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Investigating the distribution, presence, and potential for herbicide resistance of UK brome species in arable farming

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Contents

1. Abstract

This project investigated the distribution, presence, and potential for herbicide resistance of brome species on UK arable farms.

The project assessed the distribution of brome populations and how black-grass management influences bromes and resistance evolution (Objective 1). This aspect used a UK-wide online survey and further survey work, via the black-grass resistance initiative (BGRI) farm network.

Container-based experiments (Objective 2) – with great (*Anisantha diandrus*), sterile (*A. sterilis*), meadow (*Bromus commutatus*) and rye brome (*B. secalinus*) – built on knowledge of mechanisms associated with herbicide resistance evolution in grass weeds, assessed populations and investigated the processes that may lead to herbicide resistance in bromes. Seed samples, collected in the survey, were used to investigate the range in herbicide susceptibility, within and between brome species, and to test for herbicide resistant populations. Experiments also investigated if populations can be pushed towards resistance and the herbicide modes of action most at risk.

Using selected populations, herbicide application timing was investigated to identify strategies to help maintain and improve herbicide control, while also minimising the risk of resistance evolution (Objective 3). Finally, a knowledge transfer strategy was developed to disseminate results and communicate an integrated weed management system (Objective 4).

The results showed that brome was present in all UK regions and species were not localised. Bromes were also found to be more abundant than previously observed and likely to increase further – due, in part, to the change in control measures for black-grass, the move towards reduced cultivations, along with increases in areas under environmental management. Its presence could threaten soil health initiatives.

The project has published evidence of the presence of herbicide resistance to ALS-inhibitor herbicides in one great, four sterile, two meadow and four rye brome populations. The work also detected reduced sensitivity to glyphosate in one sterile and one rye brome population. The use of ineffective herbicides, or doses that allow survivors, risk driving resistance development to some herbicides. However, the link is not as marked as in some other species, most probably due to the self-pollinating nature of bromes.

There was no clear evidence of resistance to either of the two ACCase herbicides tested (propaquizafop, cycloxydim), even in populations showing resistance to ALS herbicides. This positive finding highlights the potential for good brome control in non-cereal crops within the rotation.

The results provided consistent and strong evidence on best application timing and weed growth stage for optimal efficacy of herbicides.

Integrated control, with heavy reliance on cultural measures, is important. However, the project identified a need for better dissemination of information on identifying and understanding the biology of brome species, as well as the effectiveness of cultural/integrated control measures.

2. Introduction

Bromes are highly competitive weeds that most commonly infest field margins and headland areas, but in severe cases also infest field centres (Cussans *et al*., 1994). Infestations of sterile brome (*Anisantha sterilis*) at densities of 5 plants/m² can cause a 5% yield loss (Marshall *et al*., 2003). At higher densities of 120 plants/m², wheat yields can be reduced by 35-47% and barley yields by 8-14% (Peters *et al.*, 1993). In comparison, black-grass (*Alopecurus myosuroides*) causes a yield loss of 15-25% at 100 plants/m² in winter wheat (Blair et al., 1999). High levels of brome infestations can increase costs to growers by impacting grain quality, contaminating grain, and causing lodging (Peters *et al*., 1993).

At present, there are few data on the presence, spread and economic impact of the five problematic brome species in UK cereals: great brome (*Anisantha diandrus*), sterile brome, meadow brome (*Bromus commutatus*), soft brome (*B. hordeaceus*), and rye brome (*B. secalinus*). However, there have been indications that populations of sterile brome (Smart *et al*., 2005) and rye brome (Cook *et al*., 2012) have been increasing over the past few decades and are more widespread than previously thought due to several factors. The introduction of field margin schemes has led to an increase in brome (Kellet, 2016), particularly where margin seed mixtures have failed to establish well. Additionally, the increased repeated use of glyphosate particularly in less than perfect spraying conditions prior to drilling kills off or thins margin flora leaving bare patches which again favours brome establishment, seed is subsequently moved into the field by combines and cultivation. Finally, the return to minimal tillage and direct drilling moves brome seeds just below the surface invoking dormancy, creating a long-term weed problem.

The last British survey of the distribution of brome grasses as arable weeds was conducted in 1989, where it was estimated that brome weeds infested 600,000 ha of cropped cereal in Britain, however, the survey did not include Northern Ireland (Cussans *et al*., 1994). Since 1989, surveys have only been conducted on brome weed abundance in field margins (Critchley *et al*., 2005), and only limited surveys of brome infestations in arable fields by companies and distributors. Moreover, since the 1989 survey several new herbicides have been introduced to the market and many have been removed, influencing brome control (Stobart & Ballingall, 2013).

There is a limited range of herbicides available to control brome in cereals, as a minimum a 'stack' or 'sequence' programme needs to be used including a pre and post emergence spray. Stobart & Ballingall (2013) evaluated a wide range of herbicides in winter barley and noted that where brome populations of over 15 plants/m² were present in winter barley, only 80% control was achieved with the most effective programmes as control was reliant on pre-emergence herbicides as no spring control options are available. Since this work was done, chlorotoluron has been withdrawn as a single active. In winter wheat, brome control was more successful with 90% plus control being achieved. Worryingly, all the post emergence options available contain ALS inhibitors, to which bromes have already developed resistance in France, Australia and the USA (Table 1). In areas with a high incidence of black-grass, brome control has been incidental with applications timed to suit black-grass, brome is usually at a later growth stage due to its early emergence and often escapes control. In break crops, most herbicides for brome control are ACCase inhibitors to which resistance has also already been identified in other countries (Table 1).

Natural variation between different weed populations in herbicide susceptibility to different modes of action exists in all species (Jasieniuk *et al*., 1996; Neve *et al*., 2014). The extent of this variation and the level of susceptibility compared to the recommended field rate of a herbicide can be indicators of the potential for resistance evolution (Neve *et al.,* 2009; Busi *et al*., 2013). Variation in herbicide susceptibility has previously been investigated in some brome species. Escorial *et al*., (2011) found variation and decreased glyphosate susceptibility in *Bromus diandrus* between different Spanish regions of 5.9% and 13.8%. Variation in the same populations to the herbicides chlorotoluron, diclofop-methyl and chlorsulfuron was also reported.

Research has suggested that some brome species may be naturally less susceptible to certain herbicides (Cook *et al*., 2012), with poorer control at recommended field rate compared to other grass weeds. One such example is the moderate resistance of sterile, great, and rye brome to grass weed herbicides containing mesosulfuron-methyl and iodosulfuron-methyl-sodium at doses that control susceptible black-grass and ryegrass. Even at higher label rates, these brome species are only moderately susceptible.

Poor herbicide control has the potential to lead to non-target site herbicide resistance evolution. Glasshouse experiments and practical field examples have demonstrated that grass weeds can respond to low herbicide doses that act within the variation of herbicide susceptibility, with populations becoming less susceptible and even resistant over a number of generations – for example, ACCase glasshouse selected rigid ryegrass (Neve & Powles, 2005) and glyphosate (Busi & Powles, 2009), glyphosate glasshouse selected black-grass (Davies & Neve, 2017), and rigid ryegrass exposed to low glyphosate doses in the field (Collavo & Sattin, 2014). However, no herbicide selection experiments have been conducted using brome species and it is possible that if bromes are naturally less herbicide susceptible than other grass weed species, herbicide doses used in the field could act within this natural variation leading to an increased chance of resistance evolution.

Globally, the incidence of herbicide resistance in brome is increasing and there are now seven brome species with herbicide resistant populations in six countries, with most cases of resistance discovered in the last decade (Table 1). Currently, there have been no officially reported cases of herbicide resistance in brome in the UK. However, UK sterile brome populations have been shown to have reduced glyphosate sensitivity and are in the process of evolving resistance (Davies *et al*., 2019).

Table 1 World-wide herbicide resistant brome species.

Common name	Species	Herbicide resistance	Country	Year	Reference
Sterile brome	*Anisantha sterilis*	ALS	France	2009	Heap (2021)
		ACCase	Germany	2012	Heap (2021)
Great brome	*Anisantha diandrus*	ACCase	Australia	1999	Boutsalis and Preston (2006)
		Glyphosate	Australia	2011	Heap (2021)
		ALS	Australia	2011	Heap (2021)
Red brome	*Anisantha rubens*	Glyphosate	Australia	2014	Heap (2021)
Stiff brome	*Anisantha rigidus*	ACCase	Australia	2006	Boutsalis and Preston (2006)
		ALS	Australia	2011	Heap (2021)
Downy brome	*Anisantha tectorum*	Photosystem II inhibitors	France, Spain	1981	Menendez *et al*. (2006); Heap (2021)
		ACCase	USA	2005	Ball *et al*. (2007); Park and Mallory-Smith (2005)
		ALS	USA	1997	Park and Mallory-Smith (2005), Heap (2021)
Rye brome	*Bromus secalinus*	ALS	USA	2007	Heap (2021)
Japanese brome	*Bromus japonicus*	ALS	USA	2007	Heap (2021)

2.2. Project roadmap

Work package	Component parts				Report sections for methodology (3.x) and results (4.x)
1	Surveying brome in UK arable crops	Online survey of farmers and agronomists	Seed samples of suspect herbicide resistant populations sent in	Brome species identified	3.1 & 4.1
2	Investigating the range in herbicide susceptibility within and between brome species and possible cases of herbicide resistance				3.2 & 4.2
2.1	Investigating variation in herbicide susceptibility within and between brome species	Using seed samples from WP 1	Initial herbicide resistance screen ALS, ACCase and glyphosate	Confirmation of herbicide resistant populations through dose responses	
2.2	Testing possible herbicide resistant brome populations	Seed samples sent in annually	Annual testing of suspect herbicide resistant populations	Further confirmation of herbicide resistant populations through dose responses	
2.3	Identifying the presence of target site resistance in herbicide resistant brome		Molecular analysis on herbicide resistant bromes		
3	Investigate if populations can be pushed towards resistance evolution and identify modes of action most at risk of resistance evolution				3.3 & 4.3
3.1	Herbicide selection		Selection of sterile and rye brome against ALS, ACCase and glyphosate for 3 years		
3.2	Dose-response of herbicide selected lines		Confirmation herbicide resistance development of 3-year selected populations through dose responses		
4	Adding value to the BGRI survey (Rothamsted)				3.4 & 4.4
4.1	BGRI network brome abundance		Assess the presence, distribution and abundance of brome grasses across the BGRI network (71 farms)		
4.2	BGRI brome herbicide susceptibility		Seed samples of suspect herbicide resistant populations tested for herbicide resistance		
5	Determine the best herbicide application timing to increase brome control and reduce the risk of resistance evolution		Determine optimum dose rate and timing of ALS, ACCase and glyphosate in sterile and rye brome		3.5 & 4.5

3. Materials and methods

3.1. Online brome survey (WP1)

3.1.1. Survey sampling

The online survey was made available between 12 June and 15 August 2017. The survey was distributed by five agri-chemical companies (BASF, Bayer Corteva, Monsanto (now Bayer CropScience Ltd) and UPL) farmers and agronomists, and promoted by ADAS and AHDB through social media, open days, and farming events. The survey was a non-random, self-selecting sample and, therefore, individuals experiencing problems with brome weeds were much more likely to respond than those without brome weeds.

3.1.2. Online survey method

The online survey consisted of 31 questions, initially asking for farm location, holding area, predominant soil type, and grass weed species present including the most problematic. If the respondent had brome weeds present, further details were requested. These included the predominant brome weed species, whether there had been a change in brome weed levels in the preceding three years and reasons for a change, cultural and chemical control methods used to control brome weeds, and whether there were any problems controlling brome weeds, including potential perceived resistance. Questions are detailed at Appendix 8.1.

Respondents with brome weeds also had the option of providing details of one arable cropped field affected by brome weeds. Respondents were asked to provide information on the current crop including: all brome species present in the chosen field, crop sowing date, percentage of field area affected by brome, brome incidence levels in different locations in field, how long brome had been present in the field, and where the brome may have originated from. To evaluate the bromes density in each field, respondents were asked to assess where in the field (uncropped margin, headland, and centre) and the level of brome present in each area of the field. Brome levels were based on those used by Cussans *et al*. (1994): Low - less than 10 heads panicles /m^2, Intermediate - 10–50 heads panicles /m^2, Severe - more than 50 heads panicles /m^2, and occasional - odd individuals.

Survey respondents also had the opportunity to send samples to ADAS to confirm brome species identification. Samples were identified to species level using the Identification of Brome Grasses publication by Moss (2015).

3.1.3. Statistical analysis

For the online survey field data, crop sowing dates were grouped into one of six groups (Before 15/09, 15/09-30/09, 1/10-14/10, 15/10-31/10, after October and spring). Pearson's correlation coefficient analysis was conducted on autumn crop sowing date group and percent of field affected by brome weeds. Two-way ANOVA analysis was also conducted on cereal crop and percentage area of field affected by brome species in R.

3.2. Investigating the range in herbicide susceptibility within and between brome species and possible cases of herbicide resistance (WP2)

As a result of the project, a total of 91 brome populations were received, between 2017 and 2020, from farmers and agronomists (Table 2). Fourteen percent of the samples were great brome (*Anisantha diandrus*), 31% sterile brome (*A. sterilis*), 18% meadow brome (*Bromus commutatus*) and 34% rye brome (*B. secalinus*). Only one soft brome sample was received, and this was not tested. A total of 103 samples were tested through the project with up to four standards of known herbicide resistance status included in each test.

Table 2 Number and species of brome received and tested for herbicide resistance

Species	2017		2018		2019		2020		
	received	tested	received	tested	received	tested	received	tested	Total
Great brome	12	10	1	2	1	1	-	-	15
Standards		*1*		*1*		*1*		-	
Sterile brome	20	19	2	2	8	8	-	-	30
Standards		*4*		*1*		*2*		-	
Meadow brome	15	12	-	-	2	2	1	1	18
Standards		*2*		-	-			-	
Rye brome	22	18	3	3	9	9	-	-	34
Standards		*2*		*1*		*1*		-	
Total	69	68	6	10	20	24	1	1	

Further details of individual populations can be found in Appendix 8.3

3.2.1. Investigating variation in herbicide susceptibility within and between brome species (WP 2.1)

In November 2017, populations were screened for sensitivity to Pacifica Plus (mesosulfuron-methyl + iodosulfuron-methyl-sodium + amidosulfuron, Broadway Star (pyroxsulam + florasulam), Laser (cycloxydim), Falcon (propaquizafop) and MON79379 (glyphosate). Populations showing low sensitivity were then subjected to a full dose response with Pacifica Plus, Broadway Star, Laser and

MON79379. Further screening to Pacifica Plus and Broadway Star took place in 2018, 2019 and 2020. A final dose response to GF1274 (pyroxsulam) was done in 2020.

BW18-033 ALS inhibitor, ACCase and glyphosate sensitivity screening on 2017 seed

In summer 2017, a total of 68 brome populations were sourced to assess the range in variation of herbicide response to ALS inhibitors (Table 2). Test populations were supplied by interested parties who had generally completed the survey regardless of perceived resistance status. At least one population of confirmed sensitivity to herbicides was included for each species (two for meadow and rye brome). For sterile brome an ACCase resistant from Germany and two ALS resistant populations from France were also included. Details of individual populations can be found in Appendix 8.3.

Six seeds were sown directly into 90mm square pots containing sterilized Kettering loam soil and lime-free grit (3 to 6 mm) in a 4:1 ratio, with the addition of Osmocote slow-release fertilizer (2 kg m^{-3}). Pots were placed in a glasshouse compartment with a 14-hour day length set to a temperature of 18°C with venting at 20°C with supplementary lighting, and a 10-hour dark cycle set at 12°C with venting at 14°C.

At the 2-3 leaf growth stage, plants were thinned to five plants per pot. Five herbicide treatments were done (Table 3 and Table 4). Herbicide treatments were applied using a track sprayer with a Teejet 01F110 flat fan nozzle, at a pressure of 1.3 bar, and a water volume of 200L/ha. After treatment, plants were left for 24 hours before being moved back into the glasshouse.

Pots were arranged as subplots (trays) by dose for ease of herbicide application, subplots were randomised within replicate, and populations were randomised within subplot.

Surviving plants were counted and above-ground plant material was harvested from each pot and fresh weight recorded. This was done at 35 days after treatment (DAT) for ALS treatments, at 24 DAT for glyphosate treatments, and 25 DAT for propaquizafop treatments.

Six populations were not harvested due to poor emergence, these were SD489, SD444, SD447, SD509, SD443 and SD502.

Table 3 Herbicide treatments for sensitivity screening 2017 - ALS herbicides

Treatment	Product	Active ingredient (a.s.)	Proportion of field rate	Dose rate g a.s./ha	kg/ha of product
1	Untreated	-	-		-
2	Pacifica Plus[1]	mesosulfuron-methyl	0.5x	7.5	0.25
		iodosulfuron-methyl-sodium		2.5	
		amidosulfuron		12.5	
3	Pacifica Plus[1]	mesosulfuron-methyl	1x	15.0	0.5
		iodosulfuron-methyl-sodium		5.0	
		amidosulfuron		25.0	
4	Broadway Star[2]	Pyroxsulam	0.5x	9.42	0.133
		cloquintocet-mexyl		9.42	
		florasulam		1.89	
5	Broadway Star[2]	Pyroxsulam	1x	18.76	0.265
		cloquintocet-mexyl		18.76	
		florasulam		3.76	

[1]Adjuvant Biopower 1.0L/ha, [2]Adjuvant biosyl 1% spray volume

Table 4 Herbicide treatments for the sensitivity screening 2017 - cycloxydim, propaquizafop and glyphosate

Treat No.	Product	HRAC* group	Active ingredient (g a.s./L)	Proportion of field rate	Dose rate g a.s./ ha	Amount of product (L/ha)
1	Untreated		-	-	-	-
2	Laser[1]	1	Cycloxydim 200	0.5x	100	0.5
3				1x	200	1
4	Falcon	1	Propaquizafop 100	0.5x	50	0.5
5				1x	100	1
6	MON79376	9	Glyphosate 360	0.66x	360	1
7				1x	540	1.5

[1]plus Adigor adjuvant at 0.5% spray volume *HRAC Herbicide resistance action committee

Statistical analysis

Herbicide sensitivity (% control) was assessed for each brome population at each herbicide treatment by expressing the fresh weight measured for each treatment as a percent control of the fresh weight of the untreated controls. Data were subjected to a two-way ANOVA in Genstat. Outliers were identified as those outside the LSD at 0.05 for population x herbicide.

3.2.2. Confirmation of herbicide resistant populations from seed collected in 2017

Populations were selected from seed collected in 2017 and screened in 2018, this is described in 3.2.1 and reported in 4.2.1, the selected populations are listed in Table 5. Herbicides were tested up to 2x field rate. A further dose response was done in winter 2019 with a wider range of dose rates (to 4x field rate).

BW19-021 Initial dose response on populations from 2017

A total of 1072 pots (9 cm 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 Osmocote mini)) to a depth of 2 cm below the rim. The pots were laid out in the glasshouse and watered well using an overhead watering system. Seeds were sown directly into pots (six seeds/pot) on 10 October 2018 and plant counts and thinning to a maximum of five plants per pot on 22-24 October 2018. At GS12, the pots were grouped into treatments and moved to the spray area.

There were 13 herbicide treatments plus an untreated control used (Table 23). All populations included an untreated control, and all treatments are detailed in Table 6. ALS herbicides were applied on 26 October 2018, Cycloxydim and glyphosate on 30 October 2018. There were four replicates for each treatment/population. Herbicides were applied using F02/110 nozzles at 2 bar and a water volume of 200L/ha. The treatment was allowed to dry on the foliage before the pots were placed back into the glasshouse and were not watered for at least six hours post herbicide application.

Table 5 Populations used in the dose response experiment (BW19-021), standards and selected from resistance screening in 2017.

Population	County/country	Population details and resistance status	Untreated	Pacifica Plus	Broadway Star	Laser	MON79376
				ALS		ACCase	Glyphosate
Great brome							
SD221	Hampshire	Sensitive	Y	Y	Y	Y	
SD440	East Lothian	Possible ACCase	Y	Y	Y	Y	
SD441	Shrops	Possible ALS resistant Poor field control	Y	Y	Y		
Sterile brome							
SD224	Germany	ACCase resistant	Y			Y	
SD409	France	Tested ALS resistant	Y	Y	Y		
SD454	Lincs	Possible ALS resistant Poor field control	Y	Y	Y		
SD464	Notts	Sensitive ALS, tested glyphosate tolerant	Y	Y	Y	Y	Y
SD468	Cambs	Sensitive	Y	Y	Y	Y	Y
SD478	Wilts	Possible ALS resistant	Y	Y	Y	Y	
SD479	Oxon	Sensitive to ALS	Y	Y	Y	Y	Y
SD488	Worcs	Possible ALS resistant	Y	Y	Y		
SD498	Yorks	Possible ALS resistant	Y	Y	Y		
Meadow brome							
SD466	Yorks	Possible ALS resistant Poor field control	Y	Y	Y		
SD518	Cambs	Sensitive	Y	Y	Y		
Rye brome							
SD453	Monmouth	Sensitive	Y	Y	Y	Y	Y
SD455	Surrey	Sensitive Poor field control	Y	Y	Y	Y	Y
SD470	Yorks	Sensitive ALS Poor field control	Y	Y	Y	Y	Y
SD506	Oxon	Possible ALS resistant	Y	Y	Y		

Table 6 Herbicide treatments in dose response experiment

Trt.	Product	Active ingredient	Prop dose	Dose rate (g a.s./ha)	Rate of product
1	Untreated	-	-		-
2	Pacifica Plus[1]	30g/kg mesosulfuron-methyl, 10g/kg iodosulfuron-methyl-sodium 50g/kg amidosulfuron	0.25x	3.75 1.25 6.25	0.125kg/ha
3			0.5x	7.5 2.5 12.5	0.25kg/ha
4			0.75x	11.25 3.75 18.75	0.375kg/ha
5			1x	15 5 25	0.5kg/ha
6			2x	30 10 50	1kg/ha
7	Broadway Star[2]	7.08%w/w pyroxsulam, 7.08% cloquintocet-mexyl 1.42% w/w florasulam	0.25x	4.71 4.71 0.95	0.066kg/ha
8			0.5x	9.42 9.42 1.89	0.133kg/ha
9			0.75x	14.13 14.13 2.85	0.199kg/ha
10			1x	18.76 18.76 3.76	0.265kg/ha
11			2x	37.52 37.52 7.52	0.530kg/ha
12	Laser[3]	Cycloxydim 200g a.s./L	0.25x	50	0.25L/ha
13			0.5x	100	0.5L/ha
14			0.75x	150	0.75L/ha
15			1x	200	1.0L/ha
16			2x	400	2.0L/ha
17	MON79376	Glyphosate 360g a.s./L	0.25x	135	0.375L/ha
18			0.5x	270	0.75L/ha
19			0.75x	408	1.125L/ha
20			1x	540	1.5L/ha
21			2x	1080	3.0L/ha

[1]plus Biopower adjuvant; at 1.0L/ha [2]; plus Biosyl adjuvant at 1.0% spray volume; [3]plus Adigor adjuvant at 0.5% spray volume

Plants were assessed between 3 December 2018 and 8 December 2018 by replicate. Photographs were taken of all populations in treatment order plus the untreated control. Plant counts were taken to record the number of surviving plants. To record the fresh weight, all plants in the pot were carefully cut at the base and weighed.

BW19-066 Second ALS inhibitor dose-response (4x rate)

This further dose response experiment was done in spring 2019 with a greater range of ALS herbicide rates (up to 4x field rate). The populations tested are shown in Table 7.

The dose-response followed the same method as described in the initial dose response above. There were three replicates. Pots were sown on 28 January 2019 and thinned to 5 plants per pot on 18 February 2019. The 15 herbicide treatments (Table 7) were applied on 27 February 2019. Plants were harvested on 2 April 2019.

Table 7 Brome populations tested in dose-response experiments for possible resistance to two ALS inhibitor herbicides – iodosulfuron + mesosulfuron and pyroxsulam

Population	Location	Population details and resistance status	Population	Location	Population details and resistance status
Great brome			Meadow brome		
SD441	Shrops	Possible ALS resistant Poor field control	SD466	Yorks	Possible ALS resistant Poor field control
SD523	Rutland	Sensitive	SD518	Cambs	ADAS
Sterile brome			Rye brome		
SD409	France	ALS resistant	SD0453	Monmouth	Sensitive
SD454	Lincs	Possible ALS resistant Poor field control	SD0455	Surrey	Sensitive Poor field control
SD478	Wilts	Possible ALS resistant	SD470	Yorks	Sensitive ALS Poor field control
SD488	Worcs	Possible ALS resistant	SD506	Oxon	Possible ALS resistant
SD522	Cambs	Sensitive	SD521		BRO

Table 8 Herbicide treatments in dose response

Trt.	Product	Active ingredient	Prop dose	Dose rate (g a.s./ha)	Product (kg/ha)
1	Untreated	-	-		-
2	Pacifica Plus[1]	30g/kg mesosulfuron-methyl, 10g/kg iodosulfuron-methyl-sodium 50g/kg amidosulfuron	0.125x	1.875 0.625 3.125	0.0625
3			0.25x	3.75 1.25 6.25	0.125
4			0.5x	7.5 2.5 12.5	0.25
5			0.75x	11.25 3.75 18.75	0.375
6			1x	15 5 25	0.5
7			2x	30 10 50	1
8			4x	60 20 100	2
9	Broadway Star[2]	7.08%w/w pyroxsulam, 7.08% cloquintocet-mexyl 1.42% w/w florasulam	0.125x	2.355 2.355 0.475	0.033
10			0.25x	4.71 4.71 0.95	0.066
11			0.5x	9.42 9.42 1.89	0.133
12			0.75x	14.13 14.13 2.85	0.199
13			1x	18.76 18.76 3.76	0.265
14			2x	37.52 37.52 7.52	0.530
15			4x	75.04 75.04 15.04	1.060

[1]plus Biopower adjuvant; at 1.0L/ha [2]; plus Biosyl adjuvant at 1.0% spray volume

3.2.3. Herbicide resistance screening 2018-2020

Resistance screening 2018

ALS resistance testing was carried out on 10 brome populations collected predominantly in 2018, (Appendix 8.3). Several sensitive and resistant populations were also included. There were two herbicide treatments, Broadway Star at full and half rate, (Table 9), and an untreated control, replicated four times.

For each of the seed populations, 12 pots (9cm) 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 Osmocote mini to a depth of 2 cm below the rim and watered using an overhead watering system two days before sowing. Six seeds were sown per pot and covered with 1cm of soil on 8 February 2019. Pots were thinned 20 days after sowing on 28 February 2019 and three days before herbicide treatments were applied (1 March 2019). Herbicides were applied at growth stage 12-13 in 200L/ha water. Survival counts and fresh weight was assessed 40 DAT on 9 April 2019.

Table 9 Herbicide treatments applied to brome populations in resistance screening, 2018

<table>
<tr><th>Trt No</th><th>Product</th><th>Active ingredient</th><th>Prop dose</th><th>Dose rate (g a.s./ha)</th><th>kg/ha of product</th></tr>
<tr><td>1</td><td>Untreated</td><td>-</td><td>-</td><td></td><td>-</td></tr>
<tr><td>2</td><td rowspan="2">Broadway Star[1]</td><td rowspan="2">7.08%w/w pyroxsulam,
7.08% cloquintocet-mexyl
1.42% w/w florasulam</td><td>0.5x</td><td>9.42
9.42
1.89</td><td>0.133</td></tr>
<tr><td>3</td><td>1x</td><td>18.76
18.76
3.76</td><td>0.265</td></tr>
</table>

[1]plus Biosyl adjuvant at 1.0% spray volume

The data from the test were interpreted by comparing the foliage fresh weights of herbicide treated and untreated pots for the same population and the percentage reduction in fresh weight calculated. The resistance rating ‘R’ was calculated from the % reduction relative to untreated controls for same population (Moss *et al*., 2007). The ‘R’ system assigns populations to four resistance categories (RRR, RR, R?, or S) depending on the degree of control achieved relative to the susceptible population in the same test.

Resistance screening 2019 (BW20-007)

ALS resistance testing was carried out on 24 brome populations (Appendix 8.3) with four herbicide treatments, Broadway Star and Pacifica Plus at full and half rate, (Table 10), and an untreated control, replicated four times.

For each of the seed populations 20 pots (9cm) were filled using the same materials and method described above. Seed was sown on 25 October 2019 and thinned to five plants per plot at GS 11-12. Herbicides were applied at GS 12-13 in 200L/ha water. Survival counts and fresh weight was assessed on 19 December 2019.

Table 10 Herbicide treatments applied in resistance screening, 2019

Treatment	Product	Active ingredients	Proportion of field rate	Dose rate (g a.s./ha)	product (kg/ha)
1	Untreated	-	-		-
2	Pacifica Plus[1]	30g/kg mesosulfuron-methyl 10g/kg iodosulfuron-methyl-sodium 50g/kg amidosulfuron	0.5x	7.5 2.5 12.5	0.25
3	Pacifica Plus[1]		1x	15.0 5.0 25.0	0.5
4	Broadway Star[2]	7.08%w/w Pyroxsulam 7.08%w/w cloquintocet-mexyl 1.42%w/w florasulam	0.5x	9.42 9.42 1.89	0.133
5	Broadway Star[2]		1x	18.76 18.76 3.76	0.265

[1]plus Biopower adjuvant; at 1.0L/ha [2]; plus Biosyl adjuvant at 1.0% spray volume;

Data were interpreted as for the 2018 resistance testing.

Resistance screening 2020

In 2020, a single meadow brome population (20C11, North Yorkshire) was tested for resistance using the same methodology as in previous years. The herbicide treatments were untreated and 0.265kg/ha GF-1274 (7.08%w/w pyroxsulam) + Biosyl (1% spray volume). Data were interpreted as for 2018 resistance testing.

3.2.4. Confirmation of herbicide resistant populations from seed collected in 2018 and 2019 – dose response

Six populations which were identified as resistant by screening in 2018 and 2019 were included in the final dose response experiment (Appendix 8.3), the experimental methodology is detailed in section 3.3.2. The herbicide treatments are in (Table 11).

Table 11 Herbicide treatments in dose response experiment, December 2020.

Trt.	Product	Active ingredient	Prop dose	Dose rate (g/ha)	Amount of product (kg/ha)
1	Untreated	-	-		-
2	GF-1274	7.08%w/w pyroxsulam + biosyl (1% spray volume)	0.0625x	1.176	0.0165
3			0.125x	2.355	0.033
4			0.25x	4.71	0.066
5			0.5x	9.42	0.133
6			0.75x	14.13	0.199
7			1x	18.76	0.265
8			2x	37.52	0.530

3.2.5. Identifying the presence of target site resistance in herbicide resistant brome (WP 2.3)

Leaf samples of one great brome (SD441), two sterile bromes (SD454 and SD488), one meadow brome (SD466), and one rye brome (SD506) population were collected from the ALS-inhibitor sensitivity screening. These populations were suspected of being resistant to ALS inhibitors. Leaf samples from the known sensitive populations were also collected.

Samples were collected from 15 surviving individual plants from each population, 5 treated with 15g/ha mesosulfuron and 5g/ha iodosulfuron, 5 treated with 18.8g/ha pyroxsulam, and 5 from the untreated control. Samples were taken from all plants in the replicate, and two of these populations and survival/death was recorded. Leaf samples were dried for three days before being sent to IDENTXX (Stuttgart, Germany) for target site resistance genotyping using pyrosequencing to detect point mutations in the ALS gene at positions Pro-197 and Trp-574, as described in Keshtkar *et al*. (2015)

In 2021, further samples were sent for pyrosequencing analysis at Bayer for possible mutations at the Pro197 and Trp574 position. Seed was sent of the same populations as above. Plants were grown, remained untreated and were tested.

3.3. Investigating if populations can be pushed towards resistance evolution and identify modes of action most at risk of resistance evolution

3.3.1. Herbicide selection – ACCase, ALS and glyphosate brome selection (WP3.1)

The aim of this experiment was to try and push brome populations towards herbicide resistance using lower dose selections of an ACCase, ALS, and glyphosate herbicides (Table 12). The doses were selected to provide 60-80% control as survivors were required to repeat the treatments the following year. The doses were identified selected and agreed by the Steering group. The experiment began in autumn 2018 and was repeated in 2019 and 2020 with seed collected from the survivors of the preceding year. Three populations each of sterile brome and rye brome were identified as most at risk of resistance evolution in the UK (Table 13). At the start of the project, these populations represented the variation in herbicide susceptibility in these species, with one population of each species thought to be highly susceptible, one with intermediate susceptibility, and one with low susceptibility.

The aim of the selection experiment was to produce three-herbicide selected lines for each of the six populations and one non-herbicide selected line. The cycloxydim lines were attempted in each year but control from the herbicide, even at low doses, meant no survivors remained to carry on selection. Nine lines were created from the three original sterile brome populations (Figure 1) and eight lines from the rye brome (Figure 2).

