Studentship Project: Annual Progress Report
October 2021 to September 2022

Student Name:	Jed Clark	**AHDB Project Number:**	21120187
Project Title:	Wheat contra weed: Identifying weed germplasm for enhanced competition against black-grass		
Lead Partner:	ADAS		
Supervisor:	Tom Bennett		
Start Date:	1/10/2020	**End Date:**	31/09/2024

1. Project aims and objectives

The aim of this project is to identify wheat germplasm with increased competitiveness against the common agricultural weed black-grass.

Specific objectives:

1. To establish a system to understand the basis of competition between wheat and black-grass.
2. Understand the role of roots in black-grass competition. Produce evidence for our root growth hypothesis – how does barley and wheat root growth differ and how do these differences affect their ability to compete with black-grass?
3. Identify wheat lines with increased adult root growth (and increased competitiveness?), then test these lines against black-grass in outdoor conditions.
4. Understand the role of below-ground chemical signalling in the inter- and intra-specific plant-plant interactions occurring in wheat – black-grass competition, and how as they might collectively contribute to black-grass out-competing wheat.

2. Key messages emerging from the project

- We have established a system to test wheat lines for competitiveness against black-grass.
- Our root growth hypothesis has been backed up by results obtained in container trials. Further analysis will determine the cause of differences in competitive ability of different wheat lines. Analysis including possible physical (root traits, tillering) and chemical (root exudates) components of competition.
- Our findings highlight that hybrid barley may be particularly good at competing with black-grass.
- Intra-specific chemical interactions may be less important than we previously thought.
- Possibility for differences in interspecific chemical inhibition between wheat lines and black-grass.

3. Summary of results from the reporting year

3.1 Multi-dimensional interactions between wheat and black-grass

We had previously observed that inter- and intra-specific interactions between wheat and black-grass plants may play an important role in the overall wheat-black-grass competition. Using a split-pot root barrier system, we determined that wheat grown in the presence of another wheat plant separated by a permeable root barrier were significantly smaller than wheat plants separated by an impermeable root barrier. This indicated that some chemical signal exchanged between the wheat plants affects their growth. Dissimilarly, when black-grass was grown in the same set-up, the presence of an impermeable root barrier did not result in significantly different sized black-grass plants compared to those with a permeable root barrier, indicating a different response by black-grass and wheat to chemical competition. When wheat and black-grass were grown in the same pot, separated by a permeable root barrier, both the wheat and black-grass were smaller than when they were grown with an impermeable root barrier, indicating mutual chemical inhibition between wheat and black-grass. We had also seen in this set-up that when wheat and black-grass were grown in the same pot in open competition, wheat would 'win' in competition. Given that black-grass doesn't outcompete wheat as seen in the field in this set-up we wondered whether black-grass requires all of these inter and intraspecific interactions to be present to outcompete wheat.

Here we aimed to determine whether the mutual growth downregulation seen previously when wheat plants are grown together, affected overall competition with black-grass. To test this, we grew 8 black-grass and 2 wheat plants in a single rectangular pot. Half the pots were split in half by an impermeable root barrier, with 4 black-grass and 1 wheat plant in each half. The other half of the pots were left open. The barrier prevents any belowground competition between the two wheat plants. If no root barrier was placed, full below-ground competition can occur between wheat plants. Our results (figure 1) show that there was no significant difference in crop or black-grass growth in the presence of either barrier indicating that mutual wheat competition has little effect on overall wheat-black-grass competition, at least under these conditions.

