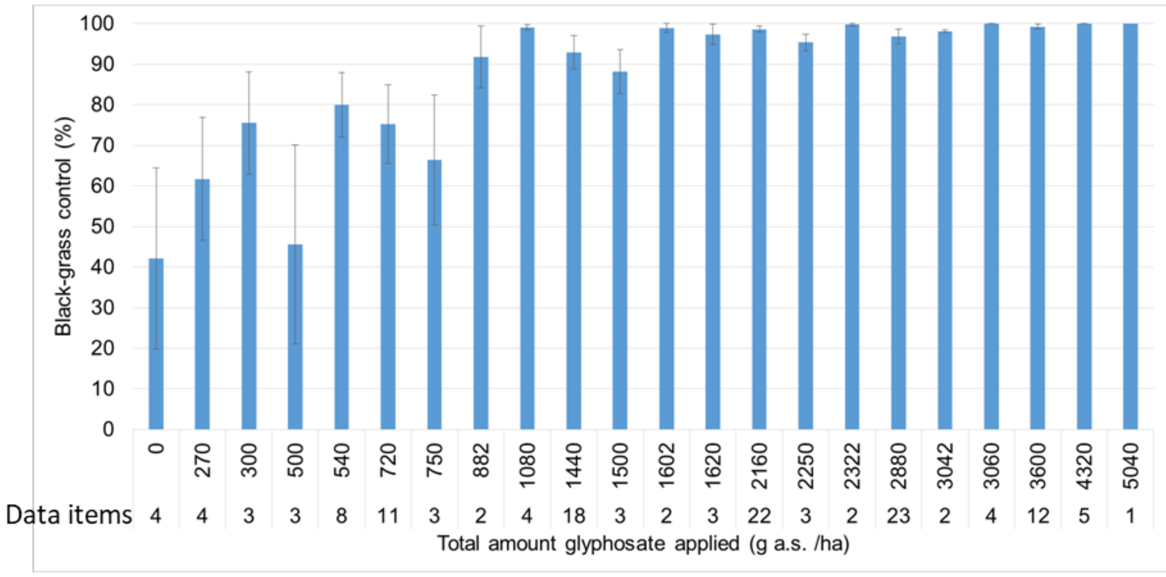

Figure 1 The total amount of glyphosate applied and the effect on the level of black-grass control. The number of collated trials for each data point is shown along the X axis under the glyphosate rate.

Number of glyphosate treatments

The data show that increasing the number of glyphosate treatments increases black-grass control, with the optimum being approximately 2-3 application timings (Figure 2).

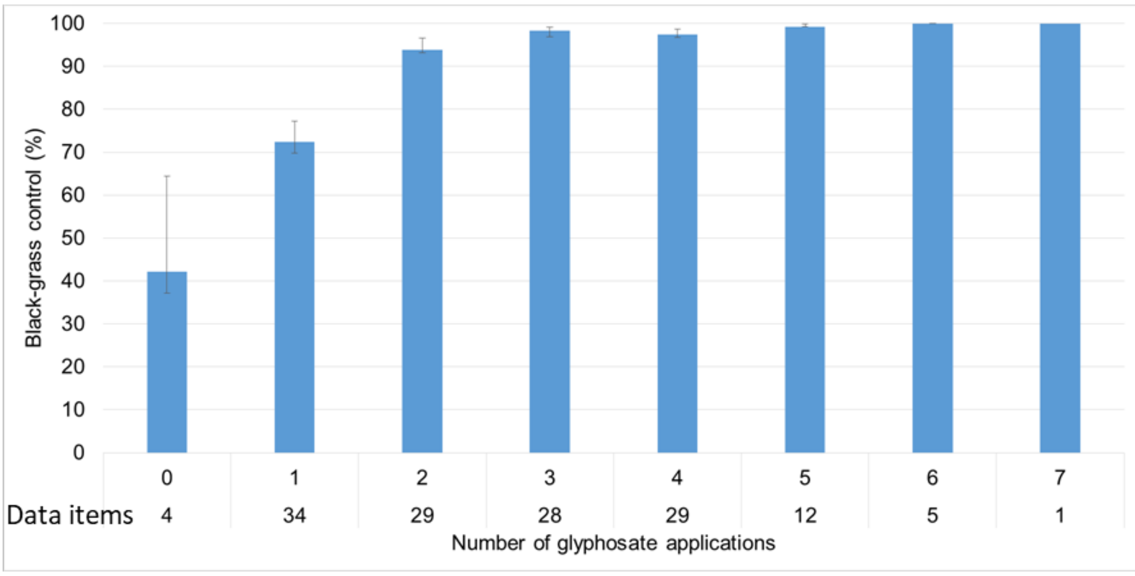

Figure 2 The number of glyphosate applications and the effect on the level of black-grass control.

Rate and number of applications

The data were further analysed to try and determine the optimum rate and timing combination for effective black-grass control. The data showed that the more application timings the higher the total amount of glyphosate applied, as would have been expected (Figure 3). There were no data available on a total of 1080g a.s./ha applied in a single application (it was always a split dose). From these data there was no more control achieved when more than two applications were made providing an overall rate of 1080g a.s./ha.

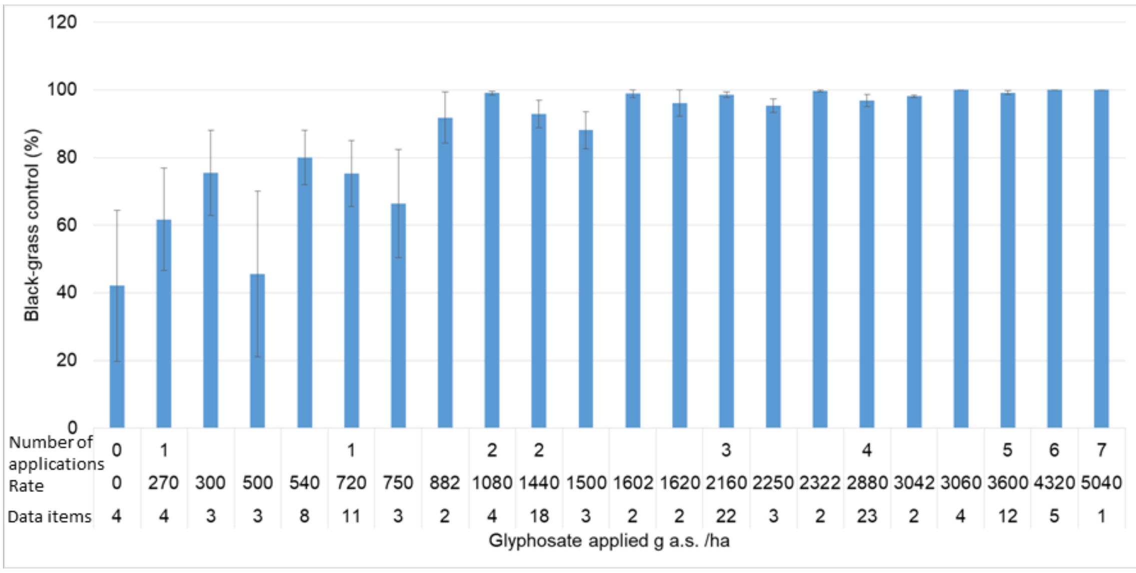

Figure 3 The number of glyphosate applications and the total amount of glyphosate applied on the level of black-grass control.

Timing and rate

The collated data set did not include many autumn only applications and were generally a combination of autumn and spring application timings. For the autumn applications data showed that maximum control only reached 75% with application rates only up to 750g a.s./ha glyphosate from these data (Figure 4).

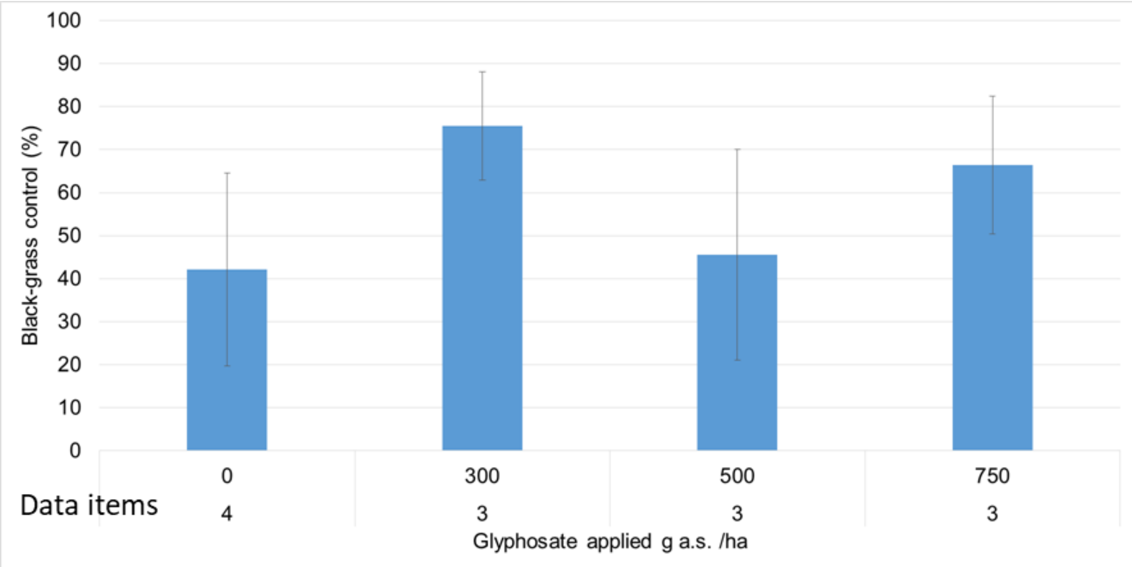

Figure 4 The effect of the total amount of glyphosate applied on black-grass control (%)

The data in Figure 5 indicated that splitting the application rates between autumn and spring a single glyphosate dose of 1080g a.s./ha to 1440g a.s./ha applied alone was more effective than splitting it.

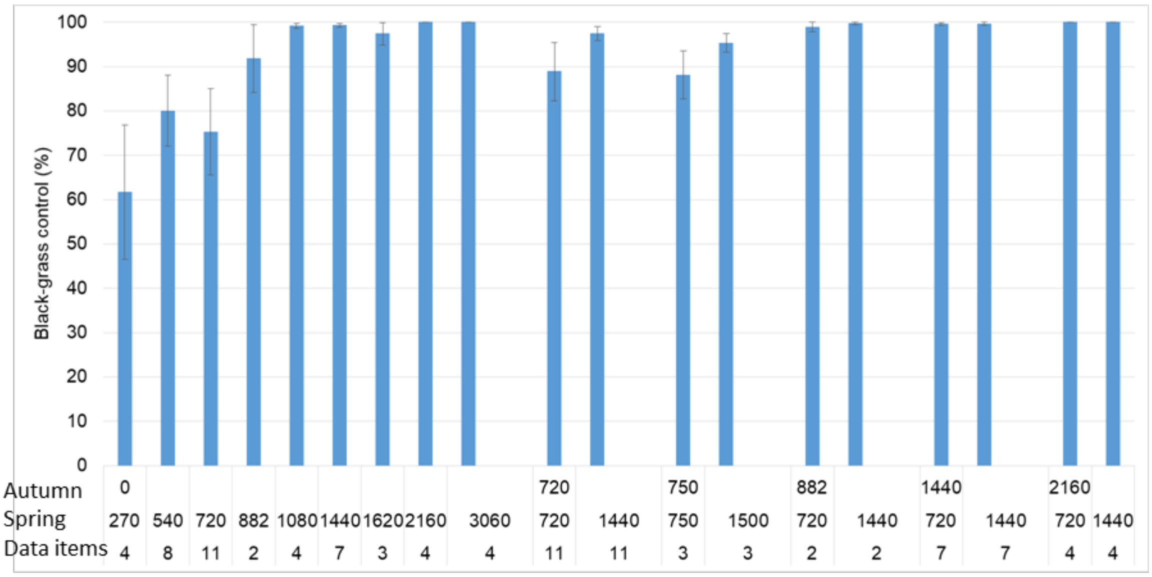

Figure 5 The effect of splitting glyphosate doses between autumn and spring.

The data sets reviewed were limited with most information available on applications made to stubbles and a single year fallow and much less on autumn, pre-drilling applications. Two key points were taken through into the project field trial treatments to help validate the following: (1) The optimum glyphosate application was around 1080g a.s./ha, generally split into two applications. (2) There was no increase in the level of black-grass control with two applications above or over 1080g a.s./ha.

4.1.2. **Field experimentation data (WP1.1)**

Field experiment 1. Glyphosate and weed flushes (winter wheat cropping 2017 & 2018)

Three field trials were established in autumn 2017 and 2018. Glyphosate was applied at three timings – early autumn, mid- autumn and late autumn, either as a single rate of 540g a.s./ha (early and mid), two applications of 540g (early followed by (fb) mid, mid fb late) or a single application of 1080g (late only). A cultivation was done just prior to or at drilling.

2017

Table 40 Black-grass population information for 2017 sites

Site name	Mean Black-grass/m² Autumn	Mean black-grass/m² Spring	Mean black-grass heads/m²
CambsH17	31	1	3
LincsF17	30	4	10
CambsA17	124	2	9

At the CambsH17 site mean black-grass numbers were 31 plants/m² when the trial was set out. All applications of glyphosate worked well (Figure 6) and reduced populations. A shallow cultivation was done on 12 October and had little effect on black-grass numbers, the site was drilled on 24 October.

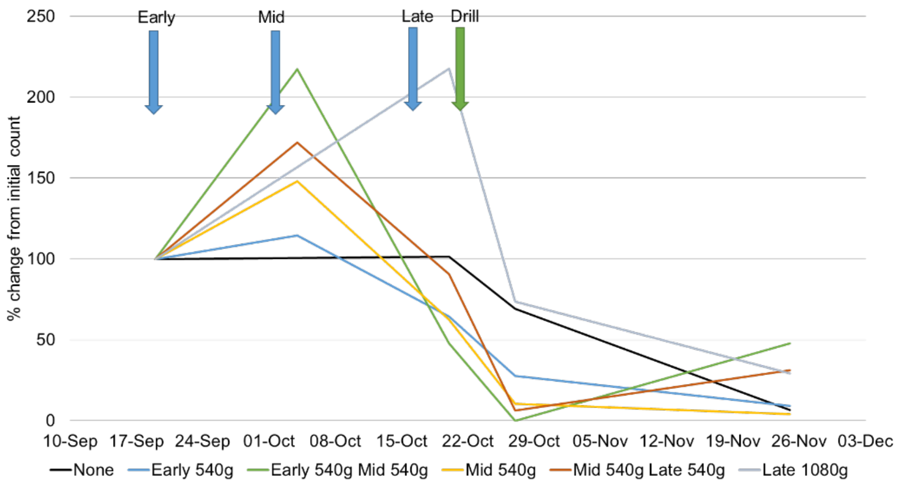

Figure 6 Emergence of black-grass through the autumn and winter in uncultivated plots, percentage change compared to initial count – CambsH17

At heading the number of heads was very low (Figure 7, Table 41) and there were no significant differences between treatments.

Table 41 Black-grass head counts CambsH17

Treatment	Cultivation	No cultivation	Mean herbicide	
None	2	1	1	
Early 540g	2	8	5	
Early 540g Mid 540g	1	0	1	
Mid 540g	3	10	6	
Mid 540g Late 540g	2	0	1	
Late 1080g	0	7	4	
Mean cultivation	2	4		
	Fpr	***d.f***	***s.e.d***	***l.s.d***
Cultivation	*NS*	*22*	*1.88*	*3.92*
Herbicide	*NS*	*22*	*3.27*	*6.78*
Cult x herb	*NS*	*22*	*4.63*	*9.59*

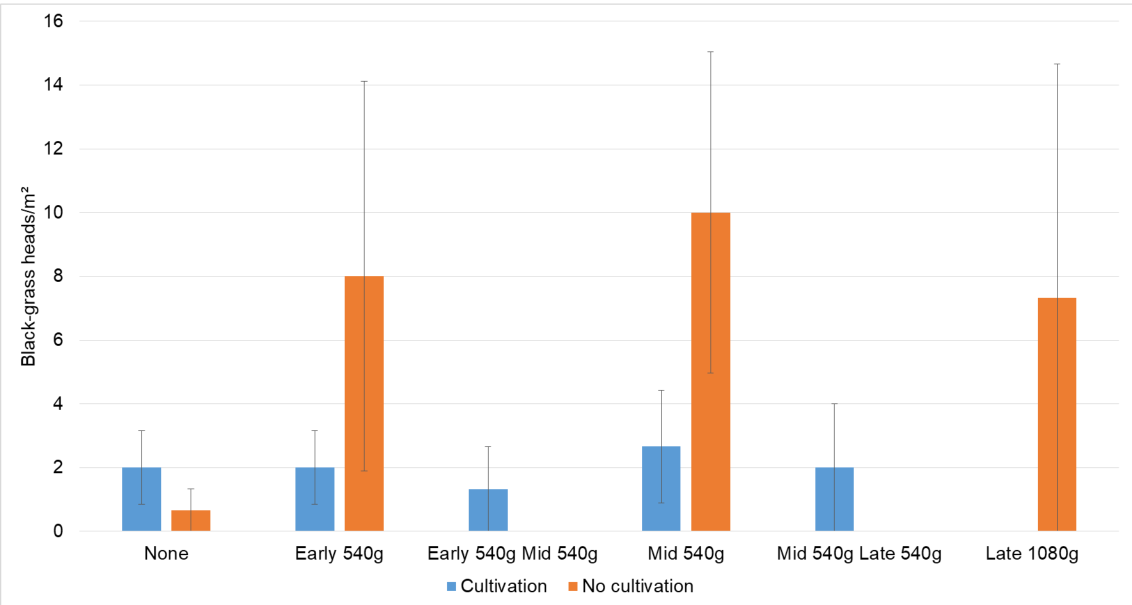

Figure 7 Black-grass head counts – CambsH17

At the LincsF17 black-grass populations were moderate (30 plants/m²) at the start of the trial. Black-grass numbers increased through September and October (Figure 8) but glyphosate applications reduced populations. The cultivation was done on the same day as drilling, 24 October 2016.

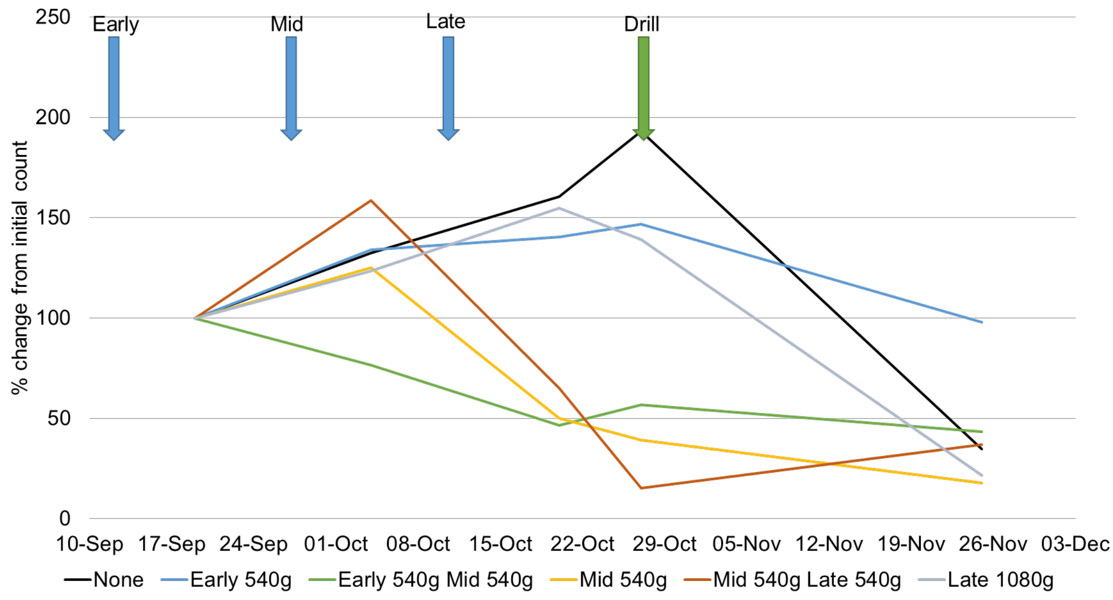

Figure 8 Emergence of black-grass through the autumn and winter in uncultivated plots, percentage change compared to initial count – LincsF17

There were significantly fewer black-grass plants in November where the cultivation were done (Table 42) and where glyphosate had been applied at the mid and late timings and where two applications were made.

Table 42 Mean black-grass plant counts on 25 November 2016 - LincsF17

Treatment	Cultivation	No cultivation	mean herbicide	
None	15	10	13	
Early 540g	11	31	21	
Early 540g Mid 540g	1	9	5	
Mid 540g	5	7	6	
Mid 540g Late 540g	3	11	7	
Late 1080g	1	7	4	
Mean cultivation	6	12		
	Fpr	***d.f***	***s.e.d***	***l.s.d***
Cultivation	*0.021*	*22*	*2.56*	*5.28*
Herbicide	*0.008*	*22*	*4.43*	*9.14*
Cult x herb	*NS*	*22*	*6.27*	*12.93*

The November plant counts were reflected in the final head counts (Table 43, Figure 9), with significantly fewer black-grass in the cultivated plots. Black-grass populations were significantly lower in the mid and late glyphosate timings and where two applications were made.

Table 43 Black-grass head counts LincsF17

Treatment	Cultivation	No cultivation	mean herbicide	
None	395	552	474	
Early 540g	392	994	693	
Early 540g Mid 540g	78	91	84	
Mid 540g	63	127	95	
Mid 540g Late 540g	14	45	30	
Late 1080g	37	62	50	
Mean cultivation	163	312		
	Fpr	***d.f***	***s.e.d***	***l.s.d***
Cultivation	*0.013*	*22*	*54.9*	*113.8*
Herbicide	*<0.001*	*22*	*95.0*	*197.1*
Cult x herb	*0.038*	*22*	*134.4*	*278.7*

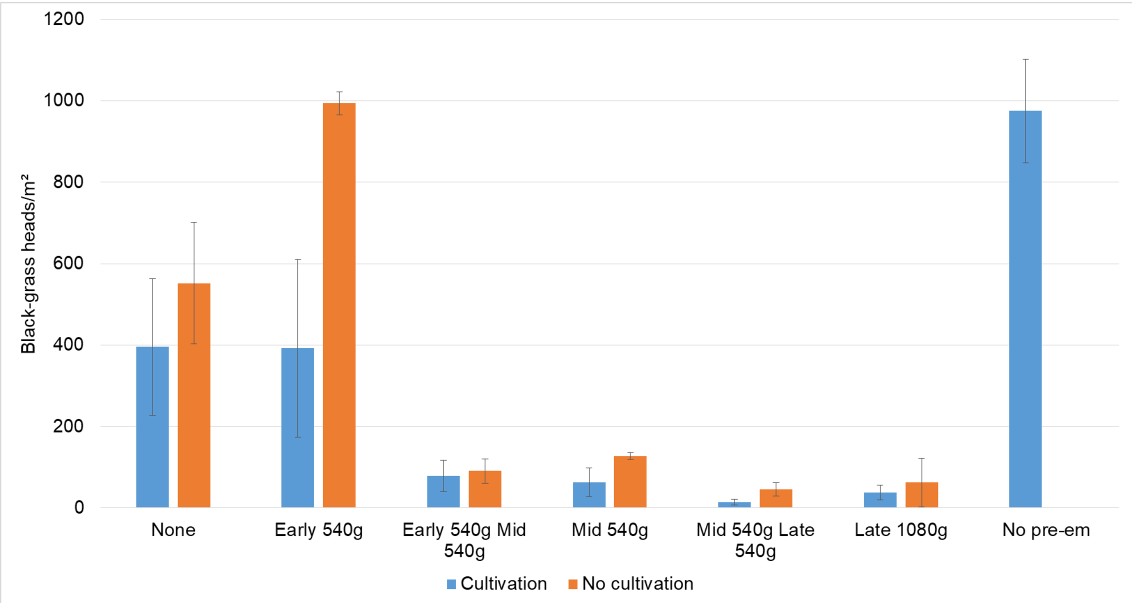

Figure 9 Black-grass head counts – LincsF17

At the CambsA17 site initial black-grass populations were high in September (124 plants/m²), but there was no indication of further emergence after glyphosate applications (Figure 10). The shallow cultivation was done on 25 October 2016 and the trial drilled on 26 October 2016. All glyphosate treatments had reduced black-grass populations to low levels by November 2016 (6 plants/m²).

