October 2016

Project Report No. 564

Preventing a widescale increase in ALS resistant broad-leaved weeds through effective management in cereal/oilseed rape rotation, using common poppy as an indicator species.

LV Tatnell, LR Davies, KA Boardman & JH Clarke

ADAS UK Ltd, Battlegate road, Boxworth, Cambridge, CB23 4NN

This is the final report of a 48 month project (RD-2012-3788) which started in October 2012. The work was funded by AHDB Cereals & Oilseeds (£120,000), and BASF, Dow AgroSciences and DuPont (£67,500). Total project £187,500.

AHDB Cereals & Oilseeds is a part of the Agriculture and Horticulture Development Board (AHDB).

Contents

1 Abstract

The project aim was to (1) identify and quantify the risks of ALS resistance in broad-leaved weeds, (2) develop the optimum management practices to manage, reduce or eliminate developing resistance, (3) raise awareness of the issue and provide information about the early warning signs and how to manage the situation in the UK. The focus weed was common poppy (*Papaver rhoeas*) which was used as an indicator species for broad leaved weeds in general.

The key results from both the field and container experiments showed that a non-ALS herbicide programme consistently provided the highest control across all experimental years and poppy populations (both ALS-resistant and susceptible populations). A mixture or programme of non-ALS + ALS herbicides also provided good control. The use of a post-emergent ALS inhibitor herbicide alone was always the weakest treatment with the poorest control of known resistant poppy populations. These results provide further evidence that common poppy populations resistant to ALS inhibitors can be controlled using well-timed applications of other herbicide modes of action.

The number of confirmed herbicide resistant broad-leaved weed populations in the UK is still relatively low compared with grass weeds. This project shows that ALS-resistant broad-leaved weeds are currently controllable with alternative modes of action and a robust herbicide-resistance management strategy is essential. It is crucial that a wide range of effective herbicide alternative modes of action are maintained to enable control of resistant populations and prevent further cases of resistance. Early detection, monitoring and removal of patches of problem broad-leaved weeds will also limit and potentially prevent resistance spread.

The project results provide evidence of the importance of retaining the availability of effective herbicides, by quantifying the value of alternative modes of action in resistance management strategies. Practical guidelines for resistance management strategies for broad-leaved weeds have been provided for agronomists, farmers and regulators, in the form of an AHDB leaflet and through the wide dissemination of results via agronomic events, workshops, national and international scientific conference papers and presentations.

2 Introduction

Recent reductions in available herbicides (including Approvals legislation 1107/2009/EC (replaced 91/414/EEC), Sustainable Use Directive 2009/128/EC, MRL requirements, Water Framework Directive 2000/60/EC and commercial pressures) has led to very limited herbicide choice across arable rotations, with many growers now relying heavily on acetolactate-synthase (ALS) products, which are a high risk resistance group (HRAC group B) (Tranel & Wright, 2002). New herbicide modes of action are extremely rare and have not been introduced for at least the last 20 years. Although most ALS products are not highly active on common poppy (*Papaver rhoeas*) their risk to resistance developing is important to determine and manage.

Where resistance occurs, growers face costly control (often with increased cultural control), major inconvenience and difficulties balancing long-term planning strategies and short-term financial constraints. World-wide, the ALS-inhibiting herbicide class has the greatest incidence of resistance as reported by 'The International survey of herbicide-resistant weeds' website (www.weedscience.com). The number of ALS-resistant broad-leaved weeds in 1997 was reported as 26; by 2011 this had risen to 133 and it currently stands as 159 species (Heap, 2016), so is rapidly increasing. Of these species 109 are dicots and 50 are monocots. Biotypes of common poppy were first reported as showing resistance to ALS inhibiting sulfonylurea herbicides in the UK in 2001 and have now been identified in nine counties of England (Moss *et al*., 2005, 2011, Hull *et al*., 2014) with greater than 70 resistant populations (Tatnell *et al.*, 2016). The primary ALS resistance mechanism in common poppy is target site resistance. In grassweeds, both target site and non-target site resistant (NTSR) mechanisms are common. To date no NTSR has been identified in any UK populations of poppy. However, in 2015, Scarabel presented the first evidence of NTSR in a common poppy population in Italy, after speculation that target site resistance was not the only mechanism present in common poppy. This NTSR mechanism of resistance has only ever been found in two other dicot weed species, charlock (*Sinapis arvensis*) and smooth pigweed (*Amaranthus hybridus*).

Research in Spain has highlighted the need for a range of different modes of action to effectively control common poppy to avoid over reliance on the high risk ALS-inhibitors (Torra *et al*., 2010). It also demonstrates the benefits of a robust herbicide programme include pre- and post-emergence herbicides. Common poppy is the most important broad-leaved weed species in north-eastern Spain and it has been reported that wheat yield can be decreased by 32% due to its highly competitive nature if not controlled effectively. However, the range of alternative modes of action available to Spanish farmers is much greater than in the UK as they still rely heavily on trifluralin and isoproturon, both of which have been withdrawn from the UK market.

Resistance to ALS-inhibiting herbicides is now widespread in black-grass populations in the UK (Moss *et al*., 2007). Resistance has built up very quickly by the repeated use of this herbicide class and once there it does not go away, but can be managed, as was demonstrated in the LINK project (3035) ‘Integrated management of herbicide resistance’. A proactive response to the lessons learnt from the black-grass resistance issues in the UK should help prevent or slow the development of widespread resistance in broad-leaved weeds.

Across a rotation, ALS inhibiting herbicides can control common poppy indirectly as a non-target weed in a grass-weed dominated management plan. Currently in the UK one of the main herbicides used directly for controlling common poppy is pendimethalin, a dinitroaniline (HRAC group K_1). Future weed resistance management strategies will rely upon maintaining approvals for a wide range of herbicide modes of action.

A literature review was carried out for the Chemicals Regulation Directorate (CRD) as part of the broad-leaved weed resistance project PS2709 (Tatnell *et al.*, 2007). The aim of the review was to predict which species were likely to develop resistance in the UK, based on chemical and biological aspects linked to each species and an understanding of resistance incidence world-wide. The key biological factor that denotes a weed as ‘high risk’ is a high level of seed production, which would include a species such as common poppy. Factors that compose the highest chemical risks for resistance development include herbicide mode of action, mode of use (i.e. mixtures and sequences), intrinsic activity and residual activity. The review produced a useful list of risk factors that could be included in a prediction tool for use by regulators however it was based on an evidence review and not validated by practical research.

The objectives of this project were to (1) identify and quantify the risks of ALS resistance in broad-leaved weeds, (2) develop the optimum management practices to manage, reduce or eliminate developing resistance levels, (3) raise awareness of the issue and provide information about the early warning signs and how to manage the situation in the UK. The project was delivered through a series of field and container-based experiments to provide more detailed data and understanding.

The proposed project outcome was to understand the need for availability of herbicides, directly by providing strategies to retain their benefit and indirectly by providing evidence of their value in resistance management strategies. As a result, practical guidelines for resistance management strategies for broad-leaved weeds have been provided for agronomists, farmers and regulators.

3 Materials and methods

The experimental work was divided up into glasshouse pot screens (year one and four), container experiments (years one to three) and field experiments (Cambridgeshire years one to three and Yorkshire year two only). Seed from one of the field experiments (CAMB-M) were used in the container and glasshouse pot screens for consistency and further validation of results. This population was known to have a high level of ALS-resistance. The CAMB-W populations was known to have a moderate level of ALS-resistance.

3.1 Pot screen dose response experiments

Two pot-based experiments were set up in the glasshouse at ADAS Boxworth in project years one and four to investigate the dose response of a range of different herbicide treatments on different populations of common poppy (*Papaver rhoeas*). The first experiment in 2013 included cereal herbicides and the second experiment in 2014 included oilseed rape herbicides. The herbicides were selected as they were either ALS-inhibitors commonly used in that particular crop and in addition a non-ALS herbicide was included as a reference.

3.1.1 Cereal herbicide treatments

A fully randomised block design (per weed population) was used with 13 treatments including untreated controls replicated six times (total 234 pots).

The common poppy seed populations used included a susceptible standard (S1, a 50:50 mixture of both 2011 and 2010 seed lots, purchased from Herbiseed, UK), and resistant populations CAMB-W and CAMB-M.

Sowing seed

Common poppy seed were sown on 04/04/13 by filling half seed trays (15cm x 20cm) with potting compost (John Innes potting compost no.1), allowing two half seed trays per population (six trays in total). Seed trays were lightly watered at least an hour before sowing seed and labelled. Poppy seed were sown by sprinkling a very small pinch of seed onto the soil surface, taking care to spread seed as evenly as possible across the tray and then covering the seed with a very small amount of potting compost. The trays were gently watered using a watering can and rose attachment. Sown seed trays were placed on a bench in a glasshouse (18^{o}C, 14 hour days (light) and 12^{o}C, 10 hour nights (no light) and were watered daily using an automatic overhead watering boom.

When the poppy seedlings were at the three leaf growth stage (BBCH 13) they were carefully transplanted (18 April 2013) into plastic plant pots (5cm x 5cm x 6cm) full of loam-based soil (Kettering loam ‘weed’ mix comprising of: 80% Kettering loam (sterilised): 20% grit: 2kg/tonne Osmacote slow release fertiliser mini), allowing one seedling per pot. All pots were placed on capillary matting on a glasshouse bench and watered as above.

Herbicide applications

When the poppy plants were at the 3-5 true leaf growth stage (BBCH 13-15) herbicide treatments (Table 1) were applied on 22/04/13 using a hand-held 1m boom and knapsack sprayer at 2 bar, F110 02 nozzles at a water volume of 200 l/ha. The soil was damp, but not wet before spraying and were left for at least 6 hours post-spray application before watering again from above.

Table 1 Cereal herbicide treatments for the common poppy dose response experiment 2013.

Treatment number	**Herbicide product**	**Dose of label rate**	**Amount of product applied**
1	Untreated		
2	Metsulfuron-methyl	Double	60g/ha
3	Metsulfuron-methyl	Full	30g/ha
4	Metsulfuron-methyl	½	15 g/ha
5	Metsulfuron-methyl	¼	7.5 g/ha
6	MCPA	Double	4.0 l/ha
7	MCPA	Full	2.0 l/ha*
8	MCPA	½	1.5 l/ha
9	MCPA	¼	0.75 l/ha
10	Mesosulfuron-methyl + iodosulfuron-methyl-sodium + adjuvant	Double	0.8 kg/ha + 1.0 l/ha
11	Mesosulfuron-methyl + iodosulfuron-methyl-sodium + adjuvant	Full	0.4 kg/ha + 1.0 l/ha
12	Mesosulfuron-methyl + iodosulfuron-methyl-sodium + adjuvant	½	0.2 kg/ha + 1.0 l/ha
13	Mesosulfuron-methyl + iodosulfuron-methyl-sodium + adjuvant	¼	0.1 kg/ha + 1.0 l/ha

*Full rate of MCPA 50 for wheat is 3.3 l/ha, rates were reduced due to higher efficacy in glasshouse conditions.
MCPA 50 (MAPP 14908) 500 g /l MCPA
Atlantis® WG (MAPP 12478) 30 g/kg mesosulfuron-methyl and 6 g/kg iodosulfuron-methyl-sodium
Jubilee SX (MAPP 12203) 200 g/kg metsulfuron-methyl

Assessments and harvesting

Pots were monitored daily to ensure they were not too wet or dry. A visual score of the plants using a 0-10 rating (where 0= dead plants and 10 = live/healthy plants) was done on 20/05/13 prior to destructive sampling. The fresh weight of plants (g) was assessed by carefully cutting the plant at the base of the stem and weighing. Data were meaned by treatment and summarised.

3.1.2 Oilseed rape herbicide treatments

The methodology for sowing (20/02/14), transplanting (17/03/14), treating (01/04/14) and assessing (24/04/14) seed was exactly the same as described above for the cereal herbicide experiment (section 3.1.1). The herbicide treatments applied are shown in Table 2 and included 17 treatments with a total of 408 pots.

Table 2 Oilseed rape herbicide treatments for the poppy dose response experiment 2014.

Treatment number	**Herbicide active ingredient**	**Dose of label rate**	**Dose of product**
1	Untreated		
2	Imazamox + adjuvant	Double	1.75 l/ha + 1.0 l/ha
3	Imazamox+ adjuvant	Full	0.875 l/ha + 1.0 l/ha
4	Imazamox+ adjuvant	½	0.437 l/ha + 1.0 l/ha
5	Imazamox+ adjuvant	¼	0.219 l/ha + 1.0 l/ha
6	Imazamox + metazachlor + adjuvant	Double	4.0 l/ha + 1.0 l/ha
7	Imazamox + metazachlor + adjuvant	Full	2.0 l/ha + 1.0 l/ha
8	Imazamox + metazachlor + adjuvant	½	1.0 l/ha + 1.0 l/ha
9	Imazamox + metazachlor + adjuvant	¼	0.5 l/ha + 1.0 l/ha
10	Metazachlor	Double	3.0 l/ha
11	Metazachlor	Full	1.5 l/ha
12	Metazachlor	½	0.75 l/ha
13	Metazachlor	¼	0.375 l/ha
14	Propyzamide + aminopyralid	Double	3.0 l/ha
15	Propyzamide + aminopyralid	Full	1.5 l/ha
16	Propyzamide + aminopyralid	½	0.75 l/ha
17	Propyzamide + aminopyralid	¼	0.375 l/ha

Imazamox (provided for experimental purposes only, not registered in the UK)
Cleranda MAPP 15036 17.5g/l imazamox and 375g/l metazachlor, Adjuvant = DASH HD
Butisan MAPP 16569 5001g/l metazachlor
Astrokerb MAPP 16184 500g/l propyzamide and 5.3 g/l aminopyralid

3.2 Container experiments

A set of container-based experiments were run over three project years, 2012-13, 2013-14 and 2014-15 starting in the autumn to mimic a winter cropping field season .

3.2.1 Populations

The poppy populations selected were 1) CAMB-M (from a field site in Cambridgeshire where the long-term field experiment was located), 2) CAMB-W (from a second field site in Cambridgeshire that was proposed for the long-term field trials but unfortunately was unavailable when the trials began (see section 3.3)) and 3) S1 standard (Purchased from Herbiseed, UK). This included seed batches from 2010 and 2011 that were mixed to enhance germination quality).

3.2.2 Herbicide treatments

Thirteen herbicide treatments combinations were used over a period of three years between 2013 and 2015 (Table 3). All herbicide treatments were applied using a hand-held 2m boom and knapsack sprayer at 2.0 bar, F110 02 nozzles at a water volume of 200 l/ha. The herbicide treatments were selected to simulate those typically used in a crop rotation. Treatments were replicated four times. The treatment principles and proposed 'crop' rotation (although no crop was included in the containers, but the herbicide selection was based on a crop choice) are listed in Table 3.

Table 3 Herbicide treatment list of poppy containers across all three treatment years (2013-2015)

Treatment number	Herbicide treatment		Crop- *no crop present but herbicide selection to mirror field treatments*		
	Pre-emergence	Post-emergence	Year 1	Year 2	Year 3
1	Untreated control		-	-	-
2	-	ALS	W. Wheat	W. Wheat	W. Wheat
3	Non-ALS	ALS	W. Wheat	W. Wheat	W. Wheat
4	Non-ALS	Non-ALS	W. Wheat	W. Wheat	W. Wheat
5	-	ALS	W. Wheat	W. OSR	W. Wheat
6	Non-ALS	ALS	W. Wheat	W. OSR	W. Wheat
7	Non-ALS	Non-ALS	W. Wheat	W. OSR	W. Wheat
8	-	ALS	W. Wheat	W. Wheat	Fallow
9	Non-ALS	ALS	W. Wheat	W. Wheat	Fallow
10	Non-ALS	Non-ALS	W. Wheat	W. Wheat	Fallow
11	-	ALS	W. Wheat	W. OSR	Fallow
12	Non-ALS	ALS	W. Wheat	W. OSR	Fallow
13	Non-ALS	Non-ALS	W. Wheat	W. OSR	Fallow

In 2013 (year 1) all herbicide treatments were for herbicide actives used to control broadleaved weeds in winter wheat crops (Table 4). In 2014 (year 2) herbicides were either for broadleaved weed control in winter wheat (Table 5) or in winter oilseed rape (Table 6). In 2015, herbicide treatments were for winter wheat (Table 8) or no herbicide was applied and the containers were left in 'fallow' (Table 3). The details for each individual year are described below.

3.2.3 Containers 2013

Containers (30cm x 25 cm x 15 cm) were filled with sterilised Kettering loam 'weed' mix (described in 3.1.1)) to a depth of 3cm below the rim, and placed in a fruit cage in a randomised block design (treatments and populations randomised within replicates) at ADAS Boxworth. Containers were watered over a period of 3 days to moisten soil before seed sowing. At sowing (13/11/12), 0.2g of poppy seed per container was weighed out, mixed with a small amount of sand and spread evenly over the soil surface. After sowing, seed was covered with a layer of sterilised loam mix no more than 1cm deep. Containers were watered as required but were exposed to rainfall.

One day after sowing (14/11/12), pre-emergence herbicides were applied to treatments 3, 4, 6, 7, 9, 10, 12, and 13 (Table 4). Containers were monitored in the fruit cage until post-emergence herbicide application the following spring. In November the containers were covered by horticultural fleece due to cold weather to promote emergence of poppies and this was removed in December 2012. Containers were fleeced again for three weeks in March 2013 due to cold weather.

In March 2013, plant numbers in containers that had been treated with a pre-emergence herbicide were extremely low and it was decided that no post-emergence herbicide application would be applied to these treatments. Post-emergence herbicides were applied to treatments 2, 5, 7, and 11 (Table 4) on 17/04/13 at BBCH 14-21.