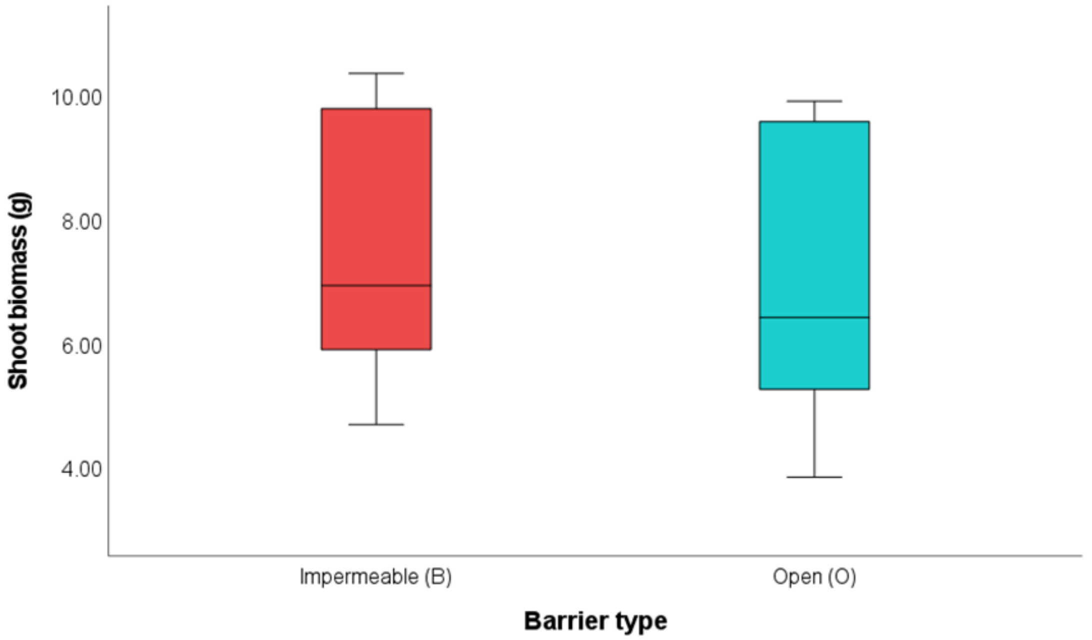

Figure 1 Multi-dimensional analysis of wheat-black-grass competition. Final dry shoot biomass of wheat in each set-up. Impermeable(B) is wheat grown in the presence of an impermeable root barrier. Open(O) refers to wheat grown in open competition without a root barrier. No significant difference can be seen between barrier types.

3.2 Developing a screen for wheat-black-grass competition

We have identified a set-up that allows the visualisation of black-grass competition on wheat. Previously, growing wheat and black-grass 1:1 under spring conditions resulted in wheat out-competing black-grass. Changing the set-up to grow black-grass at a higher density (6:1) and under winter conditions (10 degrees, 8h days) allowed visualisation of black-grass competition. However, due to the winter conditions growth was slow and it took a long time to see the effects of competition, therefore this method wouldn't be suitable for the screening of large numbers of plants. The decision was taken to maintain the higher density of black-grass but to return to spring conditions, since this might allow visualisation of competition under a shorter time frame. This set-up consists of 2L pots of soil consisting of either a single crop placed in the centre of the pot (no competition) or a single crop with six black-grass plants arranged evenly around the centre crop (competition), these were then grown in the glasshouse under spring conditions (22 degrees, 16h days). We found this to be successful in allowing us to visualize competition on much shorter timescales. This set-up will therefore allow the more facile screening of many wheat lines for competition with black-grass.

3.3 Root growth hypothesis – comparison of crop lines

The work carried out during the first year of my PhD led to the development of our root growth hypothesis. To recap, our root growth hypothesis states that black-grass gains a competitive advantage over wheat due to:

1. Increased investment in the root system.
2. Faster growth in winter conditions.
3. Prolonged period during the winter to grow roots.

These three factors allow black-grass to dominate below-ground space and resources by the spring time. Black-grass does not gain these same advantages in spring plantings; therefore we do not see the same impact of black-grass on spring wheats.

Subsequently, the focus of my work in the second year has been on testing this hypothesis and developing lines of investigation based on our findings. To test this hypothesis, we needed to test whether lines with stronger root growth were indeed more competitive against black-grass. We also needed a way of visualising both physical root growth and crop-weed competition.