Table 12 Herbicides and dose rates

Treatment	Product	Active ingredient	HRAC group	Dose (g a.s./ha)	Product rate	Proportion of field rate
1	NIL	-		-	-	
2	MON79376	Glyphosate	9	360	1.0L/ha	0.66x
3	GF-1274 + Biosyl	Pyroxsulam	2	6.25	0.083kg/ha + 1% biosyl spray volume	0.33x
4	Laser	Cycloxydim	1	75	0.375L/ha	0.5x (if rate 0.75L/ha)

Table 13 Seed populations used to evaluate the risk of resistance evolution

ADAS reference	Details
Sterile brome	
SD464	Nottinghamshire, sensitive to ALS, glyphosate tolerant
SD468	Cambridgeshire, sensitive to all herbicides
SD479	Oxfordshire, sensitive to ALS, glyphosate tolerant
Rye brome	
SD453	Monmouthshire, Sensitive to ALS
SD455	Surrey, infield control issues with ALS, sensitive to ALS, glyphosate tolerant
SD470	North Yorkshire, infield control issues with ALS, sensitive to ALS

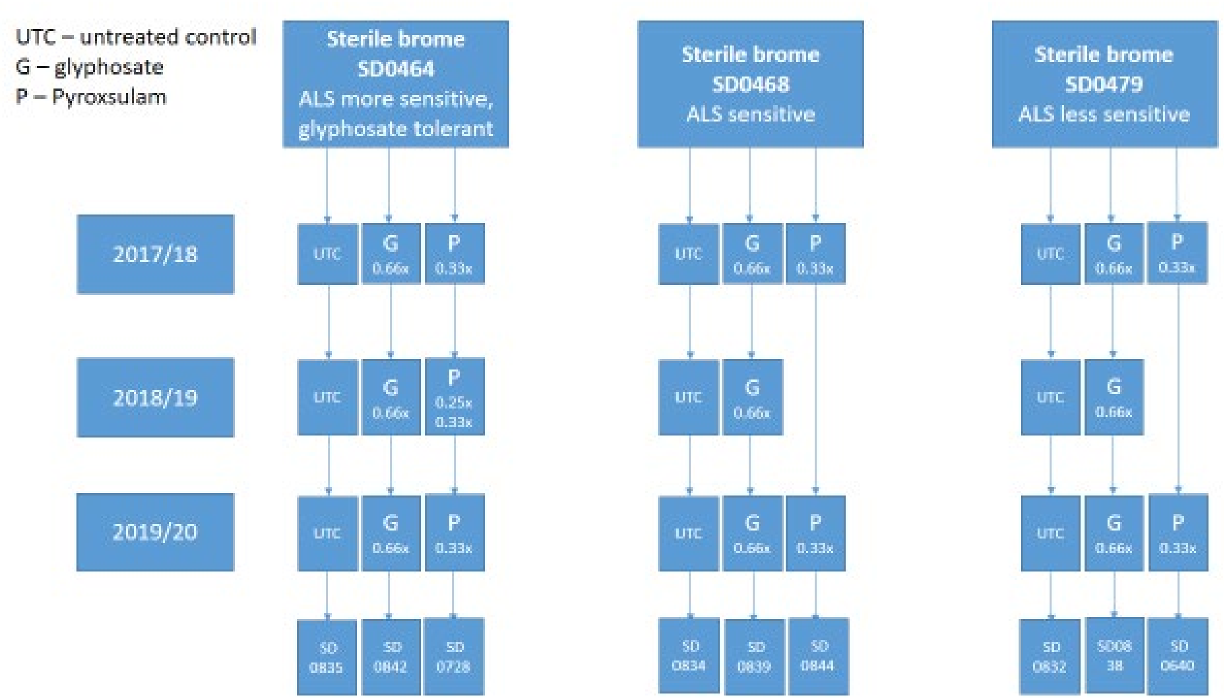


Figure 1 The selection lines for sterile brome populations from 2018-2020

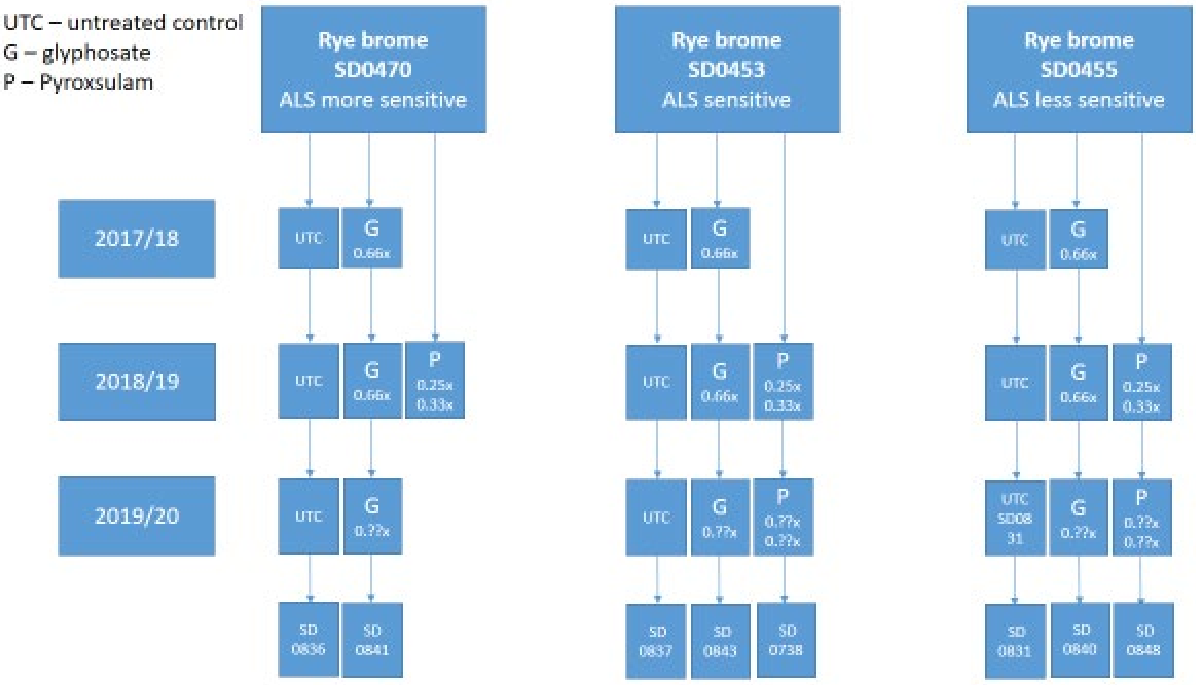


Figure 2 The selection lines for rye brome populations from 2018-2020

2017/2018 experiment

A total of 12 plug trays, 162 modules per population, were filled with sterilised Kettering loam mix (Rothamsted 'weed mix', 4:1 loam: lime free 3-6mm grit plus 2kg/m^3 Osmocote mini) and laid out in the ADAS Boxworth glasshouse and watered using an overhead watering system in early December 2017. One seed per cell was sown into moist soil and covered with a very shallow layer of soil to the top of the module. Trays were continued to be watered twice a day from above.

A pre-treatment plant count was carried out on 04 January 2018. Not all populations had 120 plants available; this was the target number required (Table 14).

Table 14 Pre-treatment plant counts of all brome populations

Species	ADAS reference	Untreated	Glyphosate	Pyroxsulam	Cycloxydim
	SD464	90	122	104	103
Sterile brome	SD468	**120**	**120**	**120**	**120**
	SD479	**120**	**120**	**120**	**120**
	SD453	58	68	72	72
Rye brome	SD455	98	100	111	110
	SD470	106	110	112	**120**

When the brome plants were at growth stage 11-13 (05/01/18), the trays were grouped into herbicide treatments and moved to the spray area. Three different herbicide treatments were applied. Doses were set to provide 60-80% control (Table 12). Herbicides were applied using air knapsack sprayer and single nozzle boom at 2.9 bar and a water volume of 200L/ha. The treatment was allowed to dry on the foliage before the containers were placed back into the glasshouse and were not watered for at least six hours post-herbicide application. The glyphosate, pyroxsulam and the untreated control trays were moved to the polytunnel on 31 January 2018 to acclimatise to the colder conditions.

The cycloxydim treatment remained in the glasshouse and a second herbicide application of Laser at 0.6L/ha (120g a.s./ha) applied on 01 February 2018 to get the plant populations to the required 20% survival rate, as it was considered that the first rate applied was too low. However, it was discovered that the adjuvant had not been applied with either cycloxydim treatment, resulting in the treatment not being effective. These populations were not continued in 2017/18 and were included in the second year of selection.

Plant counts of all remaining treatments/populations were taken on 31 January 2018 and the rye brome ALS selection (pyroxsulam) re-counted on 15 February 2018.

On 16 February 2018, plastic containers (outer 310mm x 210 mm x 145 mm. Inner 287mm x 185mm x 130mm) were filled with the same sterilised loam mix to a depth of 2cm below the rim. Containers were laid out in the polytunnel and watered well using an overhead watering system. The surviving plants were transplanted to be grown on for seed production, with up to a maximum of 12 plants per container (Table 15).

Table 15 Survival counts for each treatment, transplanted to containers on 16 February 2018.

Population		Herbicide treatment and surviving plant numbers		
		Untreated	Glyphosate	Pyroxsulam
SD464	Sterile brome	69	69	50
SD468		73	70	73
SD479		59	59	48
SD453	Rye brome	30	30	0
SD455		40	40	0
SD470		37	37	0

The containers were moved to the outdoor hard standing area on 21 February 2018 and grouped into herbicide treatment/population. On 25 May 2018, before flowering, the herbicide treatment/populations were moved into isolation groups to prevent cross-pollination between the different populations and herbicide treatments. A set of isolation cages were used consisting of a wooden frame and insect tents with each tent being a minimum of 10 metres apart to further enhance isolation. Seeds were collected between 29 June and 10 July 2018 and the population replicates were bulked together and assigned new ADAS reference (SD) numbers to be re-sown in autumn 2018.

2018/19 experiment

In total, 21 populations were carried over from the 2017-18 experiment, 12 sterile brome and nine rye brome populations (Table 16). These seeds were sown on 11 October 2018 into plug trays using the same sterilised loam mix and method as described for the 2017/18 experiment above. A set of seeds of the original sterile and rye brome populations (Table 13) were sown to repeat the failed cycloxydim treatments for year one.

Due to poor germination some populations were re-sown on 12 November 2018 to provide adequate plant numbers. They are referred to as tray 1 and tray 2.

Table 16 Populations and treatments, 2018-19 selection experiments.

Population	Previous treatment and population	Herbicide to be treated with	Treatment no. (see Table 18)
Sterile brome			
SD638	Untreated (SD479 Y1)	-	1
SD641	Untreated (SD468 Y1)	-	1
SD644	Untreated (SD464 Y1)	-	1
SD639	Glyphosate (SD479 Y1)	MON79376	2
SD642	Glyphosate (SD468 Y1)	MON79376	2
SD645	Glyphosate (SD464 Y1)	MON79376	2
SD464	Sensitive ALS, Glyphosate tolerant	Laser	3
SD468	Sensitive	Laser	3
SD479	Sensitive ALS, Glyphosate tolerant	Laser	3
SD640	Pyroxsulam (SD479 Y1)	GF-1274	4
SD643	Pyroxsulam (SD468 Y1)	GF-1274	4
SD646	Pyroxsulam (SD464 Y1)	GF-1274	4
Rye brome			
SD647	Untreated (SD455 Y1)	-	1
SD649	Untreated (SD470 Y1)	-	1
SD651	Untreated (SD453 Y1)	-	1
SD648	Glyphosate (SD455 Y1)	MON79376	2
SD650	Glyphosate (SD470 Y1)	MON79376	2
SD652	Glyphosate (SD453 Y1)	MON79376	2
SD470	Sensitive ALS Poor field control	Laser GF-1274	3 5
SD453	Sensitive	Laser GF-1274	3 5
SD455	Sensitive ALS, Glyphosate tolerant	Laser GF-1274	3 5

At growth stage 12-13, the trays were grouped into treatments and moved to the spray area. Herbicides (Table 18) were applied using the Oxford precision backpack and single nozzle boom at 2.9 bar and a water volume of 200L/ha. The treatment was allowed to dry on the foliage before the trays were placed back into the glasshouse and were not watered for at least six hours post-herbicide application. These were applied on 06 or 07 November for the early sown treatments and on 29 November 2018 or 11 December 2018 for the later sown treatments (Table 17). SD646 (sterile brome pyroxsulam line) was sown twice, each tray treated twice, first at 0.25x, secondly at 0.33x to provide adequate numbers (Table 17 &Table 18).

Table 17 Application dates for all treatments.

Population	Previous treatment and population	Trt	Tray 1 treatment date	Tray 2 treatment date
Sterile brome				
SD638	Untreated (SD479 Y1)	1	NA	
SD641	Untreated (SD468 Y1)	1	NA	
SD644	Untreated (SD464 Y1)	1	NA	
SD639	Glyphosate (SD479 Y1)	2	06/11/18	
SD642	Glyphosate (SD468 Y1)	2	07/11/18	29/11/18
SD645	Glyphosate (SD464 Y1)	2	07/11/18	11/12/18
SD464	Sensitive ALS, Glyphosate tolerant	3	07/11/18	11/12/18
SD468	Sensitive	3	07/11/18	11/12/18
SD479	Sensitive ALS, Glyphosate tolerant	3	07/11/18	11/12/18
SD640	Pyroxsulam (SD479 Y1)	4a	06/11/18	
		4b	11/12/18	
SD643	Pyroxsulam (SD468 Y1)	4a	06/11/18	
		4b	11/12/18	
SD646	Pyroxsulam (SD464 Y1)	4a	07/11/18	-
		4b	11/12/18	11/12/18
Rye brome				
SD647	Untreated (SD455 Y1)	1	NA	NA
SD649	Untreated (SD470 Y1)	1	NA	NA
SD651	Untreated (SD453 Y1)	1	NA	
SD648	Glyphosate (SD455 Y1)	2	07/11/18	29/11/18
SD650	Glyphosate (SD470 Y1)	2	07/11/18	11/12/18
SD652	Glyphosate (SD453 Y1)	2	07/11/18	29/11/18
SD470	Sensitive ALS	3	07/11/18	11/12/18
	Poor field control	5	07/11/18	11/12/18
SD453	Sensitive	3	07/11/18	29/11/18
		5	07/11/18	29/11/18
SD455	Sensitive ALS, Glyphosate tolerant	3	07/11/18	29/11/18
		5	07/11/18	29/11/18

Table 18 Herbicide treatments 2018/19

Trt	Product	Active ingredient	Dose rate (g a.s./ha)	L/ha or kg/ha of product	Proportion field rate
1	NIL	-	-	-	
2	MON79376	Glyphosate	360	1.0	0.66x
3	Laser + Adigor[1]	Cycloxydim	100	0.5	0.5x (if rate 1.0)
4a	GF-1274 + Biosyl[2]	Pyroxsulam	4.73	0.063	0.25x
4b	GF-1274 + Biosyl[2]	Pyroxsulam	6.25	0.083	0.33x
5	GF-1274 + Biosyl[2]	Pyroxsulam	6.25	0.083	0.33x

[1]0.5% spray volume [2]1% biosyl spray volume

On the 15 February 2019, surviving plants were transplanted from the plug trays into containers, filled with the same sterile loam mix. A maximum of 12 plants per container were transplanted. Containers were moved outside in late February 2019 and were grouped into population/treatment sets. Each set was isolated using wooden cages covered in mesh to prevent cross-pollination in April 2019. Seed were collected in June and July 2019 to be used for a further year of selection experiments.

2019/20 selection experiment (BW20-008 2019/20)

The third and final selection experiment included 12 sterile brome populations and 11 rye brome populations (Table 19).

Table 19 Brome populations sown for selection experiment 2019/20.

ADAS reference	Previous population, treatment and year of seed collection	Treatment no.
Sterile brome		
SD720	SD638 Untreated year 2	1
SD721	SD641 Untreated year 2	1
SD722	SD644 Untreated year 2	1
SD723	SD639 Glyphosate year 2	2
SD724	SD642 Glyphosate year 2	2
SD740	SD645 Glyphosate year 2	2
SD725	SD464 Cycloxydim year 1	3
SD726	SD468 Cycloxydim year 1	3
SD727	SD479 Cycloxydim year 1	3
SD728	SD646 Pyroxsulam year 2	4
SD640	SD479 Pyroxsulam year 1	4
SD643	SD468 Pyroxsulam year 1	4
Rye brome		
SD729	SD647 UNTREATED year 2	1
SD730	SD649 UNTREATED year 2	1
SD731	SD651 UNTREATED year 2	1
SD732	SD648 Glyphosate year 2	2
SD733	SD650 Glyphosate year 2	2
SD734	SD652 Glyphosate year 2	2
SD735	SD453 Cycloxydim year 1	3
SD736	SD455 Cycloxydim year 1	3
SD737	SD470 Cycloxydim year 1	3
SD738	SD453 Pyroxsulam year 1	4
SD739	SD455 Pyroxsulam year 1	4

Seed was sown between 16 and 18 September 2019 with one seed per cell into large plug trays, filled with sterile loam mix as per previous selection experiments. The sterile brome seeds were covered with a very shallow layer of moist soil and the rye brome seed placed on the soil surface with no cover to improve germination. Two additional populations (SD640 and SD643) were sown on 30 September 2019 to add a second year of pyroxsulam selections. Plants were thinned to 120 plants per population on 30 October 2019 and plant counts were carried out on 08 and 30 October and 27 November 2019.

At growth stage 12, the trays were grouped into treatments and moved to the spray area. Herbicides were applied using F02/110 nozzles at 1.5 bar and a water volume of 200L/ha. The treatment was allowed to dry on the foliage before the containers were placed back into the polytunnel and were not watered for at least six hours post-herbicide application. The treatments (Table 20) were applied to the initial sowings (16-18 September) on 09 October 2019 and to the later sowings (30 September 2019) on 01 and 27 November 2019.

Table 20 Herbicide treatments applied on 09 October and 01 November 2019

Treatment	Product	Active ingredient	Dose (g a.s./ha)	product L/ha or kg/ha	Proportion field rate
1	NIL	-	-	-	
2	MON79376	Glyphosate	360	1.0	0.66x
3	Laser + Adigor	Cycloxydim	100	0.5 + 0.5% spray volume	0.5x (if rate 1L/ha)
4	GF-1274 + Biosyl	Pyroxsulam	6.25	0.083 + 1% biosyl spray volume	0.33x

Plastic containers (Outer 310mm x 210 mm x 145 mm. Inner 287mm x 185mm x 130mm) were filled with the same sterilised loam mix to a depth of 2cm below the rim. Containers were laid out in the polytunnel and watered well using an overhead watering system. Transplanting of seedling survivors occurred on 18 December 2019 with 8 plants per container.

The containers were then moved to the hard standing area outside and grouped into herbicide treatment/population till just before flowering. The herbicide treatment/populations were moved into isolation groups on 25 April 2019 to prevent cross-pollination between the different populations and herbicide treatments. Applications to control aphids and mildew were applied throughout the trial period (Table 21).

Table 21 Insecticide and fungicide applications for general plant health, 2019/20.

Date applied	Treatment	Dose (L/ha)
16/12/2019	Cyflamid + Hallmark Zeon	0.5 + 0.2
15/05/2020	Biscaya	0.4
19/05/2020	Cyflamid	0.5

Seed from each surviving population was harvested in June and July 2020 to be used for the final glasshouse selection dose response experiment.

3.3.2. Final dose-response of herbicide selected lines

The dose-response was the culmination of three years of herbicide selection to determine the resistance and sensitivity status of UK brome populations to ALS herbicide and glyphosate. 31 Brome populations were used (Table 22). Populations were from the 2019/20 trial and for comparison purposes the original baseline populations from 2017/18.

Table 22. Seed populations used for the glasshouse dose response experiment 2020.

No. pots	Population	Population history (from each year of selection)	Untreated	GF-1274	Glyphosate
Sterile brome					
56	SD464	Baseline seed - ALS sensitive, glyphosate tolerant	Y	Y	Y
56	SD835	SD464 > SD644 > SD722 > UNTREATED x 3 years	Y	Y	Y
32	SD728	SD464 > SD646 > Pyroxsulam x 2 years (SD728)	Y	Y	
28	SD842	SD464 > SD645 > SD740 > Glyphosate x 3 years	Y		Y
32	SD847	SD464 > SD646 > SD728 > Pyroxsulam x 3 years	Y	Y	
56	SD468	Baseline seed - sensitive	Y	Y	Y
56	SD834	SD468 > SD641 > SD721 > UNTREATED x 3 years	Y	Y	Y
32	SD844	SD468 > SD643 > Pyroxsulam x 2 years	Y	Y	
28	SD839	SD468 > SD642 > SD724 > Glyphosate x 3 years	Y		Y
56	SD479	Baseline seed - ALS sensitive, glyphosate tolerant	Y	Y	Y
56	SD832	SD479 > SD638 > SD720 > UNTREATED x 3 years	Y	Y	Y
32	SD640	SD479 > SD640 > Pyroxsulam x 2 years	Y	Y	
28	SD838	SD479 > SD639 > SD723 > Glyphosate x 3 years	Y		Y
Rye brome					
56	SD453	Baseline seed - sensitive	Y	Y	Y
56	SD837	SD453 > SD651 > SD731 > UNTREATED x 3 years	Y	Y	Y
32	SD738	SD453 > SD738 > Pyroxsulam x 2 years	Y	Y	
28	SD843	SD453 > SD652 > SD734 > Glyphosate x 3 years	Y		Y
56	SD455	Baseline seed - ALS sensitive, glyphosate tolerant	Y	Y	Y
56	SD831	SD455 > SD647 > SD729 > UNTREATED x 3 years	Y	Y	Y
32	SD848	SD455 > SD739 > Pyroxsulam x 2 years	Y	Y	
28	SD840	SD455 > SD648 > SD732 > Glyphosate x 3 years	Y		Y
56	SD470	Baseline seed - ALS sensitive	Y	Y	Y
56	SD836	SD470 > SD649 > SD730 > UNTREATED x 3 years	Y	Y	Y
28	SD841	SD470 > SD650 > SD733 > Glyphosate x 3 years	Y		Y

A total of 1232 pots (9cm diameter) were filled with the same sterilised loam mix to a depth of 2cm below the rim. The pots were placed in the glasshouse and watered well using an overhead watering system. Seeds were sown directly into pots (seven seeds/pot) on 19 October 2020 and plant counts and thinning to a maximum five plants per pot on 12 November 2020. At growth stage 12, the pots were grouped into treatments and moved to the spray area.

There were 13 herbicide treatments and an untreated control used (Table 23). All 31 populations had an untreated control, 24 populations received all seven GF-1274 doses, and 18 populations received all six doses of glyphosate (Table 23) on 13 November 2020. There were four replicates for each treatment/population. Herbicides were applied using F02/110 nozzles at 2 bar and a water volume of 200L/ha. The treatment was allowed to dry on the foliage before the pots were placed back into the glasshouse and were not watered for at least six hours post-herbicide application. Pots were treated with Cyflamid (0.5L/ha) and Biscaya (0.4L/ha) to control mildew and aphids on 16 November 2020.

Table 23 Herbicide treatments in selection dose response experiment December 2020.

Trt.	Product	Active ingredient	Prop dose	Dose rate (g a.s./ha)	Amount of product (kg/ha or L/ha)
1	Untreated	-	-		-
2	GF-1274	7.08%w/w pyroxsulam + biosyl (1% spray volume)	0.0625x	1.176	0.0165
3			0.125x	2.355	0.033
4			0.25x	4.71	0.066
5			0.5x	9.42	0.133
6			0.75x	14.13	0.199
7			1x	18.76	0.265
8			2x	37.52	0.530
9	MON79376	Glyphosate 360g a.s./L	0.125	68	0.186
10			0.25x	135	0.375
11			0.5x	270	0.75
12			0.75x	408	1.125
13			1x	540	1.5
14			2x	1080	3.0

Plant assessments took place on 16 December 2020. Photographs were taken of all populations in treatment order plus the untreated control (one set of photos for the ALS herbicide and one set for glyphosate). Plant counts were taken to record the number of surviving plants. To record the fresh weight all plants in the pot were carefully cut at the base and weighed.

3.3.3. Statistical analysis

Dose-response analysis were performed using the *DRC* package in R (version 3.5.3). Fresh weight data were fit to three-parameter models. Lack-of-fit F-tests (model fit) were performed to assess model fit.

ALS inhibitor dose-response (GF-1274)

Due to complete control of all populations at the lowest herbicide doses used, both for iodosulfuron + mesosulfuron and pyroxsulam herbicides, dose response analysis could not be conducted on the data.

Glyphosate dose-response

A two-parameter unconstrained symmetrical log-logistic model with was used to model glyphosate survival data. A four-parameter symmetrical log-logistic model with a constrained slope was used to model glyphosate fresh weight data. As residuals were normally distributed, data was not transformed. ED_{50} and GR_{50} values for survival and fresh weight, respectively, were calculated.

3.4. Adding value to the BGRI survey (WP4) Rothamsted

3.4.1. BGRI network brome abundance (WP4.1)

Field survey using the BGRI network (2016-2017)

The BGRI Black-grass farm network was set up in the summer of 2014 as part of the BBSRC / AHDB funded project BGRI (Black-grass Resistance Initiative). In the summer of 2017, this farm network was used to map the occurrence and abundance of brome species. Figure 3 shows the location of every field on the BGRI farm network. A total of 83 cereal fields were mapped for brome species in summer 2017 between 5/6/17 and 20/7/17. 69 winter wheat, eight winter barley, four spring wheat and two spring barley fields were assessed. Only cereal fields could be mapped due to the short height of the cereal crops to aid spotting of the heads / panicles out above the crop. The occurrence and abundance mapping were conducted in three separate areas of the field, the margin, the headland (the first 20m of cropped land in from the margin) and the main body of crop. Brome species were assessed at twenty locations within each of these three field areas, with survey location at least 20m apart. The five brome species of interest were split into two groups: sterile and great brome, and the other rye, meadow and soft brome. Identification of individual species was not possible at distance for the headland or main body of the crop areas. Abundance was measured with the same categories as Cussans *et al*. (1994): very low - <5 heads / panicles m^2, low 5 – 10 heads m^2, medium – 10 – 50 heads m^2, high – 50 – 250 heads m^2 and very high >250 heads m^2.

Figure 3 Distribution of fields on the BGRI farm network

In-crop survey using BGRI farm network (summer 2018, 2019 and 2020)

Brome occurrence and abundance was again mapped in the summers of 2018, 2019 and 2020 using the BGRI farm network. Only the cropped area of the field was mapped in these three seasons. For the timing of the surveys and the numbers / types of crops mapped in 2018, 2019 and 2020 (Table 24).

Table 24 Dates and cropping information for surveyed fields in 2018, 2019 and 2020.

Year	Start	Finish	Winter wheat	Winter barley	Spring wheat	Spring barley	Spring oats	Spring linseed	Total
2017 - 2018	04/06/18	19/07/18	64	11	2	12	2	1	92
2018 – 2019	03/06/19	18/07/19	76	15	2	4	-	-	97
2019 – 2020	22/06/20	21/07/20	26	2	10	44	-	-	82
All years	-	-	166	28	14	60	2	1	271

A total of 271 fields were mapped for brome occurrence and abundance over these three years, split into 194 autumn cereals and 77 spring sown crops. Fewer fields were mapped in summer 2020 due to Covid restrictions, no fields were mapped in North Lincolnshire or Yorkshire. Many more spring crops were mapped in summer 2020 due to very wet weather in autumn 2019. The same abundance categories were used as in 2017 survey and the use of the two groups of brome species. Fields were surveyed from the field tramlines, with a minimum of 2 tramlines walked per field. Percentage area of field infested was also recorded for 2018, 2019 and 2020.

3.5. Determine the best herbicide application timing to increase brome control and reduce the risk of resistance evolution (WP5)

Container experiments were used to determine the best herbicide application timing for brome to maintain and improve herbicide control and help prevent resistance evolution. Sterile and Rye brome had previously been identified as most at risk of resistance evolution and so were chosen. Experiments began in September 2018 and were repeated in September 2019.

3.5.1. BW19-019 2018/19

Six populations, three sterile brome and three rye brome were used, the populations had different reported tolerances to the herbicides or were reported to have poor field control (Table 25).

Table 25 Brome populations tested

Population	Location and population details
Sterile brome	
SD464	Nottinghamshire, Sensitive to ALS, glyphosate (GLY) tolerant
SD468	Cambridgeshire, sensitive to ALS
SD479	Oxfordshire, sensitive to ALS, GLY tolerant
Rye brome	
SD453	Monmouthshire, Sensitive to ALS
SD455	Surrey, Infield control issues with ALS, GLY tolerant
SD470	North Yorkshire, infield control issues with ALS, sensitive to ALS

Seeds were sown directly into plastic containers (outer 310mm x 210 mm x 145 mm. Inner 287mm x 185mm x 130mm) containing sterilised loam mix (Rothamsted 'weed mix', 4:1 loam:grit) to a depth of 3cm below the rim. Containers were placed outdoors on a hard standing area. At the 1-2 leaf growth stage plants were thinned to 15 plants per container, with individuals smaller or larger than 1-2 leaves removed. The first herbicide treatments were applied 7 days later.

Treatments included an untreated control, glyphosate, cycloxydim and pyroxsulam + florasulam at three different growth stages (GS12-13, 21-23 and 25) (Table 26). Laser were applied with a 0.5% spray volume of Adigor adjuvant. Broadway Star was applied with a 1% spray volume Biosyl adjuvant (32.67% w/w alkoxylated alcohols, 1.0% w/w trisiloxane). There were four replicates. Containers were laid out in a fully randomised block design.

Herbicide treatments were applied on 26 October 2018 (GS12-23), 12 November 2018 (GS21-23) and 29 November 2018 (GS25+) using an air knapsack sprayer fitted with a two-metre boom with

01F110 flat fan nozzle, at a pressure of 1.3 bar, and a water volume of 200L /ha. After treatment, plants were left for 24 hours before being moved back to the hard standing area.

Table 26 Herbicide treatment and brome plant growth stage at herbicide application

Trt. No.	Growth Stage	Product Name	Active	Prop. Dose	Herbicide dose (g a.s./ha)
1		Untreated	-	-	-
2	2-3 true leaves (GS 12-13)	Mon79376	Glyphosate	0.75x	405
3	Tillering (GS 21-23)				
4	Tillering (GS 25+)				
5	2-3 true leaves (GS 12-13)	Laser + Adigor[1]	Cycloxydim	0.75x	150
6	Tillering (GS 21-23)				
7	Tillering (GS 25+)				
8	2-3 true leaves (GS 12-13)	Broadway Star + Biosyl[2]	Pyroxsulam + florasulam	0.75x	14.13 2.84
9	Tillering (GS 21-23)				
10	Tillering (GS 25+)				

[1]0.5% spray volume, [2]biosyl 1% spray volume

Plant counts were done on 26 April 2019 and head counts were done on 28 June 2019.

3.5.2. BW20-012 2019/20

Six populations, three sterile and three rye brome were used (Table 27). The populations were collected from seed produced by the untreated control containers in the 2018-19 herbicide and growth stage experiment (3.5.1), as there were not enough seeds remaining in the original populations (Table 25). Untreated control containers from the previous experiment were put into pollen isolation cages at stem extension to prevent any potential cross pollination between populations.

Table 27 Sterile and rye brome populations used in 2019-20 herbicide dose and growth stage experiment

Population sown	Parent population	Common name	Details
SD741 & SD464	SD464	Sterile brome	Lincolnshire, sensitive glyphosate tolerant,
SD742	SD468		Cambridgeshire, sensitive to ALS
SD743	SD479		Oxfordshire, sensitive to ALS, glyphosate tolerant
SD474		Meadow brome	Bedfordshire, sensitive to ALS
SD475		Rye brome	Bedfordshire, sensitive to ALS
SD476		Rye brome	Northants, sensitive to ALS

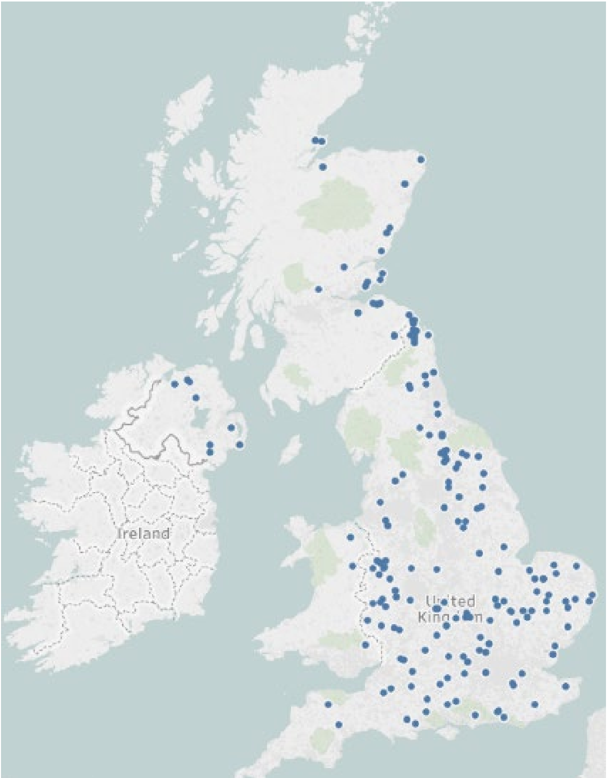
The same methods and equipment were used as the 2018-19 experiment. Seeds were sown on 26 September 2019. The GS12 treatment was applied on 24 October 2019, GS22 treatment on 19

November 2019 and GS25+ treatment applied on 7 April 2019 (actual GS26). Plant counts were done 21-28 days after treatment, on 03 December 2019 and 19 December 2019. Plants were counted again on 11 February 2020. All treatments, plants and heads were counted on 12 August 2020.

4. Results

4.1. Online brome survey

In total, there were 206 respondents to the survey from 42 UK counties, 200 of which reported the presence of brome (Figure 4). The South East had the highest number of respondents, (45), followed by the West Midlands (34), and Yorkshire & Lancashire (26). Respondents consisted of 141 farmers, 63 agronomists, and 2 of other occupation, across 129 arable, 76 mixed, and 1 other holding types.



(a) *Anisantha* sp.

(b) *Bromus* sp.

Figure 4 Reported presence of *Anisantha* and *Bromus* from an online survey of 200 respondents.