Poppy plant counts were recorded for each container on 24/05/13 and containers with surviving individuals were moved into cages covered with fine mesh to minimise pollen spread as no bees could access them so they were isolated by populations and treatments for seed production. Poppy head counts were recorded for each container on 09/07/13 and seeds were only collected from plants of treatments 1, 2, 5, 8, and 11 (Table 4) as in the other treatments there were no live poppy heads.

Table 4 Herbicide treatment, active ingredient, product, and rate for container treatments in 2013.

Treatment number	Herbicide treatment principle	Pre-emergence active ingredient	Product rate l/ha	Post-emergence active ingredient	Product rate /ha
1	Untreated control				
2	ALS alone	-		Metsulfuron-methyl	30g
3	Non-ALS + ALS	Flufenacet + pendimethalin	2.0	Metsulfuron-methyl	30g
4	Non-ALS	Flufenacet + pendimethalin	2.0	MCPA 50	1.5L
5	ALS alone	-		Metsulfuron-methyl	30g
6	Non-ALS + ALS	Flufenacet + pendimethalin	2.0	Metsulfuron-methyl	30g
7	Non-ALS	Flufenacet + pendimethalin	2.0	MCPA 50	1.5L
8	ALS alone	-		Metsulfuron-methyl	30g
9	Non-ALS + ALS	Flufenacet + pendimethalin	2.0	Metsulfuron-methyl	30g
10	Non-ALS	Flufenacet + pendimethalin	2.0	MCPA 50	1.5L
11	ALS alone	-		Metsulfuron-methyl	30g
12	Non-ALS + ALS	Flufenacet + pendimethalin	2.0	Metsulfuron-methyl	30g
13	Non-ALS	Flufenacet + pendimethalin	2.0	MCPA 50	1.5L

Crystal MAPP 13914 60g/l flufenacet + 300g/l pendimethalin
MCPA 50 (MAPP 14908) 500 g /l MCPA
Jubilee SX (MAPP 12203) 200 g/kg metsulfuron-methyl

3.2.4 Containers 2014

Containers were filled with a sterilised Kettering loam mix, placed in a fruit cage in a randomised block design and watered over a period of three days to moisten soil before seed was sown. At sowing (06/10/13) 0.1g (this amount was reduced from 2012 as it was considered too high a density for the size of the container) of poppy seed for each population-treatment-replicate combination was weighed and mixed with a small amount of sand before being evenly distributed over the soil surface in containers. After sowing seeds were covered with sterilised loam mix to a depth of 1cm.

For treatments 1, 2, 5, 8, and 11 (Table 5, Table 6) seed collected from the same treatments in the 2013 container experiment was used. For treatments 3, 4, 6, 7, 9, 10, 12, and 13 the original seed populations ('baseline') from the S1 standard, CAMB-M, and CAMB-W were sown, as in these treatments there was no seed produced in summer 2013 due to good control of the poppy from the herbicide treatments.

Two days after sowing (08/10/13) pre-emergence herbicides were applied to treatments 3, 4, 7, 9, 10, and 13 (Table 5, Table 6). For treatments 3, 4, 9, and 10 a 'winter wheat' herbicide was applied, for treatments 7 and 13 a 'winter oilseed rape' herbicide was applied (to mimic the rotations). Containers were monitored in the fruit cage until 22/11/13 and due to extremely cold weather conditions they were moved to a polytunnel to prevent them from freezing.

Oilseed rape post-emergence herbicide treatments were applied (07/01/14) at BBCH 12-17 to treatments 5, 6, 7, 11, 12, and 13 (Table 6). After herbicide treatment all containers (treated and not treated) were moved back to the fruit cage. On 21/03/14 wheat post-emergence herbicide treatments were applied to treatments 2, 3, 4, 8, 9, and 10 at up to BBCH 30 (Table 5).

Table 5: 'Wheat' herbicide treatment, active ingredient, product, and rate for container treatments in 2014.

Treatment number	Treatment principle	Pre-emergence active ingredient and rate l/ha	Post-emergence active ingredient and rate/ha
1	Untreated	-	-
2	ALS alone	-	Metsulfuron-methyl @ 30g
3	Non-ALS + ALS	Flufenacet+ pendimethalin @ 2.0	Metsulfuron-methyl @ 30g
4	Non-ALS	Flufenacet+ pendimethalin @ 2.0	MCPA 50 @ 1.5 l/ha
8	ALS alone	-	Metsulfuron-methyl @ 30g
9	Non-ALS + ALS	Flufenacet+ pendimethalin @ 2.0	Metsulfuron-methyl @ 30g
10	Non-ALS	Flufenacet+ pendimethalin @2.0	MCPA 50 @ 1.5 l/ha

Crystal MAPP 13914 60g/l flufenacet + 300g/l pendimethalin
MCPA 50 (MAPP 14908) 500 g /l MCPA
Jubilee SX (MAPP 12203) 200 g/kg metsulfuron-methyl

Poppy plant counts per container were recorded on 28/04/14 with containers moved to pollen cages to isolate populations and treatments for seed production. Poppy head counts were recorded per container on 09/06/14 and poppy seeds were collected weekly over July and August.

Table 6: 'Oilseed rape' herbicide treatment, active ingredient, product, and rate for container treatments in 2014

Treatment number	Treatment principle	Pre-emergence active ingredient and rate l/ha	Early post-emergence active ingredient and rate l/ha
5	ALS alone	-	Imazamox @ 0.875 + adjuvant @ 1.0
6	Non-ALS + ALS		(Imazamox + Metazachlor) @ 2.0 + adjuvant @ 1.0
7	Non-ALS	Metazachlor @ 1.5	Propyzamide + aminopyralid @ 0.5
11	ALS alone	-	Imazamox @ 0.875 + adjuvant @ 1.0
12	Non-ALS + ALS	Metazachlor @ 1.5	(Imazamox + Metazachlor) @ 2.0 + adjuvant @ 1.0
13	Non-ALS		Propyzamide + aminopyralid @ 0.5

Imazamox (provided for experimental purposes only, not registered in the UK)
Cleranda MAPP 15036 17.5g/l imazamox and 375g/l metazachlor, DASH HC adjuvant
Butisan MAPP 16569 5001g/l metazachlor
Astrokerb MAPP 16184 500g/l propyzamide and 5.3 g/l aminopyralid

3.2.5 Containers 2015

In 2015, an additional known herbicide susceptible population 'SSS' (sourced from Scotia seeds) was included in treatments 1, 2 and 3 to enable comparison of the selected poppy container populations against an unselected susceptible population (baseline). Seed quantity was very limited hence only a few key treatments were included.

Poppy seeds were sown (0.1g/population treatment replicate) on 13/11/14 using the same method as described above. Where possible, seeds collected in the 2014 container trial were used (Table 7).

Table 7: Seed source used to sow 2015 containers. 2014: seeds collected from 2014 treatments, 2013: seeds collected from 2013 treatments, 'baseline': original seed source, (i.e. purchased if susceptible and from the 2012 field collection if resistant population).

Treatment	S1	CAMB-W	CAMB-M	SSS
1	2014	2014	2013	Baseline
2	2014	2014	2014	Baseline
3	Baseline	2014	Baseline	Baseline
4	Baseline	2014	Baseline	-
5	2014	2014	2014	-
6	2014	2014	2014	-
7	2014	Baseline	2014	-
8	2014	2014	2014	-
9	Baseline	2014	2014	-
10	Baseline	2014	2014	-
11	2014	2014	2014	-
12	2014	2014	2014	-
13	2014	2014	Baseline	-

Pre-emergence herbicide treatments were applied to treatments 3, 4, 6, and 7 directly after sowing (Table 8). All containers were then placed in a fruit cage in a randomised block design.

Prior to post-emergence herbicide application poppy plant counts were recorded (01/04/15) for each container to assess the control provided by the pre-emergence herbicide applications. Eight days after poppy plant counts (09/04/15) post-emergence herbicide treatments were applied to treatments 2, 3, 4, 5, 6, and 7 (Table 8). Treatments 8-13 were a 'fallow' treatment for year three and so no herbicides were applied.

Containers were moved to pollen cages to isolate populations and treatments for seed production on 26/05/15. Poppy head counts were recorded for each container in June and poppy seeds were collected weekly over July and August from treatments where heads remained.

Table 8: 'Wheat' herbicide treatment, active ingredient, product, and rate for poppy container treatments in 2015 – treatments 8-13 in 'fallow' with no herbicide treatments

Treatment number	Treatment principle	Pre-emergence active ingredient and rate l/ha	Post-emergence active ingredient and rate/ha
1	Untreated	-	-
2	ALS alone	-	Metsulfuron-methyl @ 30g
3	Non-ALS + ALS	Flufenacet+ pendimethalin @ 2.0	Metsulfuron-methyl @ 30g
4	Non-ALS	Flufenacet+ pendimethalin @ 2.0	MCPA 50 @ 1.5 l/ha
5	ALS alone	-	Metsulfuron-methyl @ 30g
6	Non-ALS + ALS	Flufenacet+ pendimethalin @ 2.0	Metsulfuron-methyl @ 30g
7	Non-ALS	Flufenacet+ pendimethalin @2.0	MCPA 50 @ 1.5 l/ha

Crystal MAPP 13914 60g/l flufenacet + 300g/l pendimethalin
MCPA 50 (MAPP 14908) 500 g /l MCPA
Jubilee SX (MAPP 12203) 200 g/kg metsulfuron-methyl

3.2.6 Data analysis

Statistical data analysis was conducted in R (version 3.2.4, revised 16/03/16).

For each experimental year the percent control of poppy plants and heads compared to the untreated was calculated for every container. Means and standard errors were calculated.

For both plant count and head count percent control, a three-way ANOVA analysis was used for herbicide treatment, poppy population, and block, with an interaction between treatment and population in each separate year. Four-way ANOVA analysis was used for herbicide treatment, population, block, and year, with an interaction between treatment and population in all years together. Tukey's HSD analysis was used on ANOVA analysis to determine which, populations, treatments, year, population-treatment combination and population-treatment-year combinations were significantly different from others.

Three-way ANOVA analysis and Tukey's HSD test was also used to compare the percent control of poppy heads of S1, CAMB-W and CAMB-M for treatments 2 (ALS alone) and) and 3 (ALS + non-ALS) in 2015 against the susceptible SSS population.

3.3 Field experiments

3.3.1 Cambridgeshire field site

A three-year field trial was established in Cambridgeshire (CAMB-M) in autumn 2012 (Table 9) on a site with a known high natural population of ALS-resistant common poppy. The trial design was a randomised block, split plot (12m x 12m) design with 16 treatments (Table 10, Table 11, Table 12 and Table 13) including untreated controls replicated four times. The trial area included a central discard as a buffer zone (12m x 12m) separating the four blocks and a discard surrounded the whole trial (12m wide) to act as a buffer from the surrounding crop to minimize pollen transfer from the poppy. The different crops in the second trial year were grouped together for practical reasons, preventing it from being a fully randomised design (Section 8.1). The initial trial design included a 'fallow' treatment in year three (treatments 3, 4, 11-16), however due to the large natural weed population the host farmer was not comfortable having a non-cropped area as the potential seed return from the poppies would have been extremely high and so with agreement of the project steering group this treatment was removed for practical reasons.

Table 9: Agronomic inputs and assessments for all three cropping years

Inputs and assessments	Cropping year- date of input				
	2012-13	2013-14		2014-15	
	Wheat	Oilseed rape	Wheat	Wheat Early drill	Wheat Late drill
Drilling	12/11/12	30/08/13	30/10/13	28/10/14	06/11/14
Pre-em herbicide	15/11/12	30/08/13	31/10/13	30/10/14	10/11/14
Post-em herbicide	01/05/13	22/11/13	25/03/14	09/04/15	
Plant count	28/05/13	19/03/14	07/05/14	20/05/14	
Head count	05/07/13	12/06/14	12/06/14	14/07/14	
Seed collection*	15/08/13	08/08/14		07/08/14	

*seed were collected approximately weekly from mid-July to this date.

Herbicide treatments and cropping

Herbicide treatments were applied to plots using a 12m tractor sprayer fitted with F110 02 nozzles at a water volume of 200 l/ha. At each application date weed growth stage was recorded.

Table 10: Herbicide treatments and annual cropping (across the 3 year rotation)

Treatment number	**Herbicide treatment**		**Crop**		
	Pre-emergence	**Post-emergence**	**Year 1**	**Year 2**	**Year 3**
1	Untreated control		W. Wheat	W. Wheat	W. Wheat
2	Untreated control		W. Wheat	W. OSR	W. Wheat
3	Untreated control		W. Wheat	W. Wheat	W. Wheat
4	Untreated control		W. Wheat	W. OSR	W. Wheat
5	-	ALS	W. Wheat	W. Wheat	W. Wheat
6	Non-ALS	ALS	W. Wheat	W. Wheat	W. Wheat
7	Non-ALS	Non-ALS	W. Wheat	W. Wheat	W. Wheat
8	-	ALS	W. Wheat	W. OSR	W. Wheat
9	Non-ALS	ALS	W. Wheat	W. OSR	W. Wheat
10	Non-ALS	Non-ALS	W. Wheat	W. OSR	W. Wheat
11	-	ALS	W. Wheat	W. Wheat	W. Wheat
12	Non-ALS	ALS	W. Wheat	W. Wheat	W. Wheat
13	Non-ALS	Non-ALS	W. Wheat	W. Wheat	W. Wheat
14	-	ALS	W. Wheat	W. OSR	W. Wheat
15	Non-ALS	ALS	W. Wheat	W. OSR	W. Wheat
16	Non-ALS	Non-ALS	W. Wheat	W. OSR	W. Wheat

Table 11 Herbicide treatments in wheat field trials 2012-13

Treatment number	**Pre-emergence active ingredient and product rate**	**Post-emergence active ingredient and product rate**
1	Untreated control	Untreated control
2	Untreated control	Untreated control
3	Untreated control	Untreated control
4	Untreated control	Untreated control
5	-	Metsulfuron-methyl @ 30g
6	Flufenacet + pendimethalin @ 2.0 l/ha	Metsulfuron-methyl @ 30g
7	Flufenacet + pendimethalin @ 2.0 l/ha	MCPA 500g/l @ 1.5l/ha
8	-	Metsulfuron-methyl @ 30g
9	Flufenacet + pendimethalin @ 2.0 l/ha	Metsulfuron-methyl @ 30g
10	Flufenacet + pendimethalin @ 2.0 l/ha	MCPA 500g/l @ 1.5 l/ha
11	-	Metsulfuron-methyl @ 30g
12	Flufenacet + pendimethalin @ 2.0 l/ha	Metsulfuron-methyl @ 30g
13	Flufenacet + pendimethalin @ 2.0 l/ha	MCPA 500g/l @ 1.5 l/ha
14	-	Metsulfuron-methyl @ 30g
15	Flufenacet + pendimethalin @ 2.0 l/ha	Metsulfuron-methyl @ 30g
16	Flufenacet + pendimethalin @ 2.0 l/ha	MCPA 500g/l @ 1.5 l/ha

Crystal MAPP 13914 60g/l flufenacet + 300g/l pendimethalin
MCPA 50 (MAPP 14908) 500 g /l MCPA
Jubilee SX (MAPP 12203) 200 g/kg metsulfuron-methyl

Table 12 Herbicide treatments in oilseed rape field trials 2013-14

Treatment number	Treatment principle	Pre-emergence active ingredient and rate l/ha	Early post-emergence active ingredient and rate l/ha
2 & 4		Untreated control	
8	ALS alone	-	Imazamox @ 0.875 + adjuvant @ 1.0
9	Non-ALS + ALS		(Imazamox + Metazachlor) @ 2.0 + adjuvant @ 1.0
10	Non-ALS	Metazachlor @ 1.5	Propyzamide + aminopyralid @ 0.5
14	ALS alone	-	Imazamox @ 0.875 + adjuvant @ 1.0
15	Non-ALS + ALS	Metazachlor @ 1.5	(Imazamox + Metazachlor) @ 2.0 + adjuvant @ 1.0
16	Non-ALS		Propyzamide + aminopyralid @ 0.5

Imazamox (provided for experimental purposes only, not registered in the UK)
Cleranda MAPP 15036 17.5g/l imazamox and 375g/l metazachlor, DASH HC adjuvant
Butisan MAPP 16569 5001g/l metazachlor
Astrokerb MAPP 16184 500g/l propyzamide and 5.3 g/l aminopyralid

Table 13 Herbicide treatments in wheat field plots 2013-14

Treatment number	Treatment principle	Pre-emergence active ingredient and rate l/ha	Post-emergence active ingredient and rate/ha
1 & 3	Untreated	-	-
5	ALS alone	-	Metsulfuron-methyl @ 30g
6	Non-ALS + ALS	Flufenacet+ pendimethalin @ 2.0	Metsulfuron-methyl @ 30g
7	Non-ALS	Flufenacet+ pendimethalin @ 2.0	MCPA 50 @ 1.5 l/ha
11	ALS alone	-	Metsulfuron-methyl @ 30g
12	Non-ALS + ALS	Flufenacet+ pendimethalin @ 2.0	Metsulfuron-methyl @ 30g
13	Non-ALS	Flufenacet+ pendimethalin @2.0	MCPA 50 @ 1.5 l/ha

Crystal MAPP 13914 60g/l flufenacet + 300g/l pendimethalin
MCPA 50 (MAPP 14908) 500 g /l MCPA
Jubilee SX (MAPP 12203) 200 g/kg metsulfuron-methyl

Weed assessments and seed collection

The number weeds per plot (poppy and other significant species if present) were counted and recorded in every plot (including untreated controls) in spring (Table 9) each year. Counts were done in a central 4m x 4m 'sacred' area of the plot using 10 x 0.1m^2 quadrats/sacred area. At the same time each plot (whole plot,) was visually assessed for percentage poppy (not all weeds) cover from 0-100% cover as an additional observation assessment.