Our primary approach to this has been to carry out a series of experiments focusing on seven different crop lines. These crop lines (Table 1) consist of a hybrid winter barley and a winter barley (which are competitive against black-grass and should have vigorous root growth), three elite winter wheats (which are uncompetitive against black-grass) and two landrace winter wheats that we have identified as prospectively having more vigorous root growth.

Table 1 The seven crop lines used for experiments including the variety and source.

Seed type	Category	Variety	Source
Winter wheat	Elite	Kerrin	ADAS
Winter wheat	Elite	Elation	ADAS
Winter wheat	Elite	Barrel	ADAS
Winter wheat	Landrace	238 Turkey	University of Leeds - Watkins collection
Winter wheat	Landrace	216 USSR	University of Leeds - Watkins collection
Winter Barley		Bordeaux	ADAS
Hybrid Winter Barley		SY Kingsbarn	Syngenta seeds
Blackgrass			Childerley field, ADAS Boxworth 2021

The experiments we have performed with this set of lines are:

a) Wheat-black-grass competition under field conditions (container trial at ADAS Boxworth)
b) Wheat-black-grass competition under lab conditions (glasshouse trial at Leeds)
c) Comparison of root growth in rhizobox system
d) Comparison of chemical competition using split-box system (see above)

Results for a) are presented here; b-d) are ongoing and detailed below.

Winter container trials were carried out in collaboration with RSK ADAS at Boxworth, Cambridge, running from October 2021 to July 2022. The aim of this trial was to determine how well each line competes with different black-grass densities. We will then compare data from this trial with root data gained in the lab to see if there are any correlations between root growth and black-grass competition. 23 treatments were used in this trial (Table 2) consisting of 3 different black-grass densities. Crop density was kept constant across treatments apart from the two black-grass-only treatments.

Table 2 Container trial treatment list, treatment number, crop variety and planting density

Treatment no.	**Treatment** **Crop/variety + weed**	**Planting density – plants/container (crop/BG)**
1	WW- Kerrin	6
2	WW- Elation	6
3	WW- Barrel	6
4	WW- 238 Turkey	6
5	WW- 216 USSR	6
6	WB- Bordeaux	6
7	HWB- SY Kingsbarn	6
8	WW- Kerrin + BG	6 + 10
9	WW- Elation + BG	6 + 10
10	WW- Barrel + BG	6 + 10
11	WW- 238 Turkey + BG	6 + 10
12	WW- 216 USSR + BG	6 + 10
13	WB- Bordeaux + BG	6 + 10
14	HWB- SY Kingsbarn + BG	6 + 10
15	BG alone	10
16	WW- Kerrin + BG	6 + 20
17	WW- Elation + BG	6 + 20
18	WW- Barrel + BG	6 + 20
19	WW- 238 Turkey + BG	6 + 20
20	WW- 216 USSR + BG	6 + 20
21	WB- Bordeaux + BG	6 + 20
22	HWB- SY Kingsbarn + BG	6 + 20
23	BG alone	20

Plants were sown in October. First assessments were carried out in March 2022 where tiller number of all plants was recorded. There was no significant difference in crop tiller number between the wheat varieties tested and between the different black-grass densities, the same is also seen in the two barley lines (figure 2). There is a difference between the tiller number of the barley lines and wheat lines, but again this is conserved over black-grass densities (figure 2). Black-grass tiller number can be seen to be consistent across crop varieties, also not differing significantly between black-grass density (figure 3). We can therefore conclude that at this stage of growth there are no significant signs of competition between crop and black-grass.