4.1.1. Weed species

Of the 58 seed samples received by ADAS for brome species identification, only 34 samples (59%) were correctly identified by senders. After expert identification, 8 great (*Anisantha diandrus*), 20 sterile (*A. sterilis*), eight meadow (*Bromus commutatus*), one soft (*B. hordaceus*), 15 rye (*B. secalinus*), one mix of great and sterile, three mixes of meadow and rye, and two unidentified samples (possibly field brome (*Bromus arvensis*) were received. Senders wrongly identified five great brome samples as sterile brome, two sterile brome samples as great, two meadow brome samples as soft brome, six rye brome samples as meadow brome, two rye brome samples as soft, and one unknown sample was identified as soft brome.

Considering the level of mis-identifiation by survey respondents to the brome species level, responses were grouped into *Anisantha* and *Bromus* sp., to ensure correct reporting. The presence of both *Anisantha* and *Bromus* sp. were reported across all UK cereal growing areas and although *Anisantha* sp. were reported as the most problematic brome weed by 137 respondents (68.5%) and *Bromus* by 59 respondents (29.5%), the presence of both groups on the same holding were reported by 134 of the respondents (Figure 4 b&c). Respondents were asked to calculate the total area of their holding that was affected by bromes: *Anisantha* sp. were reported to affect 24,650 ha and B*romus* sp. were reported to affect 10,080 ha of arable land across the 200 respondent's holdings.

Across the UK, black-grass was reported as the most problematic weed (72 respondents), followed by *Anisantha* sp. (52), and annual meadow grass (40). However, the most problematic weed species varied by region. Black-grass was the most problematic weed in the East, East Midlands, and South East. *Anisantha* sp. were the most problematic weed in the South West, Yorkshire/Lancashire, and the North. Annual meadow grass was the most problematic weed in Scotland and Northern Ireland. In the West Midlands, black-grass and *Anisantha* sp. were reported equally as the most problematic weed species.

The most prevalent brome species was sterile brome (Table 28). Meadow brome was the next most reported species in all areas except Scotland. Rye brome was present at higher levels in the South East and Yorks/Lancs. Great brome was predominantly reported in the South East and soft brome in the South East, Yorks/Lancs and the East Midlands.

Soil type did not seem to be a factor in the location of brome.

Table 28 Percentage of brome species reported in each area

Region area	Sterile brome	Meadow brome	Rye brome	Great brome	Soft brome
South East	51	14	10	12	13
North	81	10	0	1	5
West Midlands	72	11	7	3	5
York/ Lancs	58	11	16	0	14
East	75	14	9	1	1
Scotland	91	0	2	1	4
South West	77	14	7	0	2
East Midlands	73	11	1	2	13
Northern Ireland	77	15	0	1	6

The survey indicated that the majority of brome was located throughout the field (Figure 5).

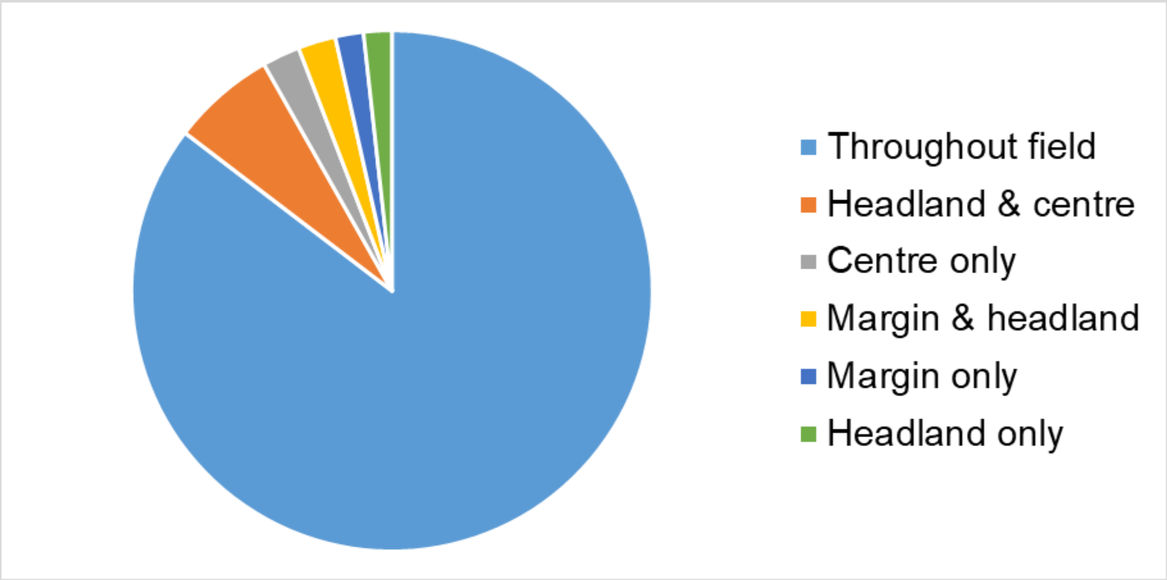


Figure 5 Location of bromes within the field

Herbicide drift into field edges has often been considered an issue as bare patches are left which brome, particularly sterile brome, colonises rapidly. Respondents took action to prevent herbicide drift with 30% spraying in ideal conditions, 27% using low drift nozzles, 17% using a low boom and low speed (11%). Nine percent had a no spray zone.

4.1.2. Perceived change in brome infestations

Of the 200 respondents that reported the presence of brome, 60% perceived that there had been an increase in the presence of brome on their holding in the last three years. Only 13% reported a decrease, and 27% reported no change. The main reasons for an increase in the presence of brome weeds were a move to minimum tillage, no tillage situation, rotations of mostly autumn sown crops, and ineffective chemistry with herbicide active substances being less effective on brome weeds than other grass weeds. The main reported reasons for a decrease in the presence of brome weeds were due to better rotations, good herbicide control, and better cultivations (Table 29).

Table 29 Reasons given by 200 survey respondents for a change in the presence of brome grass weeds in UK arable fields

Reason for change	Decrease	Increase	No change
Minimum tillage/ no tillage situation	-	50	3
Poor rotations	-	21	-
Ineffective chemistry	-	24	-
Conflict with black-grass control	-	12	-
Poor stale seedbed	-	11	-
Oats & barley in rotation	-	11	1
Grass margins	-	10	-
Climate change	-	7	2
Contaminated seed	-	7	1
Other - increase	-	18	1
Better rotation – e.g. spring cropping	13	-	10
Good herbicide control	11	-	10
Better cultivations	7	-	13
Other - decrease	9	-	9

4.1.3. Cultural control methods adopted by the online survey respondents

Respondents were asked to report the cultural and chemical control methods they used to control brome species and perceived control problems. Crop rotation and ploughing were the most commonly used cultural methods for controlling both groups of brome species (Table 30). Respondents used a wide range of herbicide modes of action, both pre- and post-emergence, to control brome weeds, including ALS inhibitors, ACCase inhibitors, glyphosate, long-chain fatty acid inhibitors, microtubule inhibitors, and lipid inhibitors (Table 31), generally at full label rates and often as part of a programme. Despite the wide range of herbicide modes of action used, many respondents reported herbicide control problems, particularly to ALS inhibitors (Table 32). The reasons for these herbicide control issues ranged from poor application and timing to possible resistance (Table 32).

Table 30 Cultural control methods used by online survey respondents to control brome weeds

Cultural control	*Anisantha*	*Bromus*	Unknown	Total
None	5	4	-	9
Shallow stubble cultivations	63	22	-	85
Min till	31	12	1	44
Plough	82	28	2	112
Crop rotation (including spring cropping)	91	41	3	135
Delayed autumn sowing	39	20	1	60
Other	8	12	-	20

Table 31 Reported modes of action where respondents of the survey had experienced herbicide control problems on brome weeds

Herbicide control problems	*HRAC group*	*Anisantha*	*Bromus*	Unknown
ALS inhibitors	2	53	21	1
ACCase inhibitors	1	32	14	1
Glyphosate	9	9	3	-
Other	-	6	8	-
No problems	-	57	23	2

Table 32 Reported reasons for herbicide control problems for brome weeds

Why herbicide control problems	*Anisantha*	*Bromus*	Unknown	Total
Application timing	40	20	1	61
Possible resistance	38	19	2	58
Ineffective products	32	13	1	46
Poor application	16	6	-	22
Poor weather	10	3	-	13
Germination timing	6	3	-	9
Herbicide dose used too low	5	4	-	9
Other	6	1	-	7

4.2. Investigating the range in herbicide susceptibility within and between brome species and possible cases of herbicide resistance (WP2)

4.2.1. Investigating variation in herbicide susceptibility within and between brome species (WP 2.1)

Herbicide sensitivity screening 2017

Sixty eight populations were tested in 2017 and comprised 19 sterile, 10 great , 12 meadow, and 18 rye bromes. Fifty five of these populations were collected in July 2017 from arable fields around the UK as part of a UK brome survey of which 48 populations were collected by growers and ten samples from the Black-grass Research Initiative (BGRI) Network collected by Rothamsted Research (Figure 6).

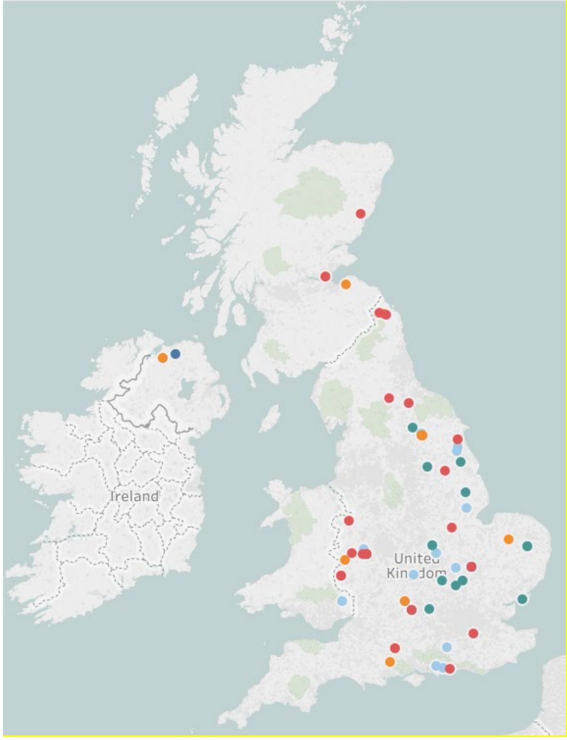
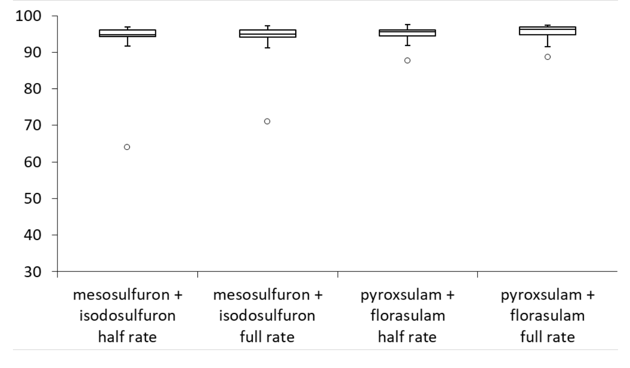


Figure 6 Collection sites of 55 UK brome spp. populations used in the herbicide sensitivity testing: (orange) *Anisantha diandrus,* (red) *Anisantha sterilis,* (green) *Bromus commutatus*, and (blue) *Bromus secalinus*

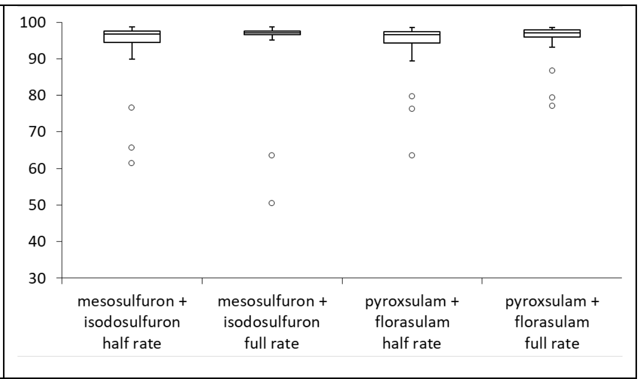
ALS inhibitor screening

All bromes were controlled to the same extent by Pacifica Plus, and Broadway Star (Figure 7) control was slightly less at the half rate. Figure 7 is a box plot, for each herbicide and rate the graph shows the most extreme values in the data set (maximum and minimum values), the lower and upper quartiles, and the mean. The boxes indicate the quartiles, the first quartile of a group of values is where 25% of the values fall at or below this value. The third quartile of a group of values is where 75% of the values fall at or below this value. The circles indicate the outliers which are values that fall outside the minimum value.

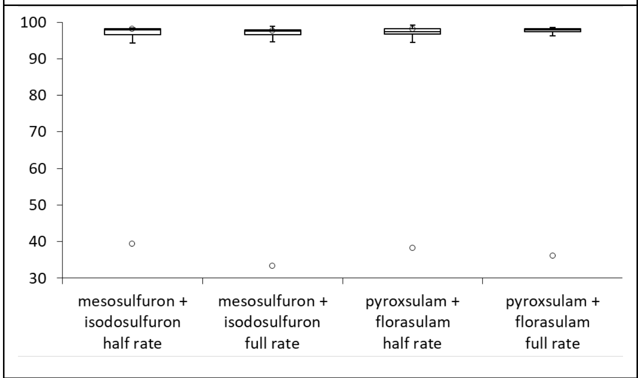
Three sterile bromes, one great brome, one meadow brome were identified as outliers. Rye brome was well-controlled, and the outliers identified were still well-controlled (>90%).



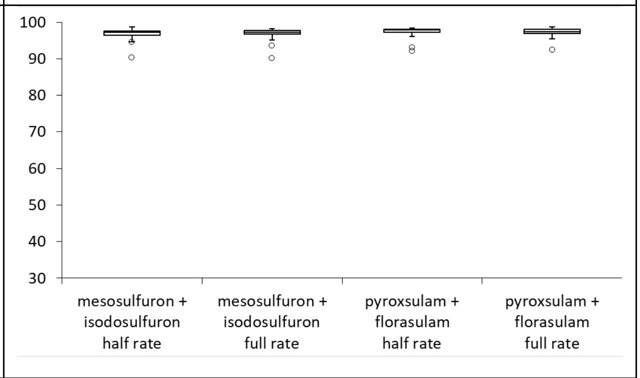
a) Great brome *Anisantha diandrus*



b) Sterile brome *Anisantha sterilis*



c) Meadow brome *Bromus commutatus*



d) Rye brome *Bromus secalinus*

Figure 7 Boxplot of mean percentage reduction in fresh weight (relative to untreated) of 21 *Anisantha sterilis*, 11 *Anisantha diandrus*, 11 *Bromus commutatus*, and 19 *Bromus secalinus* populations treated with Pacifica Plus (7.5g/ha mesosulfuron + 2.5g a.s./ha iodosulfuron (half rate), 15g a.s./ha mesosulfuron + 5g a.s./ha iodosulfuron (full rate)), Broadway Star (9.4g a.s./ha pyroxsulam + 1.9g a.s/ha florasulam (half rate) and 18.8g a.s./ha pyroxsulam + 3.8g a.s./ha florasulam (full rate)). Showing median (black line), 25th and 75th percentiles (box), minimum and maximum (line), and outliers (circles).

Three populations of sterile brome (SD454, SD478 and SD488) were identified as being significantly less sensitive to ALS herbicides (Table 33). Population SD478 only showed less sensitivity to Broadway Star (pyroxsulam + florasulam) and not to Pacifica Plus (mesosulfuron + iodosulfuron).

Table 33 Mean percentage reduction in foliage fresh weight in sterile brome (*Anisantha sterilis*) relative to untreated control. Red shading indicates resistant populations identified.

Population	7.5g a.s./ha mesosulfuron + 2.5g a.s./ha iodosulfuron (half rate)	15g a.s./ha mesosulfuron + 5g a.s./ha iodosulfuron (full rate)			9.4g a.s./ha pyroxsulam + 1.9g a.s/ha florasulam (half rate)	18.8g a.s./ha pyroxsulam + 3.8g florasulam a.s./ha (full rate)	
SD410	98.4	97.7			98.2	98.2	
SD409	97.8	97.8			97.7	98.1	
SD494	98.5	98.7			97.7	98.0	
SD224	96.8	97.4			96.6	98.0	
SD464 sensitive	97.3	97.9			97.2	97.9	
SD468	96.9	96.6			96.3	97.8	
SD484	97.2	97.9			97.5	97.7	
SD490	96.4	97.6			97.1	97.2	
SD471	94.9	97.1			96.0	97.2	
SD495	96.7	97.0			96.8	97.1	
SD442	98.0	97.3			97.3	97.0	
SD498	74.3	92.1			90.1	97.0	
SD489	92.3	96.7			94.6	96.9	
SD436	95.3	96.6			92.8	96.1	
SD522 Sensitive	95.8	96.0			96.0	96.1	
SD457	94.4	95.8			95.8	95.9	
SD479	92.6	96.6			93.2	94.2	
SD445	98.1	96.7			97.7	91.9	
SD488	54.5	40.8			81.0	85.3	
SD478	94.5	93.1			61.6	77.5	
SD454	63.8	64.5			76.9	77.0	
	F probability	SED	LSD		F probability	SED	LSD
Population	<0.001	5.09	10.07		<0.001	2.67	5.29
Herbicide	NS	1.57	3.11		NS	0.82	1.63
Population x herbicide	NS	7.19	14.24		NS	3.78	7.48
Residual df	123				123		
CV%	2.0				0.6		

One population of great brome, SD441, was identified as showing significantly less sensitivity to both herbicides (Table 34).

Table 34 Mean percentage reduction in foliage fresh weight in great brome (*Anisantha diandrus)* relative to untreated control. Red shading indicates resistant populations identified.

Population	7.5g a.s./ha mesosulfuron + 2.5g a.s./ha iodosulfuron (half rate)	15g a.s./ha mesosulfuron + 5g a.s./ha iodosulfuron (full rate)			9.4g a.s./ha pyroxsulam + 1.9g a.s./ha florasulam (half rate)	18.8g a.s./ha pyroxsulam + 3.8g a.s./ha (full rate)	
SD432	96.9	97.3			97.5	97.3	
SD481	96.3	94.7			96.8	97.0	
SD477	94.4	95.8			96.6	96.5	
SD221	95.7	96.2			95.0	96.3	
SD508	94.5	94.7			95.4	96.0	
SD497	94.6	95.6			94.8	95.7	
SD440	95.8	94.9			95.5	95.6	
SD511	93.0	93.3			93.5	94.7	
SD456	93.8	94.6			94.0	94.4	
SD523 sensitive	94.4	93.8			94.3	94.0	
SD441	61.7	68.5			87.3	89.7	
	F probability	SED	LSD		F probability	SED	LSD
Population	<0.001	2.85	5.59		<0.001	1.14	2.26
Herbicide	NS	1.22	2.43		NS	0.49	0.97
Population x herbicide	NS	4.03	8.05		NS	1.62	3.23
Residual df	63				63		
CV%	1.9				1.0		

A single population of meadow brome, SD466, was identified as showing less sensitivity to ALS herbicides (Table 35). Control of this population was particularly poor to both herbicides.

Table 35 Mean percentage reduction in foliage fresh weight in meadow brome (*Bromus commutatus)* relative to untreated control. Red shading indicates resistant populations identified.

Population	7.5g a.s./ha mesosulfuron + 2.5g a.s./ha iodosulfuron (half rate)	15g a.s./ha mesosulfuron + 5g a.s./ha iodosulfuron (full rate)			9.4g a.s./ha pyroxsulam + 1.9g a.s/ha florasulam (half rate)	18.8g a.s./ha pyroxsulam + 3.8g a.s./ha (full rate)	
SD505	98.0	98.7			98.9	98.4	
SD507	98.4	97.7			98.2	98.4	
SD486	98.0	97.6			98.0	98.1	
SD458	97.2	96.4			97.3	97.9	
SD472	94.9	95.7			95.1	97.7	
SD518 sensitive	97.9	96.5			96.6	97.5	
SD519 sensitive	96.5	97.8			96.8	97.5	
SD473	98.4	97.5			96.7	97.4	
SD474	97.8	98.1			97.4	97.4	
SD467	95.9	97.0			98.0	96.8	
SD466	41.5	32.0			34.3	32.9	
	F probability	SED	LSD		F probability	SED	LSD
Population	<0.001	4.50			<0.001	3.76	7.52
Herbicide	NS	1.92			NS	1.60	3.21
Population x herbicide	NS	6.37			NS	5.32	10.63
Residual df	63				63		
CV%	3.2				2.1		

Two rye brome populations, SD455 and SD506, were identified as showing significantly less sensitivity to ALS herbicides although the reductions in control were marginal in absolute terms. (Table 36).

Table 36 Mean percentage reduction in foliage fresh weight in *Bromus secalinus* relative to untreated control. Red shading indicates resistant populations identified.

Population	7.5g a.s./ha mesosulfuron + 2.5g a.s./ha iodosulfuron (half rate)	15g a.s./ha mesosulfuron + 5g a.s./ha iodosulfuron (full rate)			9.4g a.s./ha pyroxsulam + 1.9g a.s/ha florasulam (half rate)	18.8g a.s./ha pyroxsulam + 3.8g a.s./ha (full rate)	
SD501	98.7	98.2			98.5	98.7	
SD499	97.3	96.7			97.9	98.6	
SD496	96.4	97.0			97.6	98.4	
SD485	96.6	97.5			98.2	98.2	
SD483	97.0	97.1			97.4	98.0	
SD516	97.4	97.8			97.9	97.8	
SD476	96.7	96.1			96.4	97.7	
SD482	96.6	97.5			97.5	97.4	
SD437	97.7	97.4			98.2	97.3	
SD475	96.0	96.5			97.9	97.2	
SD453	97.3	97.7			97.1	97.0	
SD503	95.5	96.9			98.0	96.9	
SD521 sensitive	96.3	96.9			96.6	96.9	
SD500	95.4	96.5			97.1	96.5	
SD520 Sensitive	97.6	96.9			97.2	96.5	
SD512	97.4	95.7			96.6	95.9	
SD470	96.7	97.2			95.5	95.8	
SD455	94.2	92.8			92.9	94.9	
SD506	90.5	90.3			91.7	92.5	
	F probability	SED	LSD		F probability	SED	LSD
Population	<0.001	0.84	1.66		<0.001	0.96	1.89
Herbicide	NS	0.27	0.54		NS	0.31	0.61
Population x herbicide	NS	1.18	2.35		NS	1.35	2.68
Residual df	111				111		
CV%	1.0				0.8		

ACCase inhibitor screening on populations from 2017

Sterile brome was less well-controlled by cycloxydim than propaquizafop (Figure 8, Table 37), particularly at the half rate (50g a.s./ha); here, overall control was 42%. At the full rate of cycloxydim, two populations were identified as populations of interest, SD224, the known ACCase inhibitor resistant population from Germany and SD478 (Wiltshire).

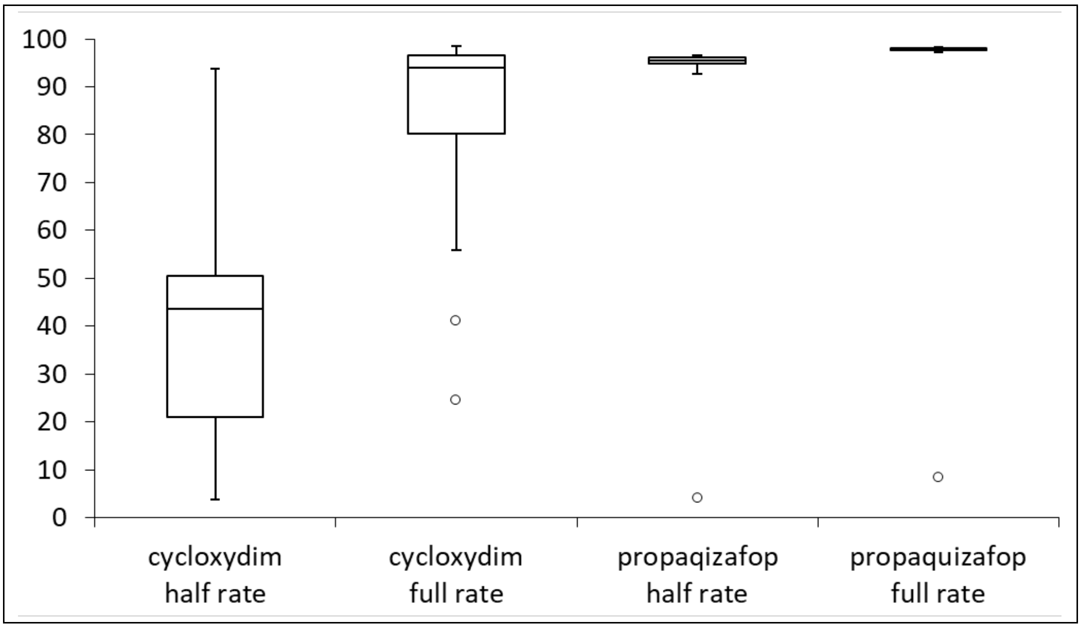


Figure 8 Boxplot of mean percentage reduction in fresh weight (relative to untreated) of 21 Sterile brome (*Anisantha sterilis)*, populations treated with 100g a.s./ha cycloxydim (half rate), 200g a.s./ha cycloxydim (full rate), 50g a.s./ha propaquizafop (half rate) and 100g a.s./ha propaquizafop (full rate). Showing median (black line), 25th and 75th percentiles (box), minimum and maximum (line), and outliers (circles).

No resistance to propaquizafop was detected in UK sterile brome populations, there was a significant reduction fresh weight at both the full rate (100g a.s./ha) and half rate (50g a.s./ha) of 98% and 96%, respectively overall (Table 37). The only population that was poorly controlled was the known ACCase inhibitor resistant population (SD224) from Germany. This population showed less than 10% reduction in fresh weight compared to the untreated control at both rates of propaquizafop.

Table 37 Mean percentage reduction in foliage fresh weight/plant in Sterile brome (*Anisantha sterilis*) relative to the untreated. Red shading indicates resistant populations identified.

Population	100g a.s./ha cycloxydim (half rate)	200g a.s./ha cycloxydim (full rate)	50g a.s./ha propaquizafop (half rate)	100g a.s./ha propaquizafop (full rate)
SD522 sensitive	20.8	24.7	95.6	98.4
SD498	34.2	80.3	96.6	98.3
SD490	18.7	78.9	96.5	98.3
SD410	5.6	98.6	96.0	98.1
SD409	45.5	97.0	95.5	98.1
SD479	50.4	76.0	96.4	98.1
SD484	36.0	63.7	96.1	98.1
SD471	71.7	97.6	94.3	98.0
SD478	3.7	41.1	95.2	98.0
SD488	82.7	96.2	95.4	97.9
SD489	36.8	87.3	95.4	97.9
SD494	51.9	96.6	96.5	97.8
SD468	43.5	83.3	96.4	97.8
SD457	20.9	97.5	94.5	97.7
SD436	93.7	96.5	95.1	97.7
SD454	71.8	94.0	94.8	97.7
SD445	27.5	81.5	95.4	97.7
SD495	19.9	77.4	96.2	97.7
SD464	49.1	96.5	94.5	97.4
SD442	49.7	94.5	94.7	97.4
SD224 ACCase resistant	20.8	24.7	4.1	8.3

	F probability	sed	lsd	F probability	sed	lsd
Population	<0.001	11.90	23.57	<0.001	1.31	2.58
Herbicide	<0.001	3.67	7.27	<0.001	0.40	0.80
Population x herbicide	0.025	16.83	33.33	1.00	1.85	3.65
Residual df	120			123		
CV%	7.7			1.1		

Great brome was less well-controlled by cycloxydim than propaquizafop (Figure 9), particularly at the half rate (50g a.s./ha), overall control was 48%.

No resistance to propaquizafop was detected in great brome, there was a significant reduction overall in fresh weight at both the full rate (100g a.s./ha) and half rate (50g a.s./ha) of 97% and 95%, respectively (Figure 9). A single population was identified with increased tolerance to cycloxydim, SD440 (East Lothian) (Table 38).

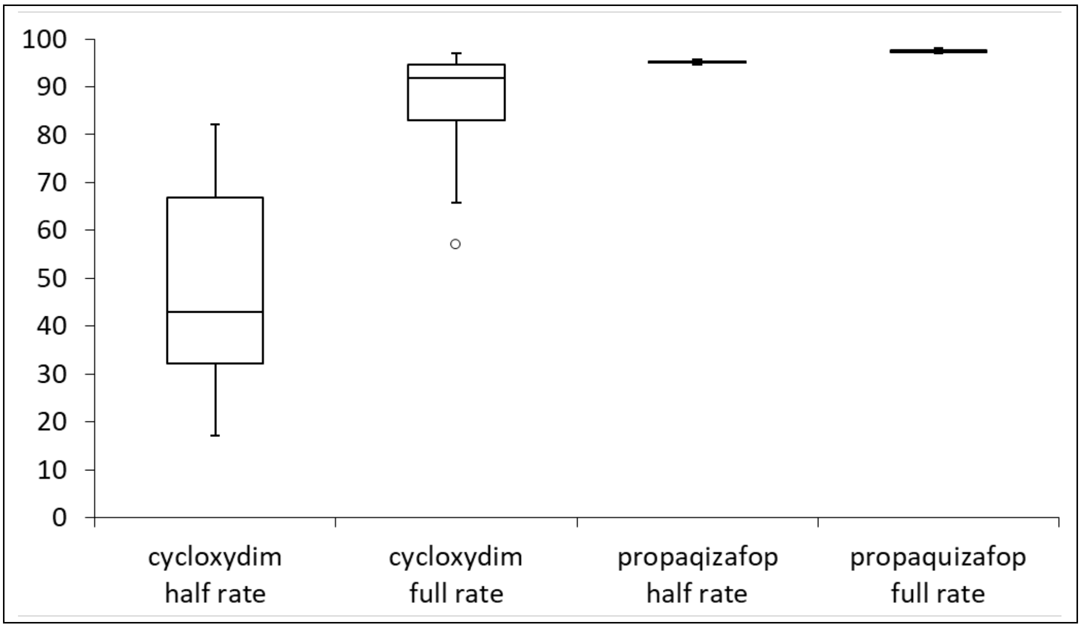


Figure 9 Boxplot of mean percentage reduction in fresh weight (relative to untreated) of 11 great brome populations treated with 100g a.s./L cycloxydim (half rate), 200g a.s./ha cycloxydim (full rate), 50g a.s./ha propaquizafop (half rate) and 100g a.s./ha propaquizafop (full rate). Showing median (black line), 25th and 75th percentiles (box), minimum and maximum (line), and outliers (circles).

Table 38 Mean percentage reduction in foliage fresh weight/plant in great brome (*Anisantha diandrus)* relative to the untreated. Red shading indicates resistant populations identified.

Population	100g a.s./ha cycloxydim (half rate)		200g a.s./ha cycloxydim (full rate)	50g a.s./ha propaquizafop (half rate)		100g a.s./ha propaquizafop (full rate)
SD440	67.4		57.1	96.5		98.1
SD497	32.4		83.0	95.3		97.8
SD432	46.7		83.0	95.1		97.6
SD477	66.5		95.3	95.2		97.6
SD508	36.4		78.8	95.4		97.6
SD481	30.4		87.6	96.4		97.5
SD456	17.1		91.8	95.0		97.3
SD511	72.1		95.1	93.9		97.3
SD221	82.2		92.8	95.0		97.2
SD523 Sensitive	32.0		93.9	95.1		97.2
SD441	42.9		97.1	94.2		97.0
	F probability	sed	lsd	F probability	sed	lsd
Population	NS	12.44	24.86	NS	0.60	1.19
Herbicide	<0.001	5.3	10.60	<0.001	0.24	0.51
Population x herbicide	NS	17.59	35.15	NS	0.84	1.68
Residual df	63			3		
CV%	12.3			63		

Meadow brome was less well-controlled by cycloxydim than propaquizafop (Figure 10), particularly at the half rate (50g a.s./ha), overall control was 72%. At either rate, no populations were identified as resistant. This species was better controlled at half rate than the other species.

No resistance to propaquizafop was detected in UK meadow brome populations, there was a significant reduction fresh weight at both the full rate (100g a.s./ha) and half rate (50g a.s./ha) of 98% and 96% respectively (Table 39).

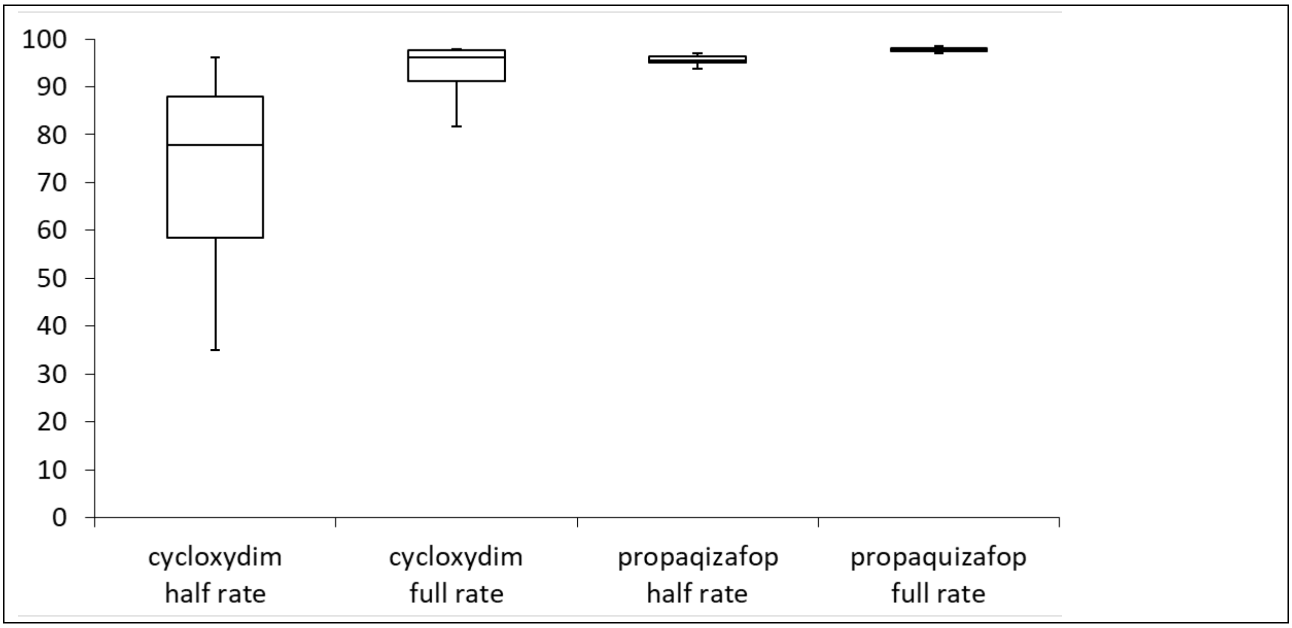


Figure 10 Boxplot of mean percentage reduction in fresh weight (relative to untreated) of 11 meadow brome (*Bromus commutatus)* populations treated with 100g a.s./L cycloxydim (half rate), 200g a.s./ha cycloxydim (full rate), 50g a.s./ha propaquizafop (half rate) and 100g a.s./ha propaquizafop (full rate). Showing median (black line), 25th and 75th percentiles (box), minimum and maximum (line), and outliers (circles).