The number of poppy heads were counted in every plot in mid-late June/early July each year (Table 9, Table 10). Head counts were also done in the 'sacred' area of the plot using 10 x 0.1m^2 quadrats.

Poppy seeds were collected on at least two separate occasions at least two weeks apart (to ensure a good range of seeds were collected as all ripen at different times) in mid-late July/very early August annually (Table 9) from the sacred 4m x 4m area of each plot. The poppy heads were cut off with scissors, placed in paper envelopes and left to air dry in the laboratory in open trays. The poppy seeds were removed from the heads when ripe. Seed were stored in paper envelopes in a seed store for future testing as required.

The data for all plant and head counts were summarised and analysed using ANOVA in Genstat.

The third field experimental year at the CAMB-M site was planned to include a split in the original plots to allow for an 'early' and 'later' drilled winter wheat crop. The hypothesis of the different drilling dates was to delay weed emergence in the later drilling date and then test the efficacy of the herbicide treatments on weeds at different growth stages. The plan was to treat the whole trial area with a non-ALS treatment only in year three as the previous two experimental years had proved that no effective control of this ALS-resistant poppy population was achieved by an ALS-inhibitor herbicide, so nothing would be gained by applying a third year of these treatments. It was also out of consideration to the host farmer as he would be left with the burden of high seed return and poppy seed has an extremely long viability. The experimental plots were marked out and the early drilling done on 28/10/14 and the pre-em Crystal (pendimethalin and flufenacet) applied by tractor and 12m boom on 30/10/14. The 'later' drilled plots were sown on 06/11/14. However, the host farmer then alerted us on that same day to the fact that there had been an error with their field spray applications and they had accidentally sprayed across the top half of our trial area, effectively resulting in half of our experiment being unusable. The project steering group were informed and a decision was made to try and salvage what we could of the original treatments for a third year. Therefore half of the experiment was continued to be sprayed as per planned treatments (pre-em on later drilled plots applied 10/11/14 and post-em (MCPA 50) applied 09/0415) and the other half was monitored but not formally counted.

A second three-year field trial site was selected (CAMB-W) and seed were collected in summer 2013. The farmer then changed his cropping pattern that autumn and so this site was unavailable until autumn 2014 and the project consortium agreed to delay this trial establishment by one year. By summer 2014 unfortunately the farmer had sold that field to become a grass paddock, so it was no longer available as a trial site in arable cropping. With agreement of the project steering group the population (CAMB-W) continued to be used in the container-based trials to provide a comparable ALS-resistant population against CAMB-M. The resources planned for this trial were re-directed into a one-year field trial in Yorkshire (2013-14) and additional glasshouse pot testing in 2015-16.

3.3.2 Yorkshire field site

A one-year field trial was established in Yorkshire (Nether Poppleton) in October 2013 on a field with a known resistant poppy population. The total trial area was restricted on this site as the field was small, but it was considered to be worthwhile investigating the four basic herbicide treatments from the CAMB-M field site, with a natural common poppy population at a different geographic location. Winter wheat (cv. JB Diego) was drilled as farm crop (13/10/13) and trial plots (3m x 12m) were marked out on 16/10/13. The trial design was a randomised block with four replicates and four treatments (Table 14).

Table 14 Herbicide treatments in the Yorkshire field trial 2013-14

Treatment number	Treatment principle	Pre-emergence active ingredient & product rate	Post-emergence active ingredient & product rate
1	untreated	Untreated control	Untreated control
2	ALS alone	-	Metsulfuron-methyl @ 30g/ha
3	Non-ALS + ALS	Flufenacet + pendimethalin @ 2.0 l/ha	Metsulfuron-methyl @ 30g/ha
4	Non-ALS	Flufenacet + pendimethalin @ 2.0 l/ha	MCPA 500g/l @ 1.5 l/ha

Crystal MAPP 13914 60g/l flufenacet + 300g/l pendimethalin
MCPA 50 (MAPP 14908) 500 g /l MCPA
Jubilee SX (MAPP 12203) 200 g/kg metsulfuron-methyl

Herbicide applications

Herbicides were applied to plots using knapsack sprayer and 3m handheld boom, F110 02 nozzles at a water volume of 200 l/ha. The pre-emergence applications were applied on 16/10/13 and the post-emergence applications were applied on 03/03/14, when the poppies were at a growth stage BBCH 14-15.

Weed assessments and seed collection

The number of all weeds (not just common poppy) per plot were counted and recorded on 30/04/14, using 10 x 0.1m^2 quadrats randomly placed per plot. At the same time plots were visually assessed for percentage poppy cover per plot from 0-100% cover. The number of poppy heads per plot were counted on 05/06/14, using 10 x 0.1m^2 quadrats/plot. Poppy seeds were not collected from this field site as the overall poppy numbers were too low.

3.3.3 Economic evaluation of Cambridgeshire field trial

An economic impact evaluation was conducted in 2016 to assess the impact each herbicide treatment used in the field trial (CAMB-M) had in terms of crop yield and herbicide treatment cost. Average wheat yield was calculated (no plot yields were taken due to the high weed burden) as 10.63 tonnes/ ha and average oilseed rape yield was calculated at 3.8 tonnes/ ha (average yearly crop yield per hectare was obtained from AHDB cereals & oilseed website 2016). The AHDB market data centre was used to provide the average price of wheat and oilseed rape for 2015 in East Anglia, the region the field study was conducted. Average feed wheat price per tonne in 2015 was £116 and average oilseed rape price per tonne in 2015 was £265.

A yield reduction calculator (MAFF funded project CE0616, 2001) was used to calculate the yield reduction for each treatment based on the average number of poppy plants per m^2 for each treatment in the CAMB-M field experiments in 2014 and 2015 (3.3.1).

Current 2016 market prices of herbicide products were used to determine the cost of herbicide treatment per hectare.

The average UK yield (wheat 10.63 tonnes, oilseed rape 3.8 tonnes) and percent yield loss per treatment were used to calculate yield per hectare in tonnes for each treatment (Equation 1).

Equation 1: $$Yield\ ha\ per\ treatment = \left(\frac{Average\ UK\ yield}{100}\right) * (100 - \%\ treatment\ yield\ loss)$$

To calculate the price received per hectare of crop for each treatment, the calculated yield per hectare in tonnes for each treatment was multiplied by the average 2015 price per tonne (feed wheat £115, oilseed rape £265). The cost of each herbicide treatment was then taken from the price received to give the profit per hectare (excluding other costs).

3.3.4 Glasshouse experiments: Validation of ALS resistance 2016

A glasshouse pot screen was carried out in January 2016 to assess any shift in resistance status of common poppy seed treated for three years with the same herbicide treatments compared to the baseline field seed and a susceptible standard (Table 15). A total of 20 replicates (individual pots) were used for each treatment (total of 1,120 pots).

Table 15 Seed populations, previous herbicide treatment and year of collection for seed used in glasshouse pot screen 2016.

Seed population and historic treatment	Year of collection
CAMB-W field baseline	2012
CAMB-W container trial Treatment 1	2015
CAMB-W container trial Treatment 2	2015
CAMB-W container trial Treatment 3	2015
CAMB-W container trial Treatment 4	2015
CAMB-M field baseline	2012
CAMB-M container trial Treatment 1	2015
CAMB-M container trial Treatment 2	2015
CAMB-M container trial Treatment 3	2015
CAMB-M container trial Treatment 4	2015
CAMB-M field untreated	2015
Susceptible standard (Scotia seeds)	2014

Seed sowing and thinning

Plastic plant pots (5cm diameter) were filled on 13/01/16 with a standard Kettering loam-based soil (Section 3.1.1) and placed in glasshouse the day before sowing and watered using an automatic overhead watering system. Poppy seed were sown (14/01/16) by sprinkling a very small pinch of seed onto the moist soil surface. Each pot was then covered with a small layer of soil (less than 0.5cm depth). Pots were left in the glasshouse (17ºC, 14 hour days (light) and 11ºC, 10 hour nights (no light)) and watered daily using the automatic overhead watering boom once a day. Poppy plants were thinned to one plant per pot from 10/02/16 to 17/02/16. Many of the populations had poor germination so the total number of replicates for spraying had to be reduced to 12 from the proposed 20 replicates. Two populations had to be removed completely from the trial as too few seeds germinated to carry out a valid test, this included the CAMB-W container T3 and T4 populations.

Herbicide applications

Poppy plants were sprayed at a growth stage of BBCH 14-16 on 23/02/16. Three herbicide treatments and an untreated control were used (Table 16). Herbicides were applied to pots using a Mardrive automated pot sprayer, F110 02 nozzles at a water volume of 200 l/ha. The soil surface was damp, but not wet before spraying and left for a minimum of six hours post-spray application before being watered again from above.

Table 16 Herbicide treatments used in glasshouse pot screen 2016.

Treatment number	Herbicide active ingredient	Product dose
1	Untreated control	
2	Metsulfuron-methyl	30g/ha
3	Imazamox	0.875 l/ha + 1.0 l/ha
4	MCPA	2.0 l/ha

MCPA 50 (MAPP 14908) 500 g /l MCPA
Jubilee SX (MAPP 12203) 200 g/kg metsulfuron-methyl
Imazamox (provided for experimental purposes only, not registered in the UK), DASH HC adjuvant

Assessments

A visual score of each individual plant was done on 04/03/16 and 05/03/16 giving a 0-10 rating (where 10 = live/healthy plants and 0= dead plants). At the same time the above soil fresh weight of each individual plant per pot (g) was assessed by carefully cutting and weighing the plants.

Data analysis

Visual score data

Means and standard errors for each treatment and population were calculated. A three-way ANOVA was used for herbicide treatment, population, and replicate, with an interaction between treatment and population. As replicate was significant (F-value = 1.945, p-value = 0.032) it was included in the analysis. Tukey's HSD analysis was used on the two-way ANOVA analysis to determine which populations, treatments, and population-treatment combination were significantly different from others.

Percent control fresh weight

For each treatment-population-replicate combination fresh weight data was converted into percent control compared to the untreated control treatment. Means and standard errors for each treatment and population were calculated. A three-way ANOVA was used for herbicide treatment, population, and replicate, with an interaction between treatment and population. As there was no significant effect of replicate (F-value = 1.57, p-value = 0.221) this factor was removed and a two-way ANOVA was used to assess the data using treatment, population, and an interaction between treatment and population. Tukey's HSD analysis was used on the two-way ANOVA analysis to determine which populations, treatments, and population-treatment combination were significantly different from others.

3.3.5 Glasshouse screening of other UK broad-leaved weed populations

In an attempt to quantify the number of resistant broad-leaved weed populations in the UK, additional seed testing of identified populations was carried out by ADAS and some of the collaborating agrochemical companies in 2013 to 2015. A total of 42 populations were tested and 22 common poppy, eight common chickweed (*Stellaria media*) and seven scentless mayweed (*Tripleurospermum inodorum*) were confirmed as ALS resistant. The methodology for testing and results were summarised in Tatnell *et al.,* 2016 (8.2).

In addition to the commercial seed samples tested the original susceptible standard seed (S1 2010 + 2011) tested in two glasshouse pot screens (3.1.1 and 3.1.2) and the container experiments in 2013 and 2014 were re-tested as two separate populations (not mixed together as in the other experiments listed) due to concerns in the results obtained against the ALS-inhibitor herbicides. The methodology for testing was identical to the Tatnell *et al.*, 2016 paper.

4 Results

4.1 Pot screen dose response experiments

4.1.1 Cereal herbicide treatments

The mean percentage reduction from the untreated control for the cereal herbicide tested, against the three poppy seed populations, in a glasshouse pot screen are presented in Table 17. There was some variation in control against the two ALS-resistant poppy populations tested (CAMB-M and CAMB-W) and generally good consistency with the susceptible standard. The CAMB-M poppy population was effectively controlled by MCPA 50 at the field rate (77% control) and double field rate (100%). A rate of half and quarter field rate of MCPA 50 gave less than 50% control, as would been expected. Jubilee SX (Metsulfuron-methyl) gave more variable control levels at the different rates against CAMB-M, with only 20.7% control at full field rate, however 29.7% control achieved at half field rate. Results for Atlantis (mesosulfuron-methyl + iodosufluron-methyl-sodium) were also variable in the dose response, with less than 15% control at full field rate, 25% control at double field rate and 17% and 16% control from half and quarter field rate respectively. The CAMB-W population was generally better controlled by all herbicides in this particular screen, with 41% control from full rate Jubilee SX, 35% full rate Atlantis and 96.5% full rate MCPA 50. All herbicides tested provided over 87% control of the susceptible standard poppy population tested, with 97% control from field rate Jubilee SX and Atlantis. Even a quarter field rate of Jubilee SX and Atlantis provided 87% and 96% control of the standard respectively.

Table 17 The percentage reduction from the untreated control for three seed populations tested against three herbicides commonly used in cereals.

Treatment	Herbicide product	Dose	Seed population % reduction from UTC		
			CAMB-M	CAMB-W	Susceptible
2	Metsulfuron-methyl	60g/ha	6.55	56.22	97.53
3	Metsulfuron-methyl	30g/ha	20.72	41.10	97.13
4	Metsulfuron-methyl	15 g/ha	29.74	23.56	89.58
5	Metsulfuron-methyl	7.5 g/ha	-1.30	27.28	87.27
6	MCPA	4.0 l/ha	100.00	99.94	100.00
7	MCPA	2.0 l/ha	77.02	96.53	87.30
8	MCPA	1.5 l/ha	41.10	77.95	44.09
9	MCPA	0.75 l/ha	10.43	52.87	11.18
10	Mesosulfuron-methyl + iodosufluron-methyl-sodium *	0.8 kg/ha	24.67	56.12	98.83
11	Mesosulfuron-methyl + iodosufluron-methyl-sodium *	0.4 kg/ha	14.67	35.12	97.32
12	Mesosulfuron-methyl + iodosufluron-methyl-sodium *	0.2 kg/ha	17.06	29.99	98.49
13	Mesosulfuron-methyl + iodosufluron-methyl-sodium*	0.1 kg/ha	16.06	29.28	96.49

**plus Biopower @1.0l/ha. Grey highlighted row is full product dose*

MCPA 50 (MAPP 14908) 500 g /l MCPA
Atlantis® WG (MAPP 12478) 30 g/kg mesosulfuron-methyl and 6 g/kg iodosulfuron-methyl-sodium
Jubilee SX (MAPP 12203) 200 g/kg metsulfuron-methyl

4.1.2 Oilseed rape herbicide treatments

Results for the glasshouse pot screen for three of the oilseed rape herbicides tested are shown in Table 18. Treatments 10-13 containing Metazachlor alone were removed as the susceptible standard was not controlled (<10%) in this experiment by full or double field rate suggesting there was an error with this treatment application and results were not valid. Cleranda (Imazamox + metazachlor) at full field rate provided 4% control of the CAMB-M population, which was lower than expected, 24% control of the CAMB-W population and 70% control of the susceptible standard. Imazamox alone (not commercially available in the UK, but included for experimental purposes allowing the ALS-component of Cleranda to be tested alone) provided a higher level of control achieving 38% control of CAMB-M, 44% of CAMB-W and 84% of susceptible standard. Astrokerb (Propyzamide + aminopyralid) provided a good level of control for all poppy populations tested at full field rate and above, with 86% control of CAMB-M, 93% control of CAMB-W and 73% control of the susceptible standard. Good levels of control (>62%) was also achieved by Astrokerb at half field rate on all poppy populations tested.

Table 18 The percentage reduction from the untreated control for three seed populations tested against three herbicides used in oilseed rape.

Treatment	Herbicide product	Dose	Seed population % reduction from UTC		
			CAMB-M	CAMB-W	Susceptible
2	Imazamox*	1.75 l/ha	31.94	43.22	94.15
3	Imazamox*	0.875 l/ha	37.86	43.71	84.00
4	Imazamox*	0.437 l/ha	-11.50	17.52	61.52
5	Imazamox*	0.219 l/ha	24.10	12.95	43.77
6	Imazamox + metazachlor*	4.0 l/ha	44.17	55.46	79.67
7	Imazamox + metazachlor *	2.0 l/ha	3.63	23.71	69.82
8	Imazamox + metazachlor *	1.0 l/ha	-4.95	13.31	47.37
9	Imazamox + metazachlor *	0.5 l/ha	-14.95	38.09	46.11
14	Propyzamide + aminopyralid	3.0 l/ha	83.76	96.29	95.74
15	Propyzamide + aminopyralid	1.5 l/ha	86.04	93.13	73.27
16	Propyzamide + aminopyralid	0.75 l/ha	62.99	85.17	62.14
17	Propyzamide + aminopyralid	0.375 l/ha	18.71	36.63	8.41

**plus DASH @ 1.0 l/ha. Grey highlighted row is full product dose*

Imazamox (provided for experimental purposes only, not registered in the UK)
Cleranda MAPP 15036 17.5g/l imazamox and 375g/l metazachlor, DASH HC adjuvant
Butisan MAPP 16569 5001g/l metazachlor
Astrokerb MAPP 16184 500g/l propyzamide and 5.3 g/l aminopyralid

4.2 Container-based experiments

4.2.1 Percent control head count data

Percent control for number of poppy heads varied between a maximum of 100% and a minimum of -77% control, with T8 (ALS alone), CAMB-M in 2014 (Figure 1, Table 19) providing the lowest control. ANOVA analysis showed that poppy population was a significant factor in all years of the container study (Table 20) with Tukey's HSD analysis showing that the S1 population significantly varied from CAMB-W and CAMB-M in 2013 and 2014 (p<0.001) and that CAMB-M significantly varied from S1 and CAMB-W in 2015. Across all years combined only S1 and CAMB-W varied significantly (p<0.01). This suggests that differences between populations can significantly affect control provided by herbicide treatment, even where all populations are resistant to the same mode of action.