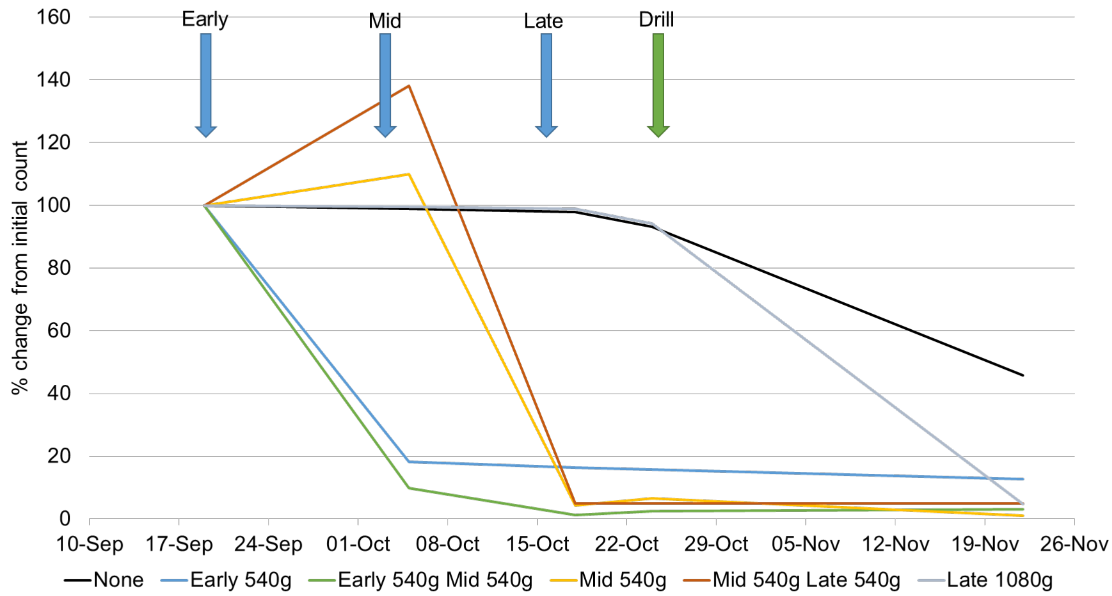

Figure 10 Emergence of black-grass through the autumn and winter in uncultivated plots, percentage change compared to initial count – CambsA17

At the November plant count (Table 44) there were significantly more black-grass in the cultivated plots. Black-grass populations were significantly lower in the mid and late timings and where two applications were made.

Table 44 Black-grass counts on 22 November 2016 - CambsA17

Treatment	Cultivation	No cultivation	Mean herbicide	
None	44	13	29	
Early 540g	14	6	10	
Early 540g Mid 540g	3	1	2	
Mid 540g	2	1	2	
Mid 540g Late 540g	5	5	5	
Late 1080g	5	4	5	
Mean cultivation	12	5		
	Fpr	***d.f***	***s.e.d***	***l.s.d***
Cultivation	*0.016*	*22*	*0.8*	*5.81*
Herbicide	*<0.001*	*22*	*4.85*	*10.06*
Cult x herb	*0.035*	*22*	*6.86*	*14.23*

At the head count (Table 45, Figure 11) there were significantly fewer heads in the cultivated plots and no difference between the glyphosate treatments.

Table 45 Black-grass head counts CambsA17

Treatment	Cultivation	No cultivation	mean herbicide	
None	49	119	84	
Early 540g	8	48	28	
Early 540g Mid 540g	23	19	21	
Mid 540g	9	11	10	
Mid 540g Late 540g	16	18	17	
Late 1080g	9	15	12	
Mean cultivation	19	38		
	Fpr	***d.f***	***s.e.d***	***l.s.d***
Cultivation	0.037	22	8.63	17.91
Herbicide	<0.001	22	14.95	31.01
Cult x herb	NS			

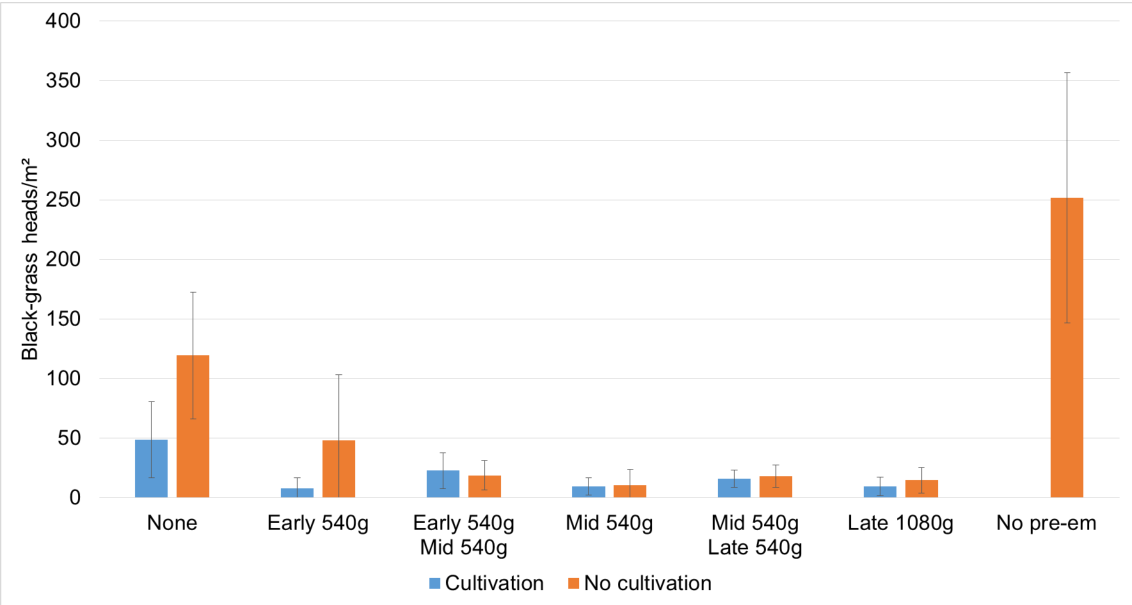

Figure 11 Black-grass head counts – CambsA17

2018

Table 46 Black-grass population information for 2018 sites

Site name	Mean Black-grass/m² Autumn	Mean black-grass/m² Spring	Mean black-grass heads/m²
LincsF18	16	0	11
CambsH18	370	21	90

The autumn of 2017 was considerably wetter than in 2016. At the LincsF18 site the shallow cultivation was done on 26 October 2017, the same day as drilling. Black-grass populations were low (16 plants/m²) at the start of the trial but there was further emergence which peaked in the untreated in early October (Figure 12). The act of drilling reduced populations in the uncultivated treatment (Figure 12).

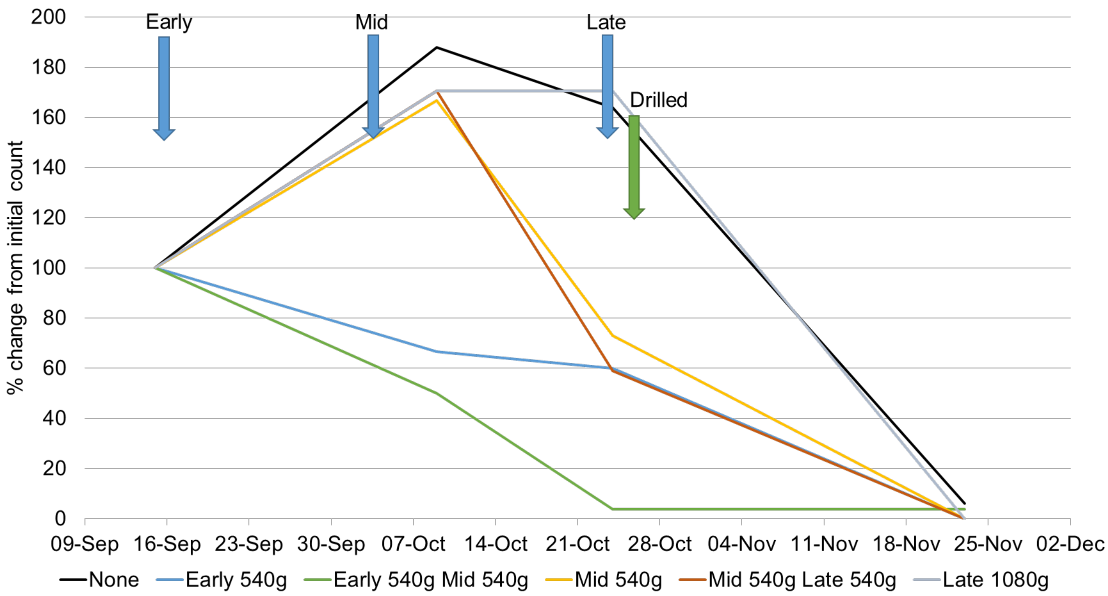

Figure 12 Emergence of black-grass through the autumn and winter in uncultivated plots, percentage change compared to initial count – LincsF18

Head numbers were low after the low autumn populations of black-grass (Table 47, Figure 13). Although there were more black-grass heads in the cultivated plots, this was not significantly different. There were no differences between the herbicide treatments.

Table 47 Black-grass head counts LincsF18

Treatment	Cultivation	No cultivation	Mean herbicide	
None	67	15	41	
Early 540g	6	5	5	
Early 540g Mid 540g	4	0	2	
Mid 540g	0	1	0	
Mid 540g Late 540g	1	0	0	
Late 1080g	4	0	2	
Mean cultivation	14	3		
	Fpr	***d.f***	***s.e.d***	***l.s.d***
Cultivation	*NS*			
Herbicide	*NS*			
Cult x herb	*NS*			

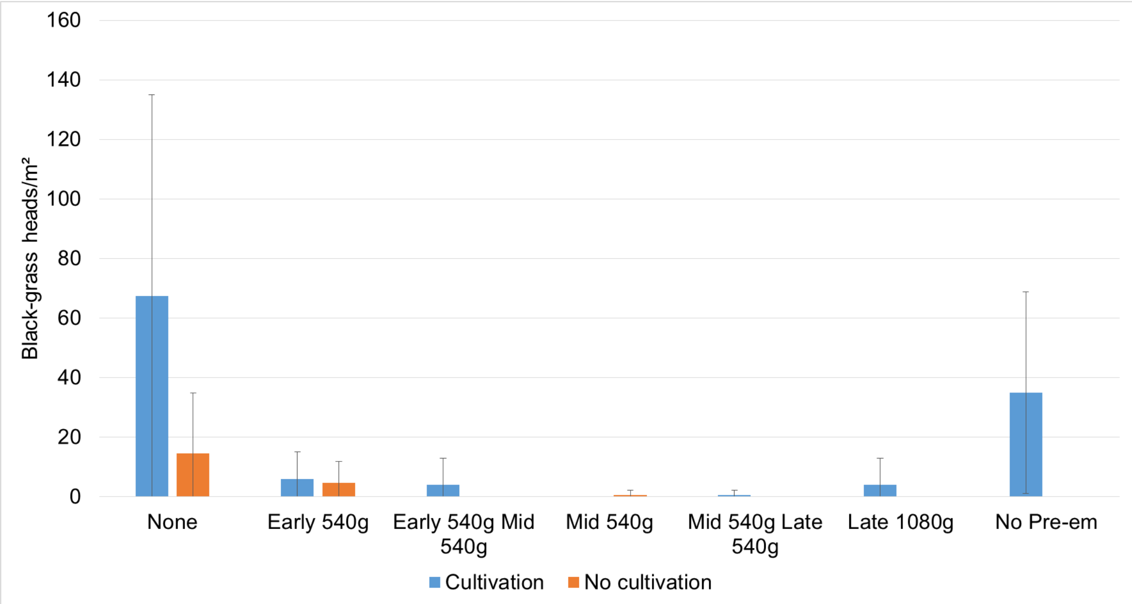

Figure 13 Black-grass head counts – LincsF18

Initial black-grass populations at the CambsH18 site were very high (370 plants/m²). The shallow cultivation was done on the same day as drilling 25 October 2017. Black-grass continued to emerge at this site until drilling (Figure 14), the early application of glyphosate was less effective due to continued emergence of black-grass. Both drilling and cultivation reduced populations.

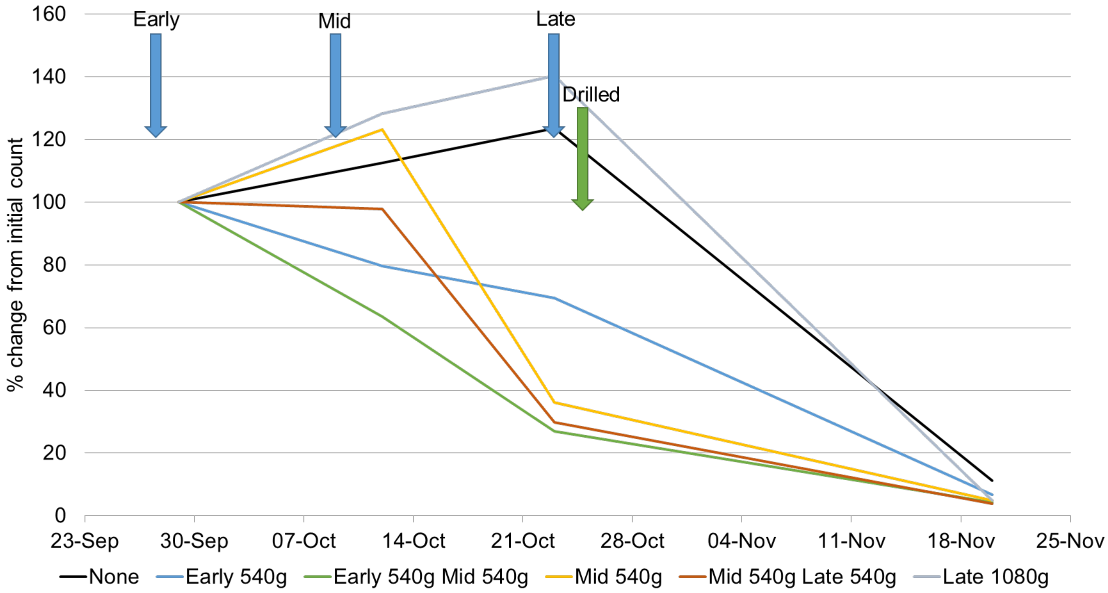

Figure 14 Emergence of black-grass through the autumn and winter in uncultivated plots, percentage change compared to initial count – CambsH18

Table 48 Black-grass counts on 20 November 2017 - CambsH18

Treatment	Cultivation	No cultivation	Mean herbicide	
None	33	36	35	
Early 540g	28	25	27	
Early 540g Mid 540g	27	17	22	
Mid 540g	24	18	21	
Mid 540g Late 540g	16	14	15	
Late 1080g	28	19	23	
Mean cultivation	26	21		
	Fpr	***d.f***	***s.e.d***	***l.s.d***
Cultivation	*0.003*	*22*	*2.35*	*4.88*
Herbicide	*NS*			
Cult x herb	*NS*			

Black-grass populations were significantly higher in the cultivated treatment in November (Table 49). There were no differences between the glyphosate treatments at this date.

At the head count (Table 49, Figure 15) there were significant differences between the untreated and the glyphosate treatments. There were no differences between the glyphosate rates and timings.

Table 49 Black-grass head counts CambsH18

Treatment	Cultivation	No cultivation	mean herbicide	
None	127	145	136	
Early 540g	35	58	46	
Early 540g Mid 540g	59	48	53	
Mid 540g	38	55	47	
Mid 540g Late 540g	30	47	38	
Late 1080g	39	39	39	
Mean cultivation	55	65		
	Fpr	***d.f***	***s.e.d***	***l.s.d***
Cultivation	*NS*			
Herbicide	*0.001*	*22*	*21.39*	*44.37*
Cult x herb	*NS*			

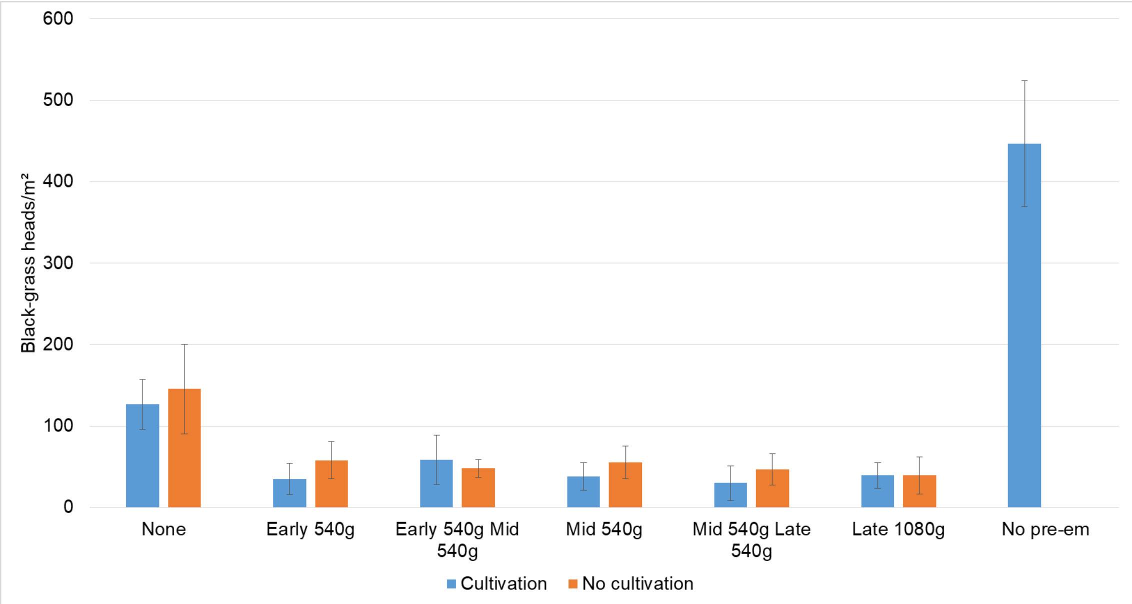

Figure 15 Black-grass head counts – CambsH18

Conclusions from winter wheat sites 2017-18

- Seasonal differences and seed bank variations had a big effect on these trials, often making them difficult to compare. There were different starting points in terms of post-harvest cultivations and seed bed establishment that again must be considered when comparing these trials.
- A cultivation at or near drilling was effective at reducing black-grass populations in 50% of cases. It could result in increased black-grass levels (1 case) or have no effect (2 cases).
- An early application (mid-September) of glyphosate was generally ineffective at reducing black-grass populations due to further emergence of the weed (4 out of 5 sites).
- In all cases a minimum of 540g a.s./ha glyphosate reduced black-grass populations compared to no glyphosate.
- A split dose of 540g a.s./ha applied at a mid and late (just before drilling) timing was generally the most effective treatment at reducing the overall black-grass numbers. This treatment also benefited by having two chances to control any later emerging weeds.
- There was no indication that a higher rate of glyphosate or two doses were more effective than a well-timed single dose in these particular experiments. However, it should be noted that the higher rate (1080g a.s./ha) was often applied at the time of drilling due to the window of opportunity to get all treatments in, making it difficult to distinguish this glyphosate treatment from the action of drilling.
- In all cases cultivation and/or an effective glyphosate timing has resulted in lower numbers of black-grass.

Field experiment 2. Optimum rate and timing of glyphosate: 2018-19

Winter wheat sites 2018-2019

There were two sites drilled to winter wheat. Glyphosate at 720g a.s./ha was applied at two timings, in mid-September (early) and mid-October (late), separately and as a sequence. The rate of 720g a.s./ha was selected by the project steering group as this was considered to be the rate that most farmers were now using in the field and was the most effective in the previous container based trials within this project. There were three cultivations - no cultivations until drilling (direct drilling), a shallow cultivation between the two glyphosate applications and a flexible treatment which was power harrowing on 8 October at LincsF19 and combination drilling at CambsA19. Autumn 2018 was extremely wet delaying the planned cultivation and drilling timings.

At the LincsF19 site cultivations began on 26 September and the crop was drilled on 9 November 2018, black-grass populations in the untreated were 20 plants/m², during this period there was little further emergence of black-grass.

At LincsF19 there were significantly fewer black-grass heads where glyphosate had been applied but there were no significant differences between the timings (Table 50, Figure 16). There were no significant effects of the cultivations on black-grass populations.

Table 50 Mean black-grass head number – LincsF19

	Herbicide rate and timing				
Cultivation	None	Early 720g	Late 720g	Early 720g Late 720g	*Cultivation[1] mean*
Power harrow 08/10/18	137	1	26	1	*42*
Direct drill 09/11/18	143	10	3	25	*45*
Straw rake 26/09/18	177	19	23	26	*61*
Herbicide[2] mean	*152*	*10*	*17*	*17*	

[1]Cultivation NS

[2]Herbicide P=0.008, SED 43.1, LSD 89.5

Cultivation x herbicide NS

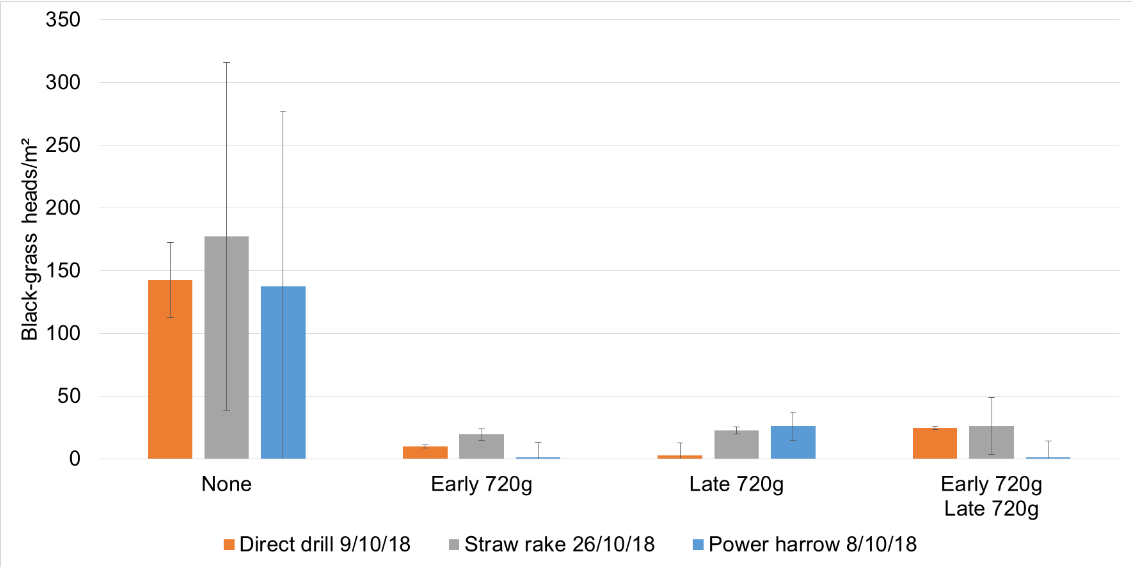

Figure 16 Mean black-grass head counts – LincsF19

At the CambsA19 site the first application of glyphosate was made on 13 September 2018, and the late application on 11 October 2018. At this site the black-grass pressure was very high as it continued to emerge after drilling, with up to 160 plants/m² present in November. As a consequence black-grass head numbers were high. There were no differences in head numbers between the untreated and sequenced dose but significantly (p=0.034) less black-grass heads in the early and late separate treatments. The late treatment having the fewest heads (Table 51, Figure 17). There were significantly (p=<0.001) fewer black-grass heads in the combi drilled treatment than the other cultivation treatments alone without any glyphosate.

Table 51 Mean black-grass head number – CambsA19

	Herbicide rate and timing				
Cultivation	None	Early 720g	Late 720g	Early 720g + Late 720g	Cultivation mean
Direct drill 12/10/18	518	461	338	541	465
Shallow 14/09/18	497	474	348	567	472
Combi drill12/10/19	479	366	356	424	406
Herbicide mean	498	434	347	511	

[1]*Cultivation P=<0.001, SED 29.4, LSD 61.0*

[2]*Herbicide P=0.034, SED 25.5, LSD 52.9*

Cultivation x herbicide NS

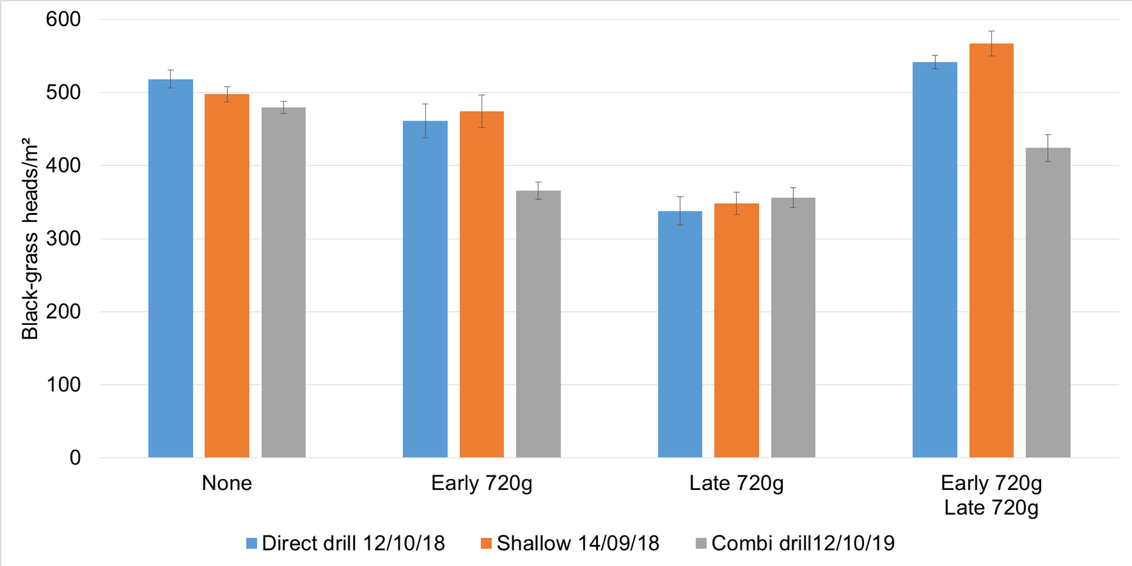

Figure 17 Black-grass head counts – CambsA19

Spring wheat sites 2018-2019

There were two field sites in spring wheat drilled in 2019 in Cambridgeshire, CambsBX19 and CambsH19. A single application of 720g of glyphosate was made, either in the autumn or in the spring pre-drilling. One treatment included a single shallow cultivation 10 days after the autumn glyphosate application, the other treatment remained uncultivated up to drilling.