Table 39 Mean percentage reduction in foliage fresh weight/plant in meadow brome (*Bromus commutatus)* relative to the untreated.

Population	100g a.s./ha cycloxydim (half rate)	200g a.s./ha cycloxydim (full rate)	50g a.s./ha propaquizafop (half rate)	100g a.s./ha propaquizafop (full rate)
SD466	77.9	97.5	97.1	98.6
SD467	81.3	97.9	96.6	98.5
SD458	49.8	77.5	96.7	98.0
SD518 Sensitive	71.8	96.6	95.7	98.0
SD486	89.5	96.0	93.7	97.9
SD474	58.7	97.8	94.8	97.5
SD505	88.6	78.1	95.2	97.5
SD473	35.1	86.4	95.5	97.4
SD507	87.2	96.0	96.0	97.4
SD519 Sensitive	58.1	96.0	95.3	97.2
SD472	96.0	97.6	94.6	97.1

	F probability	sed	lsd	F probability	sed	lsd
Population	NS	12.30	24.59	0.006	0.58	
Herbicide	<0.001	5.25	10.49	<0.001	0.25	
Population x herbicide	NS	17.40	34.78	NS	0.81	
Residual df	62			62		
CV%	10.5			0.3		

Rye brome was less well- controlled by cycloxydim than propaquizafop (Figure 11) at the half rate (50g a.s./ha), overall control was 54%. No populations were identified as resistant to cycloxydim (Table 40).

No resistance to propaquizafop was detected in UK brome populations, there was a significant reduction fresh weight at both the full rate (100g a.s./ha) and half rate (50g a.s./ha) of 98% and 96% respectively (Table 40).

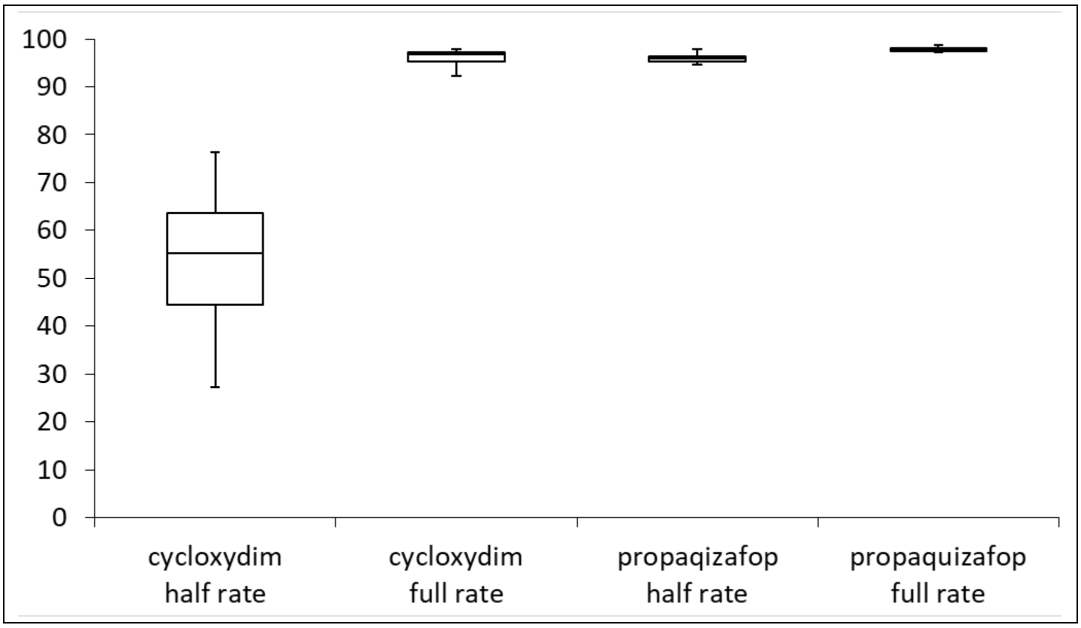


Figure 11 Boxplot of mean percentage reduction in fresh weight (relative to untreated) of 19 rye brome (*Bromus secalinus)* populations treated with 100g a.s./L cycloxydim (half rate), 200g a.s./ha cycloxydim (full rate), 50g a.s./ha propaquizafop (half rate) and 100g a.s./ha propaquizafop (full rate). Showing median (black line), 25th and 75th percentiles (box), minimum and maximum (line), and outliers (circles).

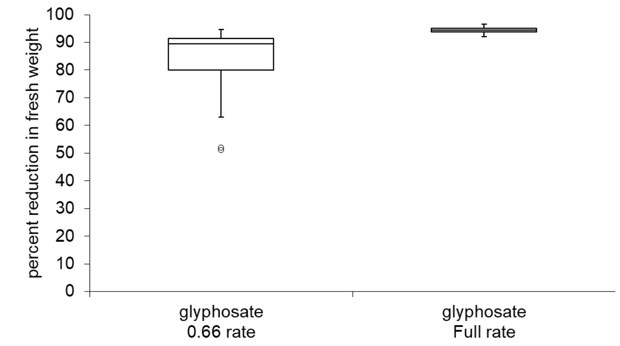
Table 40 Mean percentage reduction in foliage fresh weight/plant in *Bromus secalinus* relative to the untreated from cycloxydim and propaquizafop.

Population	100g a.s./ha cycloxydim (half rate)	200g a.s./ha cycloxydim (full rate)	50g a.s./ha propaquizafop (half rate)	100g a.s./ha propaquizafop (full rate)
SD499	49.7	94.9	97.8	98.7
SD482	39.9	80.1	96.4	98.3
SD483	63.6	97.2	96.3	98.3
SD496	36.3	97.4	96.9	98.2
SD455	59.4	96.8	96.2	98.0
SD470	60.1	97.1	96.8	98.0
SD475	35.4	97.8	94.5	98.0
SD476	53.1	90.1	96.4	97.9
SD485	67.6	97.3	95.7	97.9
SD521 sensitive	55.2	91.3	96.6	97.9
SD500	74.4	96.8	95.5	97.8
SD437	68.0	96.2	96.1	97.7
SD501	62.7	96.9	95.5	97.7
SD512	49.0	97.8	94.8	97.5
SD520	76.3	97.0	96.0	97.5
SD453	63.5	97.0	94.9	97.4
SD506	27.1	95.5	95.2	97.3
SD516	52.0	94.3	94.6	97.3
SD503	39.9	96.6	95.3	97.1
Mean				

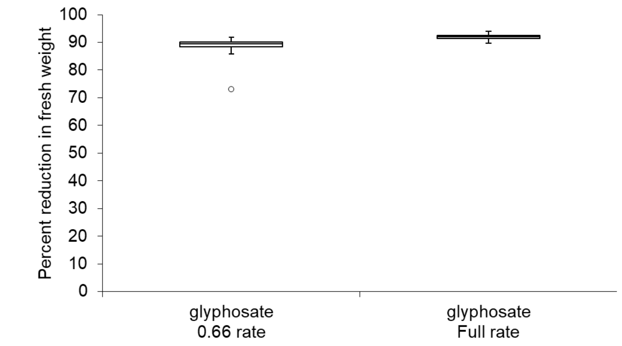
	F probability	sed	lsd	F probability	sed	lsd
Population	NS	11.97	11.97	0.002	0.53	1.05
Herbicide	<0.001	3.88	7.70	<0.001	0.17	0.34
Population x herbicide	NS	16.93	33.55	NS	0.75	1.48
Residual df	108			111		
CV%	15.2			0.5		

Glyphosate screening on populations from 2017

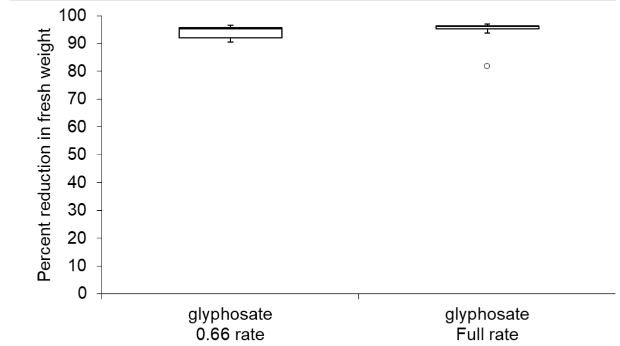
Generally, all species and populations were well-controlled by glyphosate at both half and full rate (Figure 12). The level of control of sterile brome was the most variable, particularly at the half rate. There were some populations of interest.



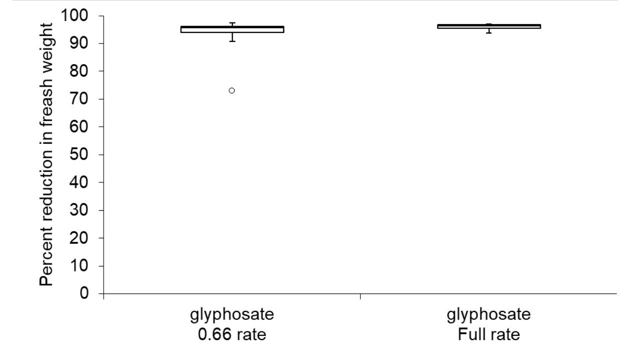
Anisantha sterilis



Anisantha diandrus



Bromus commutatus



Bromus secalinus

Figure 12 Boxplot of mean percentage reduction in fresh weight (relative to untreated) of 21 sterile brome (*Anisantha sterilis)*, 11 great brome (*Anisantha diandrus)*, 11 meadow brome (*Bromus commutatus)*, and 19 rye brome (*Bromus secalinus)* populations treated with populations treated with 360g a.s./ha cycloxydim (0.66 rate), 540g a.s./ha cycloxydim (full rate). Showing median (black line), 25th and 75th percentiles (box), minimum and maximum (line), and outliers (circles).

There were no populations of great brome showing increased tolerance to glyphosate (Table 41).

Table 41 Mean percentage reduction in foliage fresh weight/plant in great brome (*Anisantha diandrus)* relative to the untreated. Red shading indicates resistant populations identified.

Population	360g a.s./ha glyphosate (0.66 rate)		540g a.s./ha glyphosate (full rate)
SD440	91.7		92.3
SD432	90.9		90.1
SD477	90.4		92.1
SD481	90.0		95.1
SD456	89.7		91.4
SD508	89.4		91.2
SD497	89.3		92.0
SD221	89.0		92.5
SD511	87.9		92.9
SD441	85.2		91.6
SD523 sensitive	72.9		89.0
Mean	87.9		91.8
	F probability	SED	LSD
Population	NS	3.17	6.35
Herbicide	0.004	1.35	2.71
Population x herbicide	NS	4.48	8.99
Residual df	52		
CV%	1.6		

When treated with 360g/ha glyphosate, fresh weight of sterile brome was reduced by more than 84% for most populations. There were four populations of sterile brome that showed significantly increased tolerance to glyphosate at the 360g a.s./ha rate, SD224, SD409, SD464 and SD498 (Table 42). The population SD464, has been previously reported as having reduced sensitivity to glyphosate (09D118) (Davies *et al.*, 2019), had a 51% reduction and SD224 had a 52% reduction of fresh weight. There were 2 populations that had a 72% reduction of fresh weight SD409 and SD498. All the populations tested were well-controlled by 540g a.s./ha glyphosate (recommended field rate for annual grasses), including SD464, with a 94.2% reduction in fresh weight for all populations.

Table 42 Mean percentage reduction in foliage fresh weight/plant in sterile brome (*Anisantha sterilis)* relative to the untreated. Red shading indicates resistant populations identified.

Population	360g a.s./ha glyphosate (0.66 rate)	540g a.s./ha glyphosate (full rate)
SD478	94.6	94.7
SD445	94.1	95.1
SD489	94.0	96.6
SD484	92.9	95.0
SD494	92.9	94.5
SD488	91.4	94.2
SD522 (sensitive)	91.4	93.0
SD495	91.1	95.6
SD454	90.6	94.4
SD471	90.3	95.8
SD442	89.4	94.0
SD436	88.9	93.2
SD490	85.7	94.5
SD468	85.4	94.2
SD410	83.7	93.8
SD479	80.0	93.9
SD457	76.3	88.8
SD498	72.9	94.7
SD409	71.9	92.9
SD224	51.6	95.3
SD464	51.0	92.7
Mean	83.8	94.2

	F probability	SED	LSD
Population	<0.001	5.44	10.79
Herbicide	<0.001	1.68	3.33
Population x herbicide	<0.001	7.69	15.24
Residual df	102		
CV%	4.4		

There were no populations of meadow brome showing increased tolerance to glyphosate (Table 43).

Table 43 Mean percentage reduction in foliage fresh weight/plant in meadow brome (*Bromus commutatus)* relative to the untreated.

Population	360g a.s./ha glyphosate (0.66 rate)		540g a.s./ha glyphosate (full rate)
SD474	96.5		96.9
SD466	96.4		96.2
SD467	95.8		96.1
SD507	95.7		96.2
SD518 (sensitive)	95.4		94.5
SD472	95.2		96.4
SD473	95.2		96.3
SD486	92.6		96.2
SD519 (sensitive)	91.5		94.5
SD505	91.2		81.8
SD458	90.6		96.8
Mean	94.2		94.7
	F probability	SED	LSD
Population	0.012	2.50	5.03
Herbicide	NS	1.07	2.14
Population x herbicide	NS	3.54	7.11
Residual df	50		
CV%	0.6		

There was a single population of rye brome (SD475) that showed reduced sensitivity to glyphosate at 360g a.s./ha (Table 44).

Table 44 Mean percentage reduction in foliage fresh weight/plant in rye brome (*Bromus secalinus)* treated with relative to the untreated. Red shading indicates resistant populations identified.

Population	360g a.s./ha glyphosate (0.66 rate)		540g a.s./ha glyphosate (full rate)
SD499	97.3		96.8
SD482	96.7		97.1
SD483	96.7		96.0
SD470	96.4		96.2
SD500	96.2		96.6
SD476	96.0		96.7
SD453	96.0		96.5
SD496	95.9		97.1
SD501	95.8		96.8
SD485	95.6		97.0
SD512	95.6		93.7
SD521 (sensitive)	95.2		96.0
SD516	95.0		96.1
SD455	94.2		96.7
SD506	93.8		96.3
SD520	93.8		95.1
SD437	93.5		95.1
SD503	93.4		94.8
SD475	73.0		94.6
Mean	94.2		96.1
	F probability	SED	LSD
Population	NS	2.92	5.80
Herbicide	NS	0.95	1.88
Population x herbicide	NS	4.13	8.20
Residual df	90		
CV%	1.2		

4.2.2. Confirmation of herbicide resistant populations from seed collected in 2017

Initial dose response on populations from 2017

Based on the ALS screening, the resistance to ALS inhibitor herbicides in selected populations was further confirmed using a dose-response assay. The initial dose response used rate up to 2 x field rate. SD224 was excluded due to poor emergence.

The dose response identified several less-sensitive populations of sterile and meadow brome to Broadway Star (Table 45), and sterile, meadow and rye brome to Pacifica Plus (Table 46). At half the recommended field rate of Broadway Star, percent control fresh weight was 93.5% for the sensitive population SD464, 23.7% for SD454, 54.0% for SD478 and 74.8% for SD488 (Table 45). The sterile brome populations identified as sensitive in the initial screen were well-controlled by Pacifica Plus at field rate (Table 46). The populations identified as less sensitive to ALS herbicides were easily identified SD478, SD488, and SD454 (Figure 14).

The single populations of great brome (SD441) and meadow brome (SD466) identified in the screen was confirmed as tolerant in the dose response (Table 45, Table 46, Figure 13, Figure 14).

Rye brome was generally extremely well-controlled by both Pacifica Plus and Broadway Star even at the lowest dose used. Population SD506 was shown to be more tolerant of Pacifica Plus than the sensitive population (SD453) (Table 46).

A further dose response was done using a greater range of rates and this is reported below.

Table 45 Mean percentage reduction in foliage fresh weight in brome species to Broadway Star relative to untreated control. Red shading indicates less sensitive populations identified.

Population	Proportion of field rate (0.265kg/ha)				
	0.25	0.5	0.75	1	2
Great brome					
SD221 sensitive	88.1	94.5	91.3	93.7	95.1
SD440	89.8	93.2	91.4	93.2	93.5
SD441	30.6	60.7	52.7	56.6	62.3
Sterile brome					
SD409	92.4	96.1	95.4	94.6	96.4
SD464 sensitive	89.4	93.5	90.8	93.4	96.2
SD479	89.5	93.7	91.7	90.6	95.5
SD468	90.8	93.8	90.7	93.6	95.4
SD498	75.2	80.2	85.1	88.8	93.8
SD478	21.3	54.0	54.6	62.3	84.8
SD488	44.1	74.8	68.2	66.1	78.7
SD454	13.7	23.7	9.8	16.3	21.6
Meadow brome					
SD518 sensitive	94.0	96.5	94.8	95.5	97.0
SD466	2.9	17.5	6.3	7.2	22.2
Rye brome					
SD470	94.3	96.0	93.7	94.9	95.7
SD453 sensitive	91.8	94.9	92.5	94.3	95.5
SD455	86.2	91.2	87.5	89.4	93.0
SD506	79.0	84.1	86.7	90.9	90.6
	F probability	SED	LSD		
Population	<0.001	3.01	5.93		
Herbicide	<0.001	1.63	3.22		
Population x herbicide	<0.001	6.73	13.26		
Residual df	252				
CV%	4.9				

Table 46 Mean percentage reduction in foliage fresh weight in brome species to Pacifica Plus relative to untreated control. Red shading indicates resistant populations identified.

	Proportion of field rate				
Population	0.25	0.5	0.75	1	2
Great brome					
SD221 sensitive	89.8	85.5	93.3	94.3	95.7
SD440	90.4	93.3	92.7	95.2	94.2
SD441	27.8	45.6	40.5	51.3	73.7
Sterile brome					
SD409	94.3	94.0	94.8	91.2	96.0
SD479	93.1	66.8	82.1	92.4	94.8
SD468	92.1	74.4	93.9	94.1	94.3
SD464 sensitive	90.6	92.8	91.8	94.7	94.0
SD478	41.0	74.8	86.6	87.8	91.3
SD498	84.2	73.1	90.6	92.9	91.3
SD488	37.4	40.8	21.7	57.9	50.9
SD454	12.6	15.0	17.5	31.0	27.8
Meadow brome					
SD518 sensitive	94.0	94.4	93.2	97.0	96.7
SD466	8.4	4.3	2.0	17.6	21.0
Rye brome					
SD453 sensitive	92.3	91.1	92.9	95.2	94.8
SD470	81.2	92.5	93.4	95.5	94.0
SD455	86.6	85.9	88.3	90.8	91.0
SD506	62.6	74.1	54.9	73.6	86.2
	F probability	SED	LSD		
Population	<0.001	3.87	7.62		
Herbicide	<0.001	3.10	4.13		
Population x herbicide	0.003	8.65	17.03		
Residual df	252				
CV%	5.9				

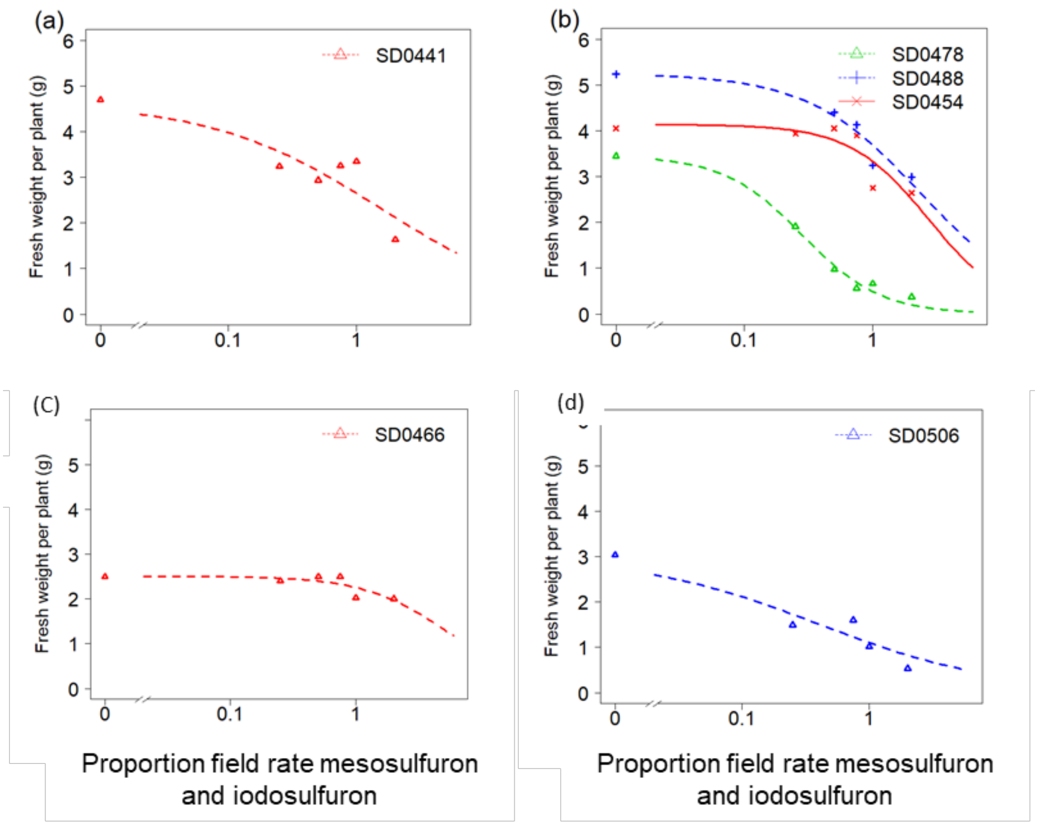


Figure 13 Dose-response curves (log-logistic 3-parameter function) for UK populations of (a) great brome (*Anisantha diandrus),* (b) sterile brome (*Anisantha sterilis),* (c) meadow brome (*Bromus commutatus)*, and (d) rye brome (*Bromus secalinus)*, treated with Pacifica Plus (mesosulfuron + iodosulfuron + amidosulfuron).

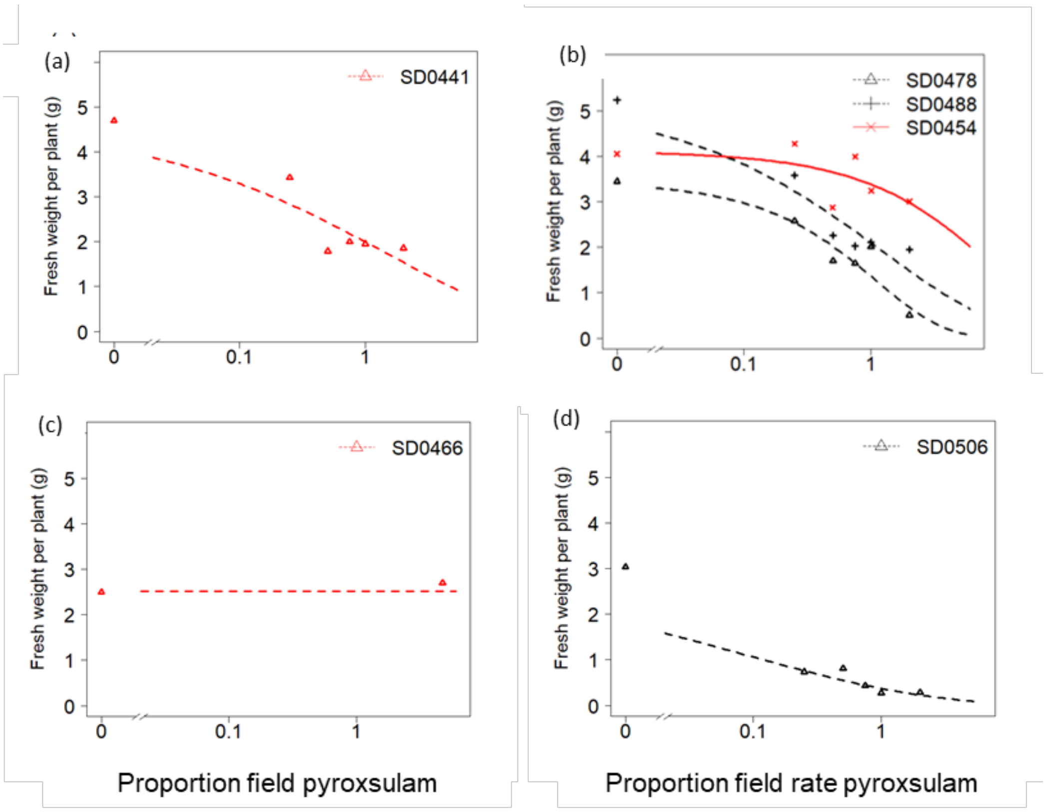


Figure 14 Dose-response curves (Weibull-1 3-parameter function) for UK populations of a) great brome (*Anisantha diandrus),* (b) sterile brome (*Anisantha sterilis),* (c) meadow brome (*Bromus commutatus)*, and (d) rye brome (*Bromus secalinus)* treated with Broadway Star (pyroxsulam + florasulam)

Populations SD0464, SD0468, SD0479 and SD0478 for sterile brome; SD0453, SD0470 and SD0455 for rye brome and SD0418 for meadow brome were totally controlled at the lowest dose (0.25x field rate) used and corresponding fresh weight reduction was >85%, hence model could not be fitted and GR_{50} values were estimated as less than the lowest dose used (Table 47).

For some resistant populations, fresh weight did not fall below 50% even at the highest dose (2x field rate) used, hence GR50 values were estimated above highest dose used.

Table 47 GR50 values and (standard error) from dose-responses using Pacifica plus (mesosulfuron + iodosulfuron + amidosulfuron), and Broadway Star (pyroxsulam + florasulam), of great brome (*Anisantha diandrus)*, sterile brome (*Anisantha sterilis)*, meadow brome (*Bromus commutatus)*, and rye brome (*Bromus secalinus)* population suspected of resistance ALS inhibitor herbicides, and one sensitive reference population of each species.

Population	Resistance status	Pacifica Plus		Broadway Star	
		GR50g a.s./ha (SE)	Resistance index	GR50g a.s./ha (SE)	Resistance index
Great brome					
SD0441	R	23.2 (12.53) + 7.8 (4.20)	6.2	10.4 (4.08)	2.2
SD0440	S	<3.75 + <1.25	-	<4.7	-
SD0221	S	<3.75 + <1.25	-	<4.7	-
Sterile brome					
SD0488	R	>30 + 10	8	10.2 (4.36)	2.2
SD0454	R	>30 + 10	8	>37.5	7.8
SD0478	R?	4.1 (1.30) + 1.4 (0.43)	1.1	13.1 (4.48)	2.8
SD0468	S	<3.75 + <1.25	-	-	-
SD0479	S	<3.75 + <1.25	-	-	-
SD0464	S	<3.75 + <1.25	-	<4.7	-
Meadow brome					
SD0466	R	>30 + 10	8	>37.5	7.8
SD0518	S	<3.75 + <1.25	-	<4.7	-
Rye brome					
SD0506	R?	6.0 (3.12) + 2.0 (1.05)	1.6	<4.7	-
SD0470	S	<3.75 + <1.25	-	<4.7	-
SD0455	S	<3.75 + <1.25	-	<4.7	-
SD0453	S	<3.75 + <1.25	-	<4.7	-

Second ALS inhibitor dose-response (4x rate)

This dose response experiment confirmed all six of the brome populations identified in the screening experiment and the first dose response as being potentially ALS resistant. The populations SD0441 (great brome), SD0488, SD0454, SD0478 (sterile brome), SD0466 (meadow brome) and SD506 (rye brome) had significantly lower percentage reductions in fresh biomass (Table 48 and Table 49).

The four populations showed high levels of resistance to Broadway Star and Pacifica Plus in the screening experiment and first dose response; SD454 and SD488 (sterile brome) SD441 (great brome), and SD466 (meadow brome) also showed the greatest resistance in the 2nd dose response experiment (Table 49, Figure 15, Figure 16). Population SD454 was a sterile brome from Lincolnshire identified as being difficult to control in the field with ALS herbicides; likewise SD488 a sterile brome from Worcestershire. SD441 was a great brome from Shropshire with a history of poor field control. The meadow brome SD466 hails from Yorkshire and was also associated with poor field control. The sterile brome SD478 (Wiltshire) and rye brome SD506 (Oxfordshire) showed partial resistance to Pacifica Plus, with survivors at field rate (Table 50). The sensitive reference populations were well-controlled, with fresh weight reduced by more than 87% at recommended field rates for Pacifica Plus, and 80% for Broadway Star.

It is noteworthy that in the ALS inhibitor screen and in the dose response, there was a large proportion of surviving plants at the field rate of Pacifica plus (mesosulfuron + iodosulfuron) for both populations, and Broadway Star (Pyroxsulam + florasulam) for SD478 population indicating herbicide tolerance.

Table 48 Mean percentage reduction in foliage fresh weight in *brome species to* Broadway Star relative to untreated control. Red shading indicates significant difference from untreated.

Population	Proportion of field rate						
	0.125	0.25	0.5	0.75	1	2	4
Great brome							
SD523 Sensitive	5.5	19.5	49.4	55.3	52.2	73.6	85.2
SD441	19.6	0.0	9.4	50.3	41.0	38.3	27.7
Sterile brome							
SD409	12.0	52.4	33.0	74.3	82.4	78.1	88.8
SD522 sensitive	3.2	15.1	40.7	38.8	79.4	74.5	83.0
SD478	0.0	10.0	17.2	1.4	39.7	63.4	63.0
SD488	0.0	4.4	13.8	0.0	13.0	34.8	62.2
SD454	5.6	9.7	11.3	6.9	14.8	16.7	3.1
Meadow brome							
SD518 sensitive	10.3	65.6	59.9	79.7	87.2	92.1	87.0
SD466	16.9	5.0	19.8	0.0	10.9	16.1	15.6
Rye brome							
SD521	17.3	38.3	59.2	46.3	80.5	87.2	90.4
SD453 sensitive	13.4	54.2	51.6	49.9	75.2	88.4	89.8
SD455	7.7	46.5	63.4	69.8	78.4	75.9	88.0
SD470	2.2	69.2	55.5	62.2	73.8	90.9	84.8
SD506	2.2	11.6	20.8	27.5	47.4	68.8	51.5
	F probability	SED	LSD				
Population	<0.001	6.53	12.89				
Herbicide	<0.001	4.62	9.11				
Population x herbicide	0.033	17.28	34.10				
Residual df	178						
CV%	3.0						

Table 49 Mean percentage reduction in foliage fresh weight in brome species to Pacifica Plus relative to untreated control. Red shading indicates less sensitive populations identified.