Treatment was a significant factor in each year and across all years combined (Table 20) with treatments containing a pre-emergence herbicide (T3, 4, 7, 9, 10, and 13) giving better poppy control across all years and all populations when containers were not ‘in fallow’ (T9, T10, T12, and T13 in 2015) (Figure 1, Table 19) (NB. T6 and T12 received a pre-emergence herbicide in 2013 and 2015, but not 2014). T4, and T7 (non-ALS) consistently provided the highest control across all years. T3 (non-ALS + ALS) also provided good control in 2013 and 2014, but control decreased in 2015 for populations CAMB-W and CAMB-M, suggesting that the control was provided by the non-ALS pre-emergence herbicide, which did not provide as high control in 2015. Treatments that only used post-emergent ALS inhibitor herbicides (T2, 5, 8, and 11) did provide limited control for some populations in some years (Figure 1) showing that a small proportion of some ALS-resistant populations may still be sensitive to ALS inhibitors. However, the proportion of sensitive individuals is not high enough to provide sufficient control, showing that other herbicide modes of action are needed.

There was a significant interaction between population and treatment in 2013 and 2015, but not in 2014 and not across all years combined (Figure 1, Table 20), suggesting that population can significantly affect the control provided by a treatment, but that this interaction can vary between years and may depend on the type of treatment applied, as treatments conditions also varied between years, for example in 2014 where there was no interaction between population and treatment oilseed rape herbicide treatments were included.

Treatment year was also significant (F-value 34.423, p-value <0.001), suggesting that control varied between the crop rotation type simulated. However, variation could also be explained by natural seasonal changes during a four year project.

Table 19 Herbicide treatment list of poppy containers across all three treatment years (2013-2015)

Treatment number	Herbicide treatment		Crop- *no crop present but herbicide selection to mirror field treatments*		
	Pre-emergence	Post-emergence	Year 1	Year 2	Year 3
1	Untreated control		-	-	-
2	-	ALS	W. Wheat	W. Wheat	W. Wheat
3	Non-ALS	ALS	W. Wheat	W. Wheat	W. Wheat
4	Non-ALS	Non-ALS	W. Wheat	W. Wheat	W. Wheat
5	-	ALS	W. Wheat	W. OSR	W. Wheat
6	Non-ALS	ALS	W. Wheat	W. OSR	W. Wheat
7	Non-ALS	Non-ALS	W. Wheat	W. OSR	W. Wheat
8	-	ALS	W. Wheat	W. Wheat	Fallow
9	Non-ALS	ALS	W. Wheat	W. Wheat	Fallow
10	Non-ALS	Non-ALS	W. Wheat	W. Wheat	Fallow
11	-	ALS	W. Wheat	W. OSR	Fallow
12	Non-ALS	ALS	W. Wheat	W. OSR	Fallow
13	Non-ALS	Non-ALS	W. Wheat	W. OSR	Fallow

This table is the same as Table 3, but has been added in the results section to assist with the treatment detail as described in Figure 1 and Figure 3.

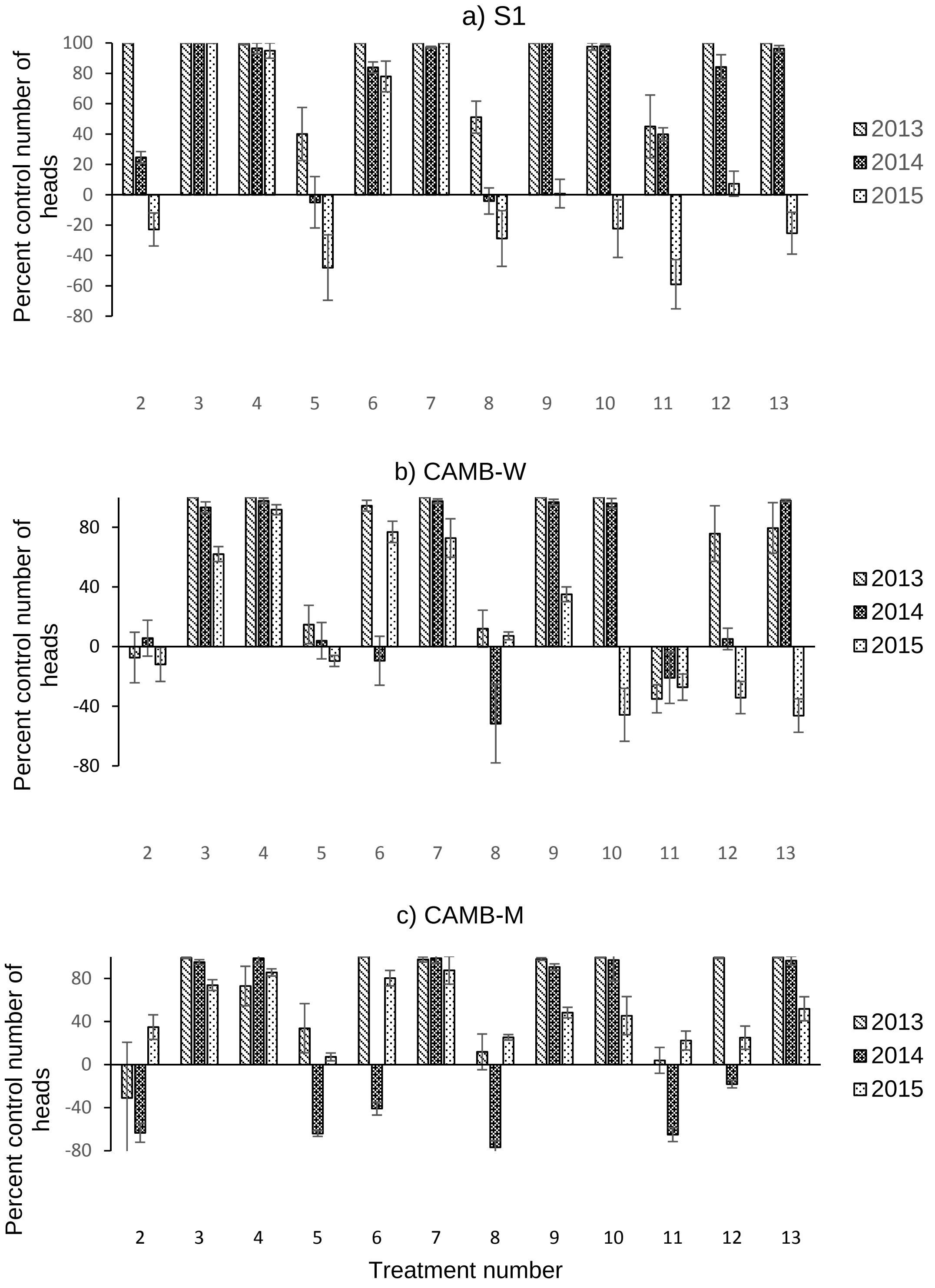


Figure 1 Percent control compared to untreated of poppy **head count** data across 12 herbicide treatments for three populations (a) S1 (b) CAMB-W (c) CAMB-M over 3 years. Treatments listed in.

Table 20 Percent control poppy head number in container-based experiments. Three way ANOVA analysis with interaction between population and herbicide treatment across three separate years (2013, 2014, and 2015) and four-way ANOVA analysis across all years combined.

Year	**Population**		**Treatment**		**Block**		**Population: Treatment**	
	F-value	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
2013	24.0	<0.001	50.3	<0.001	0.64	0.59	6.47	<0.001
2014	11.3	<0.001	17.2	<0.001	6.54	<0.001	1.27	0.212
2015	49.5	<0.001	61.3	<0.001	3.89	0.011	6.06	<0.001
All years	5.32	0.005	31.2	<0.001	2.87	0.036	1.36	0.312

The SSS susceptible population gave 97% control by T2 (ALS alone) and 100% control by T3 (non-ALS + ALS) in 2015, confirming that it is a herbicide susceptible population (Figure 2). Tukey's HSD analysis showed that there was significant difference for S1, CAMB-W and CAMB-M for T2 (ALS alone) when compared to SSS (all populations p<0.001) (Figure 2) confirming that all three experimental populations (including S1 that was purchased as a susceptible standard!) were resistant to ALS inhibitors after undergoing ALS only herbicide treatments for 2 generations.

There was no significant difference for S1 (p= 1) and CAMB-M (p=0.2) compared to SSS for T3 (non-ALS + ALS), but there was a significant difference in percent control between CAMB-W and SSS for T3 (p=0.018) (Figure 2).

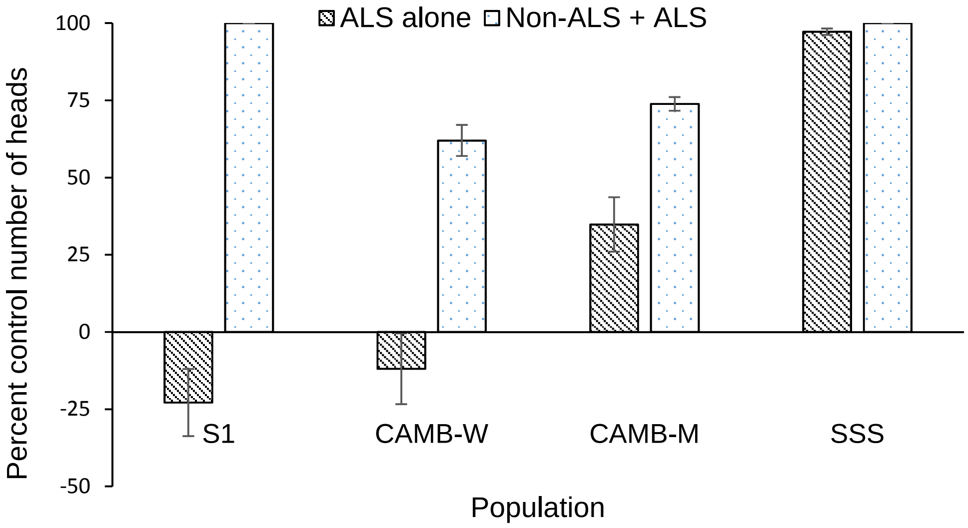


Figure 2 Percent control of poppy heads against three ALS-resistant and one susceptible population to an ALS alone and Non-ALS + ALS herbicide treatment. Mean in final year.

4.2.2 Percent control plant count data

In 2013 and 2014 percent control of plant counts ranged from a maximum of 100% to a minimum of -45%, with T5 (ALS alone) CAMB-W in 2014 having the lowest percent control (Figure *3*). ANOVA analysis showed that population was a significant factor in all years and in 2013 and 2014 combined (Table 21). In 2013 and 2014 the S1 population significantly varied from CAMB-W (2013: p<0.001, 2014: p=0.035) and CAMB-M (p<0.001) and both years combined (p<0.001). Again showing that population significantly affects herbicide control.

Treatment was also a significant factor in all years and in 2013 and 2014 combined (Table 21). Treatments that received a pre-emergence herbicide (T3, 4, 7, 9, 10, and 13) had the highest control in 2013 and 2014 (Figure *3*). Treatments that only used ALS-inhibitor herbicides post-emergence did not provide control for CAMB-W and CAMB-M in either 2013 or 2014, and provided very little control for the S1 population (Figure *3*).

In 2015, when plant counts were taken before the application of post-emergent herbicides, treatments with a pre-emergent herbicide application (T3, 4, 6, and 7) had the highest control (Figure 4). However, it was not as high as the control provided by pre- and post-emergent herbicide application (Figure *3*, Figure 4). This shows that although pre-emergent herbicides can provide good control of ALS-resistant poppy populations, pre-emergence herbicides alone do not provide sufficient control and the most effective control is a combination of pre- and a post-emergent non-ALS herbicides.

There was also a significant interaction between treatment and population in 2013 and 2015, but not in 2014 (OSR herbicides), suggesting that population can influence the effect of a treatment, but that the effect varies with treatment type. Treatment year was also significant (F-value 95.312, p-value <0.001), suggesting that control varied between the crop rotation type simulated by the use of herbicides specific to wheat or oilseed rape crops.

Table 21 Percent control poppy plant number in container experiments. Three way ANOVA analysis with interaction between population and treatment across three separate years (2013, 2014, and 2015) and four-way ANOVA analysis across 2013 and 2014 combined.

Year	Population		Treatment		Block		Population: Treatment	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
2013	26.0	<0.001	37.5	<0.001	3.53	0.017	4.86	<0.001
2014	11.3	<0.001	17.2	<0.001	6.54	<0.001	1.27	0.212
2015	18.2	<0.001	25.9	<0.001	9.48	<0.001	8.68	<0.001
2013 & 14	23.1	<0.001	43.0	<0.001	1.62	0.184	1.93	0.009

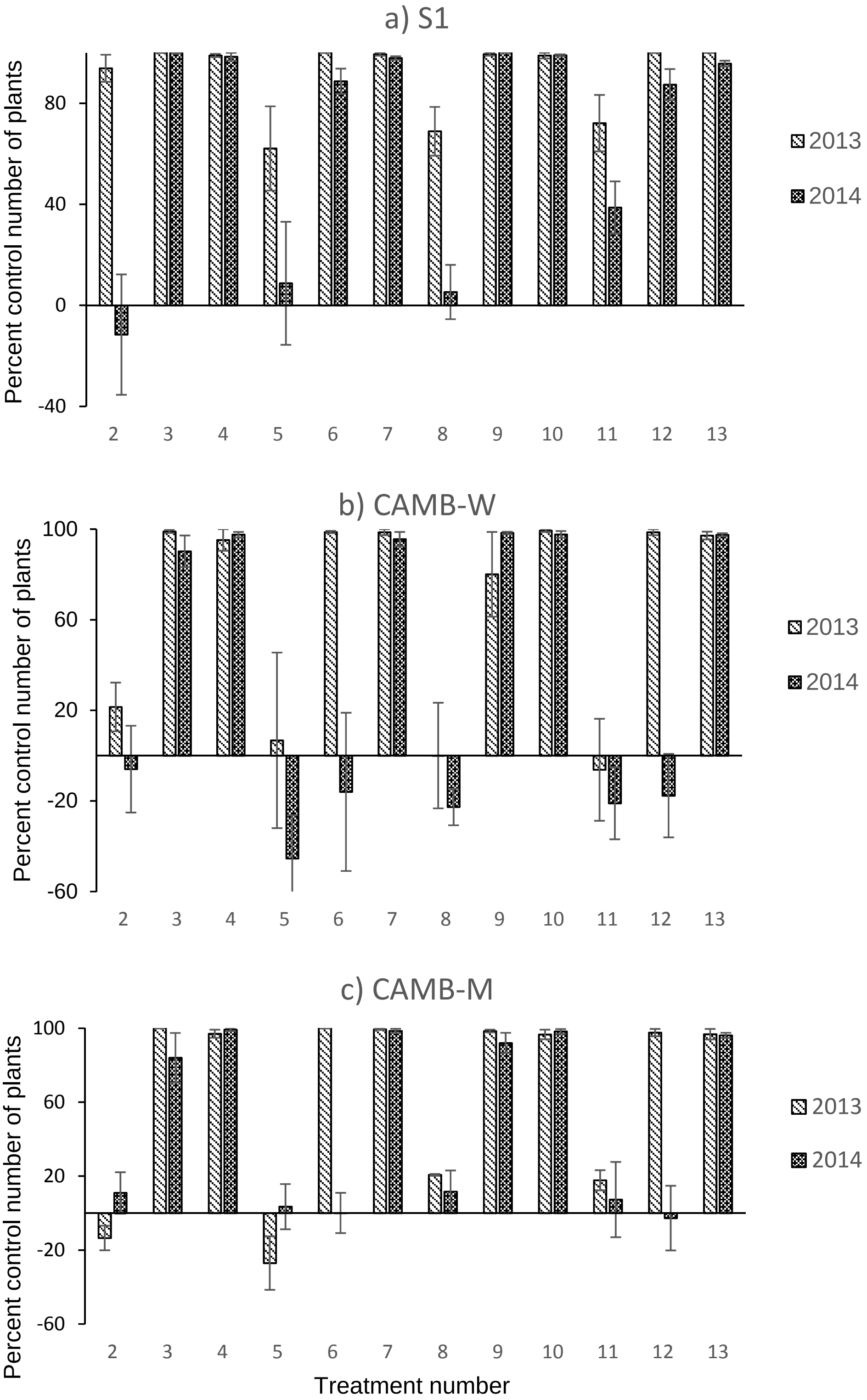
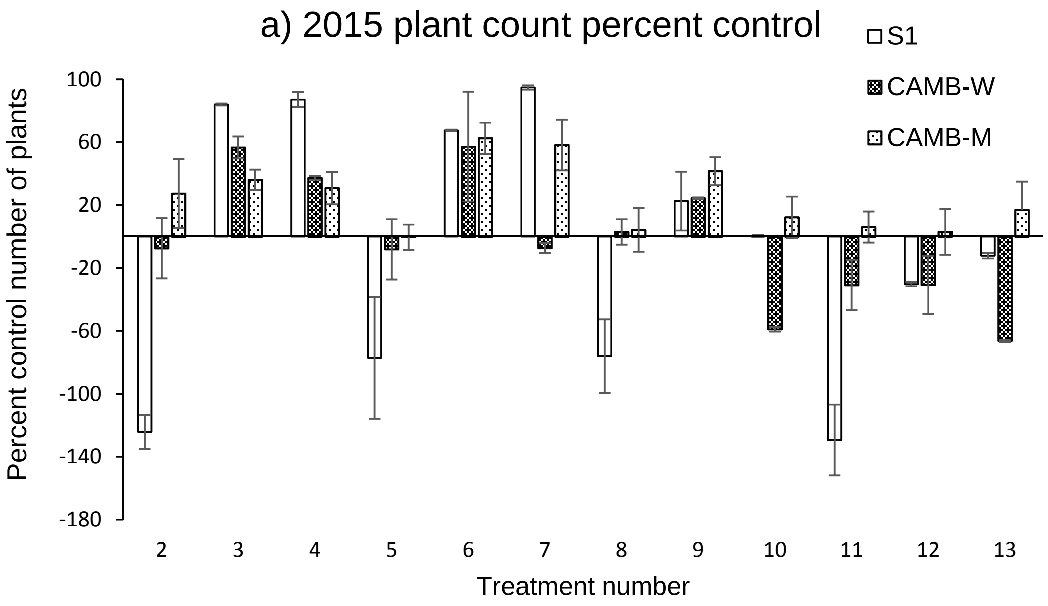