At Cambs BX19 black-grass populations were high in October (mean 1178 plants/m²). Autumn cultivation had a significantly decreased black-grass numbers and this effect was enhanced by the spring application of glyphosate (Figure 18). The effect of cultivation was greater than the effect of the glyphosate on black-grass populations.

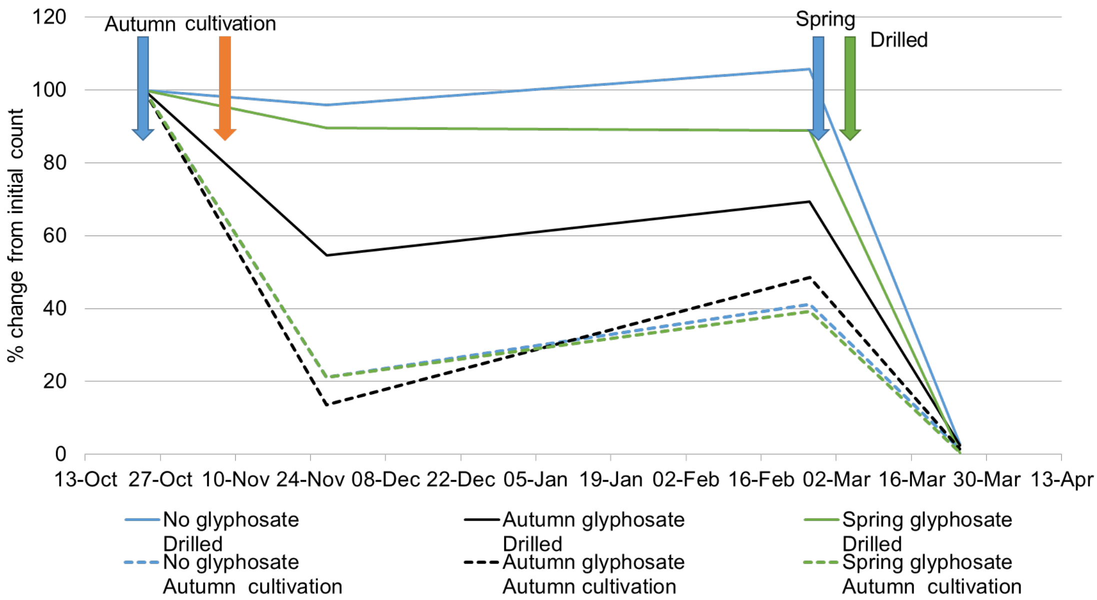

Figure 18 Emergence of black-grass through the autumn and winter, percentage change compared to initial count – CambsBX19

At the black-grass head count the value of a shallow cultivation in the autumn compared to no cultivation was a 59% reduction in black-grass head numbers (Table 52, Figure 19). There were fewest heads (14 heads/m^2) in the autumn cultivated, followed by spring glyphosate before drilling treatment. However, the spring glyphosate application before drilling with no autumn cultivation was also extremely effective at reducing the black-grass heads within the crop (40 heads/m^2) in this particular trial. A shallow cultivation was more effective at reducing black-grass head number than no cultivation and a spring glyphosate application over an autumn one.

Table 52 Mean black-grass head number – CambsBX19

	Herbicide rate and timing			
Cultivation	None	Autumn 720g	Spring 720g	*Cultivation[1] mean*
Drill 02/03/19	396	198	40	*211*
Autumn cultivation 09/11/18	184	86	14	*94*
Herbicide[2] mean	*290*	*142*	*27*	

[1]Cultivation P=<0.001, SED 26.6, LSD 56.7

[2]Herbicide P=0.034, SED 32.6, LSD 69.4

Cultivation x herbicide P=0.039, SED 46.1, LSD 98.2

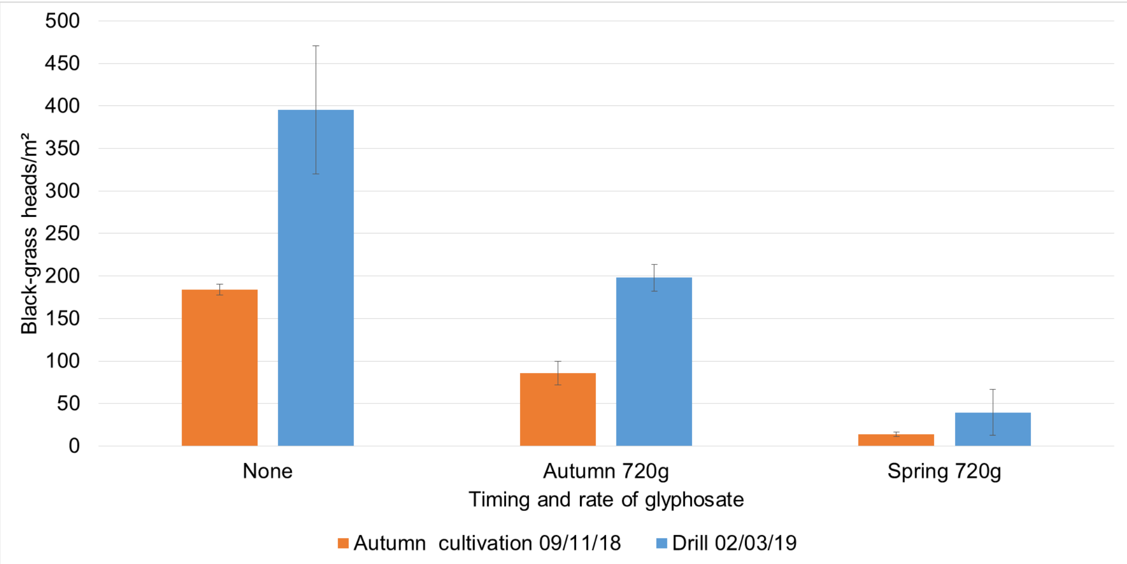

Figure 19 Black-grass head counts – CambsBX19

At the CambsH19 site the results were similar although mean black-grass population was lower (128 plants/m²). Autumn cultivation had a significant effect on black-grass numbers and this effect was enhanced by the spring application of glyphosate (Figure 20). An autumn cultivation was very effective at reducing black-grass head numbers by 51% (Table 53, Figure 21). In this particular trial and season the spring application of glyphosate was effective at removing the majority of black-grass.

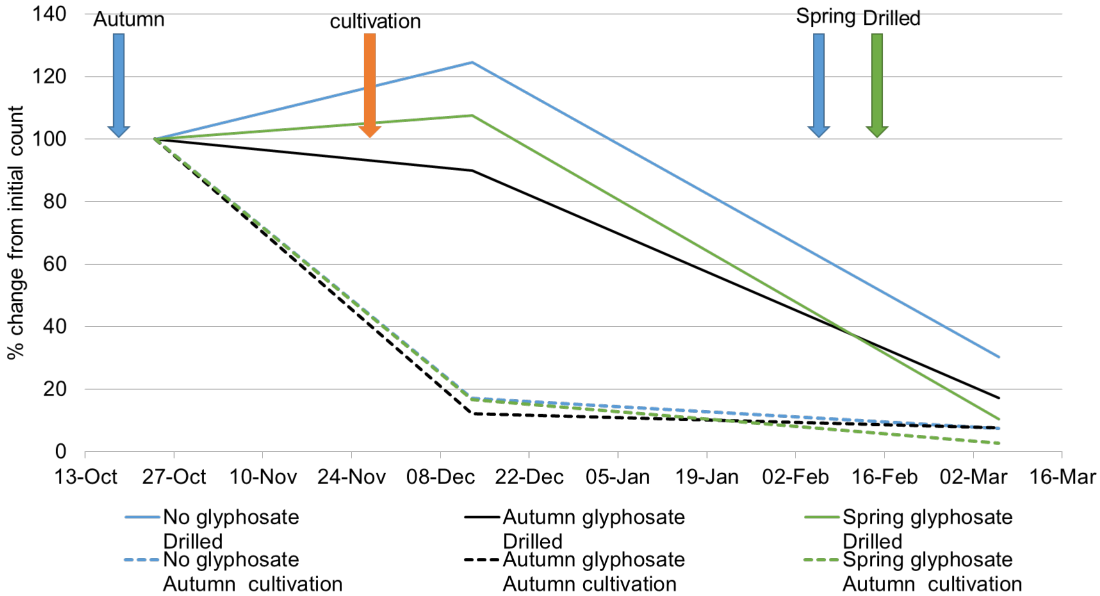

Figure 20 Emergence of black-grass through the autumn and winter, percentage change compared to initial count – CambsH19

Table 53 Black-grass head number – CambsH19

	Herbicide rate and timing			
Cultivation	None	Autumn 720g	Spring 720g	*Cultivation[1] mean*
Drill 17/02/19	21	17	0	*13*
Autumn cultivation 25/11/18	9	9	1	*6*
Herbicide[2] mean	*15*	*13*	*0*	

[1]Cultivation P=0.068, SED 3.33, LSD 6.96

[2]Herbicide P=<0.001, SED 3.84, LSD 8.04

Cultivation x herbicide NS

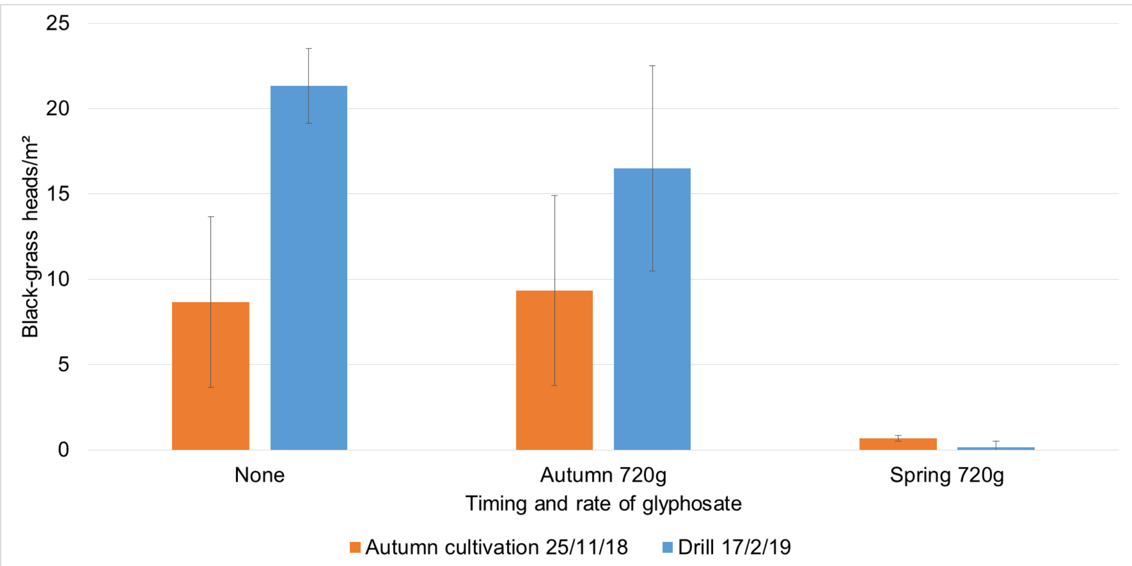

Figure 21 Black-grass head counts – CambsH19

Spring Barley 2019-2020

At the CambsBX20 the value of the autumn cultivation was shown again in a significant reduction of black-grass populations in combination with a glyphosate application (Figure 22). There was a flush of black-grass emergence between December and March at this site, more so in the cultivated treatment. The spring cultivation and application of glyphosate were done on the same day and this alone or in combination with drilling reduced populations further.

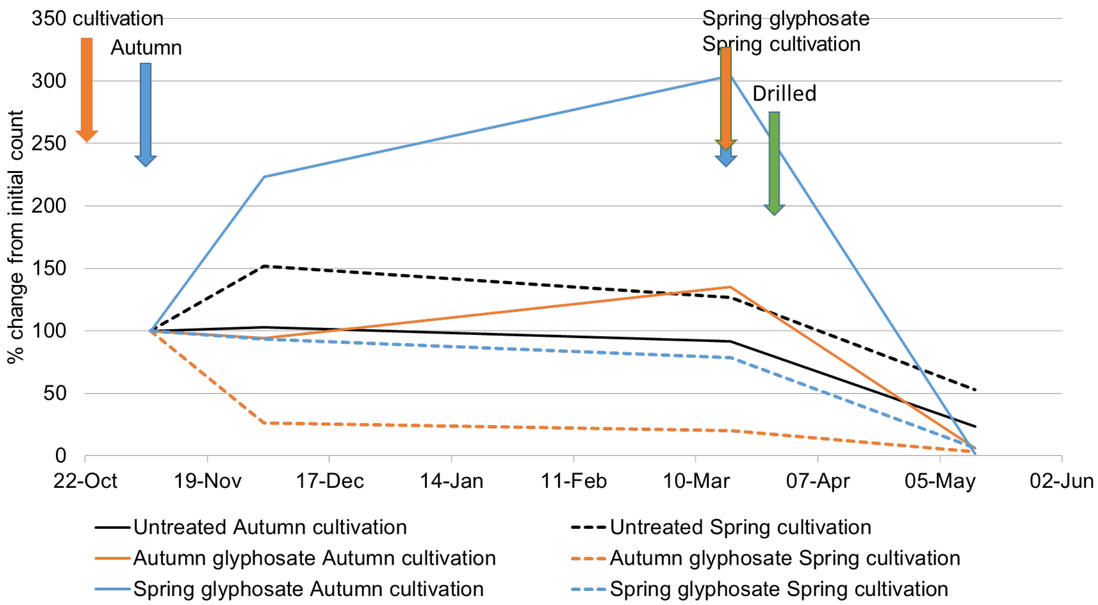

Figure 22 Emergence of black-grass through the autumn and winter, percentage change compared to initial count – CambsBX20

Head numbers followed a similar pattern to the trials in 2019, where autumn cultivation was done there were significantly reduced numbers (Table 54, Figure 23). There were significantly lower head numbers in the glyphosate treated plots but no differences between the treatments.

Table 54 Black-grass head number – CambsBX20

	Herbicide rate and timing			
Cultivation	None	Autumn 720g	Spring 720g	*Cultivation[1] mean*
Autumn cultivation 23/10/19	124	12	4	*47*
Spring cultivation 19/03/20	241	48	123	*163*
Herbicide[2] mean	*183*	*30*	*63*	

[1]Cultivation P=0.007, SED 34.4, LSD 71.16

[2]Herbicide P=0.007, SED 44.3, LSD 91.7

Cultivation x herbicide NS

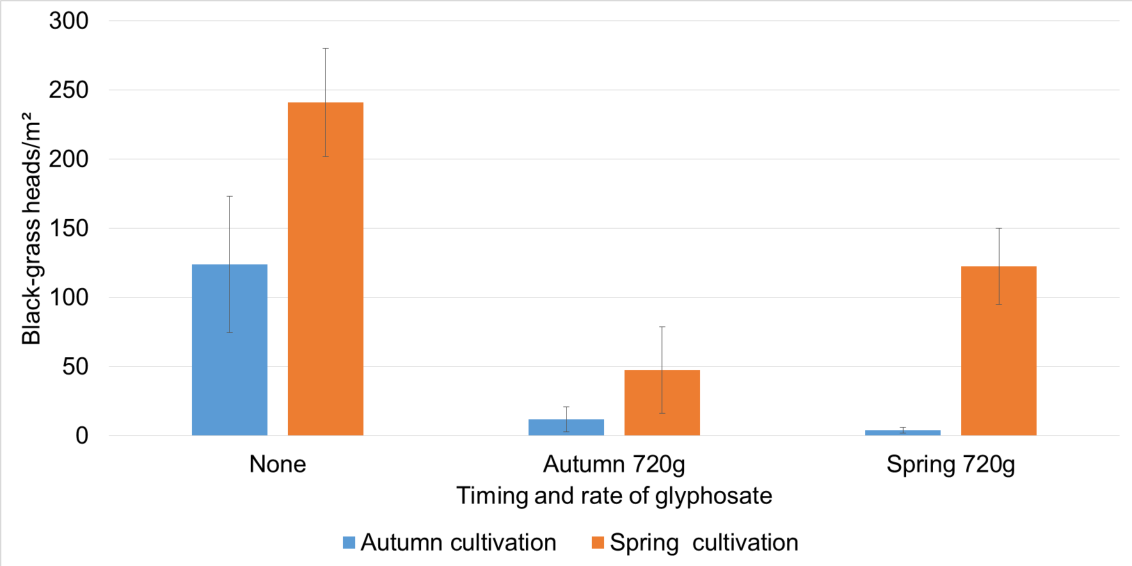

Figure 23 Black-grass head counts – CambsBx20

4.1.3. **Conclusions**

Winter wheat sites

- The effect of cultivations was difficult to conclude from the winter crop trial sites as there were seasonal challenges resulting in the cultivations not showing any significant differences from each other. At one site cultivation at drilling was effective at reducing black-grass populations compared to no cultivation.
- All glyphosate applications reduced the black-grass numbers significantly compared to no glyphosate.
- There was a very big difference in natural black-grass population between the two sites (one very low, one very high) making the overall interpretation of these results difficult.

Spring cereal sites

- These trials confirmed the value of a cultivation in the autumn for black-grass control, which reduced numbers (55% reduction on average compared to uncultivated) and selection pressure for the glyphosate application in the following spring.
- A spring application of glyphosate was the most effective in reducing black-grass numbers compared to the autumn only application of glyphosate.

4.2. Container experiments (WP1.2 and WP3)

4.2.1. Container Experiment 1. The effect of glyphosate dose against weed growth stage

The mean number of black-grass plants and heads per container are summarised in Table 55 for each of the three populations tested in 2015/16. The percentage reduction from the untreated control for black-grass plants are summarised in Figure 24.

Table 55 The mean number of black-grass plants and heads per container in 2016

Timing	Glyphosate rate g a.s./ha	Population					
		Susceptible		Moderate Resistant (BG01)		Peldon Resistant	
		Plants	Heads	Plants	Heads	Plants	Heads
GS10	-	20.33	48.30	13.67	35.70	13.33	58.70
GS 12-13	-	32.67	59.00	13.67	57.70	30.33	73.70
GS 21-22	-	18.00	27.00	10.00	43.30	27.67	52.70
GS10	360	17.00	40.00	6.00	62.30	17.67	89.00
GS10	540	8.33	43.30	8.00	46.00	11.33	25.00
GS10	720	0.00	0.00	0.33	6.70	0.00	0.00
GS 12-13	360	7.33	14.70	6.67	22.00	5.67	61.70
GS 12-13	540	0.00	0.00	0.00	0.00	0.00	3.70
GS 12-13	720	0.00	0.00	0.00	0.00	0.00	0.00
GS 21-22	360	3.00	0.00	1.33	19.30	5.67	62.30
GS 21-22	540	0.33	9.00	0.00	6.00	0.67	7.30
GS 21-22	720	0.33	0.00	0.00	0.30	0.00	0.00

		Treatment	***Population***	***Treatment*population***
d.f	*Plants*	*70*	*70*	*70*
	Heads	*69*	*69*	*69*
s.e.d	*Plants*	*1.386*	*2.772*	*4.801*
	Heads	*9.94*	*4.97*	*17.22*
l.s.d	*Plants*	*2.764*	*5.529*	*9.576*
	Heads	*19.83*	*9.91*	*34.35*
Fpr	*Plants*	*0.004*	*<.001*	*0.126*
	Heads	*<.001*	*0.006*	*0.267*

There was a significant difference between treatments, in this case glyphosate rate and timing of application, for both the number of plants (F pr 0.004) and highly significant difference for heads (Fpr <0.001). Glyphosate applied at a rate of 540g a.s./ha and 720g a.s./ha at a black-grass growth stage of GS12-13 was the most effective overall treatment for all three populations achieving 100% control. A rate of 720g a.s./ha of glyphosate was required to control the larger weed growth stage of GS21-22, tillering plants (Figure 24 and Table 55).

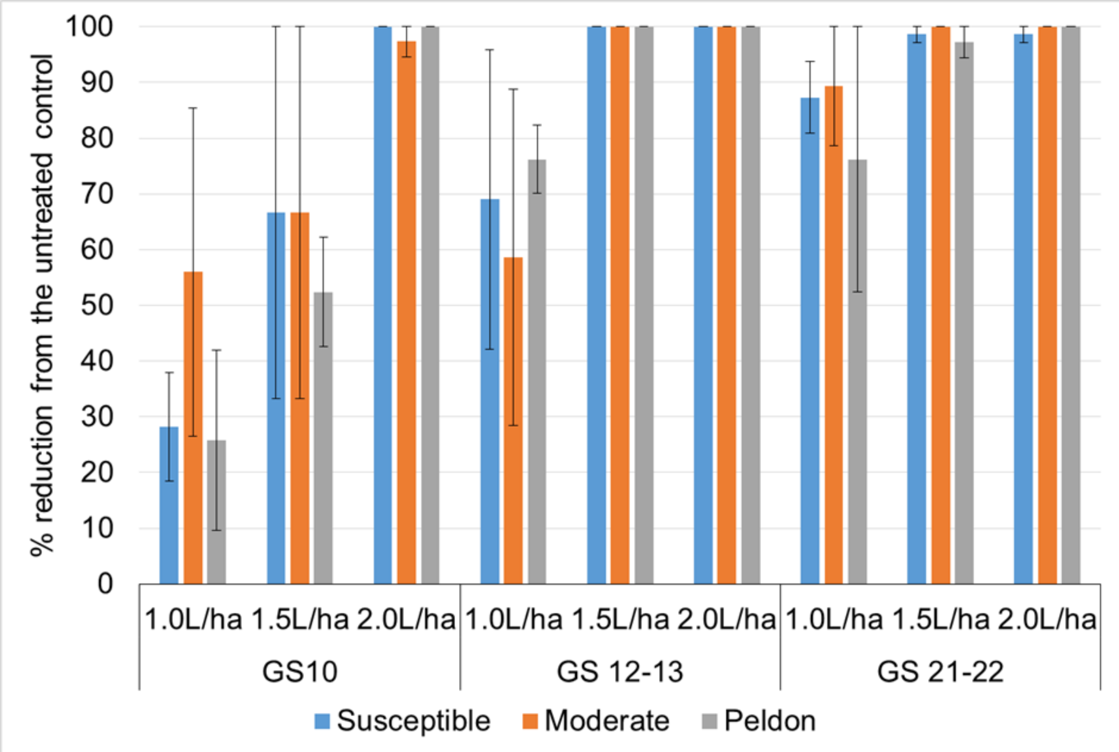

Figure 24 The percentage reduction from the untreated control of mean number of black-grass plants per container in 2016, treated with glyphosate ate different rates and timings.

The mean number of Italian rye-grass (IRG) plants and heads per container are summarised in Table 56 for each of the three populations tested in 2015/16. The percentage reduction from the untreated control for IRG plants are summarised in Figure 25.

There was a significant difference between treatment, in this case glyphosate rate and timing of application, and population of the number of heads (Fpr <0.001). Glyphosate applied at a rate of 720g a.s./ha at a weed growth stage of GS12-13 was the most effective overall treatment for all three populations achieving 100% control (Table 56 and Figure 25). Glyphosate at 540g a.s./ha at the same growth stage achieved 100% control of the susceptible and moderately resistant population, however for the IRG resistant population an extremely low number of surviving plants remained (a mean of less than one plant/container). A low glyphosate rate to a large weed growth stage achieved extremely low efficacy in all three IRG populations.