Population	Proportion of field rate						
	0.125	0.25	0.5	0.75	1	2	4
Sterile brome							
SD0409	92.7	38.6	73.0	84.6	90.1	93.5	90.8
SD0522 sensitive	89.0	71.4	78.9	84.6	87.1	82.6	87.7
SD0478	62.4	39.5	22.4	33.8	44.9	51.1	73.4
SD0488	25.0	19.5	12.8	39.1	61.3	54.9	69.3
SD0454	18.1	15.7	27.2	4.8	14.4	25.7	34.3
Great brome							
SD0523 sensitive	86.5	51.1	81.5	82.6	89.9	85.1	84.9
SD0441	57.9	11.7	25.3	25.7	46.3	46.3	64.5
Meadow brome							
SD0518 sensitive	92.6	85.5	91.9	90.2	89.8	91.4	92.5
SD0466	18.4	14.9	17.9	13.5	11.1	32.0	17.9
Rye brome							
SD0470	91.6	90.4	87.7	94.7	92.6	91.8	90.2
SD0453	89.7	84.0	89.6	90.3	90.8	89.3	91.5
SD0521 sensitive	93.2	86.8	90.9	90.3	92.5	94.0	93.9
SD0455	87.6	85.4	88.5	89.8	87.3	90.2	87.7
SD0506	58.0	43.1	69.0	59.4	71.0	60.6	68.9
	F probability	SED	LSD				
Population	<0.001	5.02	9.90				
Herbicide	<0.001	3.55	7.00				
Population x herbicide	NS	13.27	26.18				
Residual df	183						
CV%	6.8						

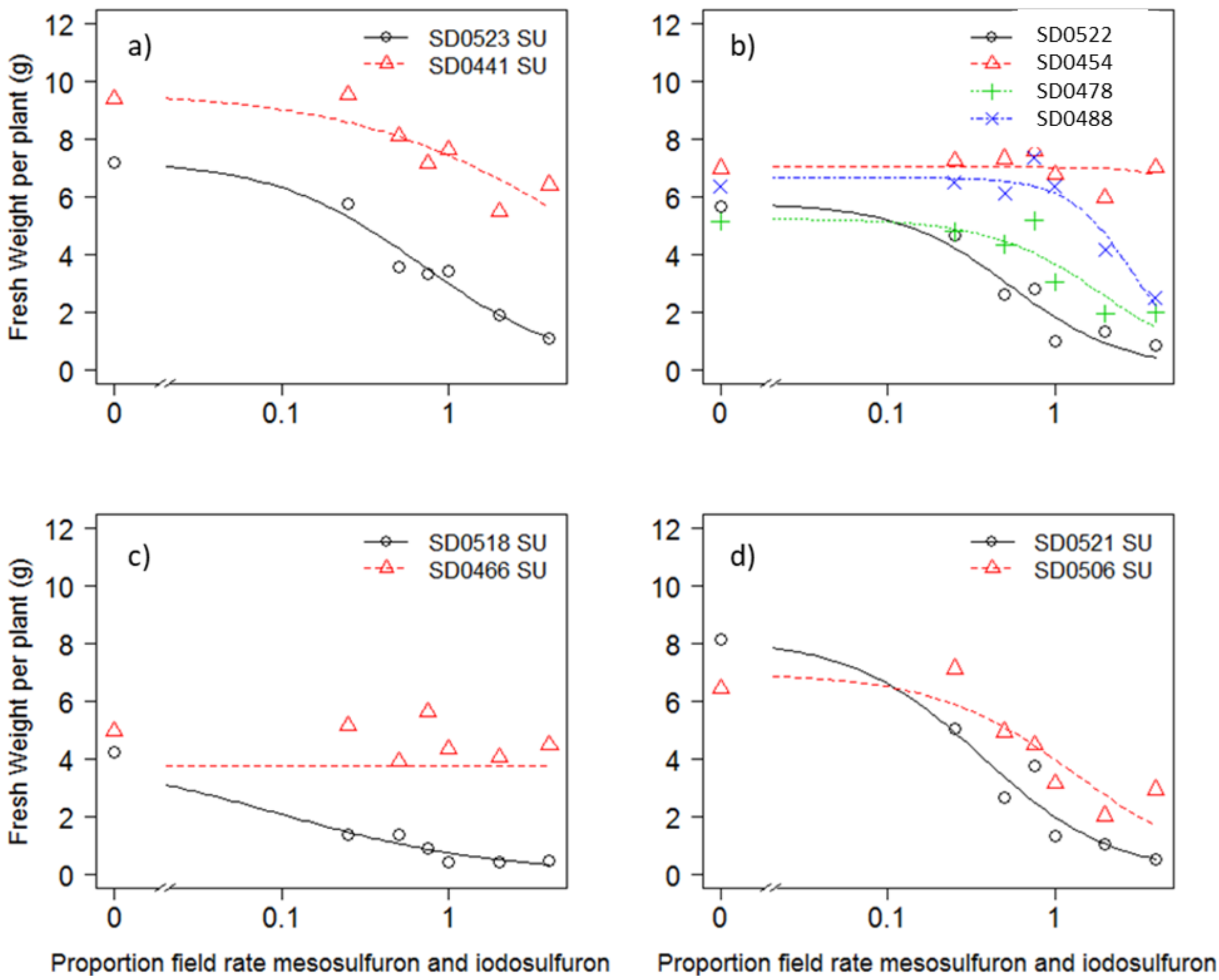


Figure 15 Dose-response curves (log-logistic 3-parameter function) for UK populations of (a) great brome (*Anisantha diandrus),* (b) sterile brome (*Anisantha sterilis),* (c) meadow brome (*Bromus commutatus)*, and (d) rye brome (*Bromus secalinus)*, treated with Pacifica plus (mesosulfuron + iodosulfuron + amidosulfuron). Known sensitive populations are in black.

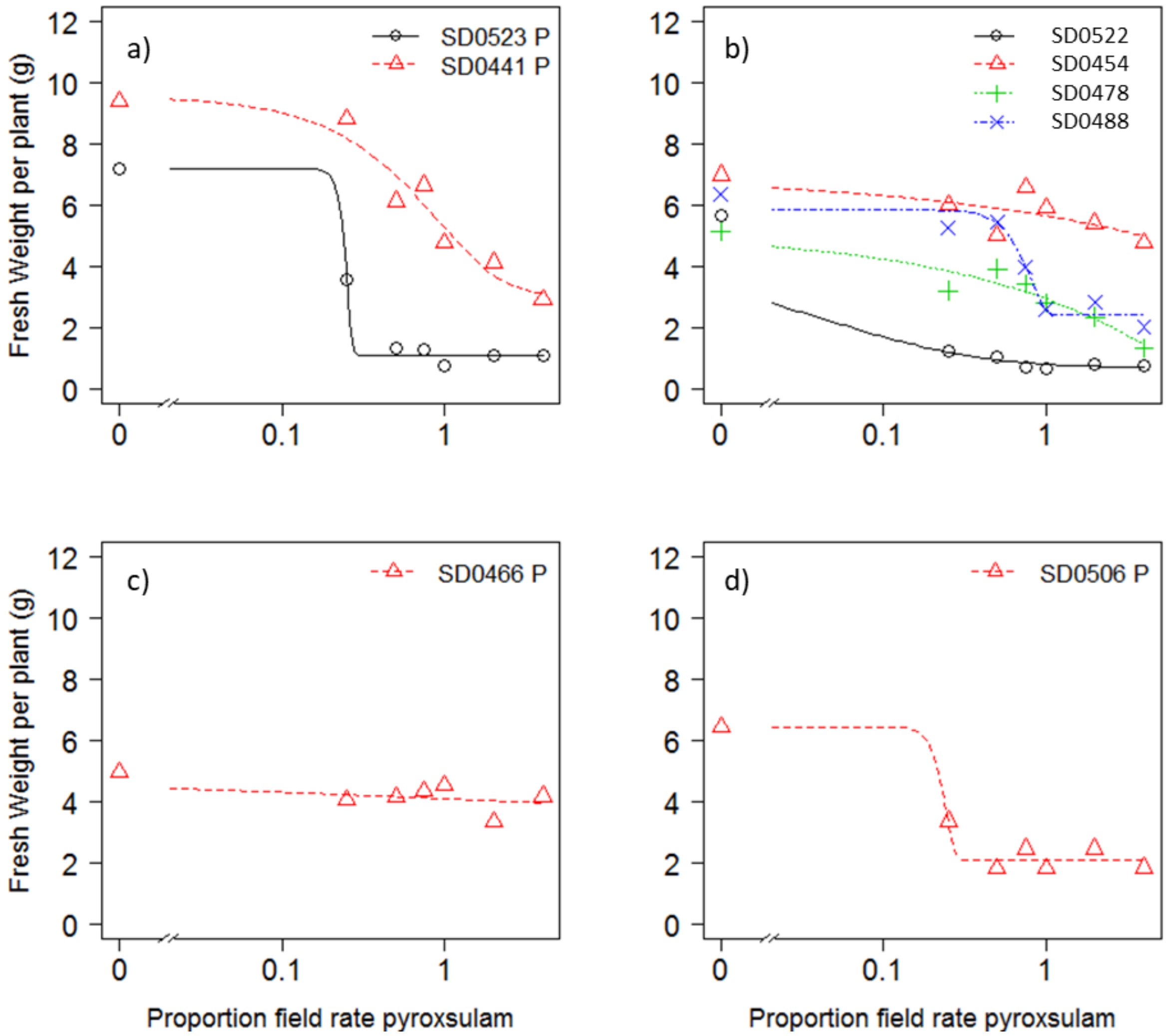


Figure 16 Dose-response curves (Weibull-1 3-parameter function) for UK populations of a) great brome (*Anisantha diandrus),* (b) sterile brome (*Anisantha sterilis),* (c) meadow brome (*Bromus commutatus)*, and (d) rye brome (*Bromus secalinus)* treated with Broadway Star (Pyroxsulam + florasulam). Known sensitive populations are in black.

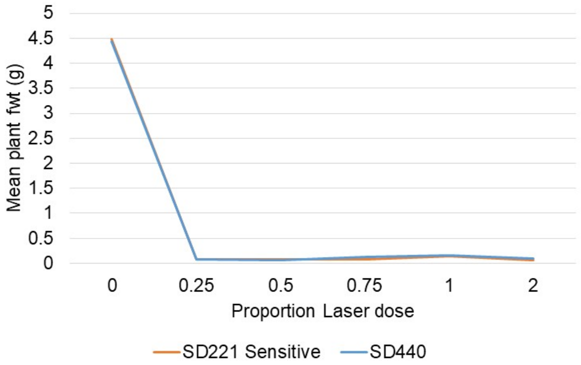
When treated with twice the recommended UK field rate of Pacifica Plus (60g/ha mesosulfuron + 20 g/ha iodosulfuron), mean fresh weight of less sensitive populations SD441, SD454, and SD466, did not fall below 50% of the fresh weight of the untreated controls. Similarly, treatment with twice the recommended rate of Broadway Star (75g/ha pyroxsulam) did not greatly reduce the fresh weight of populations SD454 and SD466 (Table 48, Table 49). Therefore, GR_{50} values for these populations were estimated to be more than four times that of recommended field rate (the highest dose used in the study) (Table 50).

Table 50 GR_{50} values and (standard error) from dose-responses using Pacifica plus (mesosulfuron + iodosulfuron + amidosulfuron), and Broadway Star (pyroxsulam + florasulam), of great brome (*Anisantha diandrus)*, three sterile brome (*Anisantha sterilis)*, one meadow brome (*Bromus commutatus)* (SD466), and one rye brome (*Bromus secalinus)* population suspected of resistance ALS inhibitor herbicides, and one sensitive reference population of each species.

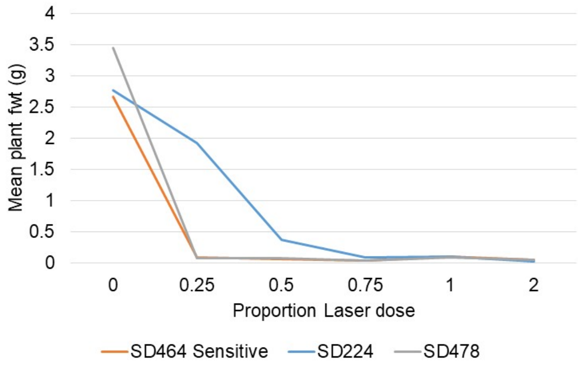
Population	Resistance status	Pacifica Plus		Broadway Star	
		GR50 g a.s./ha (SE)	Resistance index	GR50 g a.s./ha (SE)	Resistance index
Great brome					
SD441	R	>60.0 + >20.0	>5.8	12.5	2.7
SD523	S	10.4 (4.5) + 3.45 (1.5)	-	4.6 (4.5)	-
Sterile brome					
SD454	R	>60.0 + >20.0	>7.3	>75.0	>16.0
SD478	R?	28.8 (14.5) + 9.6 (4.9)	3.5	28.1 (11. 8)	>6.0
SD488	R	44.0 (11.6) + 14.7 (3.9)	5.4	13.7 (2.2)	>2.9
SD522	S	8.2 (3.5) + 2.73 (1.2)	-	<4.7	-
Meadow brome					
SD466	R	>60.0 + >20.0	>16.0	>75.0	>16.0
SD518	S	<3.75 + <1.25	-	<4.7	-
Rye brome					
SD506	R?	19.8 (8.9) + 6.6 (3.0)	3.6	4.4 (6.5)	>0.9
SD521	S	5.5 (2.2) + 2.73 (0.7)	-	<4.7	-

ACCase dose-response on selected populations from 2017

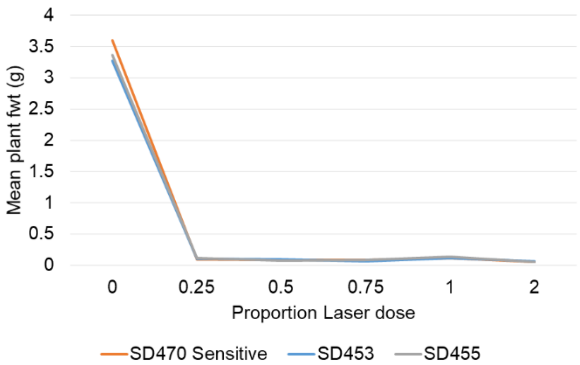
A range of populations were subjected to a dose response. All populations of great, sterile and rye brome were completely controlled at the lowest cycloxydim dose used apart from the sterile brome SD224 (Figure 17b) and a dose-response analysis was not able to be completed. SD224 is a known resistant sterile brome population from Germany.



a) Great brome



b) Sterile brome



c) Rye brome

Figure 17 Dose-response of great, sterile and rye brome populations using Laser (cycloxydim)

Glyphosate dose-response on selected populations from 2017

Several populations of sterile and rye brome were selected for the glyphosate dose response. Most populations were well-controlled by half rate glyphosate with control levels greater than 86% (Table 51). There was an indication in two populations of sterile brome, SD464 and SD479, of poorer levels of control at low rates when compared to the sensitive standard (Figure 18), SD464 is a population from Nottinghamshire with a known increased tolerance to glyphosate (Davies *et al*., 2019). SD479 is from Oxfordshire.

A single rye brome population was of interest with poorer levels of control at low rates (Table 51, Figure 18), this population originates from Surrey with a history of poor field control with ALS herbicides.

Table 51 Mean percentage reduction in foliage fresh weight in brome species to glyphosate relative to untreated control. Red shading indicates less sensitive populations identified.

	Proportion of field rate				
Population	0.25	0.5	0.75	1	2
Sterile brome					
SD468 sensitive	91.7	97.1	97.7	98.1	98.6
SD479	60.0	88.9	96.1	97.7	98.5
SD464	34.1	86.8	93.1	95.2	96.9
Rye brome					
SD453 sensitive	84.5	96.6	97.6	98.4	99.2
SD455	53.6	93.1	95.2	97.3	99.0
SD470	79.6	92.8	98.2	99.1	99.0
	F probability	SED	LSD		
Population	<0.001	2.64	5.24		
Herbicide	<0.001	2.41	4.79		
Population x herbicide	<0.001	5.90	11.72		
Residual df	87				
CV%	3.5				

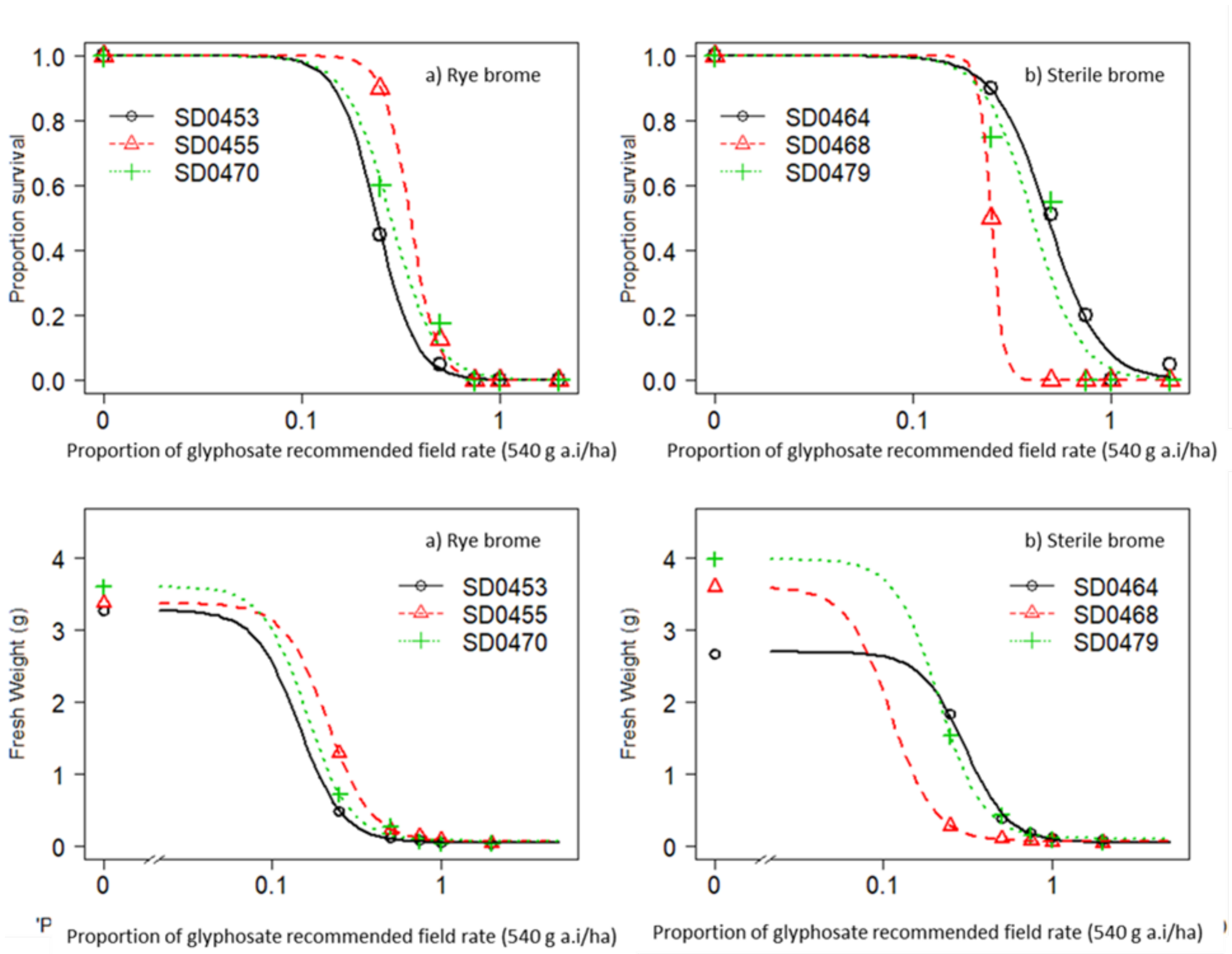


Figure 18 Dose-response curves (log-logistic 3-parameter function) for UK populations of (a) rye brome and b) sterile brome, treated with glyphosate. Known populations are in black (SD453 is sensitive, SD464 is tolerant.

A model was fitted to the dose response data (Model fit survival – 0.7437, Model fit FW – 1.00). There were significant differences in ED_{50} between the 6 populations (ANOVA P>0.001, LR value – 37.364) (Table 52). There was no significant variation in slope (ANOVA P=0.1275, LR value – 8.5693), but constraining the slope compromised model fit (model fit of constrained model – 0.0132) and, therefore, the unconstrained model (model fit of unconstrained model – 0.7473) was used to estimate ED_{50}.

For fresh weight, there was significant differences in GR_{50} between the 6 populations (ANOVA P>0.001, LR value – 60.494) (Table 52), and significant difference between sloped (ANOVA, P>0.001, LR value – 37.364), therefore, neither were constrained. The highest resistance index was 2.8 (SD464), SD479 and SD455 both had an RI greater than 1 and required a higher dose rate for control and so can be classified as less sensitive to lower rates of glyphosate.

Table 52 ED_{50} and GR_{50} values from glyphosate dose-response of three sterile brome and three rye brome populations

Species and population	ED50 (g/ha)	Std. Error	GR50 (g/ha)	Std. Error	Resistance index	Higher dose required for control
Sterile brome						
SD464	260.0	25.01	164.2	16.12	2.8	yes
SD468 Sensitive	135.0	4.33	59.7	21.31	1.0	
SD479	217.9	20.96	115.6	8.60	1.9	yes
Rye brome						
SD453 Sensitive	129.7	13.70	78.0	15.84	1.0	
SD455	191.1	15.87	115.3	9.98	1.5	yes
SD470	153.6	15.92	86.9	12.93	1.1	

4.2.3. Herbicide resistance testing

Resistance screening 2018

Of the brome populations tested for resistance in 2018, one sterile brome SD623 (Nottinghamshire) and one rye brome population SD622 (Shropshire) were found to be resistant to half and full recommended field rates of Broadway Star (Table 53).

Table 53 Resistance rating to Broadway Star (Pyroxsulam + florasulam) of great brome, sterile brome, and rye brome populations compared to known sensitive populations

ADAS reference	Fresh weight untreated (g)	Broadway Star[1] 0.25kg/ha Half recommended field rate			Broadway Star[1] 0.5kg/ha Full recommended field rate		
		Fresh weight (g)	Percent control (%)	Resistance rating	Fresh weight (g)	Percent control (%)	Resistance rating
Great brome							
SD523 Sensitive	32.6	4.0	88	S	4.1	87	S
SD502	30.5	2.6	91	S	3.0	90	S
SD625	54.9	2.2	96	S	1.8	97	S
Sterile brome							
SD522 Sensitive	29.4	1.3	96	S	1.8	94	S
SD454	31.5	19.6	38	RRR	25.5	19	RRR
SD623	33.9	22.8	33	RRR	27.3	20	RRR
Rye brome							
SD521 Sensitive	28.5	1.3	95	S	1.0	97	S
SD622	29.1	15.5	47	RR	20.6	29	RRR
SD624	28.3	1.1	96	S	1.1	96	S
SD716	25.1	1.0	96	S	1.0	96	S

[1] plus Biosyl adjuvant at 1.0% spray volume;

Resistance screening 2019

One giant brome population (SD758) did not germinate. SD454 (Lincs) ALS resistant population was included to show that the test was working correctly. One sterile brome SD753, one meadow brome SD757 and four rye brome populations SD747, SD748, SD750 and SD756 were found to be resistant to half and full recommended field rates of Pacifica Plus (Table 54).

Table 54 Resistance rating to Pacifica Plus (mesosulfuron + iodosulfuron + florasulam) of great brome, sterile brome, and rye brome populations compared to known sensitive populations, 2019

ADAS reference and species	Fresh weight untreated (g)	Pacifica Plus[1] 0.25kg/ha Half recommended field rate			Pacifica Plus[1] 0.5kg/ha Full recommended field rate		
		Fresh weight (g)	Percent control (%)	Resistance rating	Fresh weight (g)	Percent control (%)	Resistance rating
Great brome							
SD523 Sensitive	13.0	2.0	84.8	S	2.0	84.5	S
Sterile brome							
SD0522 Sensitive	8.1	4.1	49.4	S	1.0	87.5	S
SD0454 ALS resistant	12.2	7.1	41.6	R?	7.8	36.7	RR
SD0749	13.5	2.1	84.8	S	2.1	84.3	S
SD0751	12.7	3.9	69.1	S	3.5	72.2	R?
SD0752	12.7	1.3	89.9	S	1.9	85.1	S
SD0753	16.1	7.5	53.3	S	5.3	67.3	RR
SD0755	10.8	2.2	79.7	S	1.5	85.7	S
SD0761	9.8	2.0	80.1	S	2.2	78.1	R?
SD0764	15.5	3.6	77.1	S	4.3	72.1	R?
SD0786	5.4	3.5	35.9	RR	0.9	84.0	S
Meadow brome							
SD0757	4.3	2.8	36.3	RR	2.6	41.0	RR
SD0754	5.2	0.8	85.3	S	0.7	86.2	S
Rye brome							
SD0521 Sensitive	6.4	3.0	53.2	S	1.1	82.7	S
SD0747	7.6	6.6	13.9	RRR	7.7	-1.0	RRR
SD0748	8.4	5.4	35.4	RR	3.1	62.5	RR
SD0750	5.3	2.2	58.2	S	2.5	53.2	RR
SD0756	7.4	4.2	43.2	R?	4.6	37.2	RR
SD0759	9.2	1.4	85.1	S	1.2	86.5	S
SD0760	8.1	0.9	89.4	S	1.4	82.4	S
SD0762	6.1	0.9	84.6	S	0.9	85.7	S
SD0763	8.3	0.9	89.3	S	1.1	86.9	S
SD0785	8.0	1.1	85.9	S	1.0	88.0	S

[1]plus Biopower adjuvant; at 1.0L/ha

SD454 (Lincs) ALS resistant population was included to show that the test was working correctly. One sterile brome SD753 (Berwick), one meadow brome SD757 (North Yorkshire) and one rye brome population SD747 (Shropshire), were found to be resistant to half and full recommended field rates of Broadway Star (Table 55).

Table 55 Resistance rating to Broadway Star (pyroxsulam + amidosulfuron) of great brome, sterile brome, and rye brome populations compared to known sensitive populations, 2019

ADAS reference and species	Fresh weight untreated (g)	Broadway Star[1] 0.133kg/ha Half rate			Broadway Star[1] 0.265kg/ha Full rate		
		Fresh weight (g)	Percent control (%)	Resistance rating	Fresh weight (g)	Percent control (%)	Resistance rating
Great brome							
SD523 sensitive	13.0	1.4	89.2	S	1.3	89.7	S
Sterile brome							
SD522 sensitive	8.1	0.7	91.2	S	0.9	89.1	S
SD0454 ALS resistant	12.2	9.9	19.2	RRR	7.5	39.0	RR
SD0749	13.5	2.3	83.3	S	2.9	78.4	R?
SD0751	12.7	1.2	90.4	S	2.4	80.9	S
SD0752	12.7	1.9	85.0	S	1.0	92.5	S
SD0753	16.1	6.8	57.9	S	5.1	68.3	RR
SD0755	10.8	1.3	87.9	S	0.9	92.0	S
SD0761	9.8	1.4	85.8	S	0.8	92.0	S
SD0764	15.5	5.9	62.3	S	4.3	72.6	R?
SD0786	5.4	0.6	89.5	S	0.7	87.4	S
Meadow brome							
SD0754	5.2	0.6	87.8	S	0.9	81.9	S
SD0757	4.3	2.4	44.1	R?	2.5	42.0	RR
Rye brome							
SD0521 sensitive	6.4	0.7	89.1	S	0.8	87.0	S
SD0747	7.6	5.2	31.8	RR	5.2	31.7	RRR
SD0748	8.4	3.5	57.9	S	2.3	72.5	S
SD0750	5.3	1.1	78.9	S	1.8	66.3	R?
SD0756	7.4	2.8	62.5	S	2.2	71.0	S
SD0759	9.2	0.9	89.8	S	0.9	90.0	S
SD0760	8.1	0.8	89.6	S	0.8	89.6	S
SD0762	6.1	0.8	86.4	S	0.8	87.6	S
SD0763	8.3	1.0	88.4	S	0.7	92.1	S
SD0785	8.0	1.1	86.0	S	0.8	89.4	S

[1] plus Biosyl adjuvant at 1.0% spray volume.

Resistance screening 2020

There was no reduction in fresh weight in the 20C11 meadow brome population (North Yorkshire) when treated with GF-1274 (Pyroxsulam) compared to the untreated control, suggesting that this population could also potentially be resistant to pyroxsulam.

4.2.4. Confirmation of herbicide resistant populations from seed collected 2018-2019

A dose-response was conducted to test for resistance in bromes to GF-1274 (Pyroxsulam). GR_{50} values ranged from 2.2g a.s./ha to >37.5g a.s./ha for rye brome populations, 6.1g a.s./ha and 25.1g a.s./ha for sterile brome populations and was >37.5g a.s./ha for the meadow brome population tested. Three rye brome and one sterile brome populations had significantly higher GR_{50} values compared to their corresponding known sensitive populations, with R:S ratios ranging from 12.7 to >17 for rye brome populations and 4.1 for the sterile brome population, suggesting that these populations are resistant to pyroxsulam (Table 56, Figure 19).

Table 56 GR_{50} values from GF-1274 (Pyroxsulam) dose-response of potentially resistant brome species. ***P-value <0.001 compared to corresponding sensitive population

ADAS reference	Resistance status	Prop. GR50	Standard error	GR50 (g/ha)	Standard error	P-value	Resistance index
Sterile brome							
SD468	Sensitive	0.327	0.073	6.1	1.4		-
SD753	R	1.338	0.265	25.1	5.0	***	4.1
Meadow brome							
SD757	R?	>2	NA	>37.5	NA		-
Rye brome							
SD453	Sensitive	0.119	0.078	2.2	1.5		-
SD747	R	>2	0.736	>37.5	13.8	***	>17
SD748	R	1.491	0.361	28.0	6.8	***	12.7
SD750	R?	0.185	0.045	3.5	0.9		1.6
SD756	R	>2	0.164	>37.5	3.1	***	>17

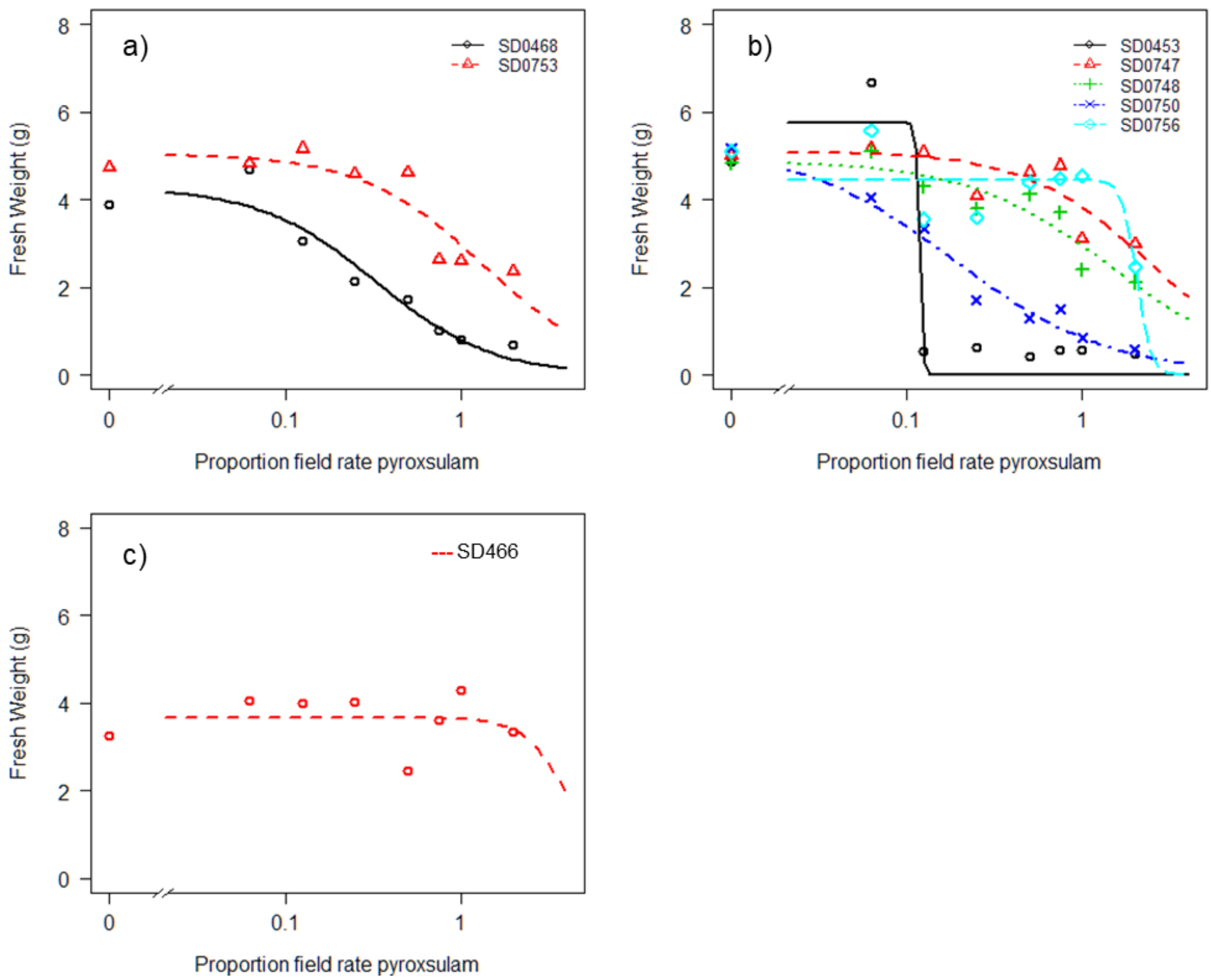


Figure 19 GF-1274 (Pyroxsulam) dose-response curves for potentially herbicide resistant brome populations. Sensitive populations are in black a) sterile brome, b) rye brome, c) meadow brome

4.2.5. ALS-inhibitor target site resistance leaf testing

The pyrosequencing analysis showed that one population, SD466 (meadow brome), contained mutations in ALS enzymes (Table 57). All 15 plants tested from SD466 population were identified to have heterozygous mutations at Trp-574 position, with a Trp/Leu substitution on the ALS protein. This was confirmed with further testing of leaf samples by Bayer in 2021, all plants tested having the Trp574 mutation.

A rye brome, SD506, in tests was shown to be tolerant to pyroxsulam, was heterozygous in 10 out of 15 plants assessed for Pro-197. Results indicated that this population may have a greater level of resistance to mesosulfuron + iodosulfuron (Pacifica Plus).

Table 57 Results of pyrosequencing analysis 2018 and 2021.

Species and Population	IDENTXX testing 2018				Bayer testing 2021
	Untreated	Pacifica Plus full rate	Broadway Star full rate	360g a.s/ha glyphosate	Untreated
Great brome					
SD441	None	None	None	-	
SD523 sensitive	None	None	None	-	
Sterile brome					
SD454	None	None	None	-	
SD464	-	-	-	None	
SD488	None	None	None	-	
SD521 sensitive	None	None	None	-	
Meadow brome					
SD466	5x Trp 574	5x Trp 574	5x Trp 574	-	all plants heterozygous for Trp 574
SD518 sensitive	None	None	None	-	
Rye brome					
SD506	3x Pro197	2x Pro197	5x Pro 197	-	all plants heterozygous for Pro 197
SD522 sensitive	None	None	None	-	

Although SD506 rye brome only showed marginal resistance to both herbicides in the initial screen (Table 36), greater resistance to Pacifica Plus compared with Broadway Star was recorded in the first dose response (Table 45, Table 46, Figure 13, Figure 14), although the differences were less obvious in the second dose response experiment (Table 48, Table 49, Figure 15, Figure 16). This population was shown in subsequent studies to possess the 197 ALS target site mutation. This mutation has been shown to confer greater resistance to sulfonylureas (like Pacifica Plus) than triazolopyrimidines (like pyroxsulam in Broadway Star) in studies with chickweed (*Stellaria media*) (Marshall *et al*., 2010).