Figure 3 Percent control compared to untreated of poppy **plant counts** across 12 herbicide treatments for three populations (a) S1 (b) CAMB-W (c) CAMB-M over 2 years. Treatment details listed in *Table 19*.


a) 2015 plant count percent control

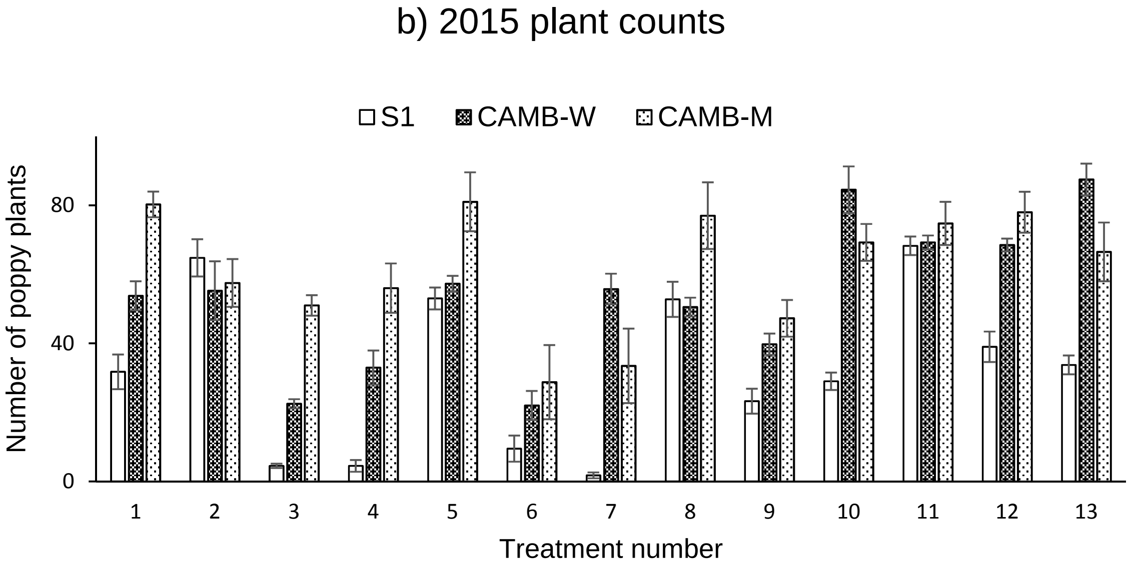

b) 2015 plant counts

Figure 4 Poppy plant count data in container experiments in 2015 across 12 herbicide treatments for three populations. Treatments listed in Table 19 (a) Percent control compared to untreated of poppy plant count data and (b) plant counts.

4.3 Field-based experiments

4.3.1 Cambridgeshire field site

The spring of 2013 was very cold and so the post-emergence herbicide application was not applied until 01/05/13, when the temperature finally increased and the weeds were actively growing again. The winter wheat crop establishment was good and there was a very high natural population of poppy on this field, with a mean of 374 heads per m^2. The mean number of poppy heads per m^2 for each herbicide treatment after one year of the three-year field experiment are presented in Figure 5. The ALS-treatment alone (metsulfuron-methyl) gave no significant control of the poppies compared to the untreated control. However, treatments containing a non-ALS + ALS and a non-ALS alone both provided a significant ($p < 0.001$) level of poppy control of 96% and 98% respectively, compared to the untreated. There was no significant difference between these latter two treatments in year one.

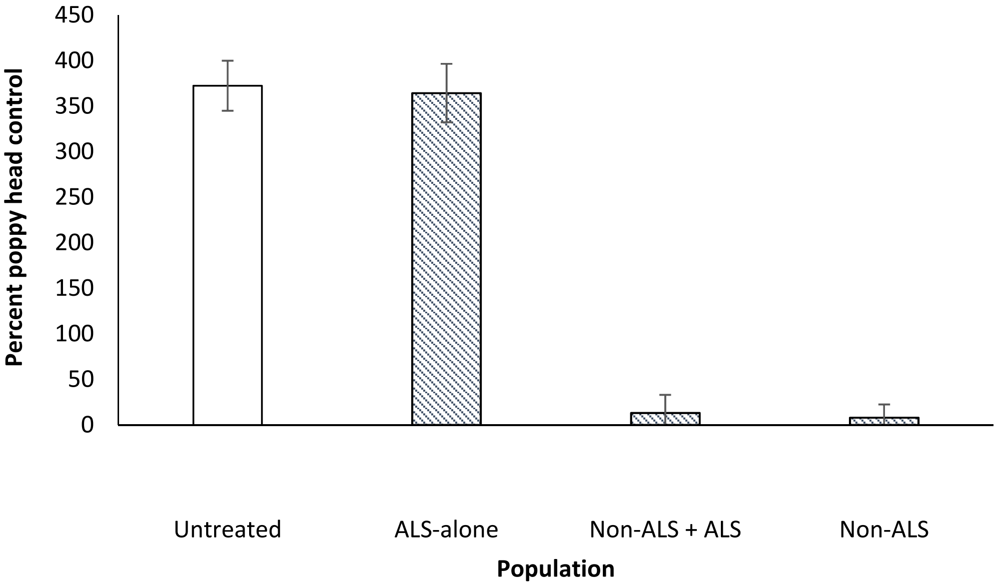


Figure 5 The mean number of poppy heads per m^2 in the CAMB-M field trial in 2013, after one year of herbicide treatments.

Other key weed species present in the CAMB-M field site included mayweed (*Tripleurospermum inodorum*), Ivy-leaved speedwell (*Veronica hederifolia*), black-bindweed (*Fallopia convolvulus*) and charlock (*Sinapsis arvenis*), however all were recorded in year one below 11 plants per m^2, so common poppy was the dominant weed on this field.

Year two of the CAMB-M field experiment included winter wheat and oilseed rape sown within the same field trial location as year one (see section 3.3.1 for detail). There was very good establishment of the oilseed rape and wheat crops and field conditions were very favourable for the autumn pre-emergence herbicides, and autumn post-emergence herbicides in oilseed rape and spring post-emergence herbicides in winter wheat. The results for the mean number of poppy heads per m^2 for all herbicide treatments in year two are presented in Figure 6. The mean number of poppy heads was always lower in the oilseed rape cropped areas (mean untreated, 252 heads per m^2) compared to the wheat (mean untreated, 537 heads per m^2). The ALS-alone herbicide treatment achieved no control compared to the untreated for both wheat and oilseed rape, with significantly ($p>0.001$) more poppy heads per m^2 in wheat and a non-significant increase, compared to the untreated control, in oilseed rape.

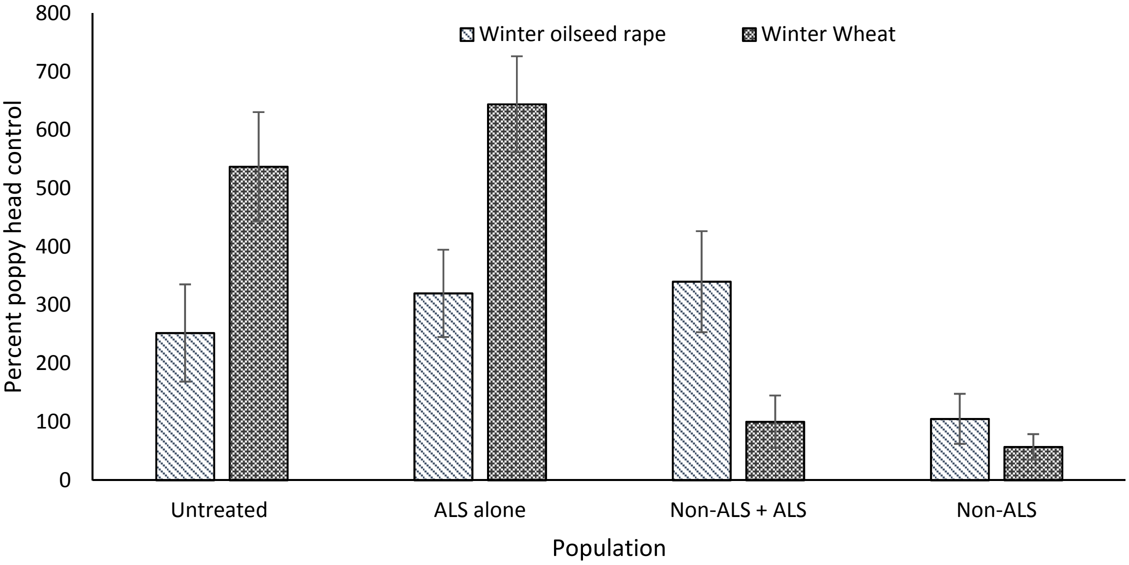


Figure 6 The mean number of poppy heads per m^2 in the CAMB-M field trial in 2014, after two years of herbicide treatments and varied crop rotation.

The oilseed rape non-ALS + ALS herbicide treatment (Cleranda) achieved no control of the CAMB-M field population, with a mean of 340 poppy heads per m^2, which was more than the ALS-alone treatment of 320 poppy head per m^2. The Non-ALS treatment in oilseed rape, Butisan (metazachlor) followed by Astrokerb (propyzamide + aminopyralid) gave a significant ($p>0.001$)

reduction in poppy heads (105 per m^2 remaining) compared to the untreated control, however this is still a large amount of poppy heads remaining in the crop that would set seed.

The wheat non-ALS + ALS treatment Crystal (pendimethalin + flufenacet) followed by Jubilee SX and the non-ALS treatment (Crystal followed by MCPA) both achieved a significant ($p>0.001$) reduction in poppy heads per m^2 compared to the untreated of 81.4% and 89.4% control respectively. This was slightly below (approximately 10%) the control level from year one of the experiment with the same herbicide treatments.

The third experimental year provided many challenges! Due to the farm spray errors it was very difficult to fully understand where the overspray area had actually been as the area described did not appear to have had any herbicide inputs as the weed populations were extremely high by spring 2015. A double drilled area was also observed on the bottom corner of block four. Headcounts were done in three blocks (two-four), but only data from block three were valid for summarising. Overall control of poppies was good, with the number of poppy heads reduced to under 150 plants per m^2 in the plots that had had winter wheat for three years and to under 106 heads per m^2 in plots that had had a wheat, oilseed rape, wheat rotation. The untreated control plots were infested with loose silky-bent grass (*Apera spica-venti*) in year three, resulting in natural competition with the poppies. It was also observed that plots that had had a non-ALS programme for the three years had increased levels of scentless mayweed, which was very visible in June when the weed was flowering and white plots could be clearly seen.

4.3.2 Yorkshire field site

The poppy numbers were generally very low at this field site compared to the Cambridgeshire site.

Mean percentage poppy cover varied from 18% to 0% (Figure 7) and mean control of other weed species varied from 7% to 100%, with groundsel treated with the non-ALS herbicide having the poorest percent control (Figure 8).

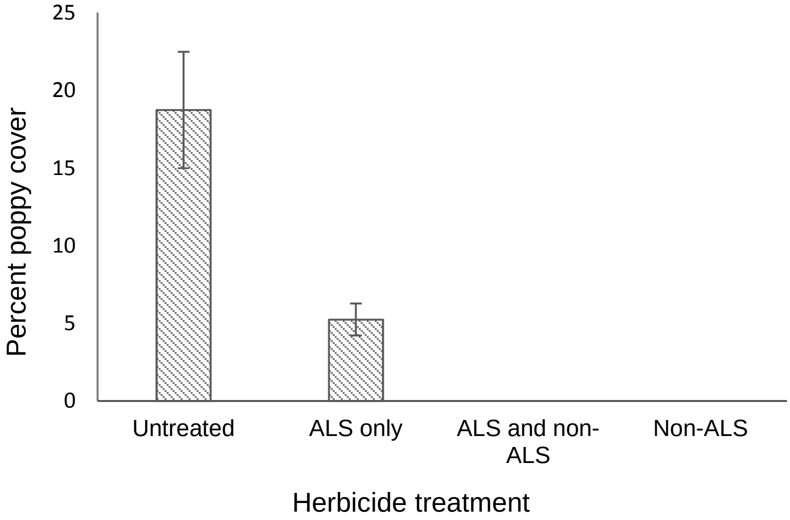


Figure 7 Poppy percent cover in Yorkshire field experiment 2013-14 across 3 herbicide treatments (see Table 14 for treatment detail).

The ALS-alone treatment reduced the percentage poppy cover compared to the untreated control (Figure 7), however it only provided 26.3% control of poppy heads per m^2 (Figure 8). Other broad-leaved weeds present were well controlled by the ALS-alone treatment, including creeping thistle (*Cirsium arvense*), field pansy (*Viola arvenis*), groundsel (*Senecio vulgaris*), and scented mayweed (*Matricaria recutita*). Poppy control from the ALS + non-ALS treatment and non-ALS treatment did not differ in this experiment, which supports the results from the Cambridgeshire field experiment. The non-ALS treatment also controlled creeping thistle, field pansy, and scented mayweed, but did not control groundsel (Figure 8), showing that including an ALS-herbicide treatment will sometimes still be necessary to control other broad-leaved weeds.

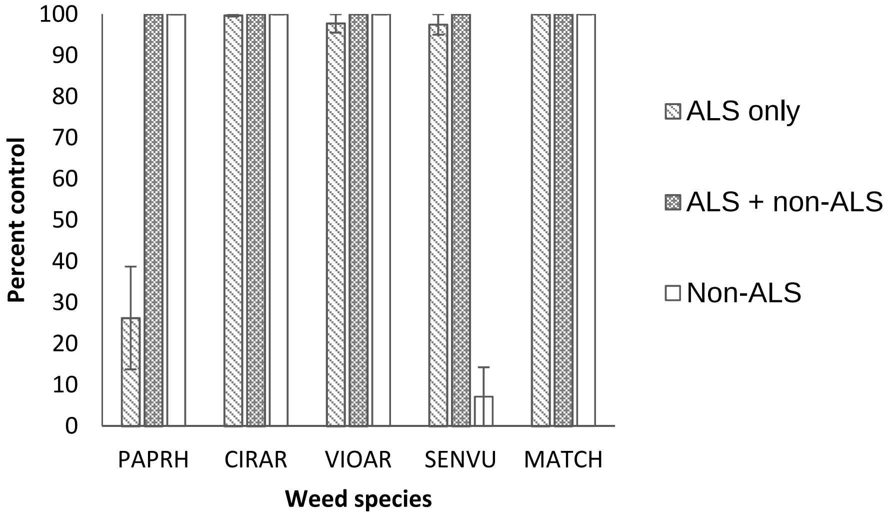


Figure 8 Percent weed control compared to untreated control treatment in Yorkshire field experiment 2013-14, across 3 herbicide treatments. Treatments details in Table 14

4.3.3 Economic evaluation of Cambridgeshire field trial

The poppy population assessed in the Cambridgeshire field trial was ALS-resistant resulting in little to no control from ALS herbicide application, an economic evaluation of an ALS-susceptible population would produce a different output, as ALS herbicide applications would provide better control. This economic evaluation excludes other costs related to producing a crop, for example the cost of labour, other pesticides, and seeds, and only focuses on costs and margin relating to herbicide application and efficacy of poppy control from the specific herbicides tested.

The most expensive treatment was the non-ALS + ALS treatment at £57.00/ha in wheat and £65.50/ ha in oilseed rape, followed by the non-ALS only treatment at £55.80/ ha in wheat and £60.30/ ha in oilseed rape. The ALS only treatment was £9.00/ ha for wheat and £25.00/ ha for oilseed rape and for both crops the untreated cost was £0/ha.

Applying an ALS only treatment to the ALS-resistant poppy population resulted in an economic loss in wheat with 100% yield loss in both 2014 and 2015 and a herbicide cost of £9/ha (Figure 9 a & b). Yield loss in oilseed rape in the ALS only treatment was lower at 64% due to increased crop competition from the oilseed rape, but due to the cost of herbicide application at £25/ha there was a lower margin compared to the untreated (Figure 9 c).

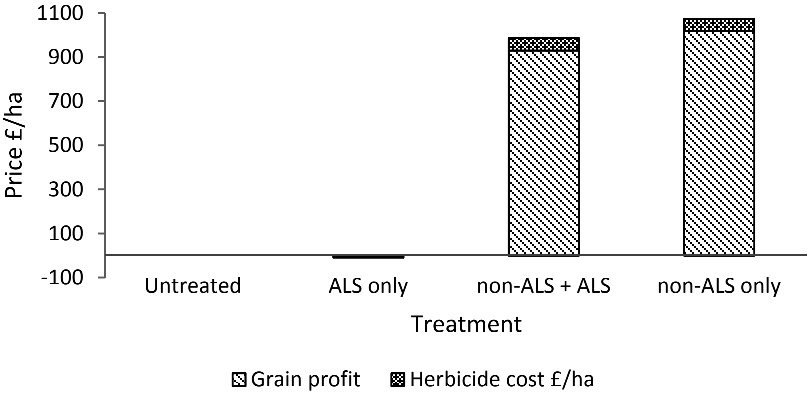
Despite having the second highest herbicide costs per hectare the most profitable treatment in both wheat and oilseed rape was the non-ALS only treatment, with low yield loss and therefore an increased margin per hectare compared to the other treatments (Figure 9 a-c). The low yield loss in the non-ALS only treatment was due to high poppy control as a result of using two herbicide modes of action that this population was not resistant to.