Table 56 The mean number of Italian rye-grass plants and heads per container in 2016

Timing	Glyphosate Rate g a.s./ha	Population Susceptible		Moderate resistance (IRG01)		Resistant	
		Plants	Heads	Plants	Heads	Plants	Heads
GS10	-	40.00	55.30	37.00	79.70	45.00	78.70
GS 12-13	-	36.70	88.30	55.70	68.70	52.30	96.00
GS 21-22	-	30.70	97.00	32.70	34.70	39.70	44.00
GS10	360	25.00	78.70	18.30	14.00	25.00	36.00
GS10	540	22.00	38.30	22.30	28.70	25.70	75.70
GS10	720	6.00	0.00	5.00	0.00	0.00	26.70
GS 12-13	360	0.70	29.00	0.00	0.00	11.00	33.30
GS 12-13	540	0.00	0.00	0.00	0.00	0.70	12.30
GS 12-13	720	0.00	0.00	0.00	0.00	0.00	0.00
GS 21-22	360	23.70	44.70	7.00	36.70	32.30	52.00
GS 21-22	540	1.00	37.30	3.00	2.00	5.30	0.00
GS 21-22	720	2.00	20.30	0.70	12.70	11.00	13.70

		Treatment	***Population***	***Treatment*population***
d.f		*70*	*70*	*70*
s.e.d	*Plants*	*3.59*	*7.18*	*12.43*
	Heads	*4.56*	*9.12*	*15.8*
l.s.d	*Plants*	*7.16*	*14.32*	*24.8*
	Heads	*9.1*	*18.19*	*31.51*
Fpr	*Plants*	*0.241*	*<.001*	*0.996*
	Heads	*<.001*	*<.001*	*0.004*

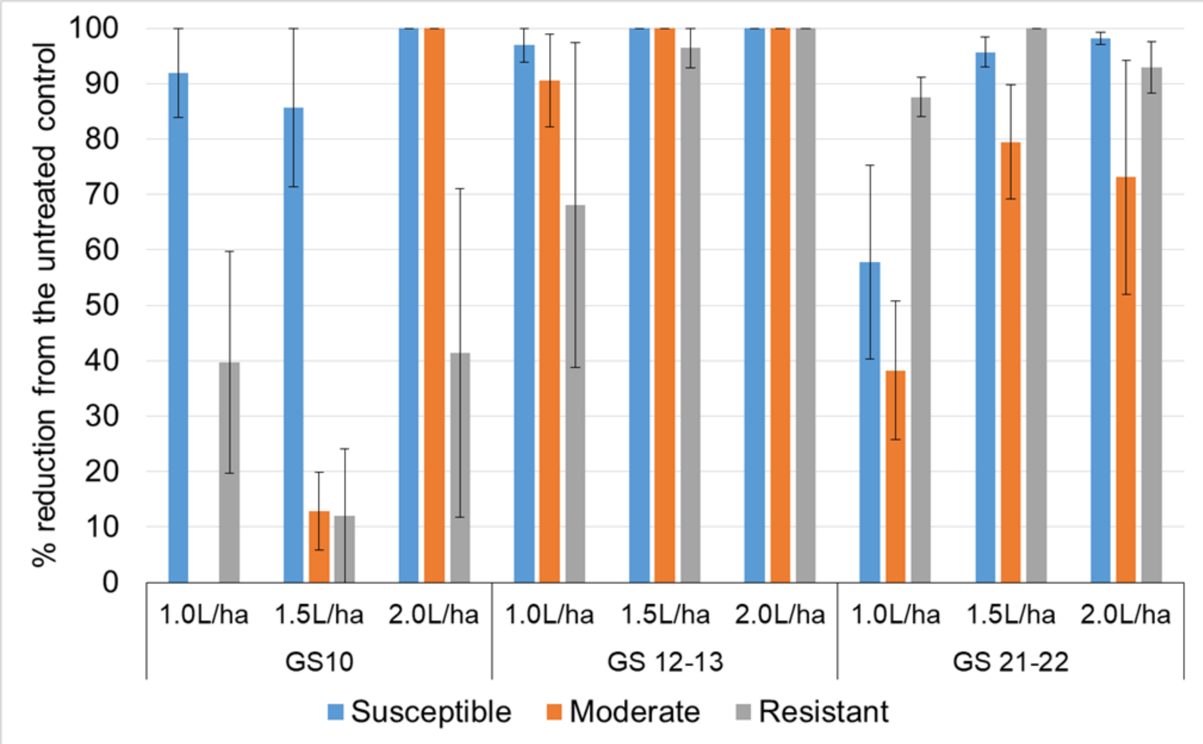

Figure 25 The percentage reduction from the untreated control of mean number of Italian rye-grass plants per container in 2016, treated with glyphosate ate different rates and timings.

The mean number of black-grass plants and heads per container are summarised in Table 57 for each of the three populations tested in 2016/17. The percentage reduction from the untreated control for black-grass plants are summarised in Figure 26. Only one untreated control treatment was included in this year reducing the treatment number to 10. Control of black-grass plants was generally much higher in this trial year.

Table 57 The mean number of black-grass plants and heads per container in 2017

Timing	Glyphosate rate g a.s./ha	Population					
		Susceptible		Moderate resistant (BG01)		Peldon Resistant	
		Plants	Heads	Plants	Heads	Plants	Heads
	-	37.00	171.70	20.67	123.30	28.33	118.00
GS10	360	0.00	0.00	0.00	0.00	0.67	14.00
GS10	540	0.00	0.00	0.00	0.00	0.00	0.00
GS10	720	0.00	0.00	0.00	0.00	1.00	16.30
GS 12-13	360	0.00	0.00	0.00	0.00	0.00	0.00
GS 12-13	540	0.00	0.00	0.00	0.00	0.00	0.00
GS 12-13	720	0.00	0.00	0.00	0.00	0.00	0.00
GS 21-22	360	18.00	146.70	16.00	78.30	16.00	137.30
GS 21-22	540	6.00	79.30	2.33	72.30	5.33	33.30
GS 21-22	720	0.33	14.00	1.33	65.70	0.00	0.00

		Treatment	***Population***	**Treatment*population**
d.f		*58*	*58*	*58*
s.e.d	*Plants*	*0.999*	*1.823*	*3.158*
	Heads	*4.47*	*8.16*	*14.13*
l.s.d	*Plants*	*1.999*	*3.65*	*6.321*
	Heads	*8.95*	*16.33*	*28.29*
Fpr	*Plants*	*0.119*	*<.001*	*0.178*
	Heads	*0.103*	*<.001*	*<.001*

Applications at GS10 and GS12-13, at all glyphosate rates for the susceptible and moderately resistant population achieved 100% control (Figure 26). The Peldon resistant population had a few survivors recorded at the smaller growth stage, GS10. When applying glyphosate at GS21-22 a rate of 720g a.s./ha was required, which controlled the Peldon resistant population by 100%, but both the susceptible and moderately resistant populations had enough survivors to produce multiple heads. A lower rate of glyphosate was not enough to control the tillering (GS21-22) plants.

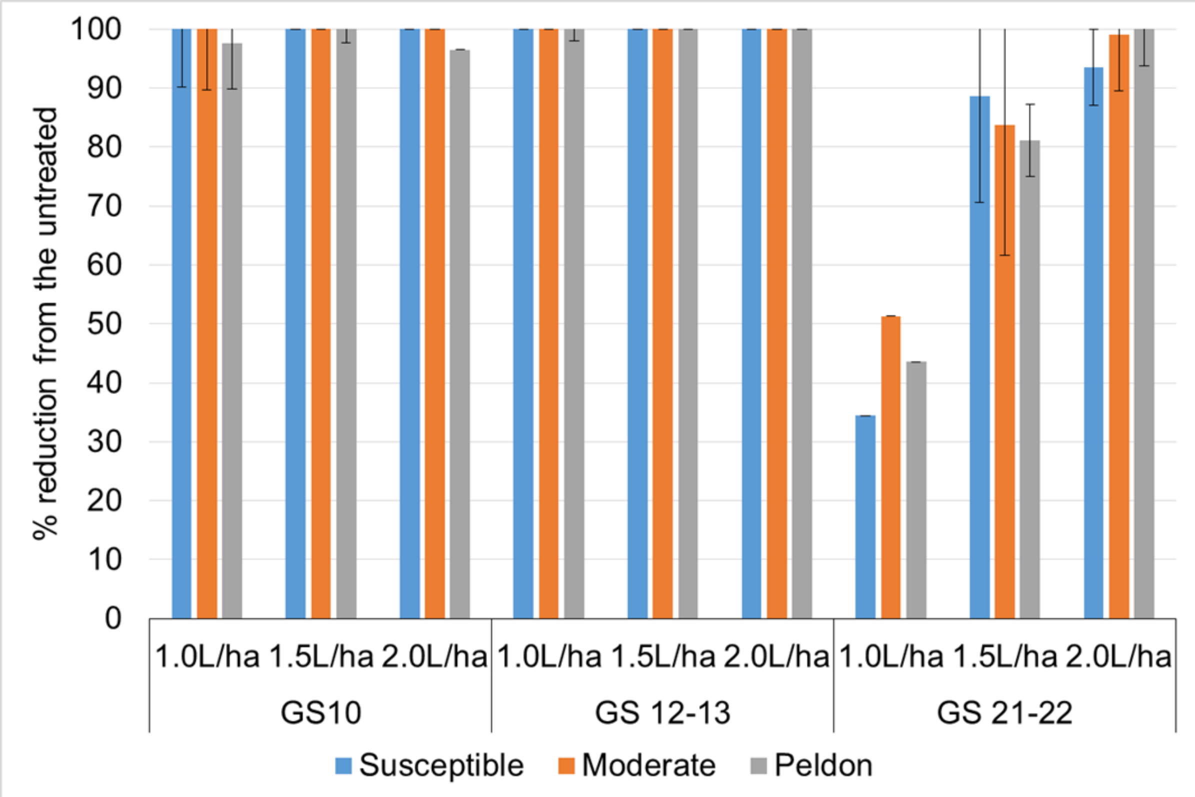

Figure 26 The percentage reduction from the untreated control of mean number of black-grass plants per container in 2017, treated with glyphosate ate different rates and timings.

The mean number of IRG plants and heads per container are summarised in Table 28 for each of the three populations tested in 2016/17. The percentage reduction from the untreated control for IRG plants are summarised in Figure 27. Plants were generally very vigorous for all populations in this season and in the untreated control treatments vast numbers of heads per plant were produced. Again, applications at a weed growth stage of GS12-13, at all glyphosate rates provided 100% control of all three Italian rye-grass populations (Figure 27). Control at GS10 required the higher rate of glyphosate for effective control, however some plants survived in the IRG resistant population.

Seeds collected in summer 2017 were saved and tested in a glasshouse glyphosate dose response experiment (section 3.4.3) to determine any shifts in glyphosate tolerance after two years of selection.

Table 58 The mean number of IRG plants and heads per container in 2017

Timing	Glyphosate rate g a.s./ha	Population					
		Susceptible		Moderate resistant (IRG01)		Resistant	
		Plants	Heads	Plants	Heads	Plants	Heads
	-	23.67	129.00	29.67	170.00	21.00	204.30
GS10	360g	7.67	47.00	1.33	46.30	9.00	234.70
GS10	540g	1.67	49.70	1.33	28.70	2.00	78.30
GS10	720g	0.00	0.00	0.00	0.00	1.00	86.30
GS 12-13	360g	0.00	0.00	0.00	0.00	0.00	0.00
GS 12-13	540g	0.00	0.00	0.00	0.00	0.00	0.00
GS 12-13	720g	0.00	0.00	0.00	0.00	0.00	0.00
GS 21-22	360g	23.33	102.00	27.67	93.70	16.67	191.70
GS 21-22	540g	4.00	30.70	5.00	62.70	5.00	48.70
GS 21-22	720g	1.33	32.70	0.33	14.00	1.33	22.00

		Treatment	Population	Treatment*population
	d.f	*58*	*58*	*58*
s.e.d	*Plants*	*1.069*	*1.951*	*3.38*
	Heads	*9.71*	*17.72*	*30.7*
l.s.d	*Plants*	*2.139*	*3.906*	*6.765*
	Heads	*19.43*	*35.48*	*61.45*
Fpr	*Plants*	*0.681*	*<.001*	*0.231*
	Heads	*<.001*	*<.001*	*<.001*

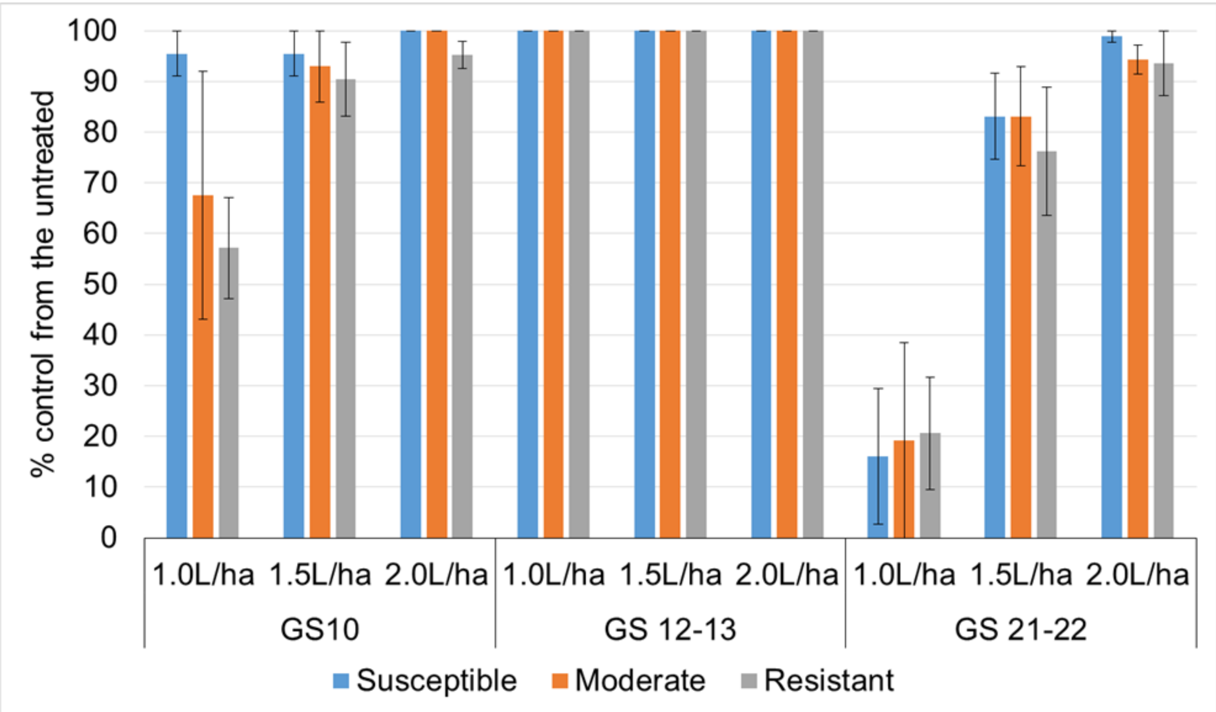

Figure 27 The percentage reduction from the untreated control of mean number of Italian rye-grass plants per container in 2017, treated with glyphosate ate different rates and timings.

4.2.2. **Containers Experiment 2. The effect of glyphosate dose, weed size and cultivations**

This experiment aimed to investigate the effect of cultivation depth (Deep, 10cm; Shallow 5cm) in conjunction with glyphosate rate (360g a.s./ha) and growth stage at application timing. In 2016/17 there was a highly significant effect (p= <0.001) of cultivation, treatment and population alone for both plant and head counts (Table 59), with the exception of population and plant counts where the significance was less (p = 0.002). All two-way interactions were significant on plant counts (Figure 28) but not on the head count data. The deep cultivation (simulating ploughing) significantly reduced the number of black-grass plants compared to shallow cultivation and no cultivation across all populations regardless of glyphosate application or no glyphosate. Shallow cultivation significantly reduced the number of black-grass plants compared to no cultivation in all treatments and populations, except the moderately resistant population at GS12-13.

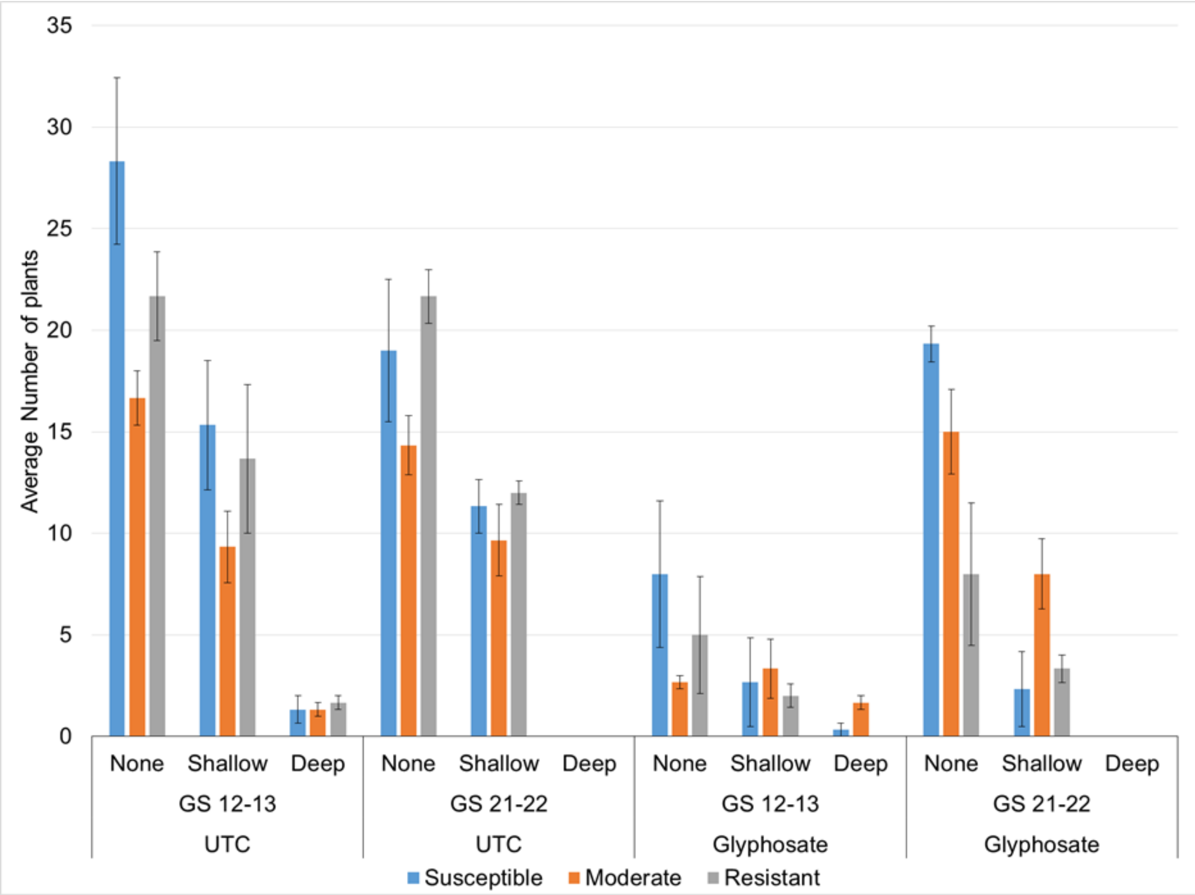

Figure 28 The mean number of plants per container for each black-grass population 2016, with or without glyphosate @ 360g a.s./L, with the addition of no, shallow (5cm) or deep cultivation (10cm).

Table 59 The number of black-grass plants and heads per container in 2016

Treatment	Population					
Herbicide, Cultivation, Growth stage of treatment	Susceptible		Moderate resistant (BG01)		Peldon resistant	
	Plants	Heads	Plants	Heads	Plants	Heads
UTC, Deep, GS 12-13	1.33	14.00	1.33	18.00	1.67	24.70
UTC, Shallow, GS 12-13	15.33	54.00	9.33	81.00	13.67	84.00
UTC, None, GS 12-13	28.33	43.30	16.67	67.00	21.67	79.30
UTC, Deep, GS 21-22	0.00	0.00	0.00	0.00	0.00	0.00
UTC, Shallow, GS 21-22	11.33	32.00	9.67	64.70	12.00	63.30
UTC, None, GS21-22	19.00	48.70	14.33	107.30	21.67	81.00
Glyphosate, Deep, GS 12-13	0.33	4.00	1.67	35.70	0.00	0.00
Glyphosate, Shallow, GS 12-13	2.67	10.30	3.33	39.70	2.00	29.70
Glyphosate, None, GS 12-13	8.00	40.00	2.67	45.70	5.00	40.70
Glyphosate, Deep, GS 21-22	0.00	0.00	0.00	0.00	0.00	0.00
Glyphosate, Shallow, GS 21-22	2.33	14.00	8.00	60.70	3.33	38.30
Glyphosate, None, GS 21-22	19.33	79.00	15.00	90.70	8.00	67.30
	Fpr Plants	*s.e.d Plants*	*l.s.d Plants*	*Fpr Heads*	*s.e.d Heads*	*l.s.d Heads*
Cultivation	*<0.001*	*1.07*	*2.14*	*<0.001*	*5.92*	*11.80*
Herbicide	*<0.001*	*0.62*	*1.23*	*<0.001*	*3.42*	*6.81*
Population	*0.02*	*0.76*	*1.51*	*<0.001*	*4.18*	*8.34*
Cultivation.Herbicide	*<0.001*	*1.51*	*3.02*	*0.001*	*8.37*	*16.69*
Cultivation.Population	*0.02*	*1.86*	*3.70*	*0.177*	*10.25*	*20.44*
Herbicide.Population	*0.00*	*1.07*	*2.14*	*0.068*	*5.92*	*11.80*
Cultivation.Herbicide.Population	*0.07*	*2.62*	*5.23*	*0.458*	*14.49*	*28.90*

Similar trends were shown for the IRG experiment (Table 60 & Figure 29). The deep cultivation always gave the highest level of control compared to the shallow or no cultivation, except for the resistant population at GS12-13. Glyphosate applied at GS12-13 significantly (p=<0.001) increased the level of control for every cultivation and for the susceptible and moderately resistant population. However, the highly resistant (to other modes of action) population showed no additional benefit from the glyphosate treatment. The rate of glyphosate applied may have been too low to effectively control this population.

Table 60 The mean number of IRG plants and heads per container in 2016

Treatment	IRG Population					
Herbicide, Cultivation, Growth stage of treatment	Susceptible		Moderate resistant (IRG01)		Resistant	
	Plants	Heads	Plants	Heads	Plants	Heads
UTC, Deep, GS12-13	0.67	8.70	0.00	0.00	5.67	65.70
UTC, Shallow, GS 12-13	36.67	54.00	17.67	77.70	11.67	78.30
UTC, None, GS 12-13	59.00	69.70	42.67	79.30	30.33	110.00
UTC, Deep, GS 21-22	0.00	0.00	0.00	0.00	0.00	0.00
UTC, Shallow, GS 21-22	21.00	59.30	23.33	53.30	14.00	72.70
UTC, None, GS21-22	56.00	83.30	51.33	74.70	19.00	106.70
Glyphosate, Deep, GS 12-13	0.00	0.00	0.00	0.00	7.67	74.30
Glyphosate, Shallow, GS 12-13	2.67	23.00	0.00	0.00	11.33	70.70
Glyphosate, None, GS 12-13	0.33	3.70	2.00	8.00	8.33	75.00
Glyphosate, Deep, GS 21-22	0.00	0.00	0.00	0.00	0.00	0.00
Glyphosate, Shallow, GS 21-22	19.67	40.00	17.67	39.30	12.33	51.30
Glyphosate, None, GS 21-22	51.00	54.70	48.33	74.70	12.00	100.00
	Fpr Plants	*s.e.d Plants*	*l.s.d Plants*	*Fpr Heads*	*s.e.d Heads*	*l.s.d Heads*
Cultivation	*<.001*	*2.71*	*5.40*	*<.001*	*4.53*	*9.04*
Herbicide	*<.001*	*1.56*	*3.12*	*<.001*	*2.62*	*5.22*
Population	*<.001*	*1.92*	*3.82*	*<.001*	*3.20*	*6.39*
Cultivation.Herbicide	*<.001*	*3.83*	*7.64*	*<.001*	*6.41*	*12.78*
Cultivation.Population	*<.001*	*4.69*	*9.36*	*<.001*	*7.85*	*15.65*
Herbicide.Population	*0.01*	*2.71*	*5.40*	*0.02*	*4.53*	*9.04*
Cultivation.Herbicide.Population	*0.06*	*6.64*	*13.23*	*0.02*	*11.10*	*22.14*

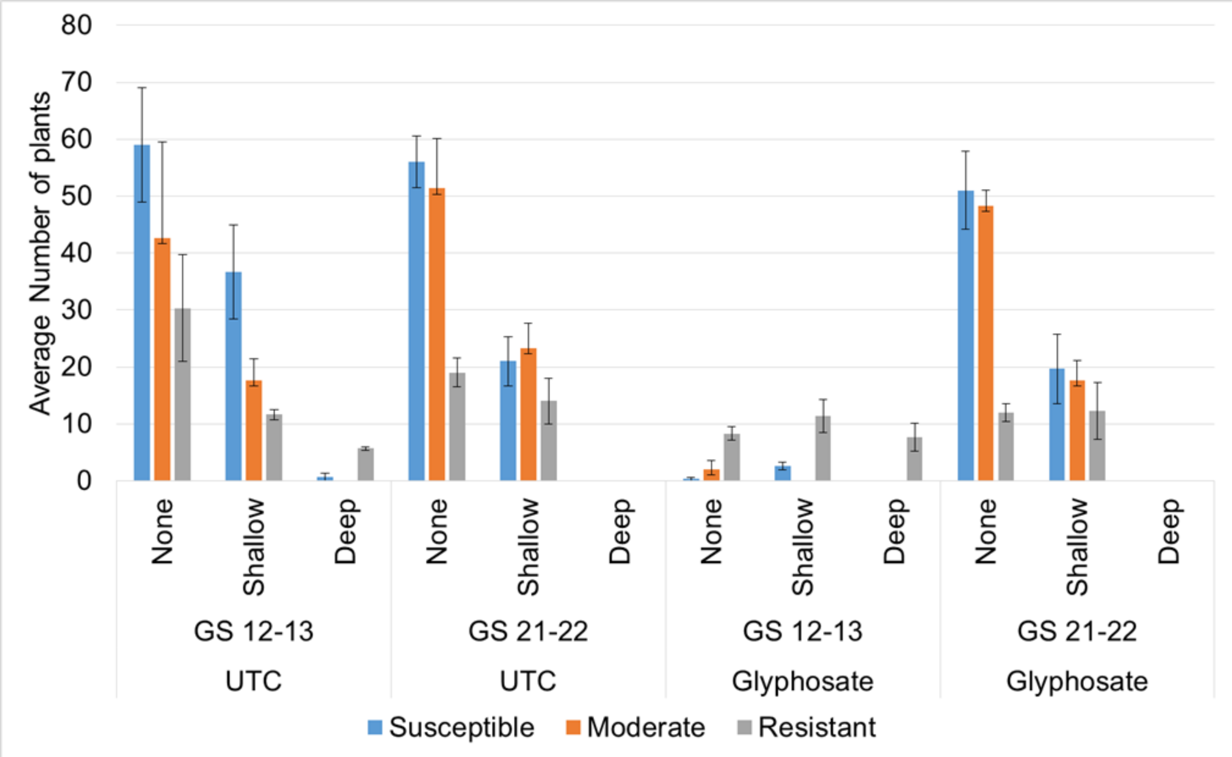

Figure 29 The mean number of plants per container for each Italian rye-grass population 2016, with or without glyphosate @ 360g a.s./L, with the addition of no, shallow (5cm) or deep cultivation (10cm).