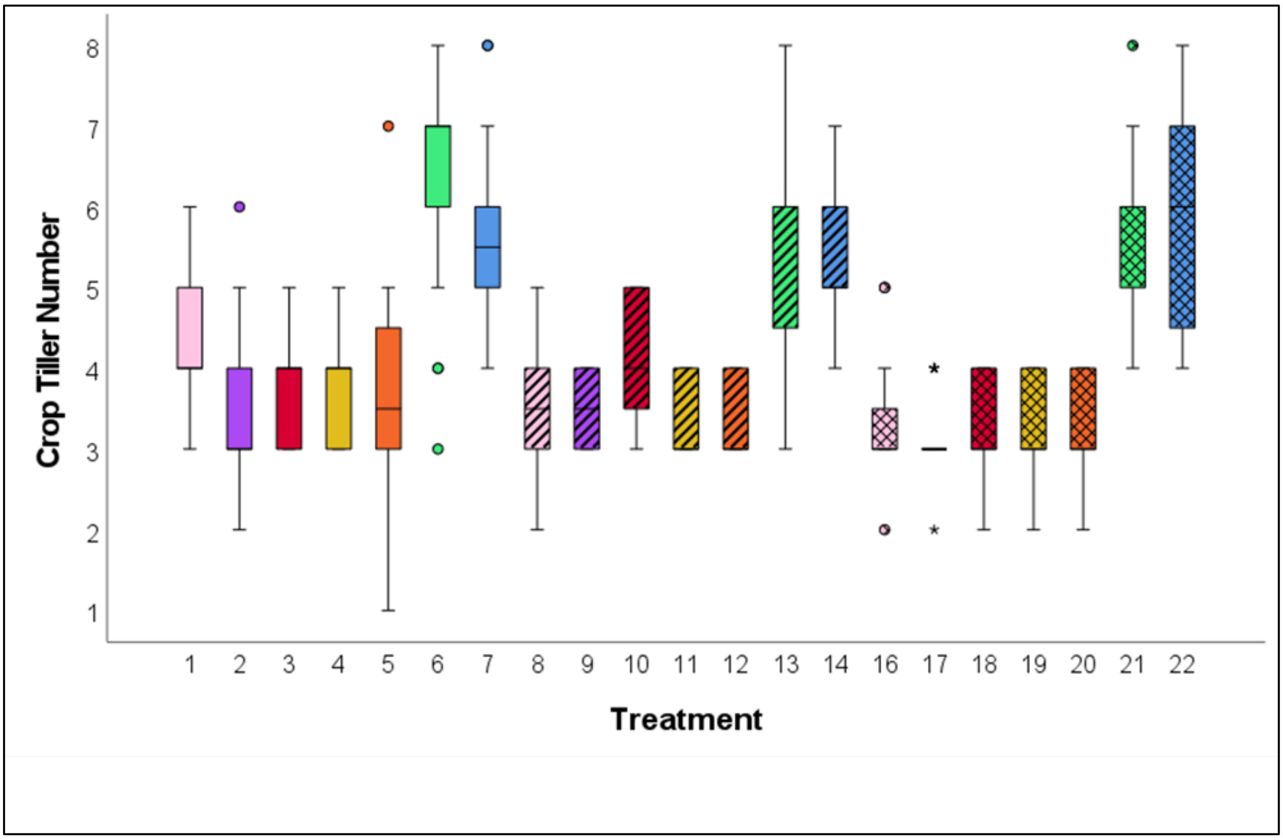

Figure 2 Crop tiller count 5 months post sowing. Crop tiller number in March show little variation between crop line and blackgrass density. For treatment numbers see table 2. Colour refers to crop variety, pattern refers to blackgrass density.

In May, we carried out a further assessment, this time recording black-grass ear number in each pot. The main finding from this data (figure 4) is that we are seeing a difference in black-grass ear number between crop lines. This would indicate competition is now occurring and that the level of competition is different between crop lines. An interesting observation is that the black-grass ear count coincides with our theoretical root growth, with elite wheats having the highest number of black-grass heads and the hybrid barley having the smallest number of black-grass ears, with the landraces and winter barley in-between.