The non-ALS + ALS treatment resulted in higher control compared to the untreated and ALS only treatment in wheat, but compared to the non-ALS only treatment yield loss was 7-15% higher and herbicide price was £1.20/ ha more. This difference resulted in a £88-186 difference per hectare, again showing that in this particular situation applying ALS herbicides to ALS-resistant poppy populations was not economically practical, even when partnered with other modes of action (Figure 9 a & b). However, it must be considered that these calculations have been based on the poppy population only and it does not take into account the effect these herbicide treatments are having on other weed species present.

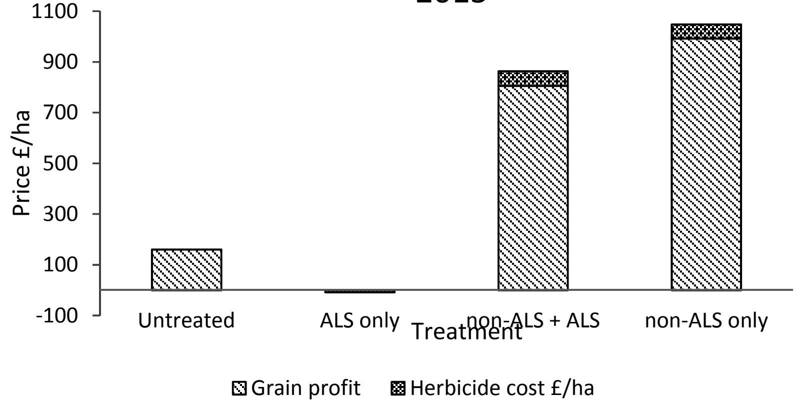
In oilseed rape the difference in profit per hectare between the non-ALS only and non-ALS + ALS treatment was £482. However, of all the treatments the yield loss was highest in oilseed rape in the non-ALS + ALS treatment (Figure 9c), which may have been a result of the seasonal weather and the size of the weed at application being above the ideal growth stage for effective control.

These results show that applying an ALS-herbicide to an ALS-resistant poppy population results in an economic cost due no effect on yield loss and the cost of the herbicide.

a) Wheat grain margin / herbicide cost 2014



b) Wheat grain margin/ herbicide cost 2015



c) OSR grain margin / herbicide cost 2015

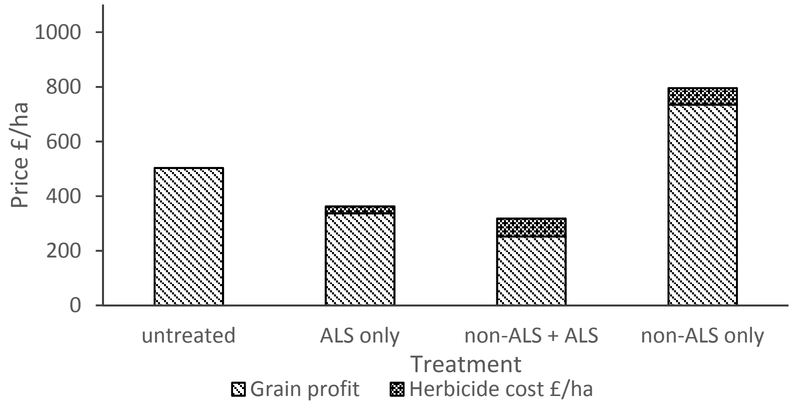


Figure 9 Estimated grain margin of wheat (a & b) and oilseed rape (c) and herbicide treatment cost per hectare of a field trial in Cambridgeshire with an ALS-resistant poppy population. Costs estimated are only those related to herbicide treatment.

4.3.4 Glasshouse experiments: seed validation tests

Visual control

Mean visual control varied from 0 (dead plants) for MCPA herbicide treatments to 10 (healthy plants) for untreated controls. Poppy population CAMB-M T2 (metsulfuron-methyl) had the lowest visual control (9.8). The susceptible population (SSS) was controlled with all herbicide treatments (Figure 10). Population (F-value = 17.64, p-value = <0.001), herbicide treatment (F-value = 618.9, p-value = <0.001) and replicate (F-value = 1.95, p-value = 0.032) were all significant factors, and there was a significant interaction between population and treatment (F-value = 8.1, p-value = <0.001).

The level of control using metsulfuron-methyl was poor for all populations and historical herbicide treatments (i.e. from seed collected from plants treated annually during the project), except for the standard susceptible SSS (Figure 10). Tukey's HSD test showed that all population and historical herbicide treatments had significantly higher visual scores compared to the susceptible SSS population (p-value CAMB-M T1 = 0.035, p-value for all other populations and historical treatments <0.001).

Although control using imazamox was also poor, there was more variation in control between populations and treatment herbicide histories (Figure 10). Control using imazamox for CAMB-M T1 (p-value = 1) was not significantly greater than control of the susceptible SSS population, but for all other populations and historical herbicide treatments control using imazamox was significantly less than that of SSS (p<0.001 for all). MCPA provided the highest visual control across all population and historic treatments, with no significant difference in control for any population compared to the susceptible SSS (p=1 for all).

Visual control using imazamox for CAMB-M T1 (p-value <0.001) and CAMB-M T4 (p-value <0.001) was significantly lower than that of CAMB-M baseline, which the populations were derived from and should therefore have had similar levels of resistance.

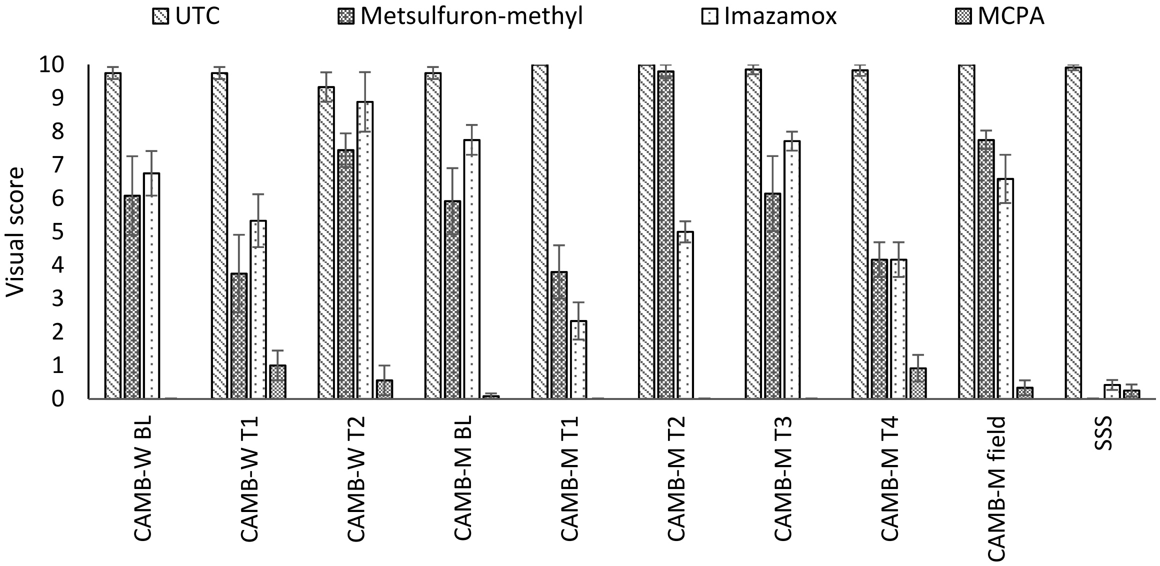


Figure 10 Poppy visual scores against three herbicide treatments and an untreated control (UTC), of two poppy populations (CAMB-M and CAMB-W) baseline (2012 seed) after four different herbicide selection treatments in the field and CAMB-M 2015 field seed, and a susceptible population (SSS).

Percent control fresh weight

Mean percent control for fresh weight varied from -38.6% to 99.4%, with CAMB-M T2 the least well controlled with metsulfuron-methyl and SSS most controlled with MCPA. Again the susceptible population SSS was well controlled in all treatments (Figure 11). There was significant variation between populations (F-value = 8.557, p-value = <0.001) and treatments (F-value = 72.752, p-value = <0.001), and there was a significant interaction between treatment and population (F-value = 2.968, p-value = <0.001).

Tukey's HSD test showed that 5 poppy seed populations and historic herbicide treatments had significantly lower control compared to the susceptible SSS population when treated with metsulfuron-methyl, and 4 had significantly lower control when treated with imazamox (Figure 11).

There were high levels of ALS-resistance (as detected by the metsulfuron-methyl and imazamox glasshouse treatments) in CAMB-W baseline, CAMB-W T2 (ALS only for three years), CAMB-M baseline, CAMB-M T2 (ALS only for three years), CAMB-M T3 (ALS and non-ALS for three years) and CAMB-M field 2015 (ALS alone for two years).

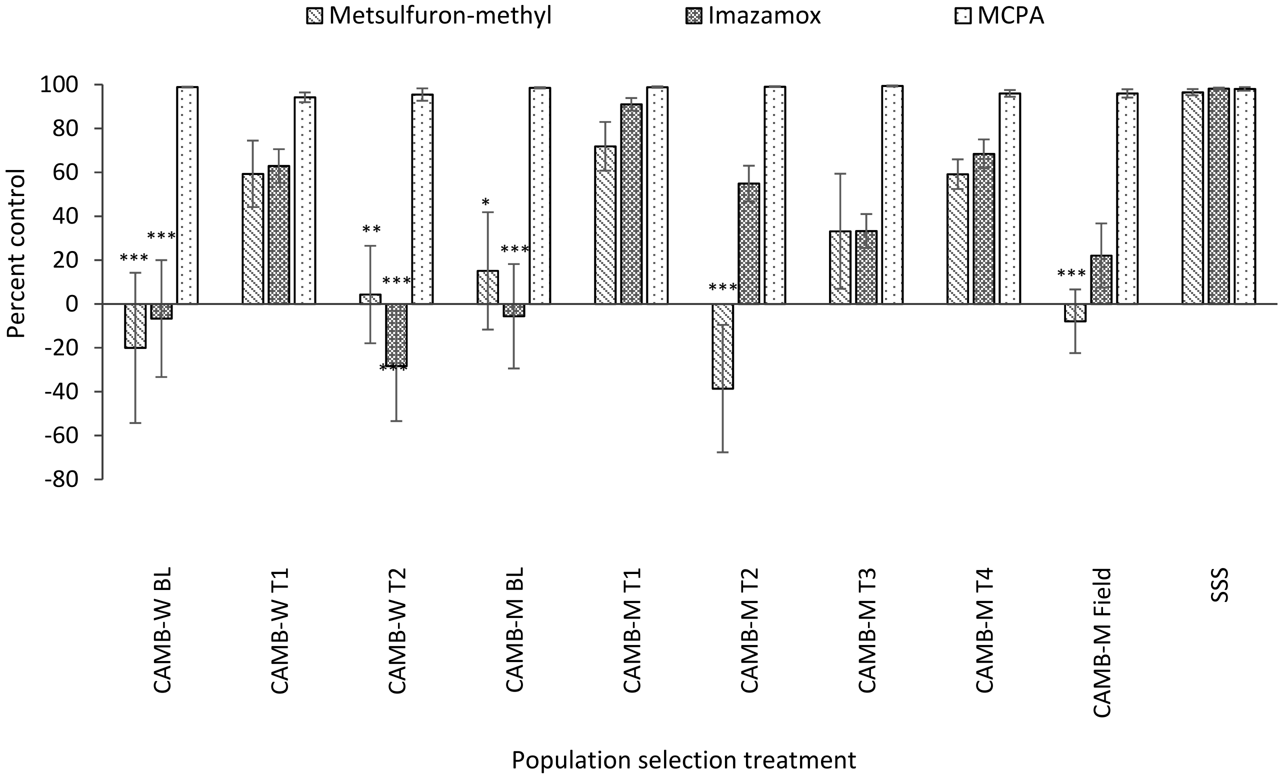


Figure 11 Poppy percent control at three herbicide treatments (metsulfuron-methyl, imazamox, and MCPA) of two poppy populations (CAMB-M and CAMB-W) baseline and after four ALS selection treatments, and susceptible population (SSS). Control significantly different from SSS treatment (p<0.05, ** <0.01, *** <0.001).*

Interestingly, for both CAMB-W and CAMB-M T1 (untreated controls for three years) the level of control obtained using metsulfuron-methyl and imazamox was higher than both baseline populations. The seed for T1 was derived from the baseline populations and sown and untreated for three experimental years and should have therefore had a similar level of resistance. All populations and historic treatments were susceptible to MCPA, with good control and no populations significantly different to SSS.

4.3.5 Glasshouse screening of susceptible standard

The original susceptible standard seed (S1 2010 + 2011) tested in two glasshouse pot screens (3.1.1 and 3.1.2) and the container experiments in 2013 and 2014 were re-tested as two separate populations (not mixed together as in the other experiments listed) due to concerns in the results obtained against the ALS-inhibitor herbicides. Both seed populations were confirmed to have ALS resistance, as when tested by metsulfuron-methyl the 2010 population achieved -26.3%

control and the 2011 population 36.4% control compared to the untreated. A new susceptible standard was included in the same test and achieved 84.2% control from metsulfuron-methyl, compared to the untreated. This result shows the importance of knowing the source of seed in weed testing programmes and a confirmation of the resistance or susceptible status of those seed must be confirmed in advance of large experiments commencing.

5 Discussion

A combination of field and container experiments proved to be an effective approach in determining resistance development and understanding management options for common poppy, as a model species for broad leaved weed ALS target site resistance. A limited number of field sites are available for conducting experiments on broad-leaved weed resistance on the scale required and are higher risk and more challenging in terms of loss of sites due to spray errors, changing land ownership, adverse weather etc. Container- based experiments provide a robust and more secure support option, additionally allowing a greater number of populations to be tested.

Results from the field experiments support the container experiment findings and provide further evidence that poppy populations resistant to ALS inhibitors can be controlled using other herbicide modes of action. It is therefore vital that effective alternative modes of action are available as they are an extremely important tool for managing resistant weed populations and ensuring growers have options available to plan weed control strategies across a crop rotation to manage or prevent resistance. This project has only explored an autumn drilled wheat and oilseed rape rotation. However many fields where broad-leaved weed resistance problems have been reported are often located in areas and with soil types favourable to more diverse crop rotations, including potatoes, sugar beet and other spring crops which all have an additional cultural benefit in successful resistance management strategies.

The results from the initial glasshouse pot screens provided a useful assessment of the different poppy populations used throughout the project, aiding the interpretation of results from both the field and container experiments. The two populations of common poppy (CAMB-M and CAMB-W) were poorly controlled by the ALS-Inhibitor herbicides as was expected, as they were known to already contain a level of ALS-resistance, but non-ALS herbicides were able to control both populations.

Results from container and field trials were generally supportive of the initial pot screen, with poor control of resistant populations by ALS herbicides but good control with non-ALS and ALS + non-ALS programmes. However, it was evident at not all non-ALS herbicide programs provided equivalent control of the experimental populations. Imazamox + metazachlor (an ALS + non-ALS oilseed rape herbicide) gave a much lower level of control in both the glasshouse pot screen and field experiment for the CAMB-M population, despite metazachlor being a non-ALS component. In the field experiment this may be due to the fact that the weed growth stage at herbicide application was at the upper end of the ideal growth stage range (up to 4 true leaves) for this herbicide. The autumn weather conditions in 2013 were very favourable for good crop and weed growth resulting in rapid growth and larger weeds early in the season. However, imazamox + metazachlor also gave poor control in the container experiment in 2014.

It is possible that the poor control may also be a result of the ALS- resistance mechanism of the CAMB-M population conveying different levels of ALS resistance to the different chemical groups within the ALS-inhibitors family. For example, the Tryptophan 574 ALS TSR mutation conveys resistance to all five ALS-chemical groups, whereas the Proline 197 ALS TSR mutation only conveys resistance to sulfonylurea ALS-inhibitors (Deyle *et al*., 2011; Deng *et al*. 2016). The ALS-inhibitor Imazamox (in Cleranda™) is part of the Imidazolinone ('IMI'), whereas metsulfuron-methyl (Jubilee SX) and mesosulfuron-methyl + iodosulfuron-methyl (Atlantis) are both part of the Sulfonylurea ('SU') group within ALS-inhibitors. If the CAMB-M population has the Tryptophan 574 mutation then herbicide products containing any ALS chemistry are likely to result in poor control of the population. Conversely, it has been shown that florasulam, a triazolopyrimidine, can control some ALS-resistant broad-leaved weeds that have the Proline 197 mutation (Deyle *et al*. 2011; Harris & Paterson, 2014). Although florasulam can provide a useful control option, it is still a high-risk ALS-Inhibitor herbicide and so should be used in addition to other herbicide modes of action and with the addition of cultural control options as appropriate.

The key results from the container experiments was that a non-ALS herbicide programme consistently provided the highest control across all years and populations. A mixture of a non-ALS + ALS herbicide programme also provided good control in 2013 and 2014, but control decreased in 2015 for the resistant populations, CAMB-M and CAMB-W, suggesting that the control was provided by the non-ALS pre-emergence herbicide, which did not provide as high control in 2015. The use of a post-emergent ALS inhibitor herbicide alone always provided the lowest amount of poppy control. Interestingly, limited control in some years showed that a small proportion of some ALS-resistant populations may still contain individuals sensitive to ALS inhibitors. However, the proportion of sensitive individuals is not high enough to provide sufficient control showing that other herbicide modes of action that are effective on the target weed, are required.