The experiment was repeated for a second year and the emergence and subsequent growth of the plants was much more vigorous in autumn 2016. Only the number of heads per container were counted in this season. There were no significant differences between the populations in this season (Table 61). Again, the deep cultivation gave the highest level of control, (highly significant) compared to the other cultivations when no glyphosate was applied. The results for the treatments with glyphosate applications were very variable. The level of control was always significantly higher in the glyphosate treatments with no cultivation, compared to the no cultivation alone. In this season glyphosate applied at GS21-22 generally gave a better level of control compared to GS12-13. This does not seem to correlate with any weather pattern difference to the previous year.

Table 61 The mean number of black-grass heads per container 2017

Treatment	Population		
Herbicide, Cultivation, Growth stage of treatment	Susceptible	Moderate resistant (BG01)	Peldon resistant
UTC, Deep, GS12-13	47.70	63.00	36.70
UTC, Shallow, 12-13	146.30	82.30	148.70
UTC, None, 12-13	154.70	120.30	125.30
UTC, Deep, GS 21-22	0.00	17.00	22.70
UTC, Shallow, GS 21-22	98.00	114.00	153.70
UTC, None, GS21-22	171.00	120.70	134.00
Glyphosate, Deep, GS 12-13	64.30	61.70	8.00
Glyphosate, Shallow, GS 12-13	30.30	41.30	34.70
Glyphosate, None, GS 12-13	30.00	64.30	35.70
Glyphosate, Deep, GS 21-22	0.00	8.70	0.00
Glyphosate, Shallow, GS 21-22	24.70	8.00	0.00
Glyphosate, None, GS 21-22	19.30	22.30	17.30

	Fpr	*df*	*s.e.d*	*l.s.d*
Cultivation	*<.001*	*70.00*	*10.06*	*20.06*
Herbicide	*<.001*	*70.00*	*5.81*	*11.58*
Population	*0.67*	*70.00*	*7.11*	*14.19*
Cultivation.Herbicide	*<.001*	*70.00*	*14.23*	*28.38*
Cultivation.Population	*0.24*	*70.00*	*17.42*	*34.75*
Herbicide.Population	*0.05*	*70.00*	*10.06*	*20.06*
Cultivation.Herbicide.Population	*0.23*	*70.00*	*24.64*	*49.15*

Control levels for the IRG populations in 2017 (Table 62) mirrored those for black-grass, with the exception of a significant difference between IRG populations. All IRG populations were very vigorous in this season, however the effect of the deep cultivation with no glyphosate significantly reduced the number of black-grass heads compared to the shallow or no cultivation treatments. The overall highest levels of control were achieved with the addition of glyphosate at GS21-22, at all cultivations and populations.

It can be concluded from these results that the deeper cultivation, with the addition of glyphosate has always achieved the highest level of black-grass and IRG control. However rate was not compared in these experiments, so a higher rate than the 360g a.s./ha tested should be investigated further with the interaction of cultivation depth and population.

Table 62 The mean number of IRG heads per container 2017

Treatment	IRG Population		
Herbicide, Cultivation, Growth stage of treatment	Susceptible	Moderate resistant (IRG01)	Resistant
UTC, Deep, GS12-13	68.30	20.70	87.00
UTC, Shallow, 12-13	194.30	112.00	158.00
UTC, None, 12-13	149.30	163.30	147.70
UTC, Deep, GS 21-22	7.70	24.00	17.00
UTC, Shallow, GS 21-22	137.70	272.70	180.30
UTC, None, GS21-22	193.00	158.00	174.30
Glyphosate, Deep, GS 12-13	63.00	14.00	85.00
Glyphosate, Shallow, GS 12-13	65.30	0.00	82.00
Glyphosate, None, GS 12-13	108.30	9.00	153.30
Glyphosate, Deep, GS 21-22	0.00	3.70	7.70
Glyphosate, Shallow, GS 21-22	32.30	41.70	32.30
Glyphosate, None, GS 21-22	10.70	20.00	49.70

	Fpr	***df***	***s.e.d***	***l.s.d***
Cultivation	*<.001*	*70.00*	*12.69*	*25.30*
Herbicide	*<.001*	*70.00*	*7.32*	*14.61*
Population	*<.001*	*70.00*	*8.97*	*17.89*
Cultivation.Herbicide	*<.001*	*70.00*	*17.94*	*35.78*
Cultivation.Population	*0.08*	*70.00*	*21.97*	*43.82*
Herbicide.Population	*0.06*	*70.00*	*12.69*	*25.30*
Cultivation.Herbicide.Population	*0.02*	*70.00*	*31.07*	*61.98*

4.2.3. **Container Experiment 3. The effect of glyphosate dose, weed size and subsequent pre- and post-emergence selective herbicide programmes.**

The results for 2018 black-grass plants and heads are shown in Table 63 and Figure 30 (plant count data only), where there was a significant (p=<0.001) interaction for plant and head count data between population, treatment and a combination of population and treatment (for heads p=0.002). These data are difficult to interpret as the treatment timing for the pre-emergence herbicide is not how it would be in the field. The experiment aim was to demonstrate the different building blocks of a robust herbicide programme in the field after stale seed bed where some plants may have survived a sub-optimal glyphosate rate at the wrong weed growth stage.

There was a big difference between the different populations tested. For treatments where no glyphosate was applied the post-emergence herbicide significantly controlled the susceptible black-grass population compared to the untreated, but has had little effect on the two resistant populations as they will have some sulfonylurea resistance.

Table 63 Black-grass plants and heads per container 2018

Treatments		Black-grass populations					
Glyphosate treatment (g a.s./ha) & application timing	Following herbicide treatment	Susceptible		Moderate resistant		Peldon resistant	
		Plants	Heads	Plants	Heads	Plants	Heads
UTC (GS 12-13)	None	23.67	148.30	20.67	124.30	24.00	109.70
	Pre-em	20.33	116.30	13.67	115.30	21.00	121.70
	Post-em	4.33	20.30	15.67	106.70	23.00	116.00
UTC (GS21-22)	None	21.33	125.00	13.00	129.00	27.00	135.30
	Pre-em	17.67	128.00	16.67	106.00	23.67	106.30
	Post-em	8.33	1.70	13.33	90.30	20.67	132.30
MON79376@ 270g, (GS 12-13)	None	12.00	103.00	14.00	92.00	18.33	102.00
	Pre-em	15.67	107.00	12.00	101.30	22.33	134.00
	Post-em	1.33	6.00	10.00	100.30	18.67	82.70
MON79376@ 270g, (GS21-22)	None	14.00	71.70	10.00	84.00	15.33	48.30
	Pre-em	15.74	78.10	11.67	40.00	17.33	77.30
	Post-em	6.33	1.70	10.33	40.70	16.00	68.00
MON79376@ 450g, (GS 12-13)	None	7.67	62.30	7.00	41.70	11.67	60.30
	Pre-em	10.33	39.70	6.33	21.00	8.67	53.30
	Post-em	1.33	6.70	5.33	48.00	12.67	81.70
MON79376@ 450g, (GS21-22)	None	2.33	5.30	1.33	2.30	9.00	15.30
	Pre-em	6.67	36.70	1.33	4.30	12.31	41.90
	Post-em	0.00	0.00	3.00	1.30	10.00	38.70
	df	*s.e.d*		*l.s.d*		*Fpr*	
Population	*104*	*0.667*	*6.11*	*1.323*	*12.12*	*<.001*	*<.001*
Treatment	*104*	*1.634*	*14.97*	*3.241*	*29.68*	*<.001*	*<.001*
Population x treatment	*104*	*2.831*	*25.93*	*5.613*	*51.41*	*<.001*	*0.002*

The pre-emergence herbicide alone showed a slight reduction in black-grass plants and heads compared to the untreated control. However it would be expected that this effect would be greater in the field when the products were applied at their recommended timing.

When comparing the rate of glyphosate to all other factors, the higher rate of 450g a.s./ha always gave the highest level of control when compared to the same growth stage at application. There were no black-grass survivors for the susceptible population when glyphosate at 450g a.s./ha was applied at a black-grass growth stage of GS21-22 and followed by a post- herbicide.

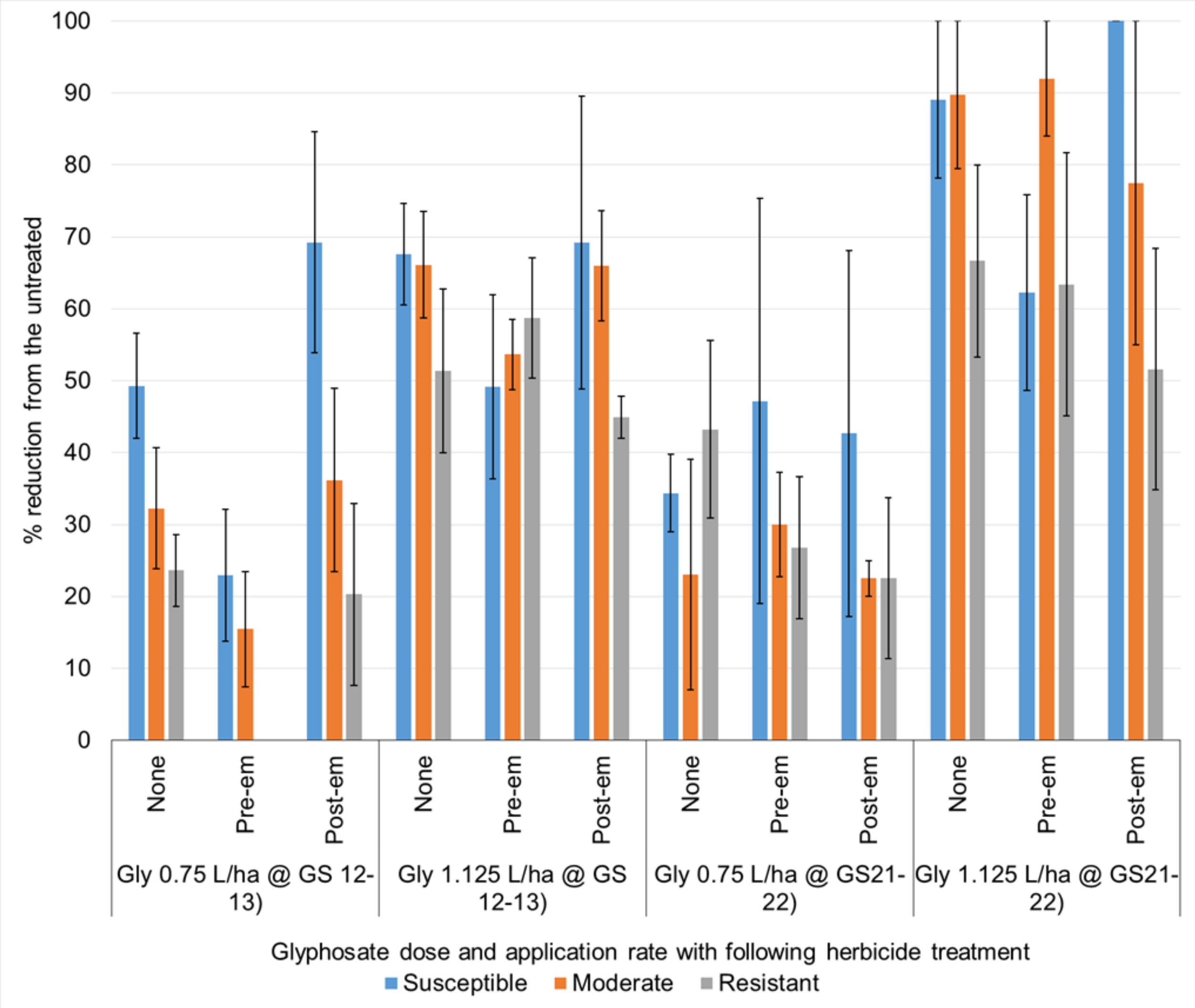

Figure 30 The percentage reduction from the untreated controls for black-grass populations in 2018.

Results for the following season (Table 64 & Figure 31) were very different, with generally higher levels of control for all treatments where glyphosate had been applied. This can be explained by the weather conditions at the time of applications in the two different seasons. In February 2019 the temperature reached an unseasonal 17 °C which is likely to have enhanced the level of glyphosate control as the weeds would have been actively growing at application (GS21-22) and pre-emergence applications.

The higher rate of glyphosate applied at GS21-22 resulted in no black-grass survivors despite population or choice of following herbicide. The addition of the pre-emergence and post-emergence applications to the 450g a.s./ha glyphosate applied at GS12-13 fully controlled the susceptible and moderately resistant population. For the same treatment on the Peldon resistant population the pre-emergence herbicide was highly effectively, however the post-emergence alone did not fully control the population, which again is due to the resistance to sulfonylurea herbicides in this particular population.

Table 64 Black-grass plants and heads per container 2019

Treatments		Black-grass populations					
Glyphosate treatment (g a.s./ha) & application timing	Following herbicide treatment	Susceptible		Moderate resistant		Peldon resistant	
		Plants	Heads	Plants	Heads	Plants	Heads
UTC (GS 12-13)	None	21.67	135.70	18.00	120.70	21.67	65.00
	Pre-em	8.33	85.30	11.00	60.00	13.00	72.00
	Post-em	1.33	12.70	17.00	59.70	23.00	106.00
UTC (GS21-22)	None	23.67	77.30	20.33	71.00	20.33	79.00
	Pre-em	17.00	84.30	19.33	89.00	20.67	74.00
	Post-em	2.67	14.70	17.67	93.30	23.72	92.90
MON79376@ 270g, (GS 12-13)	None	9.33	68.30	6.67	48.30	11.33	60.70
	Pre-em	4.67	7.00	3.67	11.30	7.33	30.00
	Post-em	0.00	0.00	6.00	32.70	14.00	66.30
MON79376@ 270g, (GS21-22)	None	0.00	0.00	0.33	2.70	0.00	0.00
	Pre-em	0.00	0.00	0.00	0.00	0.00	0.00
	Post-em	0.00	0.00	0.00	0.00	1.33	1.30
MON79376@ 450g, (GS 12-13)	None	1.00	15.30	0.00	0.00	2.67	22.70
	Pre-em	0.00	0.00	0.00	0.00	0.67	0.30
	Post-em	0.00	0.00	0.00	0.00	3.33	23.30
MON79376@ 450g, (GS21-22)	None	0.00	0.00	0.00	0.00	0.00	0.00
	Pre-em	0.00	0.00	0.00	0.00	0.00	0.00
	Post-em	0.00	0.00	0.00	0.00	0.00	0.00
	df	*s.e.d*		*l.s.d*		*Fpr*	
Population	*104*	*0.539*	*2.8*	*1.069*	*5.56*	*<.001*	*0.001*
Treatment	*104*	*1.321*	*6.87*	*2.619*	*13.62*	*<.001*	*<.001*
Population x treatment	*104*	*2.288*	*11.89*	*4.536*	*23.59*	*<.001*	*<.001*

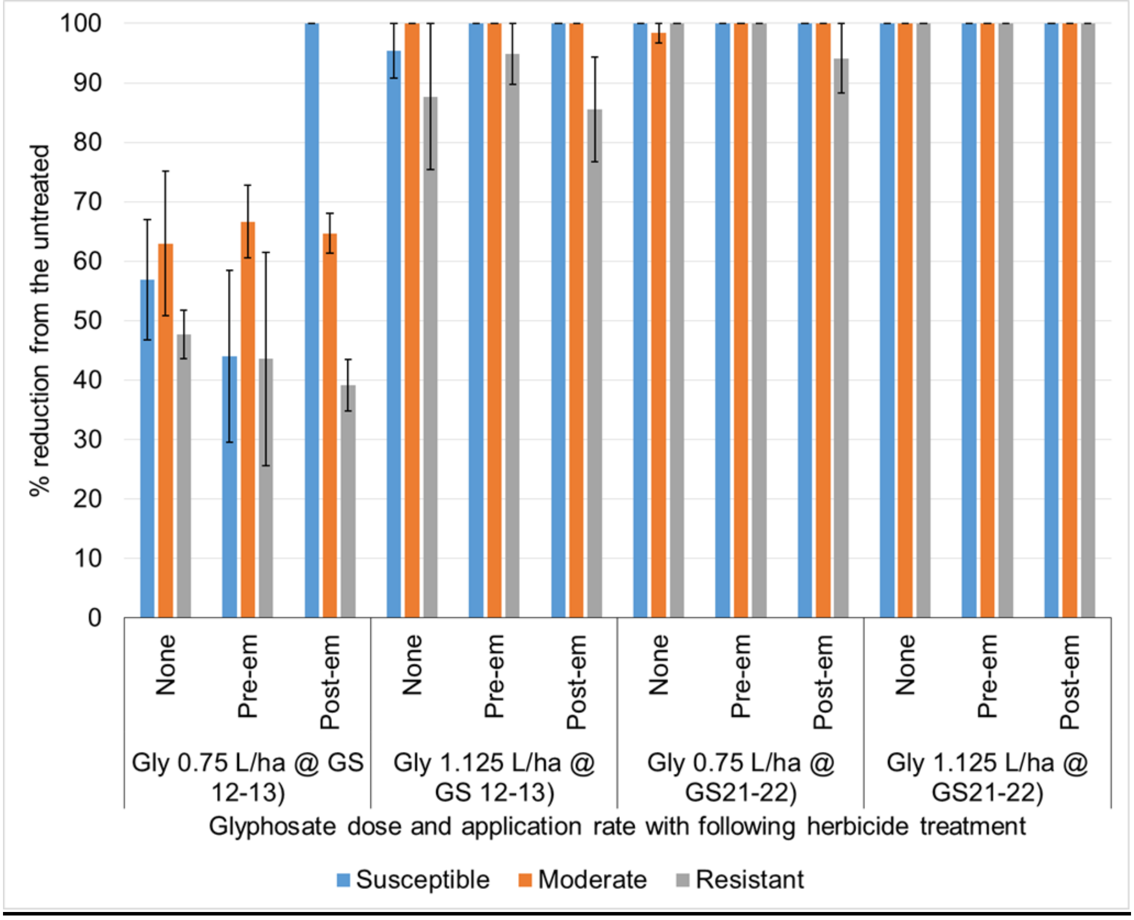

Figure 31 The percentage reduction from the untreated controls of black-grass populations in 2019.

4.2.4. **Overall conclusions from the container experiments**

- For black-grass, glyphosate applied at a rate of 540g a.s./ha and 720g a.s./ha at a growth stage of GS12-13 was the most effective overall treatment for all populations tested.
- For IRG, glyphosate applied at a rate of 720g a.s./ha at a weed growth stage of GS12-13 was the most effective overall treatment for all populations tested. Glyphosate at 540g a.s./ha at the same growth stage achieved 100% control of the susceptible and moderately resistant population, however for the IRG resistant population there were survivors.
- A rate of 720g a.s./ha of glyphosate was required to control the larger weed growth stage of GS21-22, tillering black-grass and IRG plants.
- Deeper cultivation, with the addition of glyphosate always achieved the highest level of black-grass and IRG control. This was simulating a glyphosate application followed by a cultivation in a field stale seedbed scenario before drilling. However rate was not compared in these experiments, so a higher rate than the 360g a.s./ha tested should be investigated further with the interaction of cultivation depth and population. In practice a more shallow cultivation within a stale seedbed is more likely, so glyphosate rate is extremely important for effective control.

- Results from the dose, weed size and subsequent herbicide experiment further supported the evidence that a higher rate of glyphosate is required to effectively control black-grass populations with a known resistance to other modes of action (such as Peldon resistant). The higher rate used (450g a.s./ha) was below field rate, but if field rate had been used in these controlled conditions it would have likely removed all plants preventing the control from following herbicides to be investigated. This experiment was aiming to demonstrate the need for effective pre-em and post-em herbicides to remove any black-grass plants that had potentially been exposed to glyphosate in a stale seedbed and survived.
- The warmer weather conditions in 2019 resulted in a much higher glyphosate efficacy compared to the previous trial year.

Key output of WP1 (field and container-based experiments):

Field data showed applications of glyphosate as two split rates of 540g a.s./ha provided the overall highest level of control, ensuring that there are no survivors which could help to prevent resistance evolution. The addition of a cultivation in a stale seedbed with a glyphosate application increases control of black-grass and provides an extremely valuable mitigation strategy. A spring –sown cereal provided the best opportunity to reduce black-grass numbers with an autumn cultivation and glyphosate application of 720g a.s/ha in the spring ahead of drilling.

Results from all container-based experiments show that the optimum glyphosate rate to control black-grass or IRG at GS12-13 is a minimum of 540g a.s./ha. When the weed growth stage is at tillering (GS21-22) a higher rate of glyphosate would be required for effective control. The correct glyphosate rate for the correct weed growth stage is critical to optimise efficacy and ultimately reduce the resistance risk.

The weather conditions at the time of glyphosate applications can have a big impact on the level of control. Warmer temperatures generally mean the weeds will also be actively growing so uptake has shown to be higher. When conditions are very cold the weeds will not be actively growing so uptake could be poorer.

4.3. Applications between crop rows (WP2)

4.3.1. To investigate the resistance risk of glyphosate applications to larger weed growth stages (Container experiment)

A susceptible and Peldon resistant population were compared with treatments of fairly low glyphosate rates (<360g a.s./ha) at late weed growth stages (GS23 and above). Results showed a significant difference (Fpr=<0.001) between treatments but no difference between black-grass population (Table 65). In 2018 there were survivors for all treatments, as would have been expected at these relatively low rates. However, in 2019 there was a huge seasonal difference, with very warm spring weather resulting in complete control of three treatments for both populations. In terms of resistance evolution this is very beneficial if this had been in the field, but it meant that no seed could be collected from these treatments in 2019 for a glasshouse dose response experiment.

Table 65 The mean number of black-grass heads per container in 2018 and 2019.

Treatment		Population and year					
Glyphosate rate (g a.s./ha)	Application timing (Growth stage)	2018			2019		
		Susceptible	Peldon resistant		Susceptible	Peldon resistant	
Untreated	-	172.30	162.70		124.00	114.00	
360	GS 23	132.70	111.30		9.30	5.00	
180	GS 23	162.30	140.30		0.00	0.00	
360	GS 25-28	6.30	11.70		0.00	0.00	
180	GS 25-28	93.30	78.00		0.00	0.00	
360	GS 32	75.00	130.00		19.70	16.70	
180	GS 32	128.00	127.00		107.30	90.00	
Interactions		*s.e.d*	*l.s.d*	*Fpr*	*s.e.d*	*l.s.d*	*Fpr*
Treatment		*7.85*	*16.13*	*<.001*	*9.24*	*18.99*	*<.001*
Population		*4.19*	*8.62*	*0.762*	*4.94*	*10.15*	*0.325*
Treatment x population		*11.1*	*22.81*	*<.001*	*13.07*	*26.86*	*0.955*

A combination of seed from the 2019 survivors and 2018, where complete control was achieved in 2019, were further investigated in a glasshouse dose response experiment to determine any shifts in glyphosate tolerance. The list of populations (including their original container experiment treatments) tested are shown in Table 66 and the survival curve is shown in Figure 32, along with the ED_{50} (Figure 33) and ED_{90} values (Figure 34). The results from the pairwise population comparisons using R from these figures are showing that generally all populations were controlled at field rate (540g) of glyphosate. However a few populations where a lower rate (180g) of glyphosate were used at a larger growth stage of GS25-28 in the container experiment are towards the higher end of this slope (SD 0685 and SD 0692) requiring a higher glyphosate rate for control. For the susceptible populations six out of the seven populations tested showed a significant shift in the glyphosate ED_{50} dose required to control them compared to the baseline population (SD 0805) (Figure 33). There was a significant (Fpr 0.001) difference between the baseline susceptible and baseline Peldon resistant populations.