In contrast, SD466 meadow brome showed relatively high resistance to both herbicides in the initial screen and both dose response experiments (Table 35, Table 45, Table 46, Table 48, Table 49, Figure 13, Figure 14, Figure 15, Figure 16). This population was shown to possess the 574 ALS target site mutation (Table 57). This mutation has been shown to confer resistance to both sulfonylureas (like Pacifica Plus) and triazolopyrimidines (like pyroxsulam in Broadway Star) in the same chickweed studies (*Stellaria media*) (Marshall *et al*., 2010). So, the effect of different ALS mutations on the relative efficacy of different classes of ALS inhibitors appears to be consistent in both chickweed and brome.

4.3. Investigating if populations can be pushed towards resistance evolution and the identification of modes of action most at risk of resistance evolution

After two or three years of herbicide selection to determine the resistance and sensitivity status of UK brome populations (sterile and rye bromes) to ALS herbicides and glyphosate, seeds were tested in a glasshouse dose response in autumn 2020. 24 Brome populations were used (Table 22). Populations were from the 2019/20 trial and for comparison purposes the original baseline populations from 2017/18.

4.3.1. ALS-inhibitor (pyroxsulam)

GR_{50} values ranged between 1.5 and 13.7g a.s./ha for sterile brome lines and 1.5 and 2.2g a.s./ha for rye brome (Table 58). Both slope and GR_{50} values of the populations significantly varied from each other and were, therefore, not constrained (F-value = 27.6, P-value <0.001). There were two treated sterile brome lines that had significantly higher GR_{50} values compared to their corresponding baseline population after two generations, suggesting these lines have become less sensitive to pyroxsulam during low dose herbicide selection (Figure 20). However, for the line derived from the original population SD464 (SD464>SD646>SD728>SD847), there was no significant difference between the herbicide treated and corresponding unselected lines when selected over three generations (Table 58).

No rye brome lines had significantly different GR_{50} values compared to their corresponding untreated line, with no significant change in sensitivity to pyroxsulam in these lines over two generations of selection (Table 58; Figure 21).

Table 58 GR_{50} values from GF-1274 (Pyroxsulam) dose-response of sterile and rye brome populations selected with low doses of pyroxsulam for 3 generations. *P-value <0.05 compared to corresponding untreated line

Population	Selected line	GR50 (g a.s./ha)	SE	P-value	Ratio to baseline
Sterile brome					
SD464	Baseline seed - ALS sensitive, glyphosate tolerant	3.5	1.1		1.0
SD835	SD464 > SD644 > SD722 > UNTREATED x 3 years	5.5	1.4		1.6
SD728	SD464 > SD646 > Pyroxsulam x 2 years	13.7	1.4	*	3.9
SD847	SD464 > SD646 > SD728 > Pyroxsulam x 3 years	2.4	0.4		0.7
SD468	Baseline seed - sensitive	6.1	1.4		1.0
SD834	SD468 > SD641 > SD721 > UNTREATED x 3 years	2.2	1.6		0.4
SD844	SD468 > SD643 > Pyroxsulam x 2 years	1.5	0.2		0.2
SD479	Baseline seed - ALS sensitive, glyphosate tolerant	5.7	1.2		1.0
SD832	SD479 > SD638 > SD720 > UNTREATED x 3 years	3.7	0.9		0.6
SD640	SD479 > SD640 > Pyroxsulam x 2 years	10.7	2.0	*	1.9
Rye brome					
SD453	Baseline - sensitive	2.2	1.5		1.0
SD738	SD453 > SD738 > Pyroxsulam x 2 years	1.6	0.3		0.7
SD837	SD453 > SD651 > SD731 > UNTREATED x 3 years	2.2	1.1		1.0
SD455	Baseline seed - ALS sensitive, glyphosate tolerant	1.9	0.2		1.0
SD831	SD455 > SD647 > SD729 > UNTREATED x 3 years	2.0	0.4		1.1
SD848	SD455 > SD739 > Pyroxsulam x 2 years	2.0	0.6		1.1
SD470	Baseline seed - ALS sensitive	1.7	0.2		1.0
SD836	SD470 > SD649 > SD730 > UNTREATED x 3 years	1.5	0.1		0.9

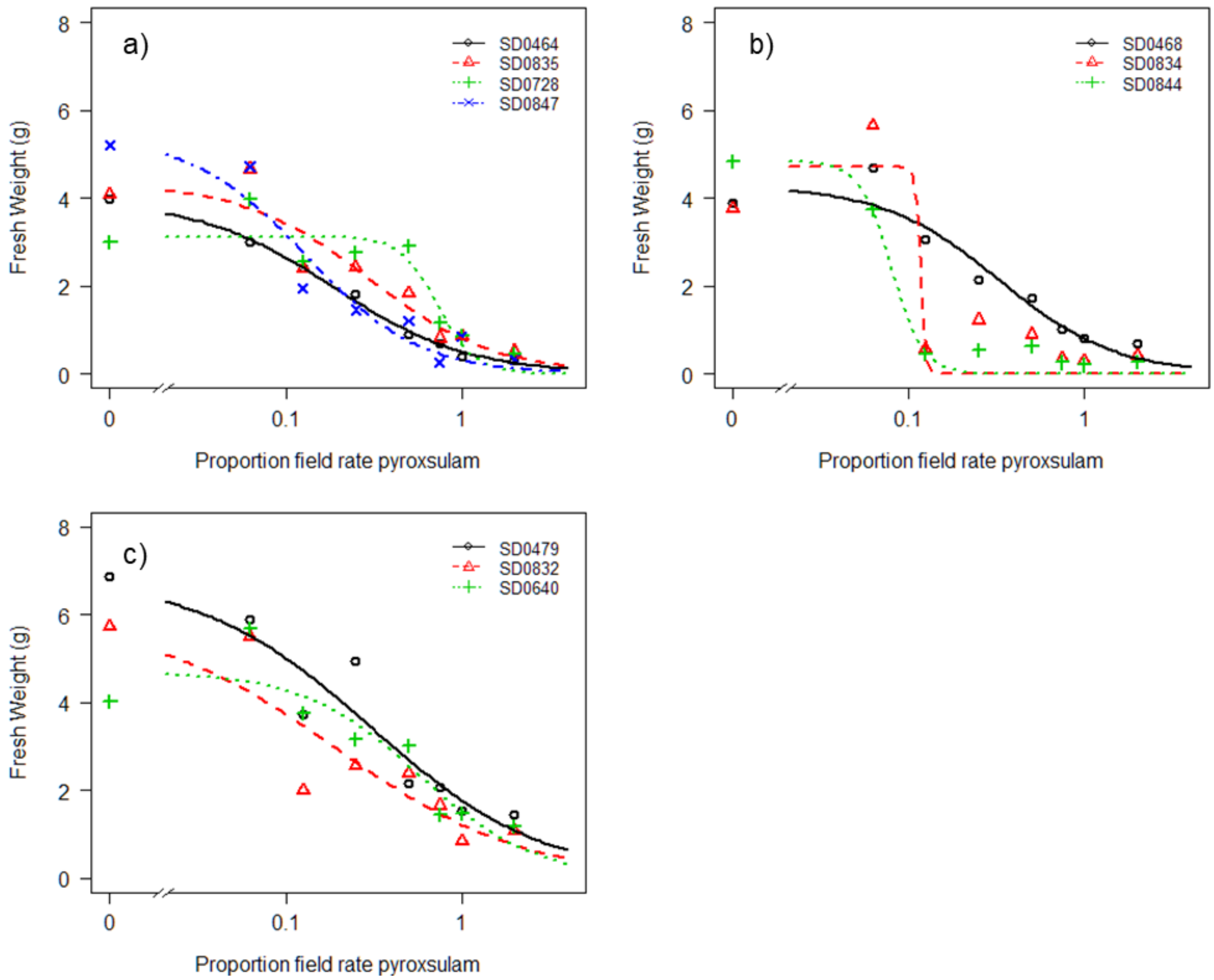


Figure 20 Sterile brome GF-1274 (Pyroxsulam) dose-response curves. Original population (black), untreated control line (red), selected line 2 years (green), selected line 3 years (blue) (a) lines derived from SD464, (b) lines derived from SD468, (c) lines derived from SD479

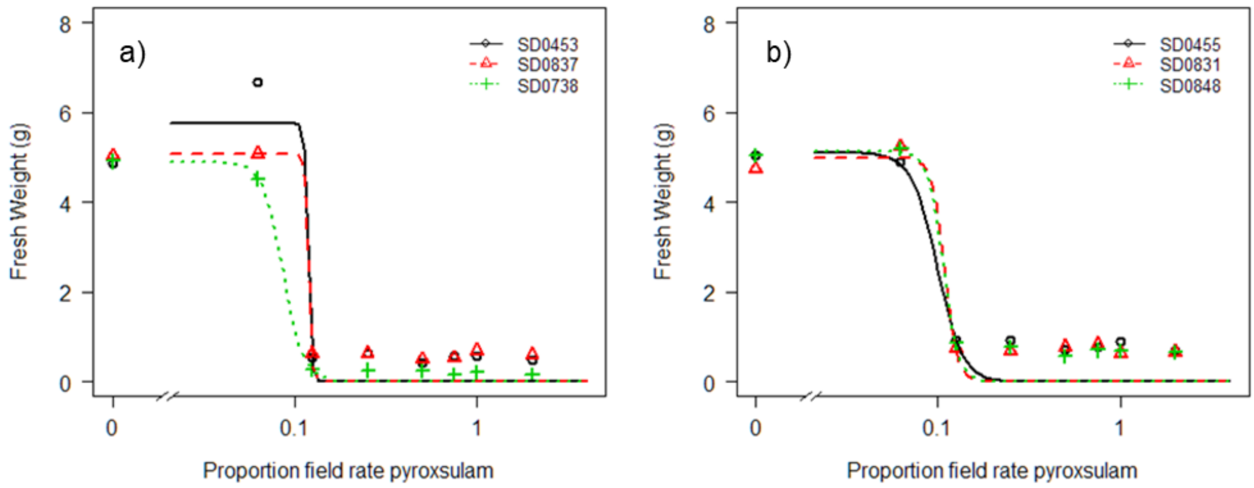


Figure 21 Rye brome GF-1274 (Pyroxsulam) dose-response curves. Original population (black), untreated control line (red), selected line (green), (a) lines derived from SD453, (b) lines derived from SD455

4.3.2. Glyphosate

GR_{50} values ranged between <135 and 358g/ha for sterile brome lines and was <135g/ha for all for rye brome lines (Figure 21). Both slope and GR_{50} values of the populations significantly varied from each other and were, therefore, not constrained (F-value = 4.33, P-value <0.001). There was one treated sterile brome line that had a significantly higher GR_{50} value compared to its corresponding baseline population, suggesting that this line has become less sensitive to glyphosate during low dose herbicide selection. However, the change in glyphosate sensitivity was small, with the GR_{50} of this line was still within the range of GR_{50} values of other populations (Figure 22).

No rye brome lines had significantly different GR_{50} values compared to their corresponding baseline population, with no significant change in sensitivity to glyphosate in these lines over 3 generations of selection (Figure 23).

Table 59 GR_{50} values from glyphosate dose-response of sterile and rye brome populations selected with low doses of glyphosate for 3 generations. **P-value <0.01 compared to corresponding untreated line

Population	Selected line	GR50 (g/ha)	SE	P-value	Ratio to baseline
Sterile brome					
SD464	Baseline seed - ALS sensitive, glyphosate tolerant	249	57.3		1.0
SD835	SD464 > SD644 > SD722 > UNTREATED x 3 years	314	57.1		1.3
SD842	SD464 > SD645 > SD740 > Glyphosate x 3 years	334	59.3		1.3
SD468	Baseline seed - sensitive	358	25.1		1.0
SD834	SD468 > SD641 > SD721 > UNTREATED x 3 years	<135	63.0		-
SD839	SD468 > SD642 > SD724 > Glyphosate x 3 years	308	91.0	**	0.9
SD479	Baseline seed - ALS sensitive, glyphosate tolerant	147	25.8		-
SD832	SD479 > SD638 > SD720 > UNTREATED x 3 years	<135	37.2		-
SD838	SD479 > SD639 > SD723 > Glyphosate x 3 years	<135	24.9		-
Rye brome					
SD453	Baseline seed - sensitive	<135	1.7		-
SD837	SD453 > SD651 > SD731 > UNTREATED x 3 years	<135	8.7		-
SD843	SD453 > SD652 > SD734 > Glyphosate x 3 years	<135	26.5		-
SD455	Baseline seed - ALS sensitive, glyphosate tolerant	<135	26.7		-
SD831	SD455 > SD647 > SD729 > UNTREATED x 3 years	<135	26.2		-
SD840	SD455 > SD648 > SD732 > Glyphosate x 3 years	<135	13.0		-
SD470	Baseline seed - ALS sensitive	<135	3.7		-
SD836	SD470 > SD649 > SD730 > UNTREATED x 3 years	<135	2.0		-
SD841	SD470 > SD650 > SD733 > Glyphosate x 3 years	<135	34.6		-

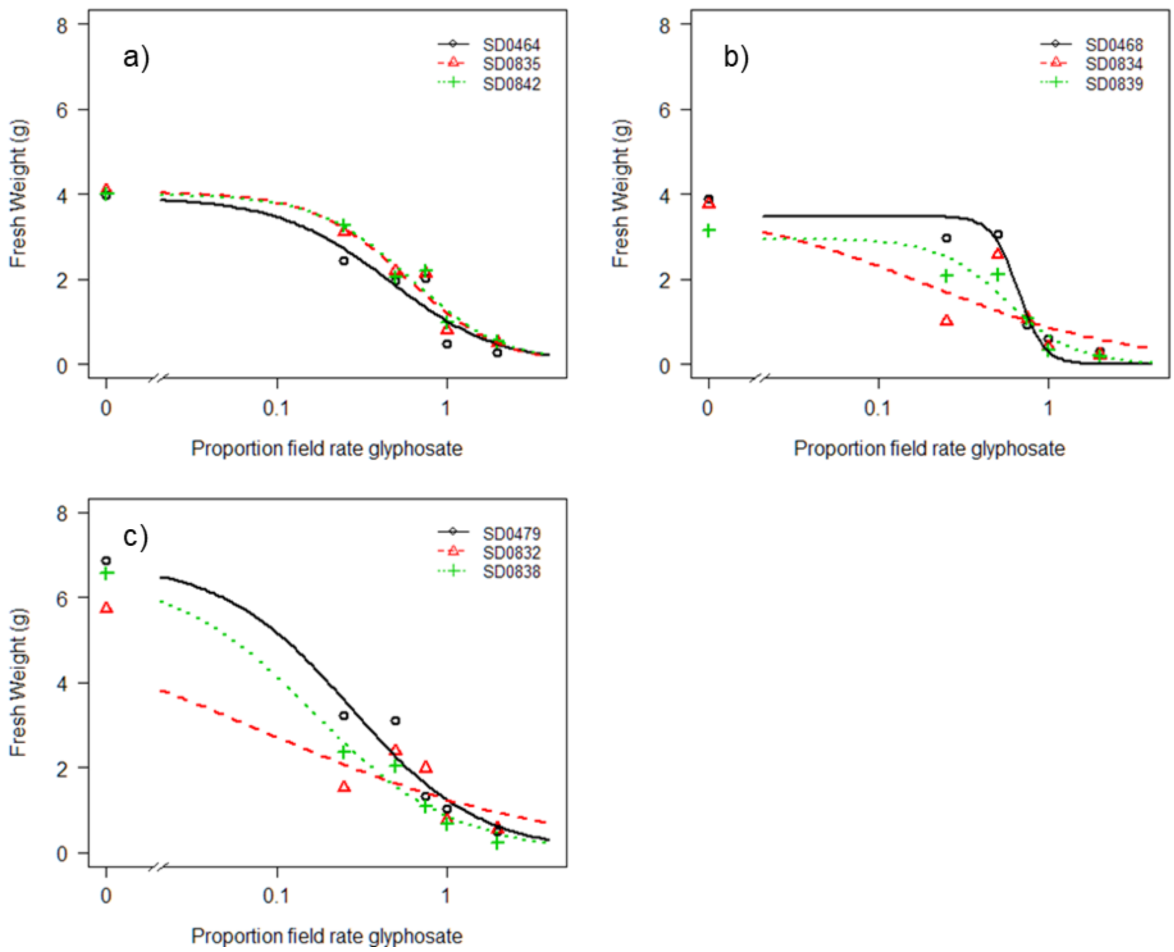


Figure 22 Sterile brome. Original population (black), untreated control line (red), selected line (green), (a) lines derived from SD464, (b) lines derived from SD468, (c) lines derived from SD479

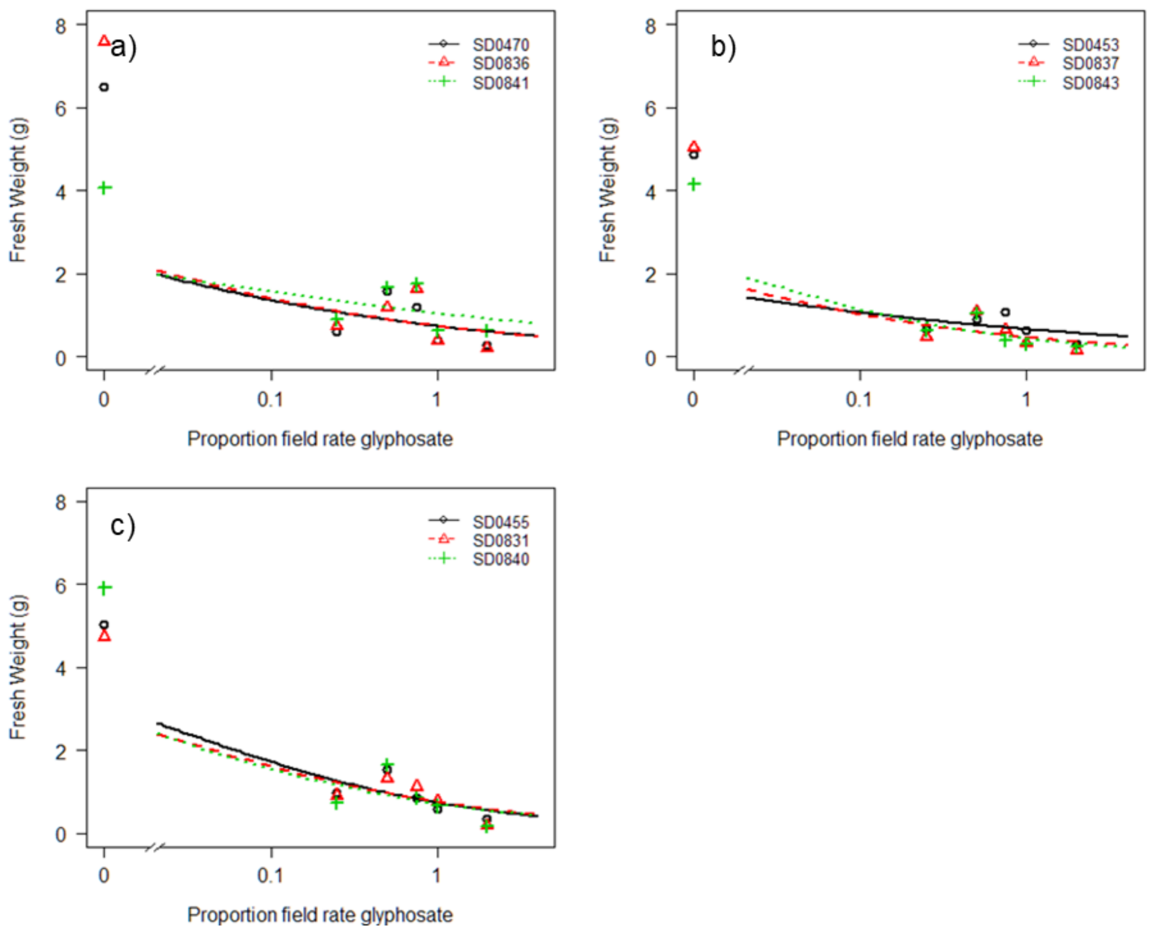


Figure 23 Rye brome. Original population (black), untreated control line (red), selected line (green), (a) lines derived from SD453, (b) lines derived from SD455

4.4. Adding value to the BGRI survey (WP4) Rothamsted

4.4.1. Field survey using the BGRI network (2016-2017)

Ninety six percent of the fields surveyed had at least one brome specie present, either in the margin, headland, or cropped area. Sterile or great brome was found in 76% of fields and 89% of the fields had rye, meadow or soft brome present somewhere in the field (Table 60).

Both groups of brome species were found most frequently in the margins of fields. 73% of fields had sterile or great brome in the margin and 87% for rye, meadow or soft brome, with densities for both groups varying from occasional plants to a few high densities (Table 60). The occurrence and density of both groups of brome species decreased as the assessments moved into cropped area (Figure 24). Only 34% of fields had sterile or great brome in the headland, predominately with only occasional plants and 61% for rye, meadow or soft brome with densities predominately very low or low. Also, most of the plants were within 1 – 2 metres of the margin (personal observation). The occurrence of both brome species decreased further moving into the main body of crop, with sterile or great brome being found in 8% of fields and 34% of fields for rye, meadow or soft brome. All densities of both brome groups were very low or low. No bromes of either group were found in the main body of the spring crops.

Table 60 Occurrence and density scores for both brome groups and field zones

	Sterile / great brome (*Anisantha* spp)			Rye / meadow/ soft brome (*Bromus* spp)		
Density	Margin	Headland	Centre	Margin	Headland	Centre
None	22	55	76	11	32	55
Very low	13	26	7	13	33	24
Low	29	2	0	36	15	4
Medium	14	0	0	20	3	0
High	5	0	0	3	0	0

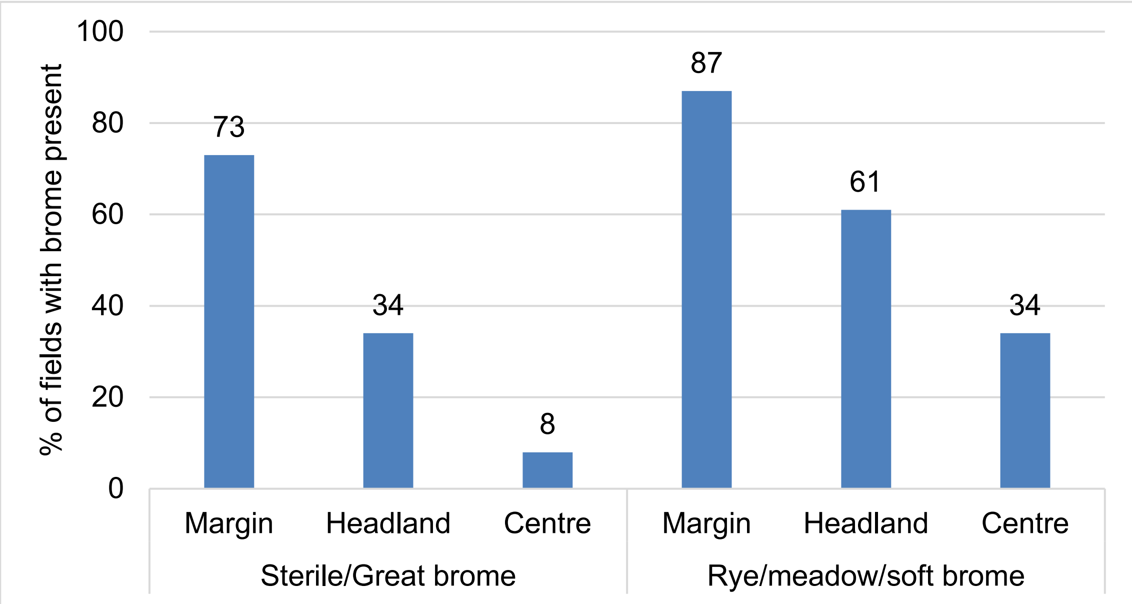


Figure 24 Percentage of brome occurrence by field area

In-crop survey using the BGRI farm network (summer 2018, 2019 and 2020)

The brome in-field surveys carried out on the BGRI network showed good consistency of results across the three years. Sterile or great bromes were only found in 2 out of 194 autumn sown crops surveyed, or 1% and 4% of fields surveyed in summer 2018 and 2020, respectively (Figure 25). No sterile or great brome were found in the spring cereals fields assessed. Rye, meadow or soft brome were present in a much higher number of fields, ranging from 18 – 23% of fields across the 3 years. A lower number of either of these three species was found in spring crops, ranging from 0 – 17% of fields.

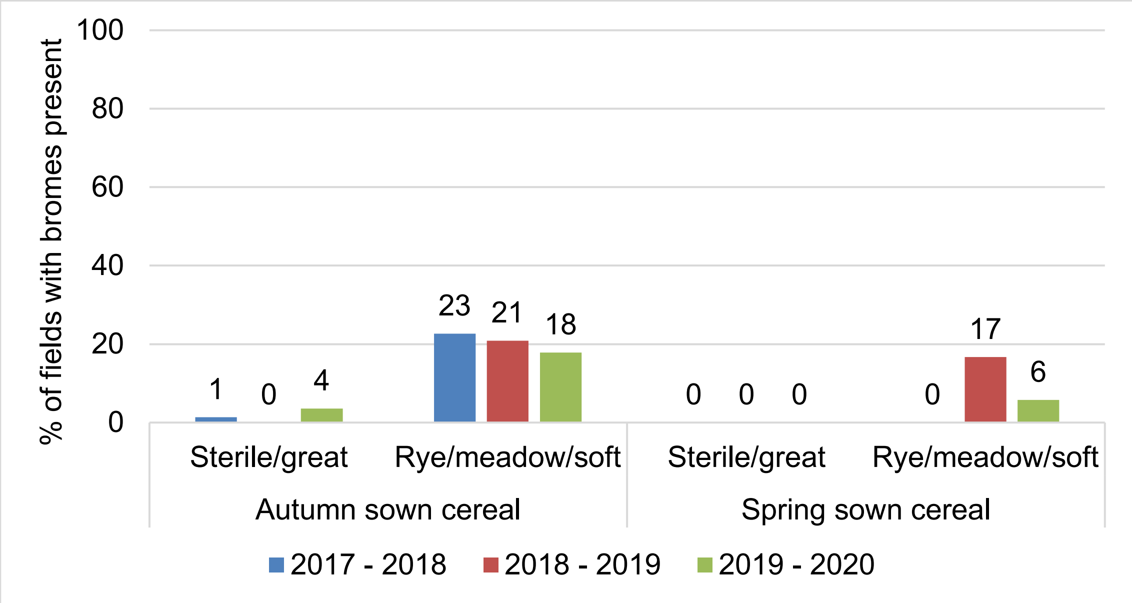


Figure 25 Percentage of fields surveyed with brome groups present for each surveying year

When averaged across all years (Figure 26), sterile or great brome was only found in 1% of autumn sown fields and none in spring cereals. Rye, meadow or soft brome was present in 21.1% of autumn cereals fields and 5.2% of spring sown fields.

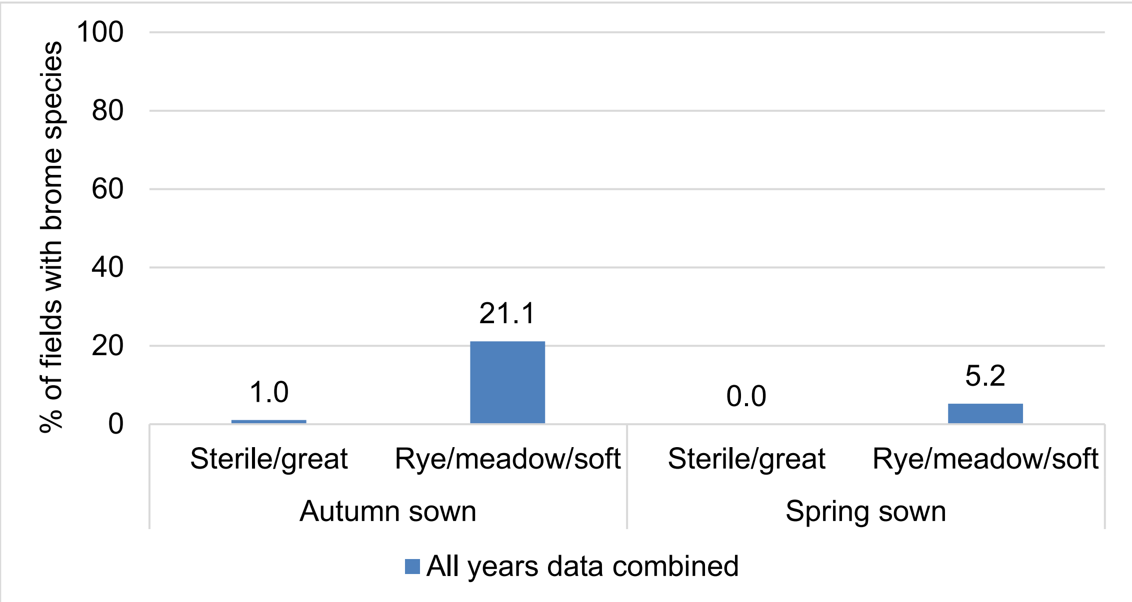


Figure 26 Percentage of fields with brome present average for all years

The brome plants found in fields occupied very small areas of the fields surveyed (Figure 27). Sterile or great brome only infested 0.1% of the fields surveyed in 2018 and 2020. Rye, meadow or soft brome had a higher level of infestation, ranging from 1.1% - 2.8% in autumn sown crops. The highest area infested in any field was 20% for autumn sown crops in 2019. The % area occupied with rye, meadow or soft brome was very low in spring crops, 0.1% and 0.4% in 2019 and 2020, respectively.

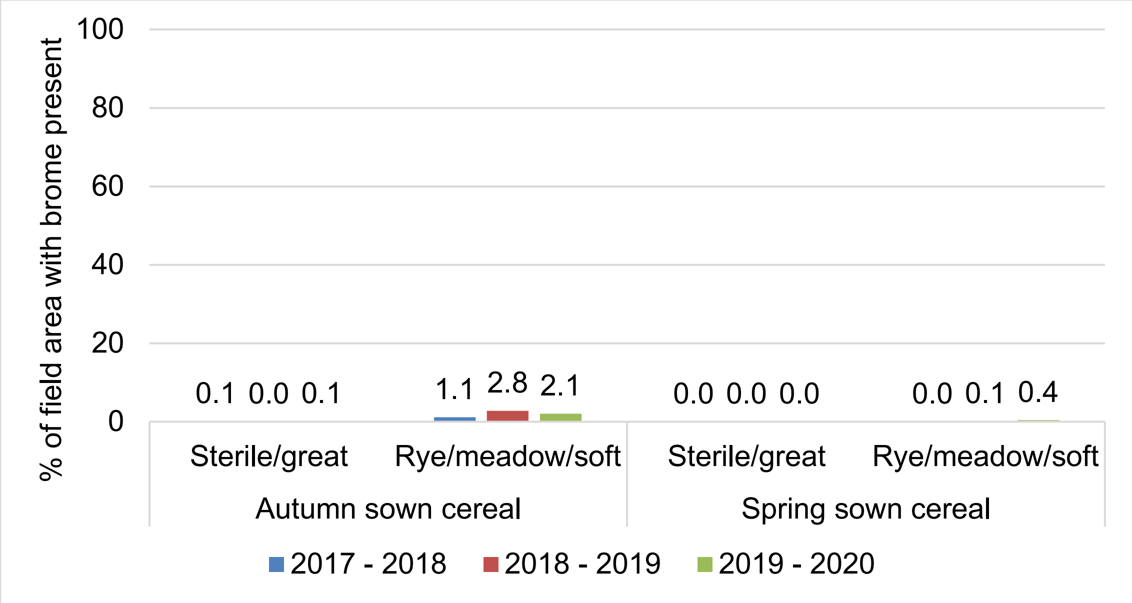


Figure 27 Percentage area of fields infested with brome species

Sterile or great brome was only found at very low densities in the survey years (Table 61). There was greater variation in the density of rye, meadow or soft bromes ranging from very low to high in autumn sown crops. Of the 41 fields that had rye, meadow or soft brome, 22 had low populations, nine fields either low or medium densities and one field with a high density. The four spring crops that had rye, meadow or soft brome were either at very low or low densities.

Table 61 Density of brome species over all surveying years

Year	Density group	Autumn sown cereal		Spring sown cereal	
		Sterile/great	rye/meadow/soft	Sterile/great	rye/meadow/soft
All years (194 autumn/ 77 spring)	VL	2	22	-	3
	L	-	9	-	1
	M	-	9	-	-
	H	-	1	-	-
	VH	-	-	-	-
Total no. of fields per density group		2	41	0	4

4.5. Determine the best herbicide application timing to increase brome control and reduce the risk of resistance evolution (WP5)

4.5.1. 2018-2019 (BW19-019)

There were significant (p<.001) differences between the glyphosate timings, the treatment at GS21-23 being the most effective (Table 62). There were also significant differences between the populations (p=0.032), with the population from Nottinghamshire shown to be tolerant to glyphosate (SD464) being more difficult to control particularly at the later growth stage (GS25+).