Other modes of action, such as synthetic auxins are considered as lower-resistance risk herbicides, but there are populations of common poppy resistant to 2,4-D in southern Europe (Rey-Caballero *et al*., 2016) and one population showed cross-resistance to another auxin, dicamba, in glasshouse pot assays. Therefore, resistance development to other modes of action always need to be taken into account and auxins should not be considered as ‘no’ risk and used in mixtures and sequences as part of a resistance management strategy.

When using herbicides it is also important to account for varying conditions in the field at application. In this study, the loam- based soil used in the container experiments may have resulted in slightly different herbicide efficacy compared to the high organic content soil in the field experiment in Cambridgeshire. Pre-emergence herbicides, such as pendimethalin, can behave differently on soils with a higher organic matter content as the herbicide can bind to this fraction lowering overall efficacy. It was considered that this had not been the case on this particular soil type as pre-emergence control levels were generally very high, but it is something that should be considered. Additionally, in the second year of the field experiments, in the oilseed rape crop in particular, the weed growth stage was very large at the time of the post-emergence herbicide application. This appeared to have reduced the overall herbicide efficacy as the weed was larger than the ideal growth stage. Missing the optimum growth stage will have resulted in a high seed return to the seed bank, a poor weed management strategy, due to the longevity of poppy seeds.

The high level of resistance in the glasshouse seed validation tests, for populations that had been treated with an ALS-inhibitor alone for three years showed that exposure to ALS herbicide modes of action in resistant population maintains and, in some cases can increase, the level of resistance. Interestingly, populations that were resistant when collected initially (baseline) and untreated with herbicides for three years showed a greater level of control from ALS-inhibitors in the absence of selection pressure. The genetic basis of the resistance mechanisms present in the poppy populations used in this study have not been investigated, but one potential cause of the reduction in ALS-resistance in the untreated lines is the possible presence of NTSR mechanisms. NTSR has been reported in some common poppy populations, but is not fully understood as most of the research focus in broad-leaved weeds has been on target site resistance mechanisms (Scarabel *et al.* 2015). The presence of a multi-gene NTSR mechanism and the removal of the ALS selection pressure may have meant that any susceptible alleles in the population could have potentially built up. However, even when the selection pressure is removed the resistant alleles are not removed completely and the potential increase in susceptible individuals is unlikely to result in practical management benefits. Alternatively, the lack of ALS selection pressure in the untreated lines may have enabled any sensitive individuals in the population to survive and reproduce, resulting in an increase in the number of sensitive individuals in the unselected lines.

In the container conditions are generally more optimal than field conditions, possibly increasing the herbicide efficacy. However, resistance is detected more quickly from these populations, as they have had no dilution from unexposed seed from the soil seedbank. Beckie (2006) showed that resistance to ALS-Inhibitors can be selected in less than 10 applications in the field and that where high resistance risk actives, such as ACCase and ALS-Inhibitors had been used regularly for over 20 years then resistance is likely to be in a high proportion of the weed population, so keeping weed numbers low in these situations is imperative. Due to potential cross-resistance occurring, no ALS-inhibitor is less risky than another ALS-inhibitor in terms of resistance development (Beckie & Tardif, 2013) and a proactive approach to managing a resistant weed population is required, with ALS-inhibitors used sparingly across a rotation and ideally tank-mixed with a lower resistance risk herbicide mode of action.

During this project a number of other herbicide resistant broad-leaved weeds have been reported. Deng *et al*. (2016) have reported ALS-resistant Flixweed (*Descurainia sophia L*.) with populations having both the Proline 197 and Tryptophan 574 TSR mutations. ALS-resistance in French populations of common groundsel (*Senecio vulgaris*) from arable fields and vineyards were reported in 2015 (Deyle *et al.*). The arable crops had been sprayed at low rates of herbicide and at large weed growth stages, which possibly allowed for resistance to spread. Non-target site resistance (NTSR) mechanisms could also be involved in this groundsel population but have not been confirmed

Cultural control options are more limited for the broad-leaved weeds as generally seed production and seedbank longevity are high. This is particularly the case for poppy where seed production has been reported on average as 20,000 seeds per plant (Hanf, 1983), but can range from 10,000 to 60,000 seeds per plant, depending on plant density and crop competition (Bond *et al*., 2006) and seeds can survive for decades. Therefore, the use of cultivation will have little effect on decreasing the weed seed bank of poppy, compared to grass weed species that may typically decline more rapidly (within 5 years) by burying them down below germination depth and preventing annual seed return. Broad-leaved weeds tend to appear in distinct patches, in particular if only target site resistant mechanisms are present. Therefore, early detection of weed patches and their subsequent monitoring and appropriate management or removal will aid in the reduction of resistance spread. The biological risk factors affecting broad-leaved weed resistance were highlighted in a CRD report (PS2709, Tatnell *et al*., 2007) as high seed producers, of which poppy and chickweed are examples. However, other broad-leaved weed species are likely to develop resistance in the UK, particularly if herbicide modes of action remain limited across a rotation and must be monitored. These could include fat hen (*Chenopodium album*), charlock (*Sinapis arvensis*) and Canadian fleabane (*Conyza canadensis*).

Testing of broad-leaved weed seed or plants to determine the presence of herbicide resistance will provide farmers with a tool to aid subsequent management. However, currently in the UK the number of samples tested annually is extremely low and one of the biggest factors affecting this is the difficulty of seed collection. A leaflet (AHDB Information sheet 54) has been produced as part of this project to provide practical guidelines for seed collection timing and method and with details of the latest management guidelines for broad-leaved weed control integrating results from the project findings. Currently the cost of seed testing may also be a prohibiting factor in the uptake of broad-leaved weed testing, but with technological advances available and potentially the development of new techniques the cost may be reduced in the future.

It is possible that NTSR resistance was present in some of the populations in this study. There appears to be limited understanding of NTSR in common poppy world-wide and so this is a research gap that needs addressing to fully understand how this type of resistance develops and spreads in broad-leaved weeds.

The number of herbicide resistant broad-leaved weeds in the UK are low and currently controllable with a robust herbicide-resistance management strategy, essentially including a range of herbicide modes of action. However, it is crucial to maintain a range of herbicide modes of action and not to further diminish the herbicide choices to enable control of resistant populations and prevent further resistance increase. Early detection, monitoring and removal of problem broad-leaved weed patches will also limit and potentially prevent resistance spread.

6 Acknowledgements

We gratefully acknowledge the funding by AHDB (project number 3788), BASF (Iain Ford), Dow AgroSciences (Andy Bailey) and DuPont (Steve Cranwell). We are very grateful for the support, guidance and consultancy of Dr Stephen Moss. We would like to thank the research support staff at ADAS Boxworth and acknowledge the support of the field trial host farmer. We would also like to thank Chris Dyer and Denise Ginsburg for statistical analysis and Dr Sarah Cook for editing.

7 References

AHDB Cereals and oilseeds website- https://cereals.ahdb.org.uk/markets.aspx

AHDB information sheet 54 (2016) Herbicide-resistance in broad-leaved weeds. https://cereals.ahdb.org.uk/media/917507/is54-herbicide-resistance-in-broad-leaved-weeds.pdf

Beckie HJ (2006) Review - Herbicide-Resistant Weeds: Management Tactics and Practices. *Weed Technology*, **20**, 793-814.

Beckie HJ, & Tardif FJ (2013) Herbicide cross resistance in weeds. *Crop Protection*, **35**, 15-28.

Bond W, Davies G & Turner R (2006) The biology and non-chemical control of Common Poppy (*Papaver rhoeas* L.). A Defra review as part of the Organic Weed Management Project, OF0315.

Deng W., Yang Q., Zhang Y., Jiao H., Mei Y., Li X., Zheng M. (2016). Cross-resistance patterns to acetolactate synthase (ALS)-inhibiting herbicides of flixweed (*Descurainia sophia L.*) conferred by different combinations of ALS isozymes with a Pro-197-Thr mutation or a novel Trp-574-Leu mutation. Pesticide Biochemistry and Physiology. In press.

Deyle C, Pernin F & Scarabel L (2011) Evolution and diversity of the mechanisms endowing resistance to herbicides inhibiting acetolactate-synthase 9ALS) in corn poppy (*Papaver rhoeas* L.). *Plant Science*, 180, 333-342.

Deyle C, Causse R & Michel S (2015) Genetic basis, evolutionary origin and spread of resistance to herbicides inhibiting acetolactate synthase in common groundsel (*Senecio vulgaris*). Pest Management Science (2015), 72, 89-102.

Hanf, M (1983) The Arable Weeds of Europe. BASF UK Ltd.

Harris D & Paterson E (2014) The use of florasulam based products for the control of ALS resistant weeds in cereals. *Proceedings of Crop protection in Northern Britain*, 2014, 143-148.

Heap IM (2016) International survey of herbicide-resistant weeds (ISHRW). Online http://www.weedscience.com

Hull R, Tatnell LV, Cook SR, Beffa R, Moss SR, 2014. Current status of herbicide-resistant weeds in the UK. Crop Protection in Southern Britain: Precision Decisions for Profitable Cropping. *Aspects of Applied Biology* 127, 261-272.

Marshall R, Hull R & Moss SR (2010) Target site resistance to ALS inhibiting herbicides in *Papaver rhoeas* and *Stellaria media* biotypes from the UK. *Weed Research*, **50**, 621-630.

Moss SR, Anderson-Taylor G, Beech PA, Cranwell SD, Davies DHK, Ford IJ, Hamilton IM, Keer JI, Mackay JD, Paterson EA, Spence EE, Tatnell LV, Turner MG (2005) The current status of herbicide-resistant grass and broad-leaved weeds of arable crops in Great Britain. *Proceeding of the BCPC International Congress - Crop Science and Technology, Glasgow*, UK, **1**, 139 -144.

Moss SR, Perryman SAM & Tatnell LV (2007) Managing Herbicide-Resistant Black-grass (*Alopercurus myosuroides*): Theory and Practice. *Weed Technology*, **21**, 300-309.

Moss SR, Marshall R, Hull R & Alcarcon-Reverte R (2011) Current status of herbicide-resistant weeds in the United Kingdom. *Aspects of Applied Biology* **106**, *Crop Protection Southern Britain*, 1-16.

Rey-Caballero J, Menéndez J, Giné-Bordonaba J, Salas M , Alcántara R, Torra J (2016) Unravelling the resistance mechanisms to 2,4-D (2,4-dichlorophenoxyacetic acid) in corn poppy (*Papaver rhoeas*). *Pesticide Biochemistry and Physiology*, **133**, 67–72.

Scarabel L, Pernin F, Délye F (2015) Occurrence, genetic control and evolution of non-target-site based resistance to herbicides inhibiting acetolactate synthase (ALS) in the dicot weed *Papaver rhoeas. Plant Science* (2015) **238**, 158–169.

Tatnell LV, Ginsburg D, Moss SR, Marshall R & Clarke JH (2007) A review of broad-leaved weed resistance 2006-2007. Web page: http://www.pesticides.gov.uk/Resources/CRD/Migrated-Resources/Documents/B/Broad20Leaf20Weed20Review20Final.pdf

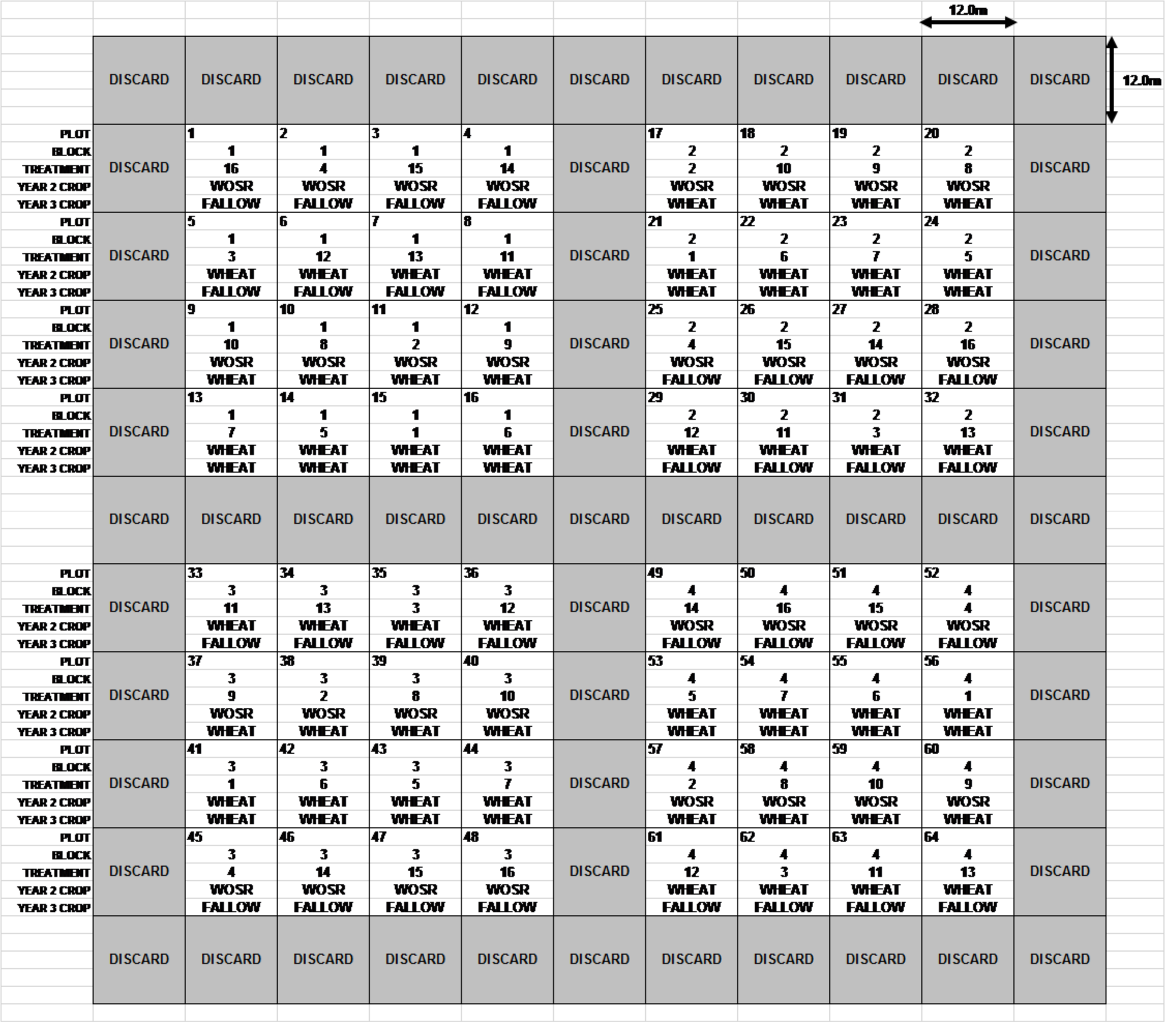
Tatnell LV, Moss SR, Clarke JH, Bailey A, Paterson E, Cranwell S, Ford I, Gosling P (2016) Quantifying the real threat of ALS-resistant broad-leaved weeds in UK arable cropping systems and developing effective management strategies, *Proceeding Crop Protection in Northern Britain* 2016, 111-116.

Torra J, Cirujeda A, Taberner A & Recasens (2010) Evaluation of herbicides to manage herbicide-resistant corn poppy (*Papaver rhoeas*) in winter cereals. *Crop Protection*, **29**, 731-736.

Tranel PJ & Wright TR (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed Science*, **50**, 700-712.