Table 66 Seed survivor populations collected from large growth stage container experiment 3.2.1 and tested in a glasshouse dose response to glyphosate.

Population reference	*Original treatment reference (see Experiment 3.2.1) Population, rate, timing*	*Year of seed collection*
SD 0795	*Susceptible UTC*	*2019*
SD 0796	*Susceptible, 360g glyphosate, GS23*	*2019*
SD 0683	*Susceptible, 180g glyphosate, GS23*	*2018*
SD 0684	*Susceptible, 360g glyphosate, GS25-28*	*2018*
SD 0685	*Susceptible, 180g glyphosate, GS25-28*	*2018*
SD 0798	*Susceptible, 360g glyphosate, GS32*	*2019*
SD 0797	*Susceptible, 180g glyphosate, GS32*	*2019*
SD 0805	*Susceptible baseline*	
SD 0800	*Peldon resistant UTC, 2019 seed*	*2019*
SD 0799	*Peldon resistant, 360g glyphosate, GS23*	*2019*
SD 0690	*Peldon resistant, 180g glyphosate, GS23*	*2018*
SD 0691	*Peldon resistant, 360g glyphosate, GS25-28*	*2018*
SD 0692	*Peldon resistant, 180g glyphosate, GS25-28*	*2018*
SD 0801	*Peldon resistant, 360g glyphosate, GS32*	*2019*
SD 0802	*Peldon resistant, 180g glyphosate, GS32*	*2019*
SD 0032	*Peldon resistant baseline seed, SD 0032*	

The susceptible baseline population (SD 0805) is on the far left-hand side of the curves (Figure 32) showing it is well controlled by glyphosate at 540g a.s./ha. For the susceptible populations six out of the seven populations tested showed a significant shift in the glyphosate ED_{50} dose required to control them after two years selection compared to the baseline population (SD 0805) (Figure 32 & Figure 33). The biggest significant (Fpr 0.001) shift is from the susceptible population treated with glyphosate at 180g a.s./ha at a growth stages of 25-28 ((SD 0685) line on the far right-hand side of the curves Figure 32). The Peldon resistant baseline population (SD 0032) requires a higher dose of glyphosate to control it and after just two years of exposing plants of the same population to glyphosate at a low rate of 180g a.s./ha sprayed at a growth stage of 25-28 has resulted in these plants requiring a significantly (Fpr 0.05) higher rate of glyphosate to now control them ((SD 0692) line on the far right-hand side of the curves).

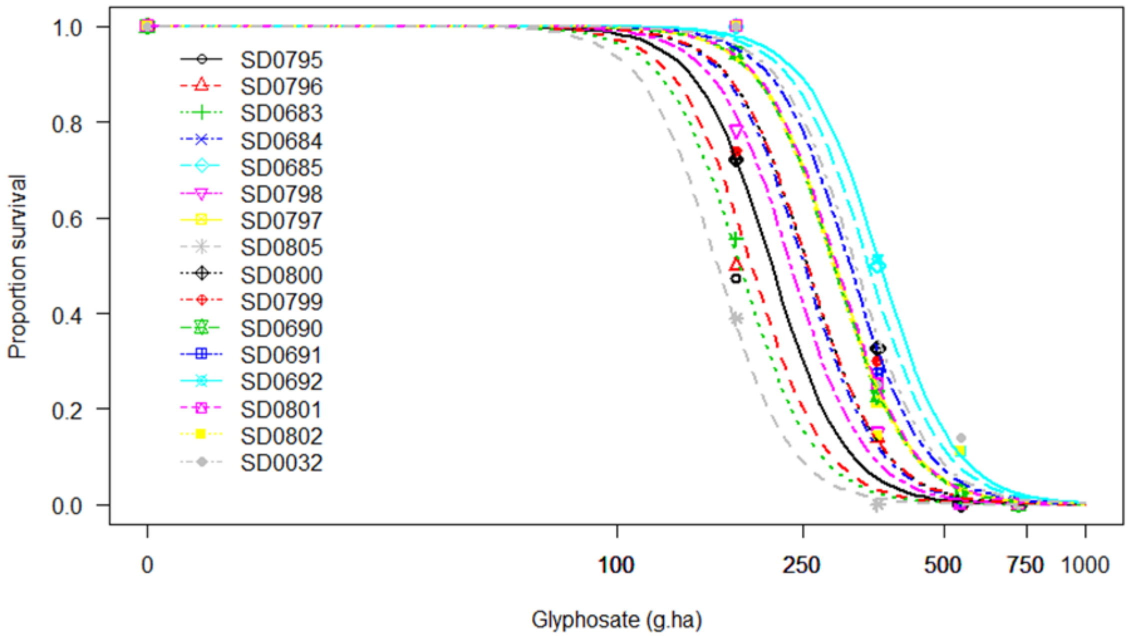

Figure 32 Survival curve from a glasshouse dose response experiment testing the surviving populations from the large growth stage container experiment. (Glyphosate rate g a.s./ha).

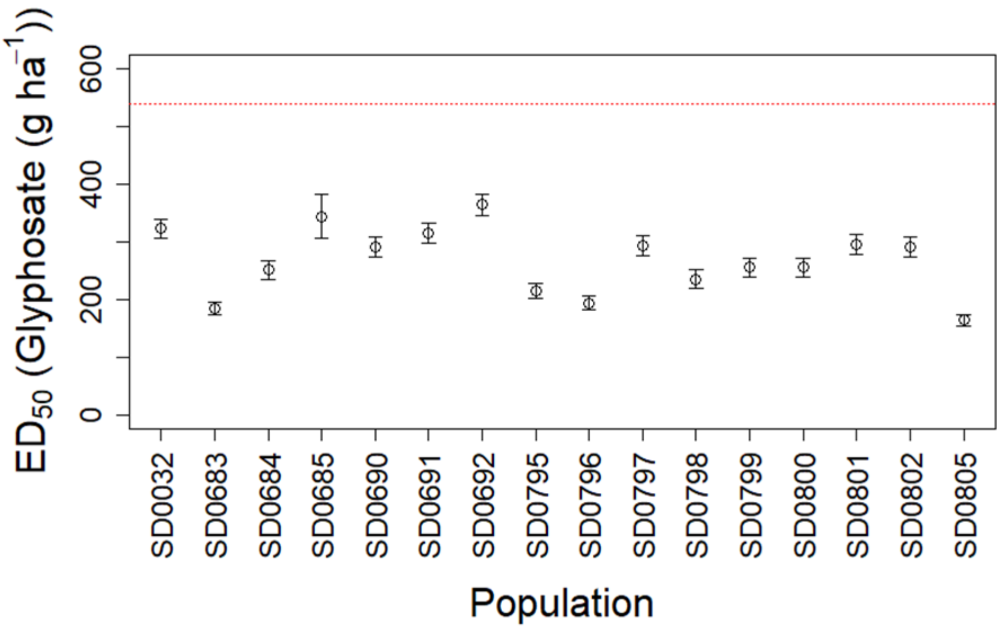

Figure 33 ED_{50} for populations from the large growth stage container dataset. Values represent the mean ± standard error. Dashed red line represents the recommended field rate of glyphosate.

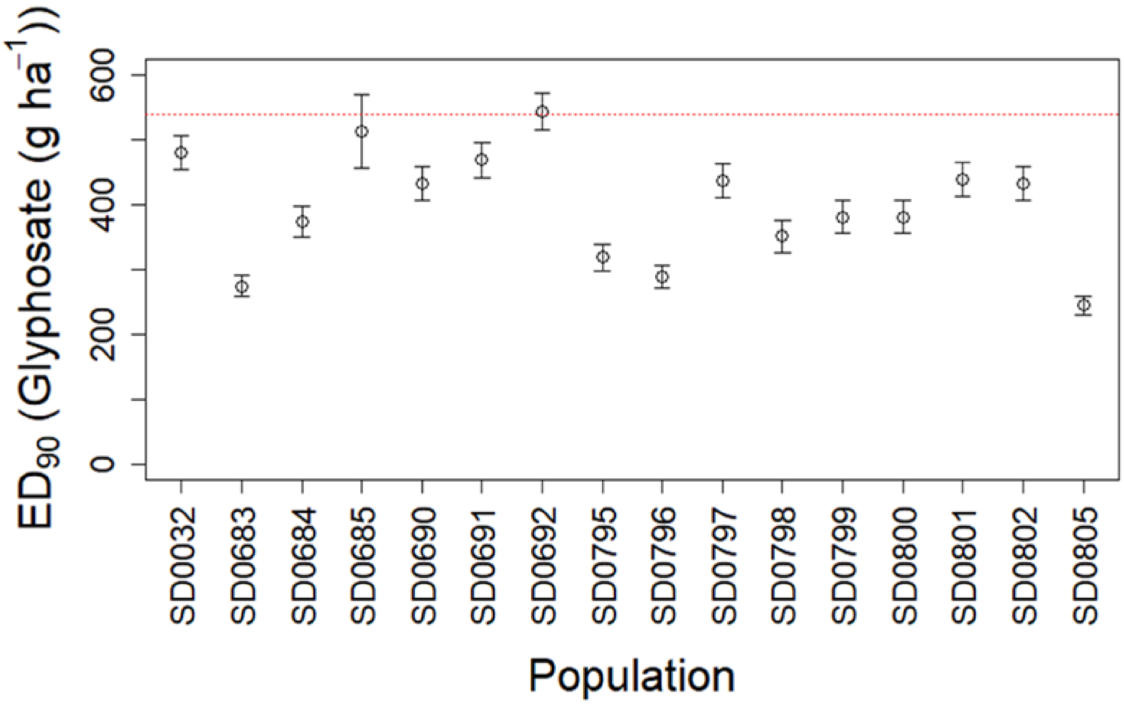

Figure 34 ED_{90} for populations included from the large growth stage container dataset. Values represent the mean ± standard error. Dashed red line represents the recommended field rate of glyphosate.

4.3.2. Conclusions for the large weed growth stage experiments

It can be concluded from the experiments on the larger black-grass growth stages that shifts in tolerance to glyphosate can occur very quickly (within two years) in controlled conditions when a sub-optimal dose for a particular weed growth stage is used. Therefore in practice the correct glyphosate rate for the weed size is even more critical to optimise efficacy and prevent survivors. This is particularly important in respect to controlling weeds in perennial crops where they are more likely to be larger, as well as other grass weed species arable crops.

4.4. Resistance testing (Glasshouse and container experiments) (WP3)

4.4.1. A glyphosate dose response experiment testing black-grass populations from a long-term field trial (Glasshouse Experiment 1).

A set of black-grass seed populations were collected from field plots in summer 2015 from different treatments from a five-year long-term field trial hosted by one of the project partners. Seed were tested in a glasshouse dose response to glyphosate (Figure 35). Results show that none of the populations gave 100% control at the recommended field rate of 540g a.s./ha glyphosate, but ranged between 55% to 90%. Increasing the glyphosate rate to 720g a.s./ha increased control of all populations to between 84% to 97% control, except for the Peldon resistant population that only achieved 65% control at this rate (Figure 35). In general populations 2 (2015AG02), 4 (2015AG04), and 5 (2015AG05) were less susceptible to glyphosate compared to the other populations tested.

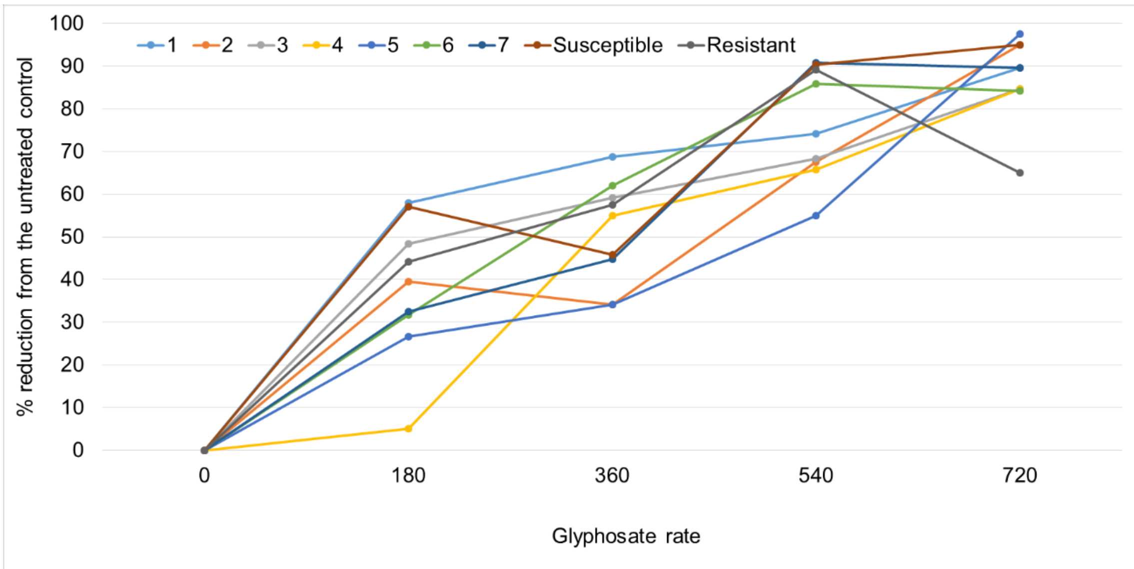

Figure 35 The percentage reduction from the untreated control for a range of CambsA15 Field populations tested in a glasshouse dose response experiment to glyphosate in 2016.

The same populations were also tested using a standard black-grass resistance test method to show ACCase resistance, enhanced metabolism resistance and ALS resistance (Table 67). Results show that all populations tested had a high level of ACCase and ALS resistance (RR or RRR). Three populations were susceptible to enhanced metabolism resistance, population 1 (2015AG01) had a five year continuous plough, population 5 (2015AG05) was sown in winter wheat and had two glyphosate applications in the stale seed bed pre-drilling, and population 7 (2015AG07) had been untreated for five years.

When considering the previous field plot history (Table 67) and comparing the glyphosate dose response results to the resistance test ratings it is difficult to determine an immediate relationship. However it has shown that black-grass populations from neighbouring plots in the same field, treated different for a five year period, do show a variation in response to glyphosate rates. The untreated control population (2015AG07) had no glyphosate for five years and yet was only 90% controlled at 720g a.s./ha in the glasshouse dose response.

Table 67 Standard black-grass resistance test results for the CambsA15 Field populations.

		Standard herbicide resistance test					
Sample number		ACCase		Enhanced metabolism		ALS resistance	
CambsA15	Previous historic field treatment*	% reduction	R rating	% reduction	R rating	% reduction	R rating
2015AG01	5 years plough	50.00	RR	78.95	S	5.41	RRR
2015AG02	5 years Claydon strip drill	50.00	RR	44.74	RR	50.47	RR
2015AG03	CC phacelia to winter W (2 glyphosates)	-31.03	RRR	51.72	RR	28.60	RRR
2015AG04	Forced fallow to spring W (4 glyphosates)	-17.95	RRR	0.00	RRR	39.38	RRR
2015AG05	Forced fallow to winter W (2 glyphosates)	31.71	RRR	78.05	S	33.60	RRR
2015AG06	15062 Full treatment	45.83	RR	8.33	RRR	23.00	RRR
2015AG07	15062 untreated	38.78	RRR	85.71	S	40.89	RR
-	Susceptible standard	100.00	S	82.46	S	100.00	S
-	Resistant standard	100.00	S	7.25	RRR	-6.79	RRR

**CC = cover crop, winter W= winter wheat, spring W = spring wheat*

Although results from this particular experiment are difficult to interpret when considering the previous history, anecdotal evidence in the industry is showing that populations that are highly resistant to many other modes of action are more likely to tolerate glyphosate. The rate and application timing in these scenarios are therefore vitally important to ensure maximum efficacy is achieved in the field to prevent survivors.

4.4.2. Annual seed testing for glyphosate resistance (Glasshouse Experiment 2).

Seed submitted for testing in 2015 and 2017 were all fully controlled by all glyphosate rates (>97% reduction compared to the untreated control) so there were no concerns of resistance. Seed tested in 2018 (Figure 36) showed a slightly more varied response to the glyphosate doses, but again all were well controlled (>93% reduction from the untreated control) at field rate (540g a.s./ha) and above so there are no concerns of resistance with any of the populations tested. The seed populations shown in Figure 36 are from very different regions of the UK. SD 0492 is from Shropshire, SD 0493 from Lincolnshire and SD 0537 is from Suffolk.

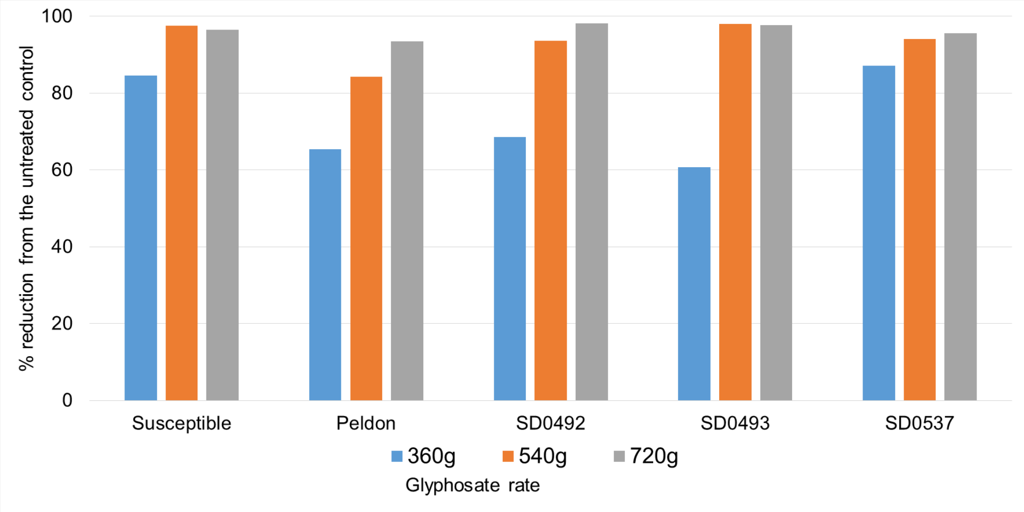

Figure 36 Results of the 2018 glyphosate resistance test in the glasshouse from farmer submitted seed. Glyphosate rate g a.s./ha.

4.4.3. **Container experiment seed survivors: Glyphosate dose response testing**

There were eight black-grass populations from the glyphosate dose and weed size container experiment that had enough seed for a dose response to glyphosate after two years of selection. Four of the populations (SD 0549, SD 0550, SD 0552 and SD 0555) showed a significant difference in the ED_{50} value, but not always the ED_{90} value (Table 68,

Figure 37 and Figure 38) compared to the baseline. Three of these populations had been exposed to a low rate (360g a.s./ha) of glyphosate at a large weed growth stage (tillering), with the Peldon resistant population (SD 0555) showing the highest ED_{90} value of 716.9.

Table 68 The ED_{50} and ED_{90} values for black-grass populations tested from container experiment 1 (dose and weed size).

Reference	Original population & treatment	ED50	*Std. Error*	*Fpr*	ED90	*Std. Error*	*Fpr*
SD 0200	*Peldon resistant baseline*	393.6	*14.8*	-	513.6	*31.0*	-
SD 0525	*Susceptible standard baseline*	240.3	*14.4*	-	325.2	*27.2*	-
SD 0548	*BG01, untreated*	286.4	*18.4*	-	462.9	*37.0*	-
SD 0549	*BG01, 720g (GS12-13)*	345.8	*21.3*	*0.020*	585.3	*47.9*	*0.021*
SD 0550	*BG01, 360g (GS21-22)*	360.0	*3.5*	*>0.001*	384.1	*289.8*	*0.823*
SD 0551	*Susceptible, untreated*	236.0	*19.3*	-	460.1	*47.5*	-
SD 0552	*Susceptible, 360g (GS21-22)*	333.4	*21.4*	*>0.001*	580.7	*49.3*	*0.0498*
SD 0553	*Peldon resistant, untreated*	335.6	*20.0*	-	540.6	*41.5*	-
SD 0554	*Peldon resistant, 720g (GS10)*	278.5	*17.8*	*0.051*	440.3	*35.4*	*0.003*
SD 0555	*Peldon resistant, 360g (GS21-22)*	420.7	*23.6*	*0.002*	716.9	*54.8*	*>0.001*

Note: there are only Fpr values when treatments were compared to the original baseline population. The gaps in the Fpr columns indicates an untreated or the baseline population.

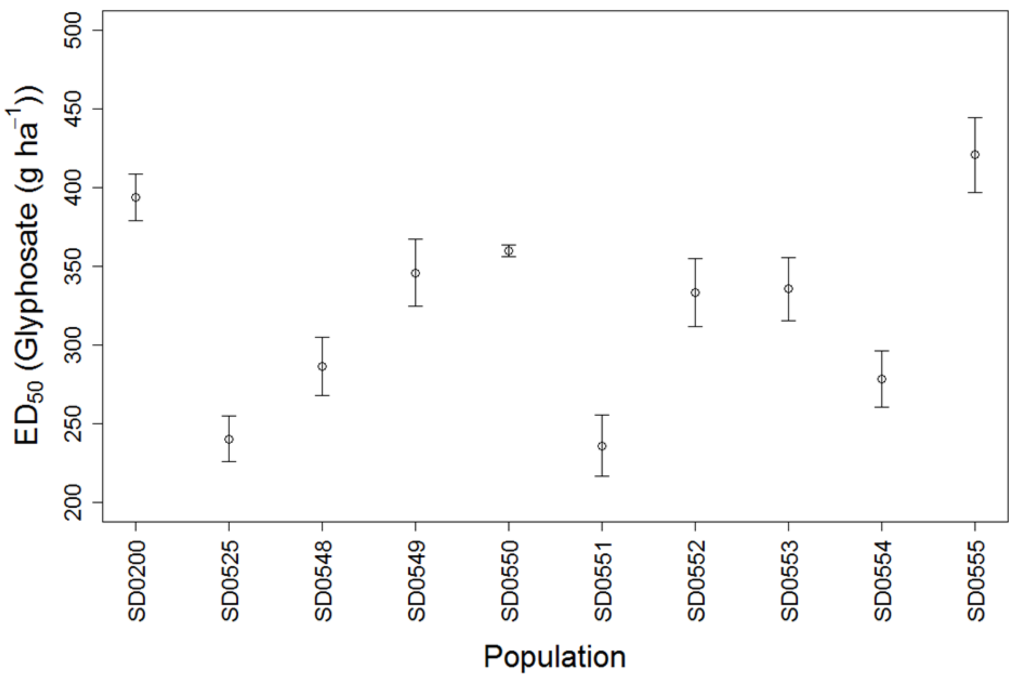

Figure 37 The ED_{50} values for black-grass populations

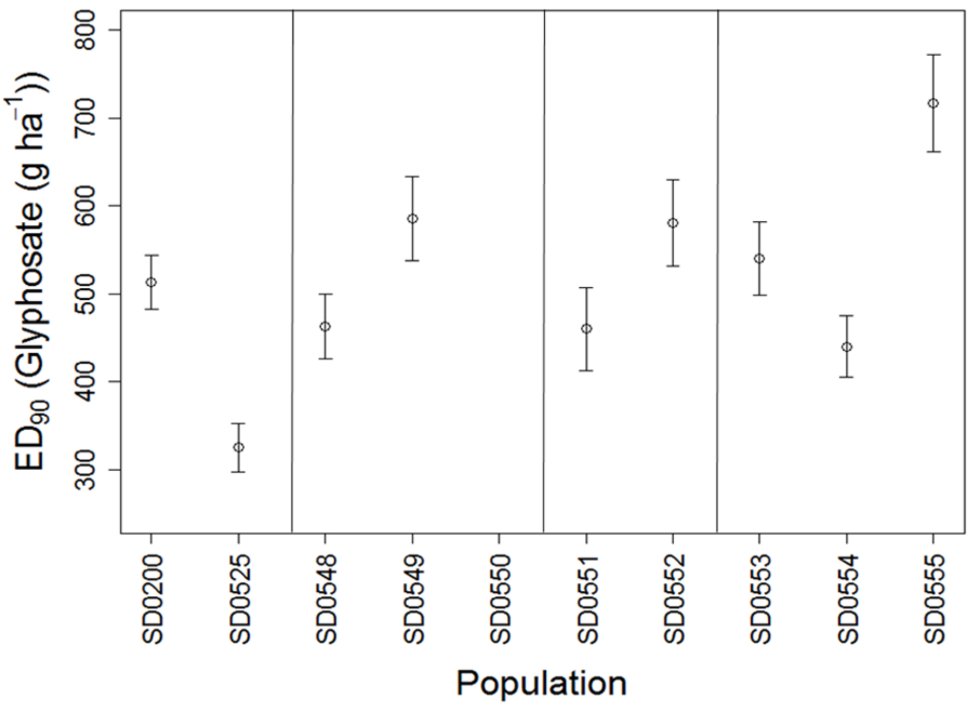

Figure 38 The ED_{90} values for black-grass populations

Results for the same dose response experiment with the survivors of the IRG populations are shown in Table 69, Figure 39 and Figure 40. Three populations (SD 0557, SD 0559 and SD 0560) out of the 10 populations tested shown a significant value for ED_{50} and ED_{90} (Table 69).

Table 69 The ED_{50} and ED_{90} values for IRG populations tested from container experiment 1 (dose and weed size).