Table 62 The mean number of sterile and rye brome plants per container in 2018-19 – Glyphosate

Herbicide timing	Sterile brome			Rye brome		
Population	SD464	SD468	SD479	SD453	SD455	SD470
	GLY tolerant	sensitive	GLY tolerant	sensitive	GLY tolerant	sensitive
Untreated	14.8	15.0	14.8	13.3	15.0	11.3
GS12-13	9.5	4.3	1.5	3.8	0.3	5.8
GS21-23	1.8	4.0	0.5	4.0	0.8	0.8
GS25+	11.5	3.5	0.5	4.5	9.5	3.5
	population	herbicide	pop x herb			
Fprob	0.032	<0.001	NS			
SED	1.5	1.22	2.98			
LSD	3.0	2.43	5.95			
df	69					
CV%	14.2					

All brome populations were killed by the Laser treatment at all timings (Table 63).

Table 63 The mean number of sterile and rye brome plants per container in 2018-19 – Laser (Cycloxydim)

Herbicide timing	Sterile brome			Rye brome		
Population	SD464	SD468	SD479	SD453	SD455	SD470
	GLY tolerant	sensitive	GLY tolerant	sensitive	GLY tolerant	sensitive
Untreated	14.8	15.0	14.8	13.3	15.0	11.3
Gs12-13	0.0	0.0	0.0	0.0	0.0	0.0
GS21-23	0.0	0.0	0.0	0.0	0.0	0.0
GS25+	0.0	0.0	0.0	0.0	0.0	0.0
	population	herbicide	pop x herb			
Fprob	NS	<0.001	NS			
SED	0.568	0.46	1.14			
LSD	1.13	0.93	2.27			
df	69					
CV%	7.4					

When Broadway Star was applied to the populations, the sterile brome SD479 was significantly less well-controlled than the other populations, this population had been shown to have a greater tolerance to glyphosate in our tests. Overall control of the populations with Broadway Star was good with the greatest control at the earliest timings (Table 64).

Table 64 The mean number of sterile and rye brome plants per container in 2018-19 – Broadway Star (pyroxsulam + florasulam)

Herbicide timing	Sterile brome			Rye brome		
	GLY tolerant	sensitive	GLY tolerant	sensitive	GLY tolerant	sensitive
Population	SD464	SD468	SD479	SD453	SD455	SD470
Untreated	14.8	15.0	14.8	13.3	15.0	11.3
Gs12-13	0.0	0.0	2.3	0.0	0.0	0.0
GS21-23	0.3	0.0	4.8	0.0	0.0	0.0
GS25+	2.5	1.8	13.8	0.0	0.0	0.0
	population	herbicide	pop x herb			
Fprob	>0.001	>0.001	>0.001			
SED	0.76	0.62	1.52			
LSD	1.52	1.24	3.03			
df	69					
CV%	5.9					

4.5.2. 2019-2020 (BW20-012)

There were significant (p<.001) differences between the glyphosate timings, the treatment at GS21-23 being the most effective (Table 65). There were also significant differences between the populations (p<0.001), with the glyphosate tolerant sterile brome (SD741, (parent SD464)) and SD475 being more difficult to control.

Table 65 The mean number of sterile and rye brome plants per container in 2019-20 – Glyphosate

Herbicide timing	Sterile brome			Rye brome		
Population	SD741	SD742	SD743	SD476	SD474	SD475
Parent population	SD464	SD468	SD479	-	-	-
	GLY tolerant	sensitive	GLY tolerant	sensitive	sensitive	sensitive
Untreated	14.3	12.3	12.3	12.5	11.0	14.5
GS12-13	10.5	6.8	5.5	9.0	6.8	11.0
GS21-23	10.5	1.8	0.8	5.0	2.5	6.0
GS25+	5.8	5.8	7.0	5.3	6.3	8.0
	population	Herbicide	pop x herb			
Fprob	<0.001	<0.001	NS			
SED	1.1	0.9	2.2			
LSD	2.2	1.8	4.5			
df	69					
CV%	12.4					

Laser was the most effective of the three herbicides. Compared to the previous year there were lower levels of control from the GS25+ timing. There were no differences between the populations (Table 66).

Table 66 The mean number of sterile and rye brome plants per container in 2019-20 – Laser (cycloxydim)

Herbicide timing	Sterile brome			Rye brome		
Population	SD741	SD742	SD743	SD476	SD474	SD475
Parent population	SD464	SD468	SD479	-	-	-
	GLY tolerant	sensitive	GLY tolerant	sensitive	sensitive	sensitive
Untreated	14.3	12.3	12.3	12.5	11.0	14.5
GS12-13	1.0	1.3	1.3	1.0	0.5	0.0
GS21-23	0.0	0.3	0.0	0.8	0.8	0.0
GS25+	4.0	5.0	7.8	6.3	4.8	6.5
	population	Herbicide	pop x herb			
Fprob	NS	<0.001	NS			
SED	0.87	0.71	1.74			
LSD	1.73	1.41	3.46			
df	69					
CV%	21.3					

In year two, Broadway Star was less effective than year one. When Broadway Star was applied to the populations, the sterile brome SD743 was significantly less well-controlled than the other populations (Table 67). Population SD473 is from the bulked-up seed of SD479 which showed the same tolerance in the experiments in the previous year. Overall control of the populations with Broadway Star was good with the greatest control at the earliest timings.

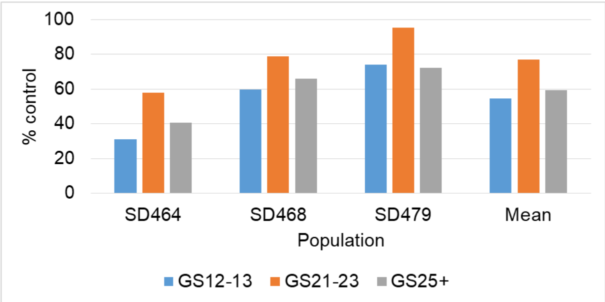
Table 67 The mean number of sterile and rye brome plants per container in 2019-20 – Broadway Star (pyroxsulam + florasulam)

Herbicide timing	Sterile brome			Rye brome		
Population	SD741	SD742	SD743	SD476	SD474	SD475
Parent population	SD464	SD468	SD479	-	-	-
	GLY tolerant	sensitive	GLY tolerant	sensitive	sensitive	sensitive
Untreated	14.3	12.3	12.3	12.5	11.0	14.5
GS12-13	4.8	3.0	10.8	0.0	2.3	4.8
GS21-23	5.0	1.0	13.0	0.0	0.0	0.0
GS25+	5.8	2.0	8.5	5.3	9.0	5.8
	Population	Herbicide	pop x herb			
Fprob	<0.001	<0.001	0.002			
SED	1.18	0.96	2.35			
LSD	2.35	1.92	4.69			
df	69					
CV%	12.5					

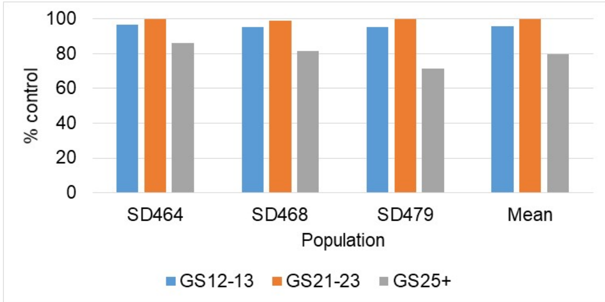
4.5.3. Summary

The data has been summarised over the two years of the trial.

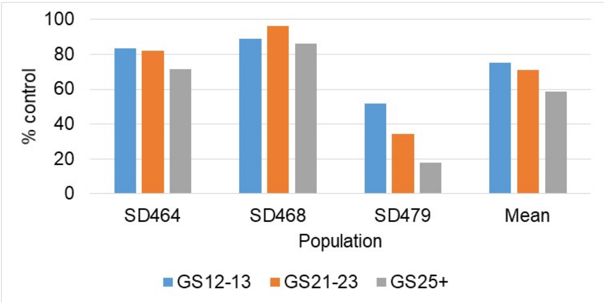
Sterile brome was generally more difficult to control than rye brome. Sterile brome control with glyphosate was greatest at GS21-23, with similar levels of control at the early and later stages even in the glyphosate tolerant populations SD464 and SD479 (Figure 28a). With the ACCase Laser, control was best at GS12-13 and GS21-23, with control falling off at the latest timing. Control from the ALS herbicide Broadway Star was more variable between the populations, with control lowest in SD479 (Figure 28b). The optimum timing was GS12-13 and GS23-23, with control falling away in late tillering applications (Figure 28c).



a) glyphosate



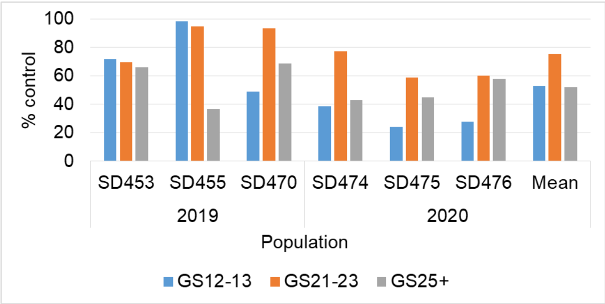
b) Laser



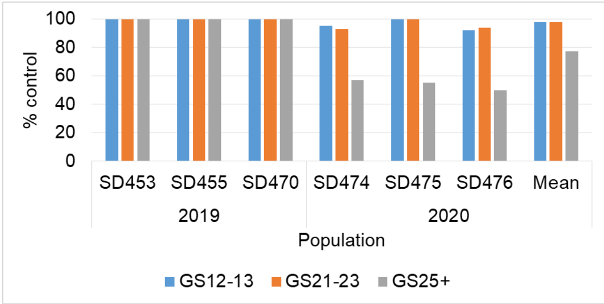
c) Broadway Star

Figure 28 A two-year (2019 and 2020) summary of percent control of sterile brome at three timings by glyphosate, Laser and Broadway Star

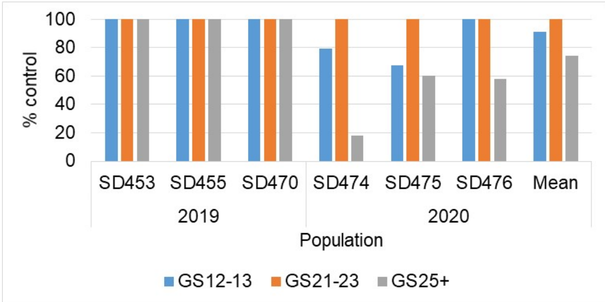
There were a wider range of populations in the rye brome experiment (Figure 29). The optimum timing was GS21-23 for glyphosate for all populations, even for the glyphosate tolerant population SD455. Overall control with Laser was very good with control falling off at the latest timing, GS25+ (Figure 29b). Control with Broadway Star varied between the years with the optimal timing being GS21-23 and levels of control declining rapidly at GS25 (Figure 29c).



a) glyphosate



b) Laser



c) Broadway Star

Figure 29 A two-year (2019 and 2020) summary of percent control of rye brome at three timings by glyphosate, Laser and Broadway Star.

5. Discussion

5.1. Surveys

The results from the two contrasting surveying strategies (online versus BGRI farm network) highlights some important issues should this be repeated in future or for other species. The online survey of farmers was good at gaining the distribution nationally of a weed species, especially if the weed species is easily identifiable. The addition of agronomic data increases our ability to try and link the distribution with farming practices. However, an online survey may only get replies from farmers that are having problems with that weed. The survey also highlighted a knowledge gap about correct identification of the five brome species. This is an ongoing issue although both a detailed four-page identification leaflet and a two-page summary are readily available online (Moss, 2015, 2017). The in-field survey using the BGRI farm network has the benefit of only one trained person carrying out all the surveying, which reduced assessor bias. However, this limits the geographical range that can be covered and is, therefore, smaller compared to online. Also, these farms are on the network because they have issues with black-grass control, which may reduce abundance levels of brome due to the many control measures in place to reduce black-grass.

The online survey results show that brome weeds are wide-spread across all cereal growing areas of the UK, were not linked to soil type and are becoming increasingly problematic and hard to control, but black-grass is still the most problematic weed in UK arable farming. *Anisantha* spp (sterile and great brome) were reported as the most problematic bromes, but *Bromus* spp (rye and meadow brome) were also reported to be problematic by a large proportion of respondents. Based on brome seed sample identification, sterile brome is still the most wide-spread species in the UK. It is likely that rye brome is more wide-spread than previously thought, particularly compared to the 1989 random survey, where no rye brome populations were identified across 733 fields with sterile brome accounting for 87% of records. The absence of records of rye brome in 1989 may reflect problems with identification, although this seems unlikely to be a complete explanation. Even if this is a partial explanation, there is still no clear reason for the much higher incidence of rye brome recorded in these recent surveys compared with 1989. The densities of brome populations were also much higher in the online survey compared to the 1989 random survey, with 16% of fields having severe infestations in the headlands and 11% in the centre in 2017 compared to 3% and 1% in 1989 (Cussans *et al*., 1994) (Figure 30).

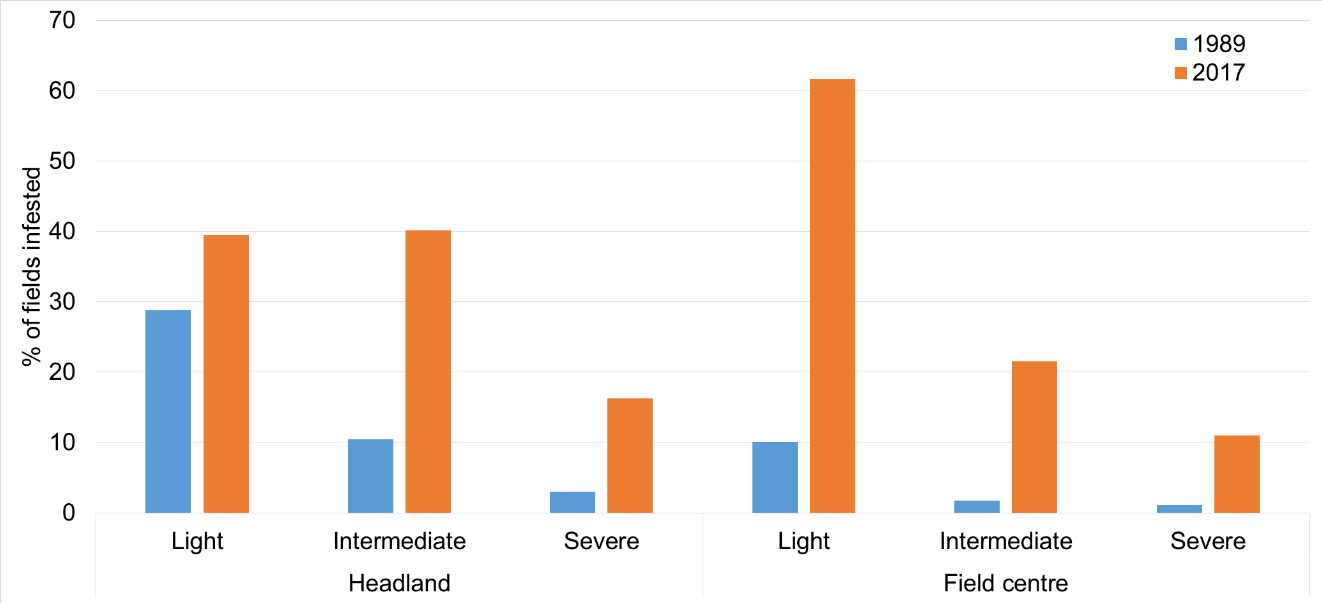


Figure 30 Position and level of infestation of brome in the field as reported in the 1989 and 2017 surveys.

Results from the survey of fields in the BGRI network farms in 2017 also found that both sterile or great brome and rye, meadow and soft brome species were wide-spread, and that all brome species were more abundant than reported in the 1989 random survey (Figure 31). Bromes of both groups were much more prevalent in field margins than in cropped areas of the field, where they form part of the natural flora, in the BGRI survey compared to the online survey, with much lower in-crop densities. This is likely due to the focus on the presence of black-grass in BGRI fields, however, these data provide a random field sample showing that although brome weeds are spread throughout all cereal growing regions in the UK, the problems they pose varies between farms even within region and that they are more likely to be a localised on-farm issue.

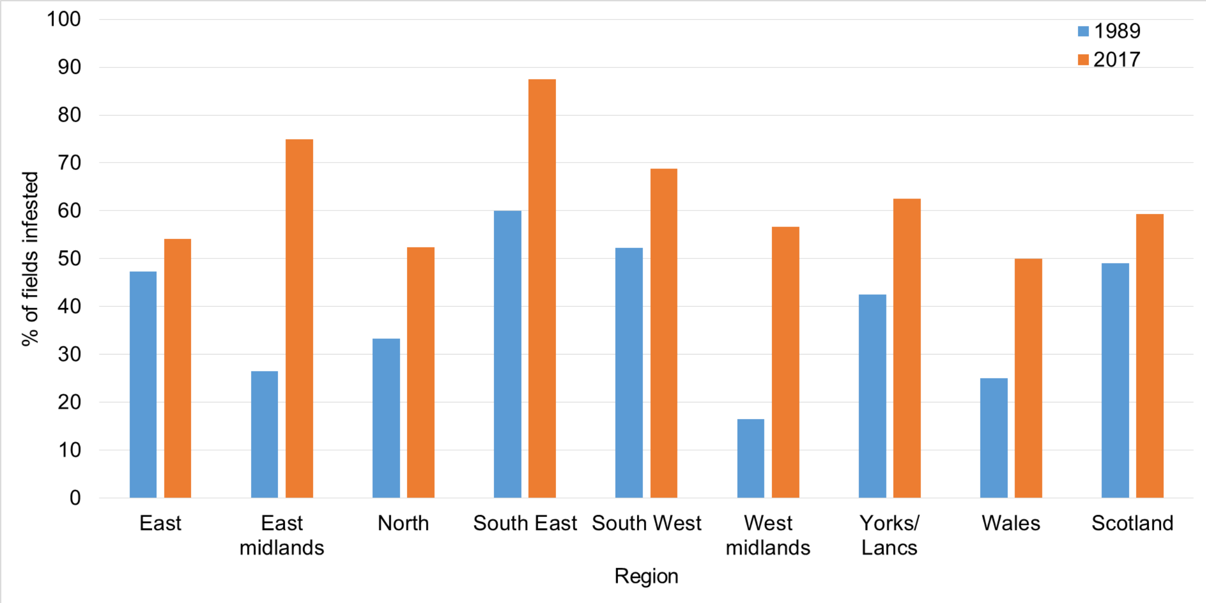


Figure 31 Abundance of all brome species by region in 1989 and 2017

Brome weed problems were perceived to be increasing by the majority of online survey respondents. Although the respondents in the online survey were self-selecting and were, therefore, likely to be biased towards the presence of bromes and problematic populations, the perceived increases in the presence of bromes in UK arable farming is consistent with recent literature that has also reported increases in both *Anisantha* and *Bromus* spp. (Smart *et al*., 2005; Davy, 2006; Cook & Ginsburg, 2012). This is a continuation of the increasing brome weed problem that was noted in the 1989 survey compared to surveys in 1981 and 1982 (Cussans *et al*., 1994). The most reported reason for an increase in the presence of brome species was a move towards minimum tillage and direct drilling, which has previously been shown lead to an increase in sterile brome and great brome infestations, resulting in the need for separate control programmes, increased herbicide use, increased costs, and potentially the abandonment of direct drilling as happened in the 1980s (Froud-Williams *et al*., 1980; Clarke *et al*., 2000; Escorial *et al*., 2011, Orson *et al*., 1998).

Worryingly, 58 survey respondents reported the poor herbicide control was suspected to be due to herbicide resistance. The term herbicide resistance is often used by the farming industry when a weed is left uncontrolled, but scientists have not confirmed the presence of herbicide resistant bromes in the UK and it is possible that the perceived herbicide resistance problems are in fact a result of herbicide selection and application issues highlighted by other respondents, but populations have been identified that are evolving resistance to glyphosate (Davies *et al*., 2019). Resistant populations have been reported in *Anisantha* spp. to ACCase inhibitors in Germany, ALS inhibitors in France, and glyphosate in Australia, and in *Bromus* spp. to ACCase inhibitors and ALS inhibitors in the USA (Heap, 2021) and this project has shown that increasing tolerance and resistance to ALS herbicides is present in five sterile bromes, one great brome, three meadow brome and six rye brome populations.

Historically, the source of in-crop brome weeds was from the field margins, with spread into the field from cultivations and combines (Rew *et al.*, 1996), and this was still the most reported source of brome weeds in-crop, followed by contaminated seed and machinery. It is, therefore, likely that the reported increase in brome weed problems is due to changes in agricultural practices in-field, such as a move towards minimum tillage and poor margin management, but that an increase in the spread of bromes between fields and farms could also be a source.

The brome survey data from the BGRI farm network during the summers of 2018, 2019 and 2020 backs up the in-crop findings from the 2017 survey. The number of fields with either sterile or great brome in-crop was very low (1% of fields) and only found at very low densities, with the area of the field infested very small. This highlights that the farmers on the BGRI network are controlling these two species very well, predominately with the control the measures that are being employed to tackle black-grass. Currently, there was no evidence that these two brome species are worse on no-till farms than land that is regularly cultivated, however, Turley *et al*. (2003) reviewed the results of straw incorporation for 11 years at six ADAS sites, three on clays and three on lighter soils. The first four years results at these sites are included in the report by Davies (1988). The test crops were continuous wheat until break crops were introduced to control brome at three of the sites. On the clays, tine/disc incorporation gave 5- 8% less yield compared with ploughing and on the lighter soils the 3-18% less. Much of this yield depression was attributed to competition from sterile and meadow brome. There has been a recent move towards a reduction in tillage and this has coincided with the introduction of effective herbicides for brome control. This project has identified herbicide resistance to these herbicides and further long-term monitoring will be needed to understand the long-term effects on all brome species under no-till regimes. A high proportion of the BGRI farmers still regularly plough, either annually or rotationally, due to most of the farms being on heavy land. This is still a very effective tactic for sterile, great or meadow brome control due to a very short seed persistence in the soil and the tendency for sterile brome to undergo synchronous suicidal germination at depths below which emergence is possible (Froud-Williams, 1983).

Rye, meadow or soft brome was much more prevalent on the BGRI network in 2018, 2019 and 2020 than sterile or great bromes, occurring in 21% of the fields. However, the densities of these patches were predominately either low or very low and infested a very small area of the fields (about 2%). The higher occurrence of this group of brome species in-crop is probably due to numerous factors. Rye, meadow and soft brome are perceived to have a longer, more protracted germination period than sterile or great brome. With the ever-increasing reliance of pre-emergence residual herbicides for control of black-grass, the longer germination period may mean more plants of rye, meadow or soft brome emerge when the control from of any effective residual actives is starting decrease. The survey data indicates that brome species of both groups are very rarely found in spring sown cereals, meaning most of the germination is in the autumn. Greater knowledge of the agroecology of all brome species, and especially the variability between populations, is needed to determine which set of non-chemical control practices are the best in combating high populations of bromes. Spring cropping, like for black-grass, seems to be a very effective strategy to reduce brome populations. There has been a decrease in the use of spring foliar acting herbicides for black-grass control with the increase of herbicide resistance. This application also played an important role in controlling other grass-weeds like bromes, wild-oats and rye-grass that may not have been the primary targets and gave good control of these grassweeds species that were either not controlled by the autumn programme or emerged later. If the germination period of rye, meadow and soft is more protracted in the autumn, and with some farmers finished with their herbicide programme for black-grass three to four weeks after drilling, rye, meadow and soft brome may become more abundant. Long-term monitoring, such as of the BGRI farm network fields, could help determine which control measures are keeping brome species in check, and what factors might lead to an increase in population abundance and density.

Anisantha and *Bromus* spp., especially rye brome, may have become more wide-spread in UK arable farming since the last UK-wide survey was conducted in 1989. This increase may be the result of a number of factors including a move towards minimum tillage, possible herbicide resistance, and poor field margin management.

5.2. Susceptibility of brome species to herbicides

Through the project, 168 brome populations were tested for their susceptibility to the herbicides Broadway Star (pyroxsulam + florasulam), Pacifica Plus (mesosulfuron-methyl + iodosulfuron-methyl-sodium + amidosulfuron), Laser (cycloxydim), Falcon (propaquizafop) and MON79379 (glyphosate). Lower sensitivity to glyphosate was found in one population of sterile brome and one population of rye brome (Table 68). Resistance and lower sensitivity were found to ALS herbicides in five sterile, one great, three meadow and six rye bromes (Table 69). It is interesting to note that the majority of the brome populations resistant or less sensitive to ALS herbicides were located in area with lower levels of black-grass.

Table 68 Summary of populations less sensitive to glyphosate

Population	Species	County	Status
SD479	Sterile brome	Oxon	Lower sensitivity at field rates
SD455	Rye brome	Surrey	Lower sensitivity at field rates

Table 69 Summary of populations resistant and less sensitive to ALS herbicides

Population	Species	County	Status
SD441	Great brome	Shrops	Resistant
SD454	Sterile brome	Lincs	Resistant
SD478		Wilts	Less sensitive
SD488		Worcs	Resistant
SD623		Notts	Resistant
SD753		Berwick	Resistant
SD466	Meadow brome	Yorks	Resistant
20C11		Yorks	Less sensitive
SD757		Yorks	Resistant
SD506	Rye brome	Oxon	Less sensitive
SD622		Shrops	Resistant
SD747		Shrops	Resistant
SD748		Beds	Resistant
SD750		Shrops	Less sensitive
SD756		Beds	Resistant

5.2.1. Glyphosate resistance

Within the project, one sterile brome population and one rye brome were found to be in the process of evolving glyphosate resistance after showing reduced sensitivity to the herbicide in a dose response assay. The populations were incompletely controlled at UK recommended field rates of glyphosate for annual grass weed control (540g a.s./ha) and were significantly less sensitive to glyphosate than 18 other sterile brome and 17 rye brome populations including known sensitive populations.

Although the populations showed reduced sensitivity to glyphosate, ED_{50} values (the estimated dose at which 50% of the population will be controlled) were 218g a.s./ha for the sterile brome and 191g a.s./ha for the rye brome, this compares to values of 420-810g a.s./ha reported by (Davies *et al*., 2019) but the population identified in that work was included in this dose response (SD464) and had an ED_{50} of 260g a.s./ha. The results show that that the populations are not currently resistant to glyphosate but are adapting to glyphosate selection.

The detection of these populations highlights the need for glyphosate stewardship to help prevent resistance evolution. Both populations were from fields with history of being difficult to control. The current move towards minimum tillage and direct drilling is a high-risk strategy for glyphosate resistance evolution, indicating the need to combine both cultural and chemical weed control to help prevent resistance. The survey has shown that all brome species have increased over the past few decades and are more widespread than previously thought. The major reason for this increase was minimum or no till, this type of farming depends primarily on the use of glyphosate prior to drilling to kill off any weeds present, such as brome. The increased repeated use of glyphosate, particularly in less than perfect spraying conditions prior to drilling kills off or thins margin flora leaving bare patches which again, favours brome establishment, seed is subsequently moved into the field by combines and cultivation. The change in agricultural support towards the environment will increase the number of field margins and will lead to a further increase in brome, particularly where margin seed mixtures have failed to establish well. The Weed Resistance Action Group (WRAG) https://ahdb.org.uk/wrag released guidelines with four key messages to help minimise the risk of glyphosate resistance in grassweeds and can be found here.

5.2.2. ALS resistance

ALS resistance was the most common resistance found in the UK brome populations. ALS herbicides control grass weeds through an inhibition of the action of acetolactate synthase, preventing the production of branched-chain amino acids valine, leucine, and isoleucine (Powles & Yu, 2010). ALS inhibiting sulfonylurea herbicides are one of the most widely used herbicide groups in UK farming, with use increasing since 2003 following the introduction of the formulated mixture of mesosulfuron + iodosulfuron for control of black-grass and other grass weed species. (Hull *et al*., 2014). Subsequently, more than 500,000 hectares of cereal crops were treated with this herbicide in 2018 with application peaking at more than 1.1 million hectares in 2010 (Garthwaite *et al*., 2019) (Figure 32). The area treated with mesosulfuron continues to decline primarily due to the high levels of resistance to this herbicide in black-grass. The use of pyroxsulam has increased since its introduction in 2010, primarily due to its effectiveness against brome and ryegrass species.

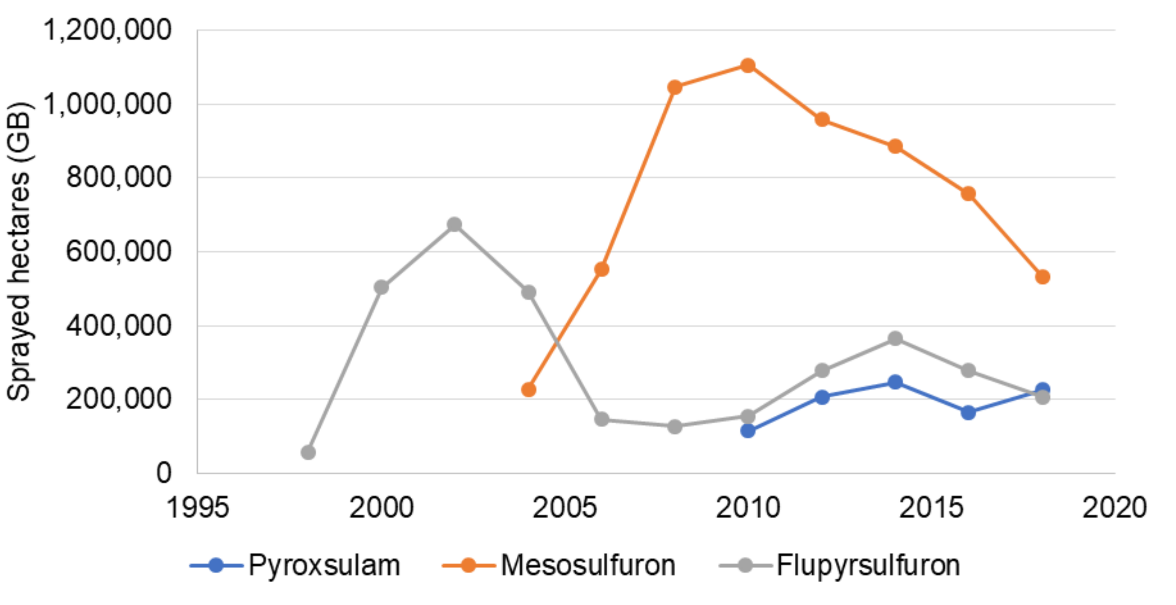


Figure 32 Area treated in Great Britain with ALS herbicides

It is no surprise that resistance and lower sensitivity were found to ALS herbicides in one great, five sterile, three meadow and six rye brome populations (Table 69). The results from the initial confirmatory dose response experiments (3.2.2) were published in 2020 (Davies *et al.*, 2020)

The results from the project confirmed clear evidence of resistance as compared to lower sensitivity to ALS inhibiting herbicides in one population of great brome (SD441), four populations of sterile brome (SD454, SD488, SD623, SD753), two population of meadow brome (SD466, SD757) and four populations of rye brome (SD622, SD747, SD748, SD756). These were the first cases of herbicide resistant brome species in the UK and the first case of herbicide resistance in meadow brome world-wide (Heap, 2021). Resistance in the meadow brome population was conferred by TSR (target site resistance) and EMR (enhanced metabolism resistance) mechanisms. The additional work to test for EMR was done outside the project by Newcastle University and the methodology is detailed in Davies *et al.*, (2020). No TSR mutations were found in the great and sterile brome populations, with the likely mechanism of resistance EMR.

TSR mutations to Pro197 were found in the majority of rye brome plants tested of the SD506 population, despite the percentage reduction in fresh weight not being statistically significant to the susceptible standard, although there was a high level of survivors. The presence of the Pro197 mutation is interesting and requires further investigation.

The final dose response identified a further sterile brome population (SD753), a meadow brome (SD757) and three rye brome populations (SD747, SD748 and SD756) as resistant to pyroxsulam. This finding needs to be supported by ALS point mutation analysis and published.

All brome populations tested were well-controlled by ACCase inhibiting herbicides and relatively well-controlled by glyphosate, enabling growers to still use other herbicide modes of action to control ALS resistant populations in a diverse cropping system. The detection of ALS herbicide resistant UK brome populations and the presence of ACCase and glyphosate resistant brome populations outside the UK, as well as populations in the UK with reduced glyphosate sensitivity (Davies *et al*., 2019), will alert UK growers of the risk of herbicide resistance evolution in UK bromes. Lessons need to be learnt from the widespread herbicide resistance in other UK grass weeds and growers need to take action to prevent widespread herbicide resistance evolution in bromes.

Evidence suggests that preventing resistance development is typically only half of the cost of controlling resistance once it has appeared (Orson, 1999). Therefore, brome populations need to be monitored for early signs of resistance evolution, and the use of non-chemical control methods and integrated weed management needs to increase for all populations to prevent further resistance evolution and control populations that are already resistant (Beckie *et al*., 2019). Knowledge of the mechanisms that are more likely to lead to resistance evolution could help reduce the costs of prevention but currently there is no evidence for this. Development of new herbicides with new modes of action may help prevent the evolution of these resistance mechanisms.

5.3. Selection experiments

There was some decrease in sensitivity to pyroxsulam in two sterile brome populations and one population to glyphosate compared to the baseline populations, but no change in sensitivity in rye brome populations. This decrease in sensitivity is significant for the populations that were originally less ALS sensitive and more ALS sensitive, suggesting that for these populations at least, the starting sensitivity to ALS was not a factor in how much the populations changed over selection generations. This could be due to the differences in original ALS sensitivity being small.