8 Appendix

8.1 Appendix 1: Experimental trial plan CAMB-M field



	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD
PLOT	DISCARD	1	2	3	4	DISCARD	17	18	19	20	DISCARD
BLOCK		1	1	1	1		2	2	2	2	
TREATMENT		16	4	15	14		2	10	9	8	
YEAR 2 CROP		WOSR	WOSR	WOSR	WOSR		WOSR	WOSR	WOSR	WOSR	
YEAR 3 CROP		FALLOW	FALLOW	FALLOW	FALLOW		WHEAT	WHEAT	WHEAT	WHEAT	
PLOT	DISCARD	5	6	7	8	DISCARD	21	22	23	24	DISCARD
BLOCK		1	1	1	1		2	2	2	2	
TREATMENT		3	12	13	11		1	6	7	5	
YEAR 2 CROP		WHEAT	WHEAT	WHEAT	WHEAT		WHEAT	WHEAT	WHEAT	WHEAT	
YEAR 3 CROP		FALLOW	FALLOW	FALLOW	FALLOW		WHEAT	WHEAT	WHEAT	WHEAT	
PLOT	DISCARD	9	10	11	12	DISCARD	25	26	27	28	DISCARD
BLOCK		1	1	1	1		2	2	2	2	
TREATMENT		10	8	2	9		4	15	14	16	
YEAR 2 CROP		WOSR	WOSR	WOSR	WOSR		WOSR	WOSR	WOSR	WOSR	
YEAR 3 CROP		WHEAT	WHEAT	WHEAT	WHEAT		FALLOW	FALLOW	FALLOW	FALLOW	
PLOT	DISCARD	13	14	15	16	DISCARD	29	30	31	32	DISCARD
BLOCK		1	1	1	1		2	2	2	2	
TREATMENT		7	5	1	6		12	11	3	13	
YEAR 2 CROP		WHEAT	WHEAT	WHEAT	WHEAT		WHEAT	WHEAT	WHEAT	WHEAT	
YEAR 3 CROP		WHEAT	WHEAT	WHEAT	WHEAT		FALLOW	FALLOW	FALLOW	FALLOW	
	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD
PLOT	DISCARD	33	34	35	36	DISCARD	49	50	51	52	DISCARD
BLOCK		3	3	3	3		4	4	4	4	
TREATMENT		11	13	3	12		14	16	15	4	
YEAR 2 CROP		WHEAT	WHEAT	WHEAT	WHEAT		WOSR	WOSR	WOSR	WOSR	
YEAR 3 CROP		FALLOW	FALLOW	FALLOW	FALLOW		FALLOW	FALLOW	FALLOW	FALLOW	
PLOT	DISCARD	37	38	39	40	DISCARD	53	54	55	56	DISCARD
BLOCK		3	3	3	3		4	4	4	4	
TREATMENT		9	2	8	10		5	7	6	1	
YEAR 2 CROP		WOSR	WOSR	WOSR	WOSR		WHEAT	WHEAT	WHEAT	WHEAT	
YEAR 3 CROP		WHEAT	WHEAT	WHEAT	WHEAT		WHEAT	WHEAT	WHEAT	WHEAT	
PLOT	DISCARD	41	42	43	44	DISCARD	57	58	59	60	DISCARD
BLOCK		3	3	3	3		4	4	4	4	
TREATMENT		1	6	5	7		2	8	10	9	
YEAR 2 CROP		WHEAT	WHEAT	WHEAT	WHEAT		WOSR	WOSR	WOSR	WOSR	
YEAR 3 CROP		WHEAT	WHEAT	WHEAT	WHEAT		WHEAT	WHEAT	WHEAT	WHEAT	
PLOT	DISCARD	45	46	47	48	DISCARD	61	62	63	64	DISCARD
BLOCK		3	3	3	3		4	4	4	4	
TREATMENT		4	14	15	16		12	3	11	13	
YEAR 2 CROP		WOSR	WOSR	WOSR	WOSR		WHEAT	WHEAT	WHEAT	WHEAT	
YEAR 3 CROP		FALLOW	FALLOW	FALLOW	FALLOW		FALLOW	FALLOW	FALLOW	FALLOW	
	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD	DISCARD

8.2 Appendix 2: CPNB Conference paper, February 2016

QUANTIFYING THE REAL THREAT OF ALS-RESISTANT BROAD-LEAVED WEEDS IN UK ARABLE CROPPING SYSTEMS AND DEVELOPING EFFECTIVE MANAGEMENT STRATEGIES

L V Tatnell[1], S R Moss[2], J H Clarke[1], A Bailey[3], E Paterson[3], S Cranwell[4], I Ford[5], P Gosling[6]

[1]*ADAS UK Ltd, Boxworth, Cambridge, CB23 4NN*

[2] *Stephen Moss consulting, Harpenden, Hertfordshire, AL5 5SZ*

[3] *Dow AgroSciences, Latchmore Court, Brand Street, Hitchin, Hertfordshire, SG5 1NH*

[4] *DuPont (UK) Ltd, Wedgewood Way, Stevenage, Hertfordshire, SG1 4QN*

[5] *BASF plc, Earl Road, Cheadle Hulme, Cheadle, Cheshire, SK8 6QG*

[6] *AHDB, Stoneleigh Park, Kenilworth, Warwickshire, CV8 2TL*

E-mail: *lynn.tatnell@adas.co.uk*

Summary: The number of populations of broad-leaved weeds tested annually and confirmed as ALS-resistant in the UK remains low. Over a four-year period less than 50 ‘suspect’ UK populations of broad-leaved weeds were tested of which 22 common poppy (*Papaver rhoeas*), 8 common chickweed (*Stellaria media*) and 7 scentless mayweed (*Tripleurospermum inodorum*) were confirmed as ALS-resistant. The collection of broad-leaved weed seed is more complicated than for grass weeds which may be inhibiting the number tested. The use of ALS-inhibiting herbicides remains high across the rotation and so there is a risk of resistance developing. However, trial results show that ALS-resistant populations of common poppy can be effectively controlled by herbicides with alternative modes of action. Non-chemical options are limited and therefore it is essential that a wide range of herbicide actives remain available to farmers to enable effective broad-leaved weed management and reduce an over reliance on ALS-inhibitor herbicides.

INTRODUCTION

The current state of herbicide resistance in broad-leaved weeds in the UK was summarised by Hull *et al*., (2014) reporting acetolactate synthase (ALS)-resistant common poppy (*Papaver rhoeas*) on over 40 farms in nine counties, common chickweed (*Stellaria media)* on more than 50 farms in 13 counties and scentless mayweed (*Tripleurospermum inodorum)* on five farms in three counties (two England and one Scotland). In the UK the main cases of broad-leaved weed resistance are target site resistance (Marshall *et al*., 2010) to ALS-inhibiting herbicides, however cases of mecoprop-resistant chickweed were reported by Lutman & Snow (1987), but this now appears to be quite isolated with no further cases in nearly 30 years. Across Europe broad-leaved weeds have shown resistance to other herbicide modes of action (Torra *et al*., 2010). The risk of broad-leaved weed resistance increasing rapidly in the UK is potentially high as there is an increased use of ALS herbicides (Heap, 2015) and a lack of herbicides available with alternative modes of action. Broad-leaved weeds generally produce a high number of seeds, and are long-lived in the seedbank, particularly in the case of common poppy, which are high risk for developing resistance (Tatnell *et al*., 2007). However, in reality there has not been a rapid increase in reported cases of resistance over the last 15 years since the first report of broad-leaved weed resistance, which is contrary to what may have been expected.

Herbicide resistance in UK grass weeds is now widespread (Hull *et al*., 2014, Moss *et al*., 2011) and by learning the lessons from black-grass resistance in particular, and applying the knowledge gained for its management or prevention, it is hoped that broad-leaved weed resistance will not develop to the same extent as the grass weeds.

Broad-leaved weed resistance is being monitored closely and data from ADAS and crop protection company resistance testing from the last four years have been amalgamated to quantify the current extent of broad-leaved weed resistance in the UK. A current research project, in its final year, aims to develop practical solutions to prevent a wide-scale increase in ALS resistant broad-leaved weeds, focussing on common poppy, through effective management in a cereal/oilseed rape crop rotation (Tatnell *et al*., 2014). Results from the broad-leaved weed seed testing for herbicide resistance are presented in this paper, along with guidelines for effective management of broad-leaved weeds from the research project field experimental data.

MATERIALS AND METHODS

Seed testing of selected broad-leaved weeds

Populations of broad-leaved weeds, including common poppy, common chickweed and mayweed were identified in UK field sites where control levels had been poor for more than two cropping seasons. Seed collected by ADAS in July 2014 were tested for resistance to a range of herbicides using a standard glasshouse pot test method detailed below (crop protection company test methods may vary). Plastic plant pots measuring 9cm diameter were filled with Kettering loam ‘weed’ mix (80% sterilised loam + 20% grit + 2kg Osmacote slow release fertiliser) to 2 cm below the pot rim and placed in trays on the glasshouse bench and watered to field capacity over a period of 24 hours before sowing seed. Pots were labelled and weed seeds were hand sown with six replicates per weed population.

Pots were placed in a glasshouse with a temperature/light regime of 18^{o}C for 14 hours with lights and 12^{o}C for 10 hours no lights. Weeds were thinned to three plants/pot at the 1-2 leaf (BBCH 11-12) stage.

In the ADAS tests weeds were sprayed at the 4-6 true leaf stage (BBCH 14-16) using the treatments shown in Table 1, an untreated control was included for each weed population. Herbicides were applied in 200 l/ha water using a Mardrive automated pot sprayer, with two F110 nozzles at 2 bar. Plants were assessed 4 weeks after treatment with a visual score of the plants using a 0-10 rating (where 10 = live/healthy plants and 0= dead plants) and a foliage fresh weight (g) of plants per pot. A total of 12 poppy, two chickweed and six mayweed samples were tested by ADAS in 2015.

Table 1. Herbicide treatments and dose used against broad-leaved weeds tested by ADAS.

Herbicide treatments		Weed species		
Active ingredient	Dose g a.i./ha	Poppy	Chickweed	Mayweed
Metsulfuron-methyl	6g	✓	✓	✓
MCPA	1000g	✓		
Fluroxypyr	200g		✓	
Mecoprop-p	1380g		✓	
Florasulam	0.25g		✓	✓
Clopyralid	100g			✓

Crop protection companies also tested a number of broad-leaved weed populations from sites where resistance was ‘suspected’, so it is important to note that these were not random samples, but they had been identified as sites with control issues. It was not possible to determine whether there was any overlap between the ADAS and company samples, however the chance of more than one test from the same field site was considered negligible. In total, less than 10 populations of each weed species were tested annually by the companies between 2012 and 2015.

Field experimentation on common poppy

A three-year field experiment was established on a site with known ALS-resistant common poppy in Cambridgeshire in 2012. Four simple treatments were tested 1) untreated control, 2) ALS-inhibitor alone, 3) ALS-inhibitor + non-ALS and 4) non-ALS herbicide, which were replicated four times and the specific herbicides were selected depending on the crop present. Plots measured 12m x 12m, with buffer strips between each replicate block to minimise pollen transfer. The plots remained in the same position each year to ensure the resistance pressures remained constant and the crop rotation included wheat (2013), wheat and oilseed rape (2014) and wheat (2015). Poppy heads were counted in the June of each season to assess the level of weed control for each treatment. After the first two experimental years a decision was taken to remove the ALS-inhibitor herbicide treatments and to manage the weed population with a non-ALS herbicide only, due to the very high weed numbers and lack of control from any ALS-inhibitors on this known resistant population. Results are therefore presented for two experimental years.

RESULTS

Seed testing

There were a relatively small number (20) of broad-leaved weed populations available for seed collection in 2014, despite a large effort to find populations where control had been poor over the previous few seasons. Of the seed tested by ADAS eight common poppy (all from England) and four mayweed populations (one Scotland, three England) were confirmed ALS-resistant (tested with metsulfuron-methyl), however both chickweed samples (from England) tested were fully controlled by metsulfuron-methyl (89% and 94% control) and greater than 97% control was achieved by the other three herbicides actives tested (Table 2).

Table 2. Number of broad-leaved weeds tested and level of control from herbicides.

Herbicide active ingredient	Species (Populations tested)					
	Poppy (12)		Chickweed (2)		Mayweed (6)	
	Resistant populations	Range % control	Resistant populations	Range % control	Resistant populations	Range % control
Metsulfuron-methyl	8	0-45	0	89-94	4	44-57
MCPA	0	88-98	-	-	-	-
Fluroxypyr	-	-	0	97-98	-	-
Mecoprop-P	-	-	0	97-99	-	-
Florasulam	-	-	0	97-99	0	100
Clopyralid	-	-	-	-	0	98-99

- *Not tested*

The level of control in all non-resistant populations was greater than 81%.

A total of 25 populations were confirmed to have ALS-resistance by crop protection companies over the four-year period. These included 14 common poppy, 8 chickweed and 3 mayweed populations. All common poppy populations were from England. All chickweed populations, except one were from Scotland and two out of the three mayweed populations were also from Scotland. There were a total of nine populations collected in 2015 which are currently being tested.

Field experimentation results

Data from the two-year poppy field experiment are shown in Figure 1. An ALS-inhibitor herbicide alone achieved no control of this resistant poppy population compared to the untreated controls.

However, a non-ALS herbicide alone gave good levels of control (mean 89%) compared to the untreated controls of this ALS-resistant population.

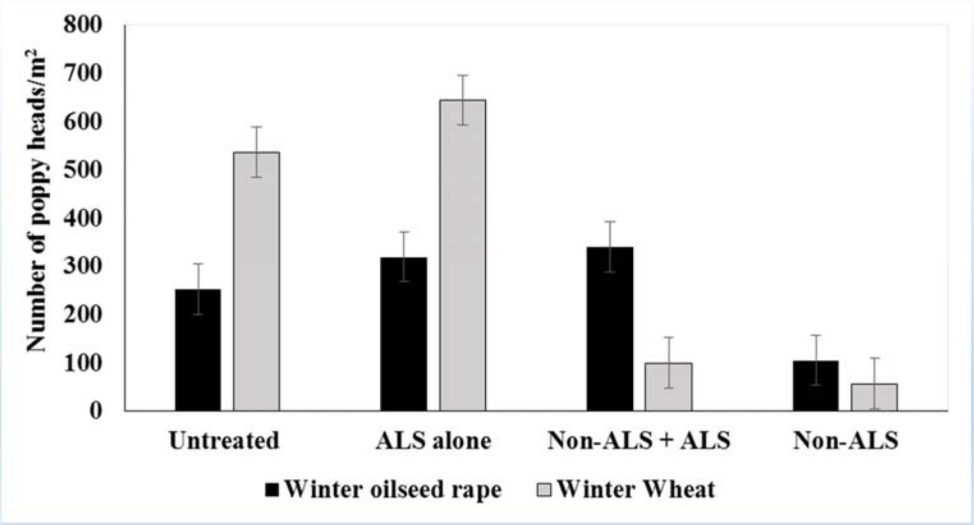


Figure 1. Mean number of common poppy heads per m^2 for different crops and herbicide treatments.

DISCUSSION

The number of populations of broad-leaved weeds tested annually in the UK for resistance remains low and there are three possible reasons for this. 1) Broad-leaved weeds seed collecting is much more difficult than for grass weeds due to their biology. Except for common poppy, where capsules above the crop canopy ripen together making collection simple. For mayweed and chickweed seeds mature over an extended time period and are often below the crop canopy. 2) The mechanism of resistance identified to date in UK broad-leaved weeds is target site, whereas the resistant grasses also have enhanced metabolism resistance. Therefore finding resistant individuals and selecting those in broad-leaved weed populations is less likely than if enhanced metabolism mechanisms were involved. However, this might change if enhanced metabolism resistance is detected in UK populations. 3) Control levels remain good due to availability of alternative modes of action and this results in only limited, often seasonal, poor control concerns. Confirmed ALS-resistant populations now include 12 mayweed, more than 70 poppy and more than 40 chickweed in the UK.

In addition to the low numbers of ‘suspected’ resistant seed tested, only a small proportion of broad-leaved weed populations have confirmed resistance, despite an increasing reliance on the high risk mode of action ALS-inhibiting herbicides. This is also likely to be because although there is a reduced number of herbicides available, of those remaining there are still many options to effectively control broad-leaved weeds in an arable rotation (Marshall *et al.*, 2010). This is illustrated by the results of the two-year rotational poppy field trial, where a highly ALS-resistant population was well controlled by a robust herbicide programme, including pre-emergence and post-emergence non-ALS herbicides. However, if legislation or other changes remove non-ALS herbicides the effective management of key broad-leaved weed such as common poppy will be threatened. In addition to product availability, the timing of a post-emergence herbicide for effective broad-leaved weed control must be at the correct weed growth stage, otherwise control will be reduced and false resistance identified. Seasonal weather variations will affect the weed growth in the spring and so the timing of the post-emergence herbicide must be tailored to ensure maximum herbicide efficacy. Non-chemical control is not as effective for weed management of broad-leaved weeds compared to the grasses due to their high seed production and seed longevity.

Resistant broad-leaved weeds are currently manageable in the UK if a robust herbicide programme including alternative modes of action are available and applied at the correct timings. However, any loss of active substances and the increased reliance on a smaller group of herbicides, in particular the ALS-inhibitors, will lead to increased resistance development. As early identification is essential to reduce risks broad-leaved weed patches must be monitored closely and appropriate management, such as the removal of small patches with a non-selective herbicide or by hand, should be administered to combat the spread of resistance.

ACKNOWLEDGEMENTS

This work is funded by AHDB (project number 3788), BASF, Dow AgroSciences and DuPont. We would like to thank the research staff at ADAS Boxworth and acknowledge the support of the host farmers of the field trials for their patience. We would also like to thank Chris Dyer and Denise Ginsburg for statistical analysis.

REFERENCES

Heap IM, 2015. International survey of herbicide-resistant weeds (ISHRW). Online http://www.weedscience.com

Hull R, Tatnell LV, Cook SR, Beffa R, Moss SR, 2014. Current status of herbicide-resistant weeds in the UK. Crop Protection in Southern Britain: Precision Decisions for Profitable Cropping. Aspects of Applied Biology 127, 261-272.

Lutman PJW, Snow HS, 1987. Further investigations into the resistance of chickweed (*Stellaria media*) to mecoprop. Proceedings of the 1987 British Crop Protection Conference-Weeds, 901-908.

Marshall R, Hull R, Moss SR, 2010. Target site resistance to ALS inhibiting herbicides in *Papaver rhoeas* and *Stellaria media* biotypes from the UK. Weed Research 50, 621-630.

Moss SR, Marshall R, Hull R, Alcarcon-Reverte R, 2011. Current status of herbicide-resistant weeds in the United Kingdom. Crop Protection in Southern Britain 2011. Aspects of Applied Biology 106, 1-10.

Tatnell LV, Ginsburg D, Moss SR, Marshall R, Clarke JH, 2007. A review of broad-leaved weed resistance 2006-2007. PSD Project report: PS2709

Tatnell LV, Clarke JH, Moss SR, 2014. Preventing a wide-scale increase in ALS resistant broad-leaved weeds through effective management in a cereals/oilseed rape cropping rotation in the UK. Crop Protection in Southern Britain: Precision Decisions for Profitable Cropping. Aspects of Applied Biology 127, 65-70.

Torra J, Cirujeda A, Taberner A, Recasens J, 2010. Evaluation of herbicides to manage herbicide-resistant corn poppy (*Papaver rhoeas*) in winter cereals. Crop Protection 29, 731-736.

8.3 Appendix 3 HRAC classification of ALS-inhibitors

HRAC classification of Inhibition of ALS (branched) chain amino acid synthesis (HRAC group B) mode of action herbicides

ALS group	Common examples
Imidazolinones	Imazamox
Sulfonylureas	Metsulfuron-methyl
Sulfonylamino-carbonyl-triazolinones	Propoxycarbazone-sodium
Triazolopyrimidines	Florasulam
Pyrimidinyl (thio) benzoates	None registered in UK