Reference	Population history	ED50	*Std. Error*	*Fpr*	ED90	*Std. Error*	*Fpr*
SD 0215	*Susceptible standard*	243.3	*15.7*	-	389.1	*26.0*	-
SD 0556	*IRG01, Untreated*	316.8	*18.3*	-	506.6	*30.1*	-
SD 0557	*IRG01, 360g (GS21-22)*	399.5	*20.6*	*>0.001*	638.8	*34.5*	*0.037*
SD 0558	*Susceptible, untreated*	252.8	*16.2*	-	404.3	*26.7*	-
SD 0559	*Susceptible, 360g (GS21-22)*	365.3	*19.5*	*>0.001*	584.1	*32.5*	*>0.001*
SD 0560	*Susceptible, 720g (GS21-22)*	345.7	*19.0*	*>0.001*	552.8	*31.5*	*>0.001*
SD 0561	*Resistant, untreated*	336.3	*18.8*	-	537.8	*31.0*	-
SD 0562	*Resistant, 720g (GS10)*	306.6	*18.1*	*0.141*	490.3	*29.7*	*0.754*
SD 0563	*Resistant, 360g (GS21-22)*	306.9	*18.0*	*0.047*	490.8	*29.7*	*0.209*
SD 0564	*Resistant, 540g (GS21-22)*	375.0	*19.8*	*0.272*	599.6	*33.1*	*0.013*
SD 0565	*Resistant, 720g (GS21-22)*	321.6	*18.4*	*0.317*	514.4	*30.3*	*0.674*

Note: there are only Fpr values when treatments were compared to the original baseline population. The gaps in the Fpr columns indicates an untreated or the baseline population.

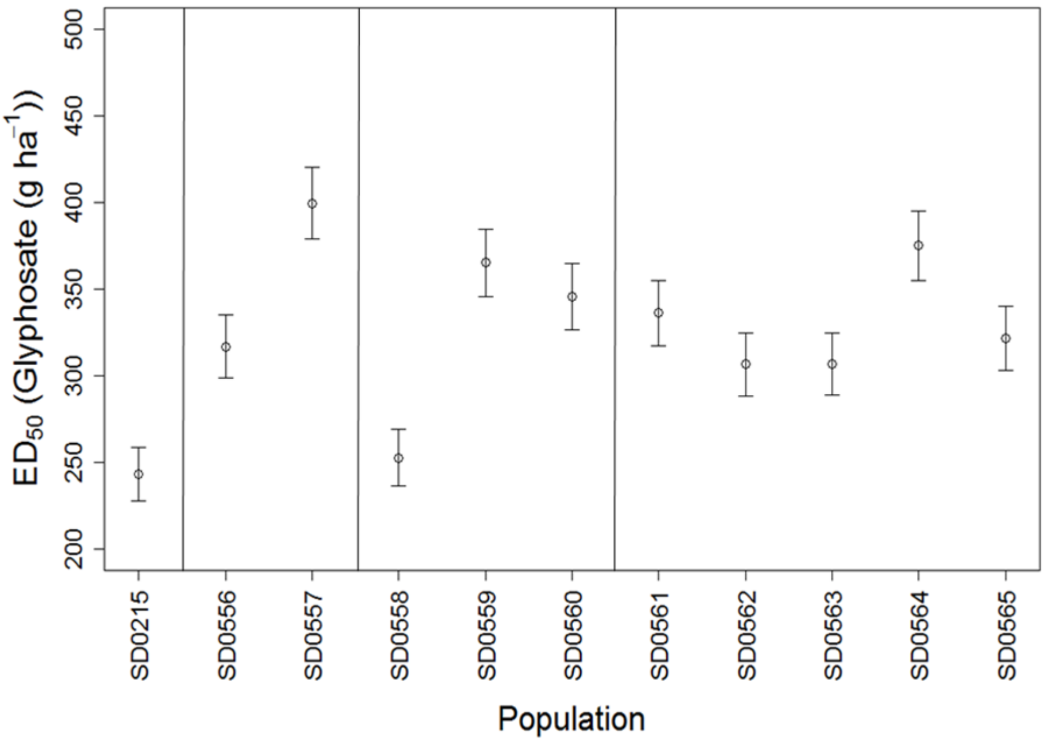

Figure 39 The ED_{50} values for IRG populations

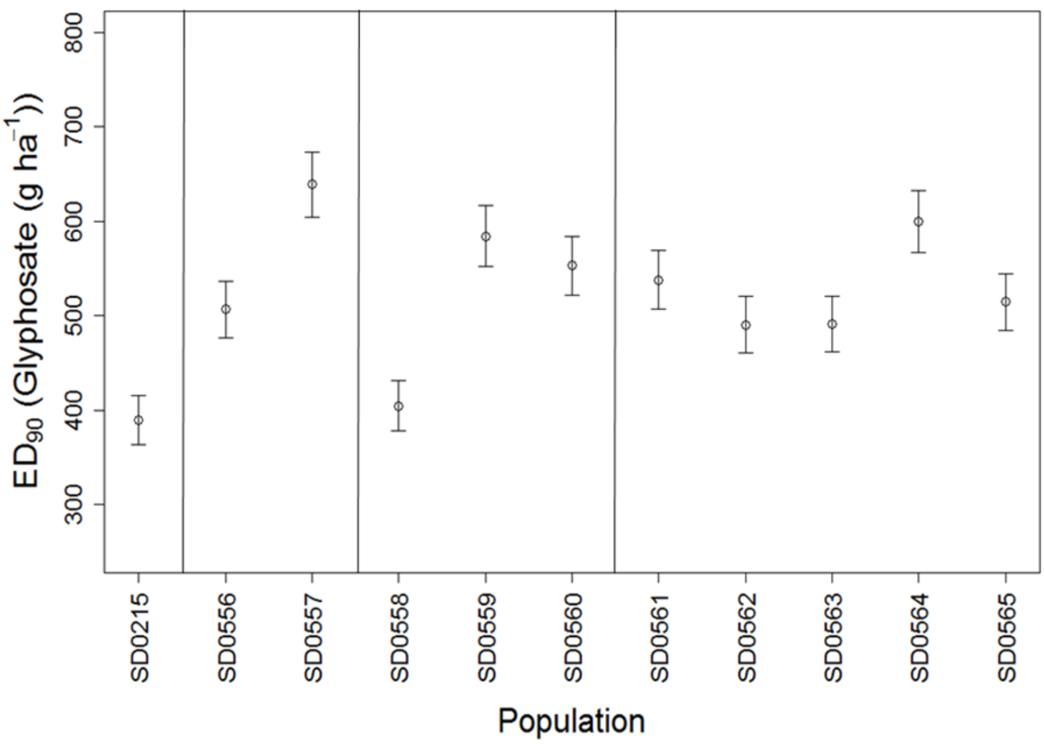

Figure 40 The ED_{90} values for IRG populations

The same trend was shown for IRG as for black-grass, where the previous treatment of a lower glyphosate rate (360g a.s./ha) at a higher weed growth stage (GS21-22), resulted in the biggest and most significant shift in glyphosate tolerance.

It can be concluded that both black-grass and IRG exposed to glyphosate at a sub-optimal rate and at a weed growth stage that is above GS12-13 for two years in controlled conditions required a higher rate of glyphosate for optimum control. These results are in controlled conditions and it would be highly unlikely to be detected this quickly in field conditions with the dilution effect of the weed seed bank and crop competition. However, this is an early warning system and validates the need for the correct rate and weed growth stage to optimise efficacy and potentially slow or prevent resistance evolution.

4.4.4. **Whole plant testing of survivors: RISQ test method**

All RISQ test experiments were very successful and provided valuable additional data to verify and further enhance this method for glyphosate. The test assessment requires scoring the root growth by observing the underneath of the Petri dish and not the appearance of leaves. Roots are assessed on Syngenta scale of 1-3: 1 Alive, lots of new growth, 2 Yellow, some new growth, 3 Dead, no new growth. However, ADAS assessors added a further category (4) as some roots look 'very' dead! Results for the initial comparison of black-grass populations that were susceptible or resistant to other herbicide modes of action concluded that optimal glyphosate rate is 50 µm and it not effected by the different populations (Figure 41), which both scored above level 3. This test was repeated to validate the result.

A second experiment included the same black-grass populations with the addition of plant size as a factor. It can be concluded from the results (Figure 42) that the larger plants (GS21-22) were equally well controlled in this test than the smaller plants (GS12-13). In this particular test both the populations at GS12 did not respond to the rate of 50 µm of glyphosate quite as effectively as in other tests, however we are still confident with this rate in the repeats of experiment one.

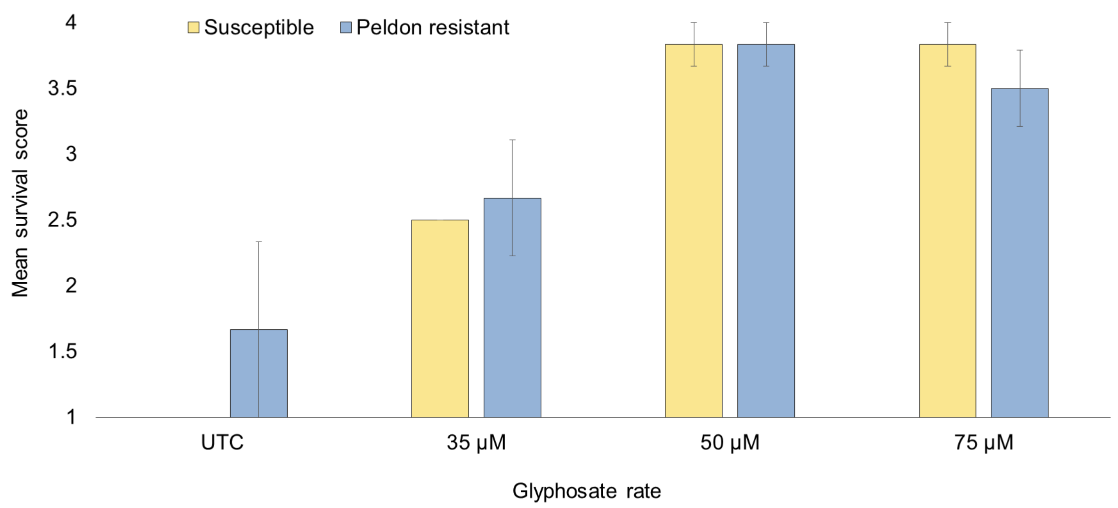

Figure 41 Level of control for the susceptible and resistant black-grass populations tested with different glyphosate rates at a growth stage of GS12 in 2016.

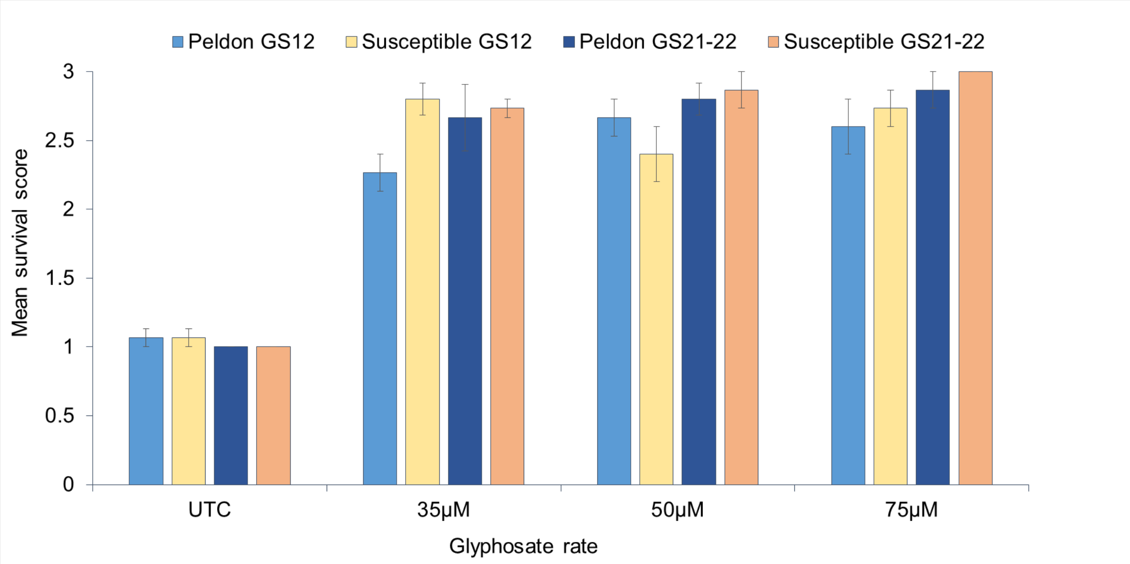

Figure 42 Level of control for the susceptible and resistant black-grass populations tested with different glyphosate rates at two different growth stages, GS12 and GS21-22 in 2016.

The RISQ test method was also used on IRG populations to validate dose and plant growth stage. The first experiment included a susceptible standard population and a known ALS-inhibitor resistant population. The results for the growth stage experiment (Figure 43) has shown that both of the populations respond better when tested at the larger growth stage of GS21-22 compared to GS12. The test was then repeated a final time using a glyphosate resistant IRG population sourced from Australia, to demonstrate the effectiveness of the test method for detecting glyphosate resistance. Results show (Figure 44) that the glyphosate resistant population produced new root growth at all doses tested. The susceptible populations tested required the higher dose of 75 µm to fully control both of them, so this higher rate should be used for further IRG testing.

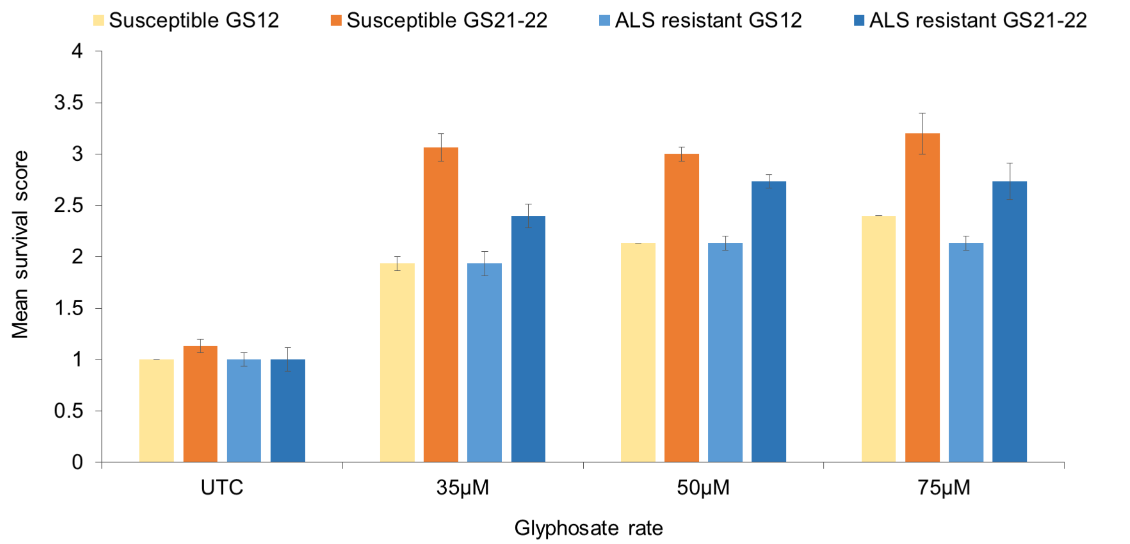

Figure 43 Level of control for the susceptible and ALS-resistant Italian rye-grass populations tested with different glyphosate rates at two different growth stages, GS12 and GS21-22 in 2018.

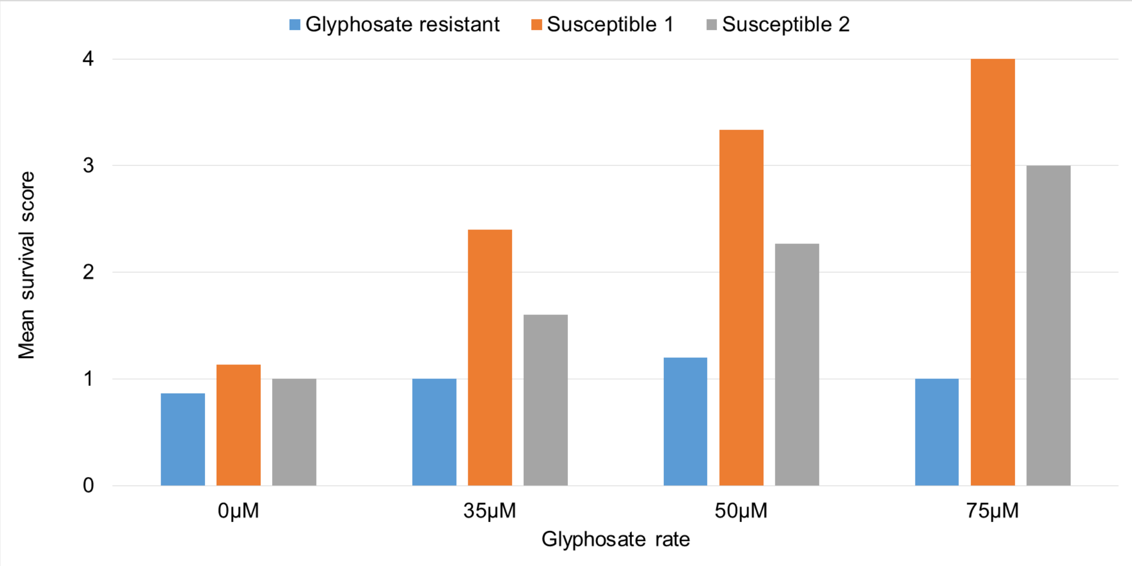

Figure 44 Level of control for IRG populations, included two herbicide susceptible and one glyphosate resistant in 2020.

Results from this project have further validated the use of the RISQ test method for black-grass and IRG with glyphosate. The optimum glyphosate rate for testing has been confirmed and the successful inclusion of larger plants will now enhance the practicalities of the test. It will provide a further option for a quick test method when resistance is suspected, but should be followed up with a glasshouse pot test if any resistance is detected.

4.4.5. Selection experiment: To determine how quickly black-grass populations shift their glyphosate tolerance

The selection experiments ran for a four-year period in containers. In 2016 and 2017 three glyphosate rates were tested, however by 2018 there were not enough survivors from the 360g a.s./ha rate to continue with this selection line so it was removed. The mean number of heads per container are shown for each year in Figure 45. Control levels were extremely high in 2018/19 resulting in some seed lots from 2018 being required for the dose response experiments, particularly for the 180g a.s./ha rate.

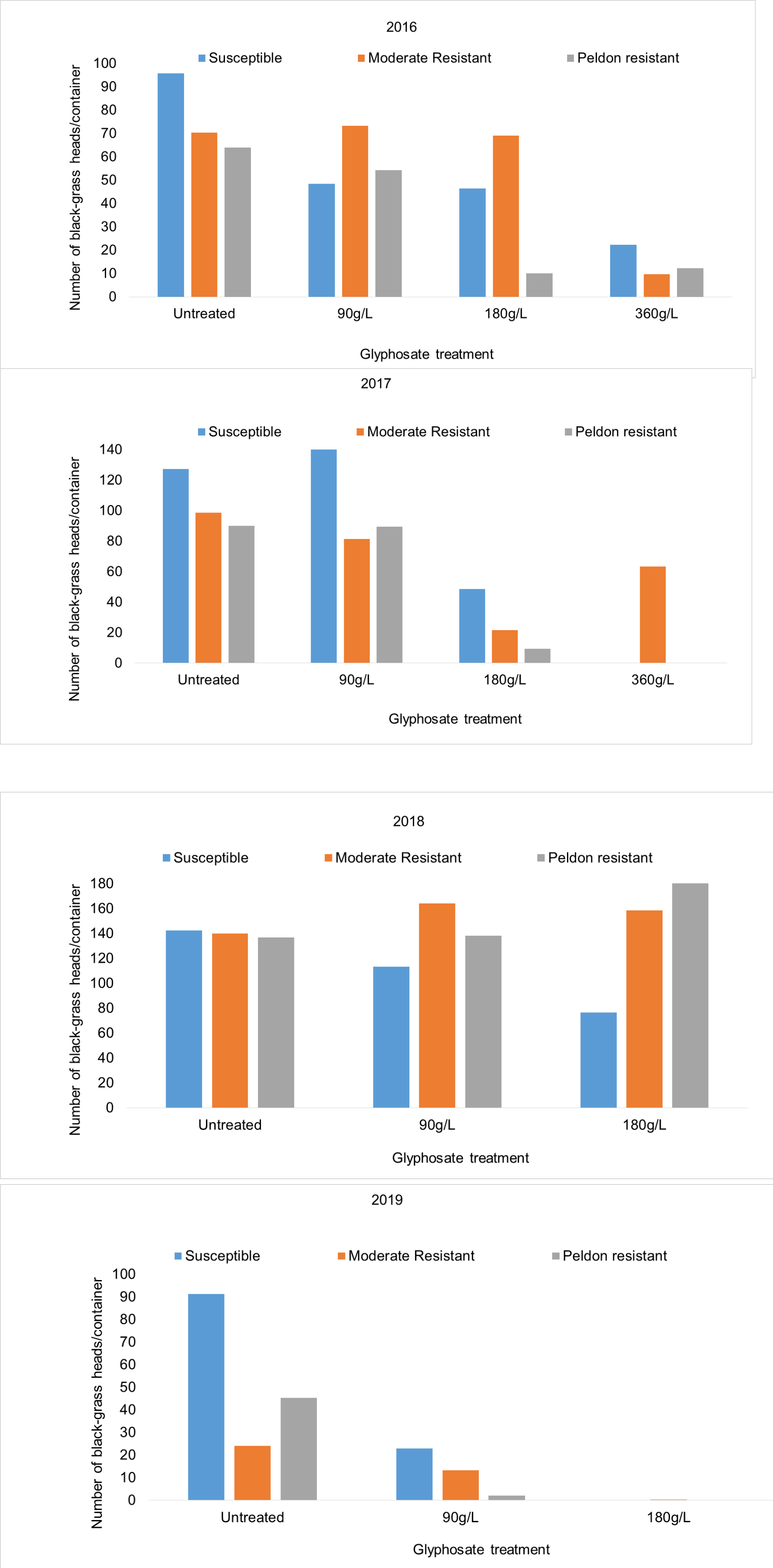

Figure 45 The mean number of black-grass heads per container for black-grass populations tested in the selection experiment for four-years, 2016-2019.

The populations tested in the glasshouse dose response experiment are show in Table 70. The survival curves, along with the ED_{50} and ED_{90} values are shown in Figure 46, Figure 47 and Figure 48 respectively.

Table 70 Population reference and original seed source for the selection experiment

Population reference	Population & historic treatment rate and timing	Year of seed collection
SD 0032	Peldon resistant baseline seed	Baseline
SD 0172	BG01 baseline seed	Baseline
SD 0673	BG01, 180g Glyphosate	2018
SD 0676	Susceptible, 180g Glyphosate	2018
SD 0679	Peldon resistant, 180g Glyphosate	2018
SD 0680	Peldon resistant, 90g Glyphosate	2018
SD 0790	Susceptible, UTC	2019
SD 0791	Susceptible, 90g Glyphosate	2019
SD 0792	BG01, UTC	2019
SD 0793	BG01, 90g Glyphosate	2019
SD 0794	Peldon resistant, UTC	2019
SD 0805	Rothamsted susceptible	2019

The results from the pairwise population comparisons using R from these figures are showing that generally all populations were controlled at field rate (540g) of glyphosate (Figure 46, Figure 47 & Figure 48). The baseline Peldon resistant population (SD 0032) required the highest glyphosate rate compared to the other populations tested, which was significantly different (Fpr 0.001) compared to the baseline susceptible population (SD 0805). The Peldon resistant populations that had been exposed to 90g (SD 0680) and 180g (SD 0679) for three-years were the next highest ED_{50} and ED_{90} levels (Figure 47 & Figure 48) showing that they required a significantly higher glyphosate rate (90g Fpr=0.1 and 180g Fpr =0.05) to control them after repeated low rate exposure in controlled conditions. There were no significant shifts from the baseline population for the BG01 population or the susceptible population in this particular experiment.

These results have not shown a very high shift in population tolerance to glyphosate, as we might have expected over four-years in controlled conditions, but have indicated a trend towards a repeated lower rate, on a black-grass population with known resistance to other herbicide modes of action (such as Peldon resistant), to then require a higher glyphosate rate for optimum control.