The shift in increasing resistance in the two populations to pyroxsulam and one to glyphosate was significant but small compared to low dose herbicide selection experiments done on outcrossing species (e.g. Davies & Neve 2017, Busi *et al*., 2012; Busi *et al.,* 2013; Lynch, 2014). However, the shift is similar to that of low dose selection with diclofop-methyl of wild oats (*Avena fatua*), which are also a self-crossing species (Busi *et al*., 2016). Additionally, similar to the lack of shift in rye brome species in this study, selection studies over five years in the UK with another self-pollinating species, wild oats (*Avena ludoviciana*) also found no evidence of any shift towards increasing resistance (Moss *et al*., 2001). In that study, the population used (T/11) was shown subsequently to have resistance conferred by enhanced metabolism and both ACCase (fenoxaprop and tralkoxydim) and ALS (imazamethabenz) inhibiting herbicides were used. Surprisingly, five annual applications of herbicides to which partial resistance already existed in the T/11 population, did not result in any increase in level of resistance. However, these studies also showed that resistance did not decline when herbicide use was reduced and that the presence of resistance increased the variability in herbicide activity between years.

Rye brome is a predominantly self-crossing species (Smith, 1944) so there would be less chance for there to be a build-up of alleles related to EMR and polygenic resistance, as suggested by Neve *et al.,* (2014). Sterile brome is mostly a self-crossing species, but outcrossing does occasionally occur (Green *et al*. 2001) and gene flow between plants can increase the frequency of alleles related to EMR traits (Busi *et al*., 2011).

As bromes are generally self-crossing species, low dose herbicide selection for resistance will be less likely to lead to a build-up of EMR polygenic resistance, as there is much less to no gene flow among a population. However, EMR has evolved in brome species (Davies *et al.*, 2020). This raises the question of how, if it not through gene flow resulting in an increase in the frequency of alleles related to resistance? There is the potential for small shifts in herbicide sensitivity in brome species, and it is possible that with a large enough population the limited genetic exchange between individuals could be counter balanced, leading to EMR (Busi *et al*., 2015). Therefore, there is a need to control population size to help prevent resistance. This technique is used for control of herbicide resistant black-grass and with this weed, greater use of cultural control measures allows grass weed populations to be reduced by non-chemical means, resulting in less dependence on herbicides and less selection pressure for herbicide resistance (Moss *et al*., 2007).

The implication for industry is that low dose appears to have less of an impact in the development of resistance in bromes, but this report has shown that there is potential for shifts using low dose herbicides and as resistance has now been demonstrated, low dose implications still need to be considered. It may be that population size and plant size at the time of treatment may be more influential in resistance evolution compared to low dose selection – as shown by the plant size experiments.

5.4. Optimum herbicide timing

It is important to control brome; compared to black-grass, the effects on crop yield are greater. Infestations of sterile brome at densities of 5 plants/m² can cause a 5% yield loss (Marshall *et al*., 2003). At higher densities of 120 plants/m², wheat yields can be reduced by 35-47% and barley yields by 8-14% (Peters et *al*., 1993). In comparison, black-grass causes a yield loss of 15-25% at 100 plants/m² in winter wheat (Blair *et al*., 1999). High levels of brome infestations can increase costs to growers by impacting on grain quality, contaminating grain, and causing lodging (Peters *et al*., 1993).

For autumn drilled crops, control of brome begins soon after harvest of the previous crop. The weed seed is unattractive to carabid beetles (Marshall *et al*., 2007; Tooley *et al*.,1999) and all species have a low level of innate dormancy that is unaffected by the weather during ripening (Cook & Ginsburg, 2012; Peters *et al*., 2000), although dormancy has been shown to vary between populations. Germination and emergence of sterile brome is inhibited by light, dry conditions and high temperatures (>23°C) (Froud-Williams, 1981). With sterile and great brome cultivating shallowly to place seeds into moisture and darkness is the best strategy although germination does occur beneath high levels of straw. Meadow and rye brome germinate later in the autumn and their seed should be left on the soil surface to mature for a month as early burial will encourage the development of longer-term dormancy (Orson et al., 1998). Their control is more problematical as emergence is more likely to occur after the crop has been drilled.

Because dormancy of brome seeds is relatively low and the seeds are relatively large, many seedlings can emerge from seeds in the surface 10cm of soil. Thus, minimum tillage and early sowing tend to favour brome resulting in many seedlings emerging both before sowing as well as in the crop. This makes glyphosate an important herbicide for the control of brome pre-sowing, both before autumn-sown and spring-sown crops.

Sterile brome was generally more difficult to control than rye brome with glyphosate, both bromes have hairy leaves, and we have no information on the level of hairiness or whether contact could be improved by the use of adjuvants. This could form an area for further research.

The optimum growth stage for control of sterile brome with glyphosate as GS21-23, the early tillering stage. This was consistent over the two years and for both the glyphosate tolerant and sensitive populations. Control was lower with the glyphosate tolerant population SD464 indicating that this trait makes it more difficult to control this weed.

Control with Laser (cycloxydim) was best at GS12-13 or at GS21-23, this herbicide was much more effective than glyphosate or Broadway Star. Falcon (propaquizafop) was also very effective at controlling sterile and rye brome, including those populations which showed resistance to ALS herbicides. This is good news, as the inclusion of a break crop in the rotation should allow good control of this weed through a wide window and makes them very valuable due to the lack of ACCase resistance. It is important to maintain the full rate as the resistance screening experiments showed high variation in the levels of control at half rate for cycloxydim.

Control from Broadway Star was best at GS12-13 and GS23-23, with control falling away in late tillering applications. Control varied between populations and will be less so where tolerance or resistance is present.

With rye brome, the response to herbicides was similar to sterile brome. Control was more variable with glyphosate, and this was exacerbated by tolerant populations. Laser (cycloxydim) was very effective at the full rate, but the screening experiments showed very poor control at half rate (54% control).

Broadway Star was very effective on rye brome, although there was variation between the years; the optimum timing was GS21-23.

A summary of the most effective timings with factors to consider is in Table 70.

Table 70 Summary table of the optimum timing for herbicide use in sterile and rye brome.

Herbicides tested	Brome species tested	GS 12–13	GS 21–23	GS 25+	Factors to consider
MON79379 (glyphosate) HRAC Group 9* (EPSP synthase)	Sterile		+++		One population detected with reduced sensitivity to glyphosate.
	Rye		+++		One population detected with reduced sensitivity to glyphosate.
Laser (cycloxydim) HRAC Group 1* (ACCase)	Sterile	++	+++	+	No tolerance to the ACCase herbicide tested in this study.
	Rye	+++	+++	+	
Broadway Star (pyroxsulam + florasulam) HRAC Group 2* (ALS)	Sterile	+++	+++	++	Some populations affected by increased tolerance to the ALS herbicides tested in this study.
	Rye	++	+++	+	

*Herbicide groups based on the Herbicide Resistance Action Committee (HRAC) Mode of Action Classification Map (2021). Group 1 = Inhibition of Acetyl CoA Carboxylase (ACCase). Group 2 = Inhibition of Acetolactate Synthase (ALS). Group 9 = Inhibition of Enolpyruvyl Shikimate Phosphate Synthase (EPSP synthase)

The lower levels of control from glyphosate and presence of tolerant populations suggests that the ACCase herbicide cycloxydim is more suitable for control of bromes on green cover on land not being used for crop production. This has the added advantage of being selective for a range of grass species and less likely to leave bare patches to be colonised by brome, but it is unlikely to kill resistant black-grass or broad-leaved weeds

6. Conclusions

- Online surveys provided an up-to-date snapshot of the prevalence of UK weed species.
- Correct identification of brome species is crucial for their optimum control.
- Accurate identification from many survey respondents was poor, although good online diagnostic information is available (Moss, 2015 & 2017).
- Brome species are slightly more abundant than earlier surveys, especially rye brome which has increased considerably in the last 30 years for reasons that remain unclear.
- Brome species are not limited to the margins and headlands but found throughout the field.
- All five main brome species occur in all regions of the UK.
- Brome levels are likely to get worse, particularly in low/no-till situations and with an increase in field margins/environmental areas.
- Individual brome populations varied considerably in their response to herbicides, although true resistance is still rare.
- Effective control requires effective products and needs good application conditions – e.g. size of weed, full rates and environmental conditions.
- There was no evidence of widespread or severe resistance to glyphosate in any brome species. However, marginal resistance was detected in one population of sterile brome and one population of rye brome.
- Brome populations showing resistance to ALS herbicides were identified for the first time in the UK, although currently the incidence of resistance is low in all brome species.
- Resistance to ALS herbicides was identified in in one population of great brome (SD441 (Shrops), four populations of sterile brome – SD454 (Lincs), SD488 (Worcs), SD623 (Notts) SD753 (Berwick), two populations of meadow brome – SD466 (Yorks) and SD757 (Yorks) and four populations of rye brome – SD622 (Shrops), SD747(Shrops), SD748 (Beds), SD756 (Beds). Additional populations of sterile, meadow and rye brome were found to be less sensitive or were identified as difficult to control and await further testing.
- Both the Trp574 point mutation and EMR were linked to high ALS resistance in one population of meadow brome (SD0466) from Yorkshire. The Pro197 mutation was detected in one rye brome (SD0506) population from Oxfordshire although, surprisingly, this conferred only partial resistance at the whole plant level. These were the only two resistant populations in which ALS target site resistance was detected, although there are other populations that could be tested.
- In the majority of ALS resistant populations, the primary mechanism of resistance appeared to be enhanced metabolism, not ALS target site, resistance. However, the mechanisms of

resistance were only investigated in a relatively small number of brome populations, so this conclusion should be considered tentative.

- There was no clear evidence of resistance to either of the two ACCase herbicides tested (propaquizafop, cycloxydim), even in populations showing resistance to ALS herbicides. This finding highlights the potential opportunities to get good control of brome in non-cereal crops within the rotation.
- The use of ineffective herbicides, or use of doses that allow survivors, risk driving resistance development to some herbicides. However, the link is not as marked as in some other species, most probably due to the self-pollinating nature of bromes.
- Reduction in population size and ensuring effective herbicide application and timing are important control measures. The use of adjuvants and possibly water conditioners to improve herbicide efficacy is an area for further work.
- Integrated control, with a reliance on cultural measures, is important. There is a need for better information on understanding the biology of brome species and the effectiveness of cultural control measures. In particular, the variation between farms in the seedling emergence patterns of brome species is poorly documented. This has a direct bearing on the effectiveness of delayed autumn drilling and spring cropping, two of the most widely used methods for controlling grass weeds.
- Many farmers thought they had resistance, but there was no real evidence that it is widespread. There is a need to better communicate what resistance development is, highlight the importance of knowing species susceptibility and maximising integrated measures for effective control/management. It is important that farmers have ready access to resistance testing facilities so that any suspect populations can be investigated without delay.

7. Acknowledgements

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9. Appendices

9.1. Appendix 1 Survey questionnaire

Q1. Where is the farm based? (Post code or nearest town/ village with county)

Q2. What is your business type?

Q3. What is the holding type?

Q4. What is the size of the holding? (In ha)

- Q4.1. Arable
- Q4.2. Grassland
- Q4.3. Woodland
- Q4.4. Other

Q5. What is the predominant soil type?

Q6. What grass weeds are present in the arable area of the holding? (Tick all that apply) Please refer to brome ID leaflet to distinguish brome species present https://croprotect.com/weeds/which-brome-is-that

- Q6.1. Black-grass
- Q6.2. Sterile brome
- Q6.3. Great brome
- Q6.4. Meadow brome
- Q6.5. Rye brome
- Q6.6. Soft brome
- Q6.7. Brome - Unknown
- Q6.8. Italian ryegrass
- Q6.9. Perennial ryegrass
- Q6.10. Wild oats
- Q6.11. Rat's tail fescue
- Q6.12. Annual meadow grass
- Q6.13. Other (please specify):

Q7. Of these, what is the MAIN grass weed present in the arable area of your holding? (Tick one) Please refer to brome ID leaflet to distinguish brome species present https://croprotect.com/weeds/which-brome-is-that

Q8. What is the predominant brome weed on your holding?

Please refer to brome ID leaflet to distinguish brome species present https://croprotect.com/weeds/which-brome-is-that
If the species is unknown, there is the option at the end of the survey to send in seed for brome species identification

Q9. What area of your holding is affected by each brome species? (In ha)

Q9.1. Sterile brome

Q9.2. Great brome

Q9.3. Meadow brome

Q9.4. Rye brome

Q9.5. Soft brome

Q9.6. Unknown - Sterile or Great

Q9.7. Unknown - Meadow, Rye, or Soft

Q9.8. Unknown

Q10. What is the predominant soil type in the areas affected by brome?

Q11. Have brome weeds become more of a problem in the last 3 years on the holding?

Q12. Why do you think there has been an increase/ decrease/ no change in the brome population?

Q13. What cultural control methods do you use for brome control?

Q13.1. None

Q13.2. Shallow stubble cultivations

Q13.3. Min tillage

Q13.4. Plough

Q13.5. Crop rotation (including spring cropping)

Q13.6. Delayed autumn sowing

Q13.7. Other (please specify):

Q14. Do you use herbicides to control brome weeds?

Q15. If yes, which herbicides do you use on brome weeds?

Q15.1. Glyphosate – before crop sowing

Q15.2. Pyroxsulam & Florasulam (e.g. Broadway Star/ Palio)

Q15.3. Pyroxsulam & Pendimethalin (e.g. Broadway Sunrise)

Q15.4. Iodosulfuron-methyl-sodium & mesosulfuron-methyl (e.g. Pacifica)

Q15.5. Flufenacet & DFF (e.g. Liberator)

Q15.6. Cycloxydim (e.g. Laser)

Q15.7. Propyzamide (e.g. Kerb)

Q15.8. Fluazifop-P-butyl (e.g. Fusilade Max)

Q15.9. Tri-allate (e.g. Avadex Excel 15G)

Q15.10. Sulfosulfuron (e.g. Monitor)

Q15.11. Other (please specify):

Q16. Do you use the label recommended field rate of these herbicides for bromes?

Q17. Do you use programmes of the above herbicides?

Q18. How do you control for herbicide drift into field edges?

Q18.1. Low drift nozzles

Q18.2. Spray in suitable weather/ wind conditions

Q18.3. Low boom

Q18.4. Coarse spray quality

Q18.5. Low speed

Q18.6. No spray zone

Q18.7. None

Q18.8. Other (please specify):

Q19. Do you have problems controlling brome with herbicides?

Q19.1. Yes, ACCase (fops and dims)

Q19.2. Yes, ALS (sulfonylureas and triazolopyrimidines)

Q19.3. Yes, Glyphosate

Q19.4. No problems

Q19.5. Other (please specify):

Q20. If yes, why do you think there are problems controlling brome with these herbicides?

Q20.1. Too late with applications

Q20.2. Poor application

Q20.3. Ineffective products

Q20.4. Possible resistance

Q20.5. Herbicide dose used too low

Q20.6. Other (please specify):

Q21. Field name/ number

Q22. Brome species present https://croprotect.com/weeds/which-brome-is-that

Q22.1. Sterile brome

Q22.2. Great brome

Q22.3. Meadow brome

Q22.4. Soft brome

Q22.5. Rye brome

Q22.6. Unknown - Sterile or Great

Q22.7. Unknown - Meadow, Soft, or Rye

Q22.8. Unknown

Q23. Field size (In ha)

Q24. Current crop

Q25. Current crop establishment method

Q25.1. Plough

Q25.2. Min till

Q25.3. Direct drilling

Q25.4. Other (please specify):

Q26. Sowing date of current crop

Q27. Previous crop

Q28. Where and to what level is brome present in this field? Low - less than 10 heads/ panicles m^2. Intermediate - 10 – 50 heads/panicles m^2. Severe - more than 50 heads/panicles m^2.

Q28.1. Margin

Q28.1.1. Odd individual

Q28.1.2. Low

Q28.1.3. Intermediate

Q28.1.4. Severe

Q28.2. Headland

Q28.2.1. Odd individual

Q28.2.2. Low

Q28.2.3. Intermediate

Q28.2.4. Severe

Q28.3. Centre

Q28.3.1. Odd individual

Q28.3.2. Low

Q28.3.3. Intermediate

Q28.3.4. Severe

Q29. What percentage of the field is affected by brome?

Q30. How long has brome been present in this field?

Q31. Where do you think the brome came from?

Q32. If you would like to send brome panicles and seed in for ID, please post a sample in a paper envelope

Q32.1. Name

Q32.2. Email

Q32.3. Phone

Q32.4. Address

Q32.5. Brome species

Q32.6. Issues with control?

9.2. Survey data

Q1. Where is the farm based?

County	Sterile brome	Great brome	Meadow brome	Rye brome	Soft brome	Brome - unknown	**Total**
Yorkshire	22	3	8	6	13	5	**57**
Northumberland	15	4	6	3	5		**33**
Herefordshire	14	5	4	6	7	1	**37**
Essex	9	2	5	2	5	1	**24**
Norfolk	9	2	5	4	4	2	**26**
Cambridgeshire	8	2	4	1	2		**17**
Northern Ireland	8	1	2	2	3		**16**
Kent	5	2	3	1	2	1	**14**
Lincolnshire	5		3				**8**
Oxfordshire	5	3	3	1	2	1	**15**
Aberdeenshire	4		2		2	1	**9**
Berkshire	4	1	3	1			**9**
Fife	4		1	1	2		**8**
Hampshire	4	2	2	1	2		**11**
Midlothian	4	1			3		**8**
Northamptonshire	4	2	2	1	3		**12**
Co. Durham	3	1	2		1		**7**
Gloucestershire	3		3	1	3		**10**
Inverness	3	1	1		2		**7**
Lancashire	3		2	1	2	2	**10**
Nottinghamshire	3	2	2	1	2	1	**11**
Shropshire	3	2	1	1	1		**8**
Somerset	3		1	1	2		**7**
Sussex	3	1	1	2	3	1	**11**
Warwickshire	3				1		**4**
Bedfordshire	2		2	2	1		**7**
Cheshire	2		2		1		**5**
Devon	2	1	1	1	1		**6**
Dorset	2		2		1		**5**
Hertfordshire	2			1		1	**4**
Scottish Borders	2	2	2		1	1	**8**
Worcestershire	2	1	1		1		**5**
Buckinghamshire	1		1		1		**3**
Denbighshire	1		1	1			**3**
Derbyshire	1	1	1	1			**4**
Monmouthshire	1		1	1			**3**
Perthshire	1				1		**2**
Staffordshire	1		1	1	1		**4**
Stirling	1			1			**2**
Surrey	1		1		1	2	**5**
West Midlands	1	1	1	1	1		**5**

Wiltshire	1		1	1			3
Grand Total	**175**	**43**	**84**	**48**	**83**	**20**	**453**

Q5. What is the predominant soil type?

	Number of responses
Medium loam	63
Clay loam	57
Sandy loam	27
Heavy clay	20
Silty loam	15
Sand	6
chalk	7
Cotswold brash	3

Q6. What grass weeds are present in the arable area of the holding?

Grass weed present	Number of responses
Wild oats	177
Sterile brome	175
Annual meadow grass	164
Black-grass	131
Meadow brome	84
Soft brome	83
Italian ryegrass	67
Perennial ryegrass	57
Rye brome	48
Great brome	43
Brome - Unknown	20
Rat's tail fescue	20
Other	11

Q7. Of these, what is the MAIN grass weed present in the arable area of your holding?

Main grass weed	Number of responses
Black-grass	72
Sterile brome	51
Annual meadow grass	40
Wild oats	11
Italian ryegrass	8
Meadow brome	8
Rye brome	7
Perennial ryegrass	4
Soft brome	3
couch	1
Great brome	1

Q8. What is the predominant brome weed on your holding?

Species	No. responses	Area covered (ha)
Sterile brome	162	22613
Meadow brome	55	3943
Soft brome	55	2769
Rye brome	37	2369
Great brome	30	1646
Unknown - *Bromus*	19	998
Unknown - *Anisantha*	10	391
Unknown	4	18

Q10. What is the predominant soil type in the areas affected by brome?

Soil type	Sterile brome	Great brome	Meadow brome	Rye brome	Soft brome	Brome - unknown
Medium loam	55	13	22	15	36	8
Clay loam	42	12	27	12	12	3
Sandy loam	22	5	12	6	9	3
Heavy clay	13	2	7	3	7	1
Silty loam	12	5	5	5	4	1
Sand	6	2		1	4	1
Chalk loam	4		2		1	
Silt clay loam	2	1	2	1	1	
chalk	2		1	1	2	
Cotswold brash	2	2	1	1	1	

Q11. Have brome weeds become more of a problem in the last 3 years on the holding? And Q12. Why do you think there has been an increase/ decrease/ no change in the brome population?

Reason for change	Decrease	Increase	No change
Min till/ no til		50	3
Poor rotations		21	
Ineffective chemistry		19	
Conflict with BG control		12	
Poor stale seedbed		11	
Oats & barley crops		11	1
Grass margins		10	
Climate change		7	2
Contaminated seed		7	1
Possible resistance		4	
Lack of spring crops		2	
Poor chemical control		1	
Other		18	1
Better rotation - eg spring cropping	13		10
Herbicides	11		10
Better cultivations	7		13
Other	4		3
Good stale seedbed	3		1
More focus on control	2		5

Q13. What cultural control methods do you use for brome control?

Cultural control	Sterile brome	Great brome	Meadow brome	Rye brome	Soft brome	Unknown	Unknown - bromus	Unknown - anisantha
None	5		1	1	2			
Shallow stubble cultivations	62	1	8	6	4		4	
Min till	28	2	8	1	3	1		1
Plough	76	3	9	10	3	2	6	3
Crop rotation (including spring cropping)	88	2	15	8	7	3	11	1
Delayed autumn sowing	36	1	10	1	4	1	5	2
Other	7	1	3	2	1		6	

Q14. Do you use herbicides to control brome weeds? And Q15. If yes, which herbicides do you use on brome weeds?

Herbicides	Sterile brome	Great brome	Meadow brome	Rye brome	Soft brome	Unkn own	Unknown - bromus	Unknown - anisantha
Glyphosate	103	3	18	11	8	3	10	2
Flufenacet & DFF	83	4	15	9	6	2	11	3
Pyroxsulam & Florasulam	86	2	11	11	8	1	7	1
Propyzamide	65	1	13	8	2	2	8	1
Iodosulfuron-methyl-sodium & mesosulfuron-methyl	47	3	9	9	6	1	6	1
Fluazifop-P-butyl	37	2	7	7	3	1	1	1
Tri-allate	33	1	3	4	4	1	3	1
Cycloxydim	16		8	2			2	
Other	14		3	2	2		1	
Sulfosulfuron	11	1	2	2	2			
Pyroxsulam & Pendimethalin	6	2	2		1			

Q16. Do you use the label recommended field rate of these herbicides for bromes?

	Number of responses
Yes, always	160
No, sometimes below	19
No, sometimes above or below	13
No answer	7
No, always below	6
No, sometimes above	1

Q17. Do you use programmes of the above herbicides?

	Number of responses
Yes, always	105
Yes, sometimes	65
No	26
No answer	10

Q18. How do you control for herbicide drift into field edges?

Drift control	Sterile brome	Great brome	Meadow brome	Rye brome	Soft brome	Unkn own	Unknown - bromus	Unknown - anisantha

Low drift nozzles	94	4	14	8	10	2	10	3
Spray in suitable weather/ wind conditions	103	5	17	12	9	2	12	1
Low boom	63	1	8	6	7	2	6	1
Coarse spray quality	15	1	1	2	1	1	1	
Low speed	39	1	6	3	2	2	6	1
No spray zone	26	3	6	5	3	3	3	2
None2	1							
Other (please specify):3	7		2	1		1		

Q19. Do you have problems controlling brome with herbicides?

Herbicide control problems	Sterile brome	Great brome	Meadow brome	Rye brome	Soft brome	Unkn own	Unknown - bromus	Unknown - anisantha
No problems	51	2	11	5	4	1	1	1
ALS (sulfonylureas and triazolopyrimidines)	46	2	5	5	5	1	5	1
ACCase (fops and dims)	31	1	4	1	3	1	5	
Other	16		2	2	1		5	1
Glyphosate	8			2	1			1

Q20. If yes, why do you think there are problems controlling brome with these herbicides?

	Sterile brome	Great brome	Meadow brome	Rye brome	Soft brome	Unkn own	Unknown - bromus	Unknown - anisantha
Possible resistance	36	1	6	6	2	2	5	1
Too late with applications	33	1	5	2	5	1	7	2
Ineffective products	30	2	4	2	3	1	4	
Other	23		2	3			4	
Poor application	15		3	1	2			1
Herbicide dose used too low	5		1	1			2	

Q22. Brome species present

Species	Number of responses
Sterile	132

Great	11
Meadow	36
Soft	30
Rye	17
Unknown - *anisantha*	7
Unknown - *bromus*	16
Unknown	1
Total	250

Q24. Current crop

Current crop	Sterile brome	Great brome	Meadow brome	Soft brome	Rye brome	Unknown - anisantha	Unknown - bromus
Winter wheat	88	8	22	23	13	6	14
Winter barley	15	3	6	3	3		2
winter oats	5		2	1			
Spring barley	4		2	2			
sugar Beet	4						
Spring Beans	3		1				
Spring oats	2						
Triticale	2						
Winter beans	2						
Potatoes	1					1	
Ryegrass	1				1		
Stewardship	1		1				
Winter oilseed rape	1		2				
Winter Triticale	1						

Q25. Current crop establishment method

Cultural control	*Anisantha*	*Bromus*	Unknown
Crop rotation (inc. spring cropping)	91	41	3
Plough	82	28	2
Shallow stubble cultivations	63	22	
Delayed autumn sowing	39	20	1
Min till	31	12	1
None	5	4	
Other	8	12	

Q26. Sowing date of current crop

Date	Number of responses
Before 15/09	8

15/09-30/09	26
1/10-14/10	18
15/10-31/10	13
After October	0
Spring	13

Q27. Previous crop

Previous crop	Sterile brome	Great brome	Meadow brome	Soft brome	Rye brome	Unknown - anisantha	Unknown - bromus
Winter wheat	50	4	21	15	10	2	6
Winter oilseed rape	25	2	4	2	3	2	4
Winter barley	12	2	1	1			
Spring beans	11	1	2	5	1		
Fallow	6		3	2			1
Spring barley	5	1	2			1	2
Winter oats	4		2		1	1	
Potatoes	3			1			
Spring Oats	3			1			
Spring linseed	2				1		1
Stubble turnips	2						
sugar beet	2			1			
Triticale	2						
Vining peas	2			1	1		1
50:50 Winter beans & sugar beet	1						
Winter beans	1	1				1	1
Spring wheat			1	1			

Q28. Where and to what level is brome present in this field? Low - less than 10 heads/ panicles m². Intermediate (inter) - 10 – 50 heads/panicles m². Severe - more than 50 heads/panicles m².

Field area	Margin				Headland				Centre			
Species	Odd	Low	Inter	severe	Odd	Low	Inter	severe	Odd	Low	Inter	severe

Sterile	15	30	52	26	15	33	59	22	34	45	30	17
Great	1	2	3	4	1	3	3	3	4	3	3	1
Meadow	5	9	12	6	3	9	14	8	9	13	8	3
Soft	5	6	13	5	6	8	14	2	12	11	5	1
Rye	5	2	9	1	5	3	6	3	8	3	5	1
Unknown – anisantha	2	1	2	2	1	2	3	1	3		2	2
Unknown - bromus	3	3	5	3	1	5	5	4	3	5	6	2

Q30. How long has brome been present in this field?

Time in field	Anisantha	Bromus
first year	6	8
2-4 years	42	36
5-7 years	36	16
8-10 years	18	9
More than 10 years	32	22
Unknown	16	8

Q31. Where do you think the brome came from?

Source	Number of responses
Margin	83
Not known	43
Seed	20
Machinery	14
Other	11
FYM	9
Straw	3

9.3. Information on brome populations

9.3.1. Great brome

Population	Comment	County	Collected	Screened	Dose response*	Leaf samples
SD221		Hampshire	2016	2017	2018A	
SD440		East Lothian	2017	2017		

SD441		Shropshire	2017	2017	2018B	yes
SD432		Northumberland	2017	2017		
SD456		Co. Londonderry	2017	2017		
SD477		Wiltshire	2017	2017		
SD481		Oxon	2017	2017		
SD497		Yorkshire	2017	2017		
SD508		Suffolk	2017	2017		
SD511		Yorkshire	2017	2017		
SD523	Sensitive standard	RUT	2016	2017, 2018, 2019	2018B	
SD502		Northumberland	2017	2018		
SD625		Wiltshire	2018	2018		
SD758		North Yorkshire	2019	2019		

*2018A = initial dose response (3.2.2), 2018B = 2nd dose response (3.2.2), 2020 = final dose response (3.2.4), selection dose response (3.3.2)

9.3.2. Sterile brome

Population	Comment	Place of origin	Collected	Screened	Dose response	Leaf testing
SD224	ACCase resistant, 2041 mutation (Dicke & Wagner 2014)	Germany	2008	2017		
SD409	ALS resistant (Heap, 2021)	Veronnes	2010	2017	2018A, 2018B	
SD410	ALS resistant (Heap, 2021)	Vilecomte	2012	2017		
SD436		Shropshire	2017	2017		
SD442		Herefordshire	2017	2017		
SD443	Did not germinate	Herefordshire	2017	2017		
SD445		Co. Durham	2017	2017		
SD454		Lincolnshire	2017	2017, 2018, 2019	2018B	yes
SD457		Northumberland	2017	2018		
SD464	Sensitive standard	Lincolnshire	2017	2017	2018A, 2018B, 2020S	
SD468		Cambridgeshire	2017	2017	2018A, 2018B, 2020S	
SD471		Yorkshire	2017	2017	2020	
SD478		Wiltshire	2017	2017	2018A, 2018B	
SD479		Oxon	2017	2017	2018A, 2018B, 2020S	
SD484		Sussex	2017	2017		
SD488		Worcestershire	2017	2017	2018A, 2018B	yes
SD489		Shropshire	2017	2017		
SD490		Shropshire	2017	2017		
SD494		Aberdeenshire	2017	2017		
SD495		Surrey	2017	2017		
SD498		Yorkshire	2017	2017	2018A	
SD502	Did not germinate	Northumberland	2017	2017		
SD522	Sensitive standard	ADAS Cambridgeshire	2016	2017, 2018, 2019	2018B	
SD623		Nottinghamshire	2018	2018		
SD749		Unknown		2019		
SD751		Shropshire	2019	2019		
SD752		Berwick	2019	2019		
SD753		Berwick	2019	2019	2020	
SD755		Lincolnshire	2019	2019		
SD761		Lincolnshire	2019	2019		
SD764		Berwick	2019	2019		
SD786		Northants	2019	2019		

*2018A = initial dose response (3.2.2), 2018B = 2nd dose response (3.2.2), 2020 = final dose response (3.2.4), selection dose response (3.3.2)

9.3.3. Meadow brome

Population	Comment	County	Collected	Screened	Dose reponse	Leaf samples
SD444	Did not germinate	Cambridgeshire	2017	2017		
SD447	Did not germinate	Essex	2017	2017		
SD458		Lincolnshire	2017	2017		
SD466		Yorkshire	2017	2017	2018A, 2018B	yes
SD467		Cambridgeshire	2017	2017		
SD472		Cambridgeshire	2017	2017		
SD473		Bedfordshire	2017	2017		
SD474		Bedfordshire	2017	2017		
SD486		Lincolnshire	2017	2017		
SD505		Oxfordshire	2017	2017		
SD507		Norfolk	2017	2017		
SD509	Did not germinate	Yorkshire	2017	2017		
SD518	Sensitive standard	BOX Cambridgeshire	2016	2017	2018A, 2018B	
SD519	Sensitive standard	MEE	2016	2017		
SD757		North Yorkshire	2019	2019		
SD754		Dorset	2019	2019	2020	
20C11		North Yorkshire	2020	2020		

*2018A = initial dose response (3.2.2), 2018B = 2nd dose response (3.2.2), 2020 = final dose response (3.2.4), selection dose response (3.3.2)

9.3.4. Rye brome

Population	Comment	County	Collected	Screened	Dose response	Leaf samples
SD437		Lincolnshire	2017	2017		
SD453	Sensitive standard	Monmouthshire	2017	2017	2018A, 2018B, 2020 & 2020S	
SD455		Surrey	2017	2017	2018A, 2018B, 2020S	
SD469	Did not germinate	Shropshire	2017	2017		
SD470		Yorkshire	2017	2017	2018A, 2018B, 2020S	
SD475		Beds	2017	2017		
SD476		Northamptonshire	2017	2017		
SD482		Oxon	2017	2017		
SD483		Sussex	2017	2017		
SD485		Sussex	2017	2017		
SD496		Surrey	2017	2017		
SD499		Yorkshire	2017	2017		
SD500		Yorkshire	2017	2017		
SD501		Londonderry	2017	2017		
SD503		Leicestershire	2017	2017		
SD506		Oxfordshire	2017	2017	2018A, 2018B	yes
SD512		Yorkshire	2017	2017		
SD516		Yorkshire	2017	2017		
SD520	Sensitive standard	HOH	2016	2017		
SD521	Sensitive standard	BRO	2016	2018, 2019	2018B	
SD622		Shropshire	2018	2018		
SD624		Cheshire	2018	2018		
SD716		BGRI, Leics	2018	2018		
SD747		Shrops	2019	2019		
SD748		Beds	2019	2019	2020	
SD750		Shrops	2019	2019	2020	
SD756		Beds	2019	2019	2020	
SD759		Notts	2019	2019	2020	
SD760		Notts	2019	2019		
SD762	Rye brome (with some meadow)	Cambs	2019	2019		
SD763		Cambs	2019	2019		
SD785		Herts	2019	2019		

*2018A = initial dose response (3.2.2), 2018B = 2nd dose response (3.2.2), 2020 = final dose response (3.2.4), selection dose response (3.3.2)