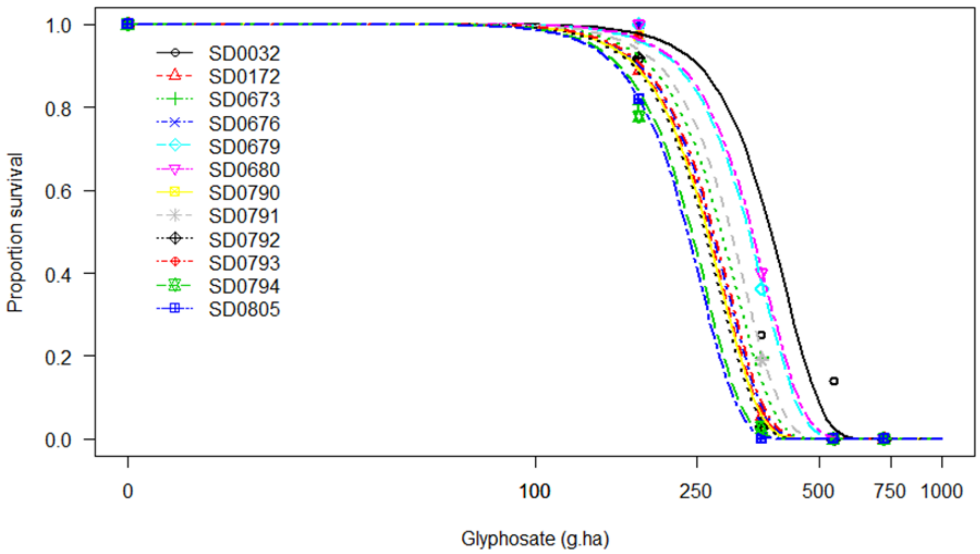

Figure 46 Survival curve for the populations included in the selection container experiment

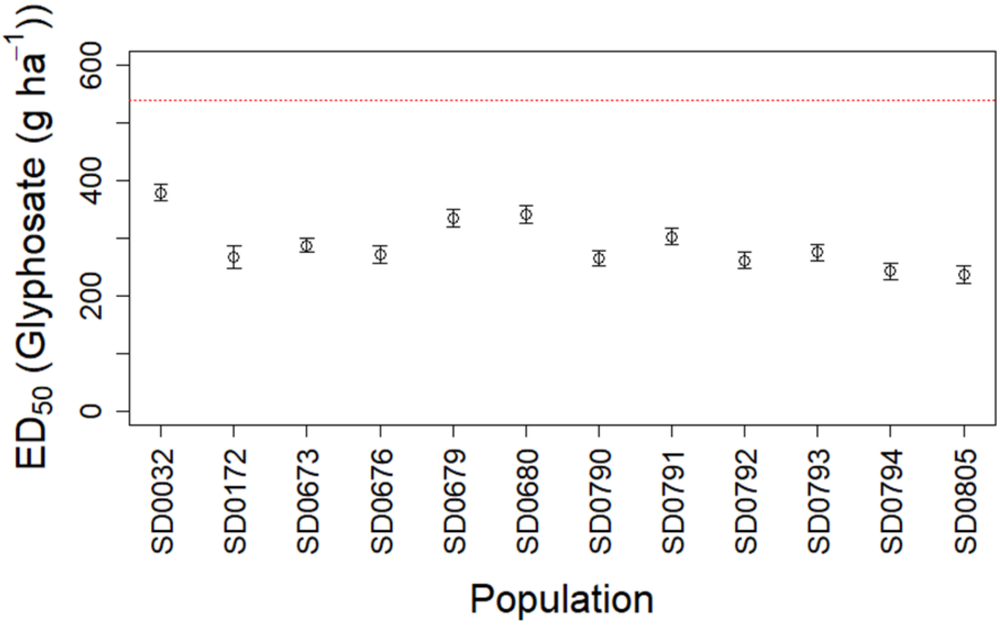

Figure 47 ED_{50} for populations included in the "Selection" dataset. Values represent the mean ± standard error. Dashed red line represents the recommended field rate of glyphosate

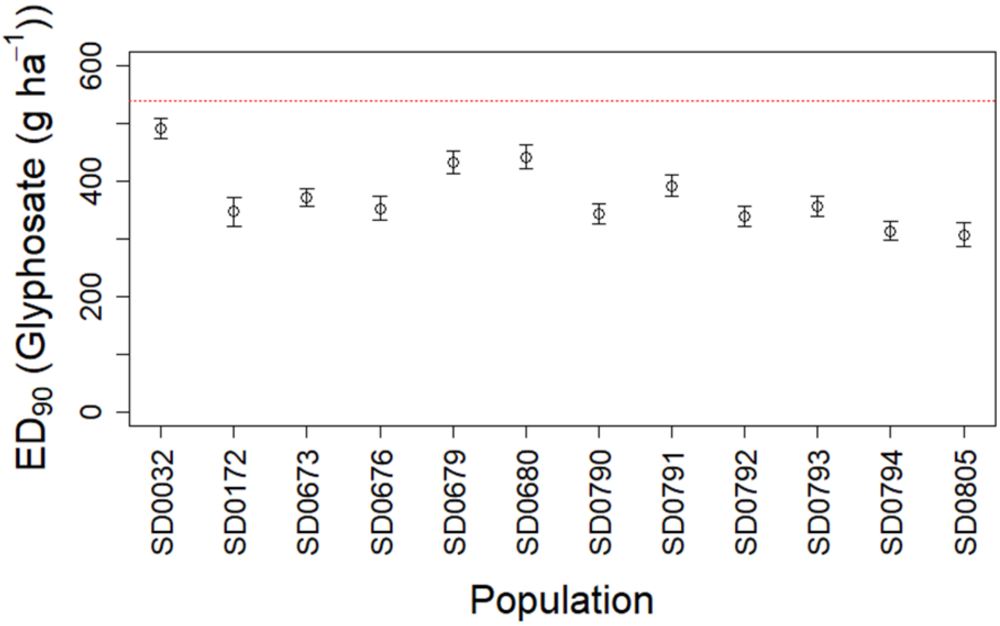

Figure 48 ED_{90} for populations included in the selection containers dataset. Values represent the mean ± standard error. Dashed red line represents the recommended field rate of glyphosate

5. Discussion

This project began when the WRAG guidelines were put together in 2015. They were a collation of the best knowledge of the experts at the time, but often presumptions were based on limited, or no, evidence, so tended to be risk averse. The initial project phase included a more in depth review of data available, but unpublished by some of the partner companies. These data enabled us to determine the gaps in knowledge or highlighted areas where further validation were required to optimise glyphosate efficacy and at the same time reduce the risk of resistance evolution. As the project developed experimental evidence showed that the majority of information in the guidelines was correct, but vitally provided increased confidence in the measures to ensure glyphosate stewardship.

Results of the multiple field and container-based experiments concluded consistent strong evidence on correct application timing, rate and weed growth stage for optimal efficacy, therefore preventing survivors. Seed collected from the dose and weed size experiment, the four-year selection experiment and the large growth stage experiments, showed a trend towards populations requiring a higher glyphosate rate when they had been exposed to a low rate at a large weed growth stage. In field situations these survivors are likely to gradually build up the resistance to glyphosate in a population over time.

The overall key messages were:

- *Optimum glyphosate application timing for black-grass & Italian ryegrass GS12-13.*
- *Glyphosate rate >540g critical for optimal control (maximum efficacy).*
- *If target weeds are tillering (>GS21-22) a higher glyphosate rate (720g +) required.*
- *Temperature at application is extremely important, enhancing or inhibiting control.*
- *Cultivation of stale seedbed essential to increase black-grass control.*
- *Maximum of two glyphosate application timings for a stale seedbed.*
- *In controlled conditions a shift in glyphosate sensitivity was detected in seed survivors of treatments at low rates and at large growth stages, therefore validating the need for optimal control to prevent survivors and reduce resistance risk.*

Field experiments were variable across the years with seasonal differences affecting the results. It was not always possible to practically get as many stale seedbeds in, or spread apart, due to the weather in a given season, or, for example, the soil was too wet to cultivate when we had planned. Therefore being too prescriptive of the number of cultivations or glyphosate applications in a stale seedbed is not practical in reality. We have been able to suggest 'best practice' based on the evidence that has been generated through the experiments, but soil type, geographic location, previous cropping and weather conditions during this phase will all have a large impact on overall success. Results from the spring sown experiments showed very strong evidence that an autumn cultivation was vital to reduce black-grass numbers ahead of a spring sown crop. All field experiments have been limited to black-grass only, so further work on Italian rye-grass is required.

Container-based experiments strongly concluded the importance of correct weed size and glyphosate rate, for both black-grass and Italian rye-grass populations, despite resistance status to other herbicide modes of action. The populations for both grassweeds that were most highly resistant to other modes of action were most often showed a higher level of tolerance to glyphosate than other populations. This was most evident in the Italian rye-grass populations tested.

The use of the RISQ test for early detection of glyphosate tolerant populations would be a very valuable and quick method for early detection, followed by a robust glasshouse pot test. The monitoring of grassweed patches of concern and immediate removal is recommended to reduce the risk of glyphosate resistance evolution, especially where glyphosate usage is high and continual.

Evidence on development of resistance evolution was more difficult to demonstrate within the experimental timeframes. One glasshouse dose response experiment did conclude that for selected populations, the use of a low dose at a higher weed growth stage, for both black-grass and Italian ryegrass, resulted in a higher rate of glyphosate required to control those survivors. It is important to note that this was from seed collected from controlled conditions with no dilution effect from a weed seed bank or crop competition. It is a concern, and due warning, that these shifts in glyphosate tolerance can be detected in just two years in controlled conditions, but within a field scenario it would be many years before these tolerance shifts would be detected. This is in line with the work by Neve *et al.,* (2002) where simulation models for rigid rye-grass predicted that resistance could evolve in the field in 10-15 years, in reduced tillage situations reliant on glyphosate pre-drilling. Results from the dose response of seed collected from the four-year selection container experiment did not show any clear shifts in glyphosate tolerance. It is a positive result that glyphosate resistance evolution is evidently not a quick process, so further enhances the messages of optimising control from glyphosate at all stages of a crop rotation.

It can be concluded that this programme of work provides information to further underpin the WRAG guidelines to manage resistance, in particular quantifying the four key principles:

Prevent survivors: Container-based experiments of repeated low glyphosate rate or applications at the wrong weed growth stage (too large for the rate used) lead to plant survivors. Seed collected from those survivors were further tested in glasshouse dose response pot experiments. Results from some of the populations tested concluded that plants exposed to a low rate applied at a large weed growth stage then required a higher rate of glyphosate for optimal control than previously. This has validated this principle.

Maximise efficacy: Container-based experiments quantifying the optimum glyphosate rate and application timing for both black-grass and Italian ryegrass successfully validated this principle. Data generated consistent conclusions that using the correct glyphosate rate at the correct weed growth stage for that target weed optimised control. Weather factors at the time of application were also important in maximising efficacy, as in one trial year a very warm spring enhanced the glyphosate efficacy. It is important to note that this was in outdoor container-based experiments where water was not limited, but may not have been exactly the same in field due to the dry weather conditions.

Use alternatives: Multiple field experiments integrating cultivations in conjunction with glyphosate applications proved that that the cultivation increased the level of black-grass suppression in the stale seed bed. This alternative method of control is important as a non-chemical input helping to reduce the reliance on glyphosate alone. This principle will be modified in the guidelines.

Monitor success: Conclusions from this project will now be used to update the WRAG guidelines. Continued promotion of the key messages will highlight the importance of monitoring future weed control programmes, in respect to glyphosate and how effective individual treatments are.

The WRAG guidelines will now require some minor modifications to include the evidence generated. The key area is stale seedbed management, which should say a maximum of two timings is ideal rather than >2 timings as currently included. However, on occasion three timings (or even just one timing) could be beneficial and this would be very seasonally dependant. The addition of a cultivation within a stale seedbed again can be complicated depending on the timing of that action. If it is early in the phase of the stale seedbed, allowing a big enough window for any flush of grassweeds to be controlled by a glyphosate application pre-drilling then it has been proven to be extremely valuable. If it is a very dry, or extremely wet autumn there may be very few weeds emerging, so advice would need to be tailored to the seasonal conditions. However, even the act of drilling can move the soil enough for another flush of grassweeds to emerge into the crop. The depth of cultivation is most beneficial when it is no more than 5cm deep. The guidelines will be reviewed to see if this can be any clearer.

There are still no glyphosate resistant grassweeds in the UK, but populations have been highlighted where sub-optimal rates show a rapid decline in control ((Davies *et al*., 2017; Davies *et al*., 2018). This was shown in some of the container-based experiments in this project, including the selection experiment, where the Peldon resistant black-grass population (resistant to other herbicide modes of action) exposed to either 90g or 180g of glyphosate for three-years in controlled conditions required a higher glyphosate rate for optimum control.

The environmental consequence of the loss of glyphosate due to resistance or legislative changes are highlighted in Clarke, 2018, including an increase in greenhouse gas emissions due to cultivation and inputs changes, increased land requirements and reductions in yield. The first cases of glyphosate resistant grassweeds were recorded in Australia (Powles *et al*., 1998) in *Lolium rigidum* in arable crops. In New Zealand cases of glyphosate resistance Italian rye-grass (*Lolium multiflorum*) and perennial rye-grass (*Lolium perenne*) were recorded in 2013 in perennial vineyard (Ghanizadeh *et al*., 2013). Further work on the ryegrass populations in New Zealand (Ghanizadeh *et al*., 2016) showed that the mechanism of glyphosate resistance loses its efficacy in cooler conditions, so practical advice there has been to spray glyphosate when cooler. However our work has shown that warmer conditions enhances the glyphosate efficacy, so where no glyphosate resistant populations exist optimising control should be best advice. A resistance management and prevention strategy has been produced in New Zealand (Harrington *et al*., 2016), similar to our UK WRAG guidelines, although they are now in a reactive mode to a problem that has already begun. The guide includes control in perennial crops such as vineyards and orchards, amenity areas, arable cropping and pasture land, which is something the UK should now consider to increase advice beyond arable cropping. The first case of glyphosate resistance in an arable crop in Europe was reported in Italy (Collavo + Sattin, 2014) to ryegrass (*Lolium* spp.), which also showed cross resistance to ACCase and ALS-inhibitor herbicides.

One of the conclusions from a recent survey carried out by the ENDURE project (Antier *et al*., 2020) highlighted the need for more research to fully capture the total glyphosate use in arable cropping across the EU, including the UK, as data are currently very variable and difficult to extrapolate exact use area, as it may be underestimated to date. It is therefore vital that in the UK accurate records of glyphosate use, including rates and timings are recorded.

The key part of optimal glyphosate management is reducing survivors and maximising the outcome of any glyphosate application. Any survivors will go on to generate a resistance risk. The stale seedbed phase is often not considered part of the herbicide inputs for that next crop. Therefore, it must be promoted clearly to arable growers that it is an extremely important phase of the cropping year that can have a huge impact on the weed pressure and subsequent herbicide choices and cost inputs in the crop. The whole glyphosate strategy has to become part of an overall integrated weed management plan and be considered across a cropping rotation.

Future work should include focus on communicating the key messages from this project and to update the WRAG guidelines as outlined, continuing to pro-actively highlight the risk of glyphosate resistance. This was discussed further by Clarke (2018). The glyphosate label, or technical supporting literature, would benefit from including specific details on optimum growth stage for application for key weeds. The labels state the risk of glyphosate resistance is low, but perhaps this should be increased to a medium risk with evidence from these results. This project has only included two grassweeds in arable crops, so investigations on other high risk weed species, other crops, particularly perennial crops need to be considered. Perennial crops may involve weeds being at much larger growth stages when treated, so optimising glyphosate rate will be essential. Only one glyphosate formulation has been used within the project for consistency, but in reality farmers will be using a wide range of different product choices, water conditions and adjuvants, so all these factors would need further investigation to be more specific about their impact. As weather conditions have such a large influence on soil, weed emergence, crop establishment and herbicide efficacy, there needs to be further work to continue to validate the optimum stale seed bed advice over multi-seasons.

Glyphosate is essential to grassweed control in arable crops in the UK. It can be maintained as a key tool for grassweed management through resistance prevention and good stewardship to prevent loss through legislation, if it is respected and used wisely.

6. Acknowledgements

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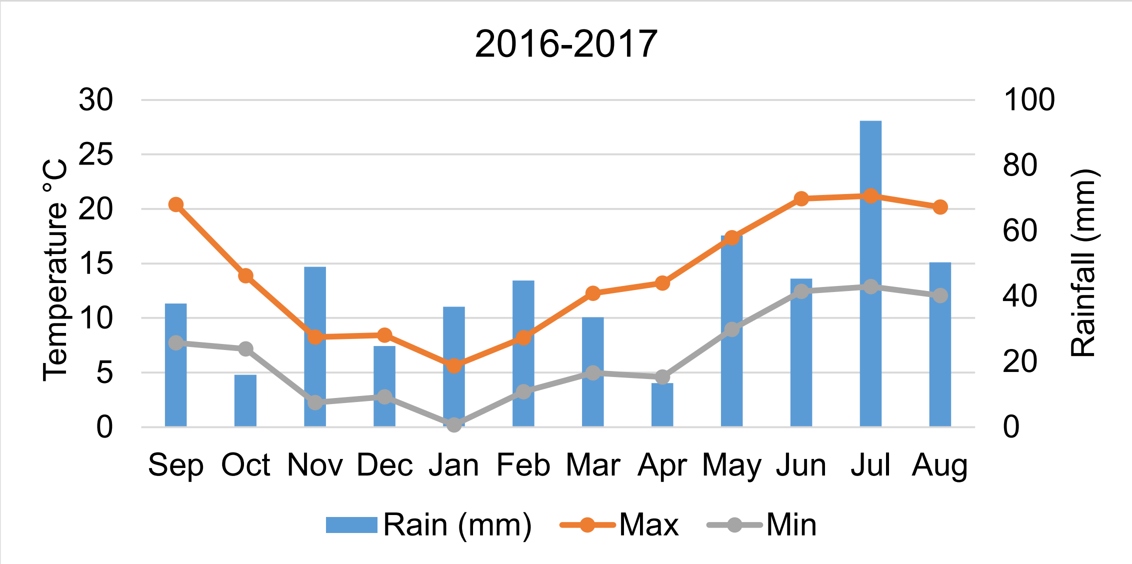
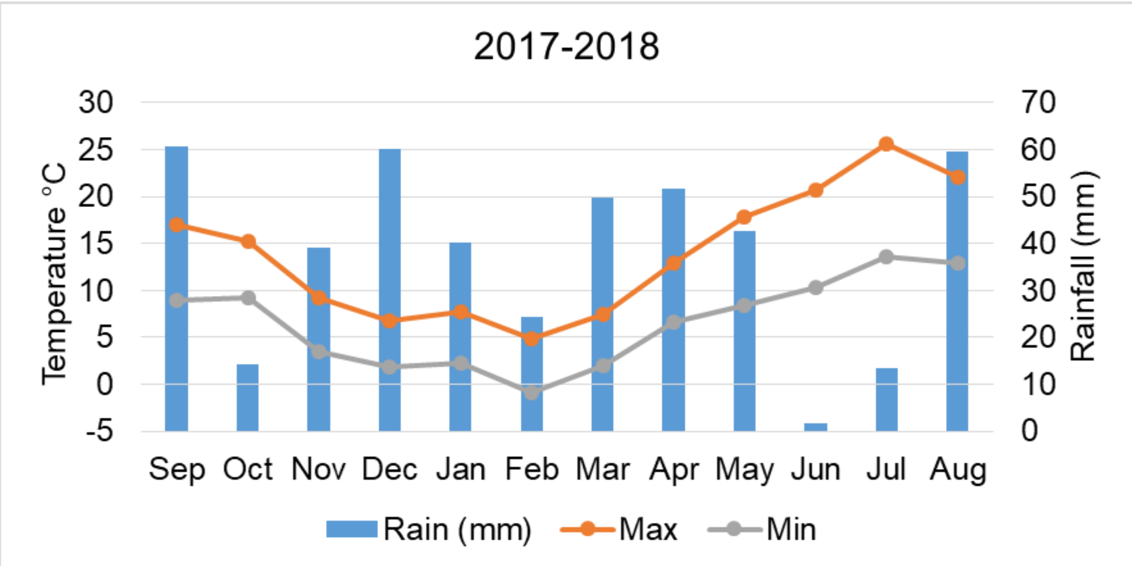
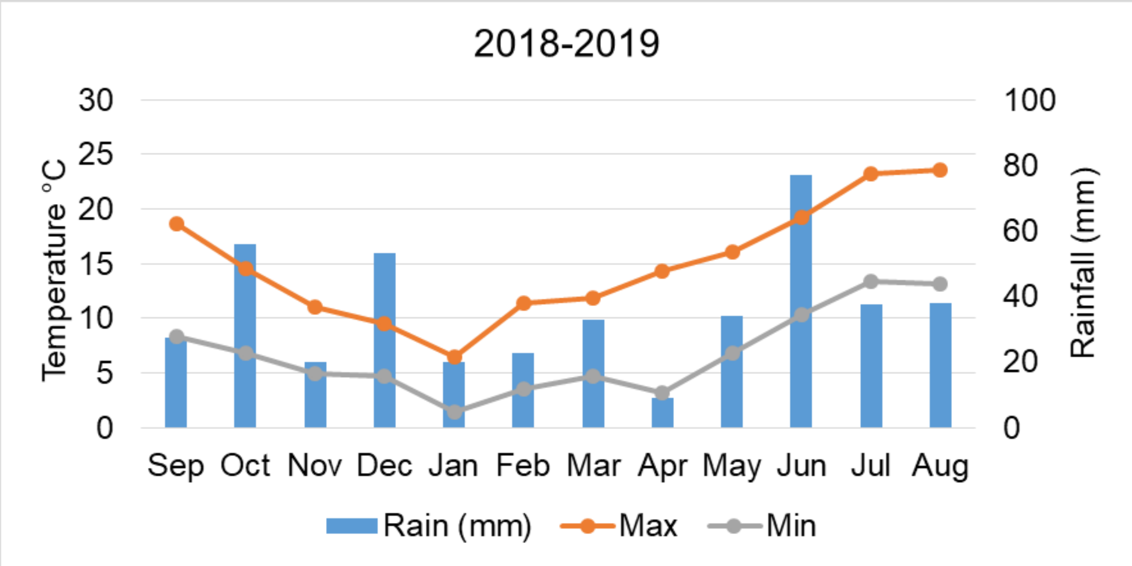
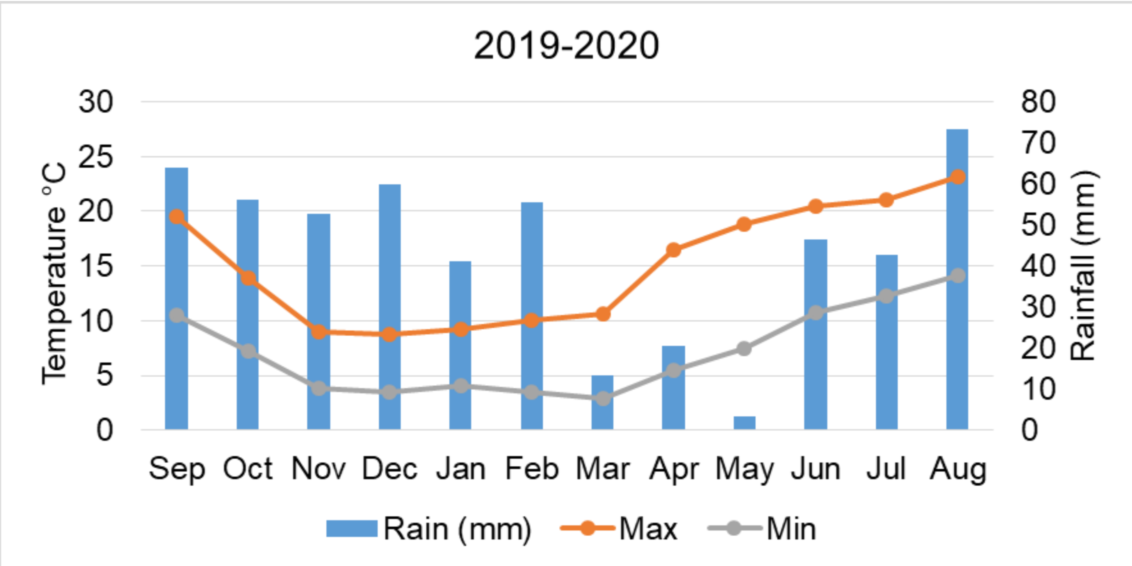
8. Appendices

8.1. Appendix 1 Weather data for ADAS Boxworth, Cambridge UK






2018-2019
Temperature °C
Rainfall (mm)
30
25
20
15
10
5
0
100
80
60
40
20
0
Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug
Rain (mm)
Max
Min


2019-2020
Temperature °C
Rainfall (mm)
30
25
20
15
10
5
0
80
70
60
50
40
30
20
10
0
Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug
Rain (mm)
Max
Min

8.2. Appendix 2 Resistant status for weed populations tested

Table 71 Resistant status of the black-grass and Italian rye-grass populations used in experiments

Weed population name and reference code	Herbicide tested and R Rating*				
	Cycloxydim**	Pendimethalin***	Mesosulfuron + Iodosulfuron	Pinoxaden	Chlorotoluron
Peldon resistant (SD0032)	S	RRR	RRR	-	-
BG 01 (SD0040/2014C81)	RRR	S	RR	-	-
IRG 01 (PS6757/SYN2011IRG003)	RRR	-	RRR	RRR	S
IRG resistant (SD0388/2015C33)	RRR	RRR	RR	RRR	-

*The R rating system for resistance testing classification is explained in Moss *et al*., 1999 & Moss, 2007.

** Cycloxydim is used to indicate ACCase target site resistance.

***Pendimethalin is used to indicate enhanced metabolism resistance.