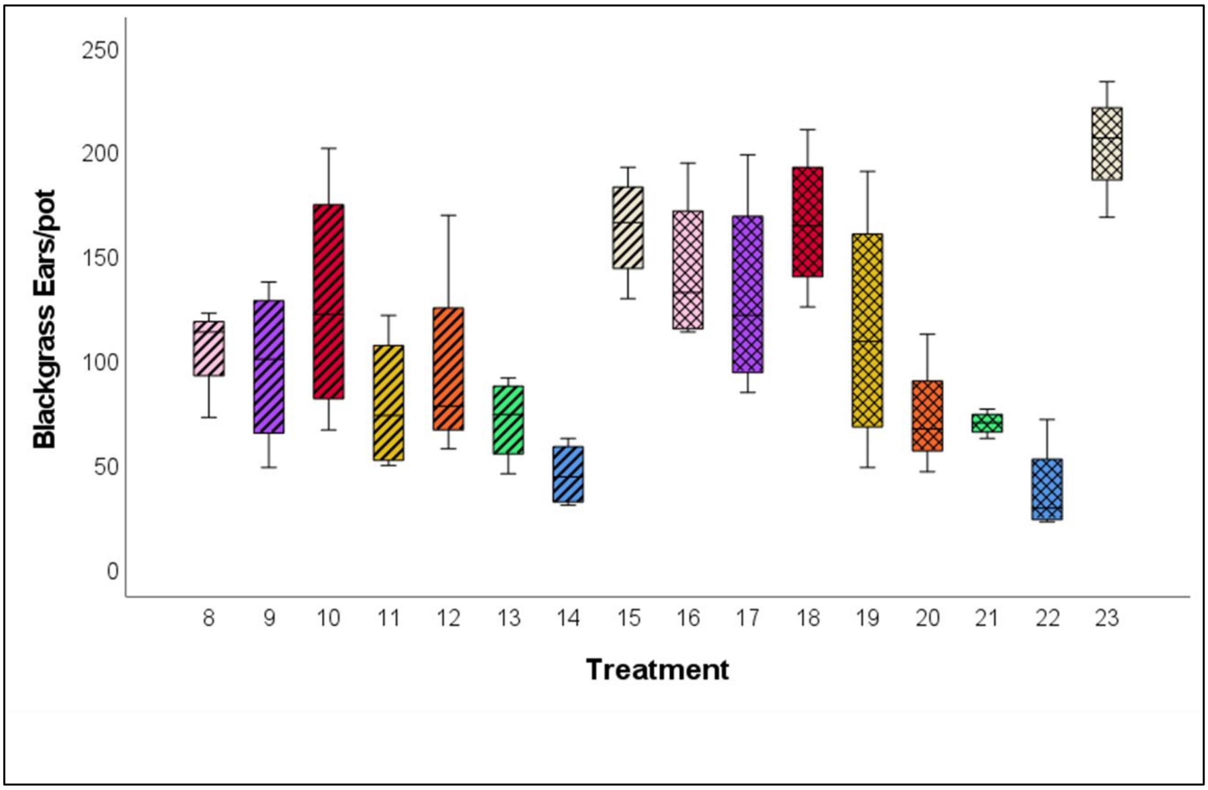

Figure 4 Blackgrass ear count 7 months post sowing. Blackgrass ear count per pot in May indicates differences in competition between crop lines. For treatment numbers see table 2, colour refers to crop variety, pattern refers to blackgrass density.

In July, all pots were harvested. Final wet shoot biomass was recorded for both crop and black-grass in each pot. For wheat, ear number and wet ear biomass was also recorded. For barley, final fertile tiller number was recorded, ear number could not be accurately recorded for barley due to bird predation of ears prior to harvest.

The total biomass of each pot was recorded (figure 5), differences can be seen in total biomass between crop lines, with elite winter wheat generally producing the smallest biomass/pot. Interestingly biomass per pot doesn't increase as black-grass density increases, given that there are more plants at high black-grass density we may have expected a general increase in total pot biomass as density increases however it seems that each treatment reaches a biomass limit independent of black-grass density. It is important to note that the biomass of barley will be an underestimate due to the loss of ears. In black-grass only pots, biomass is greater in the higher density pots, generally going against the trend of crop-black-grass treatments.

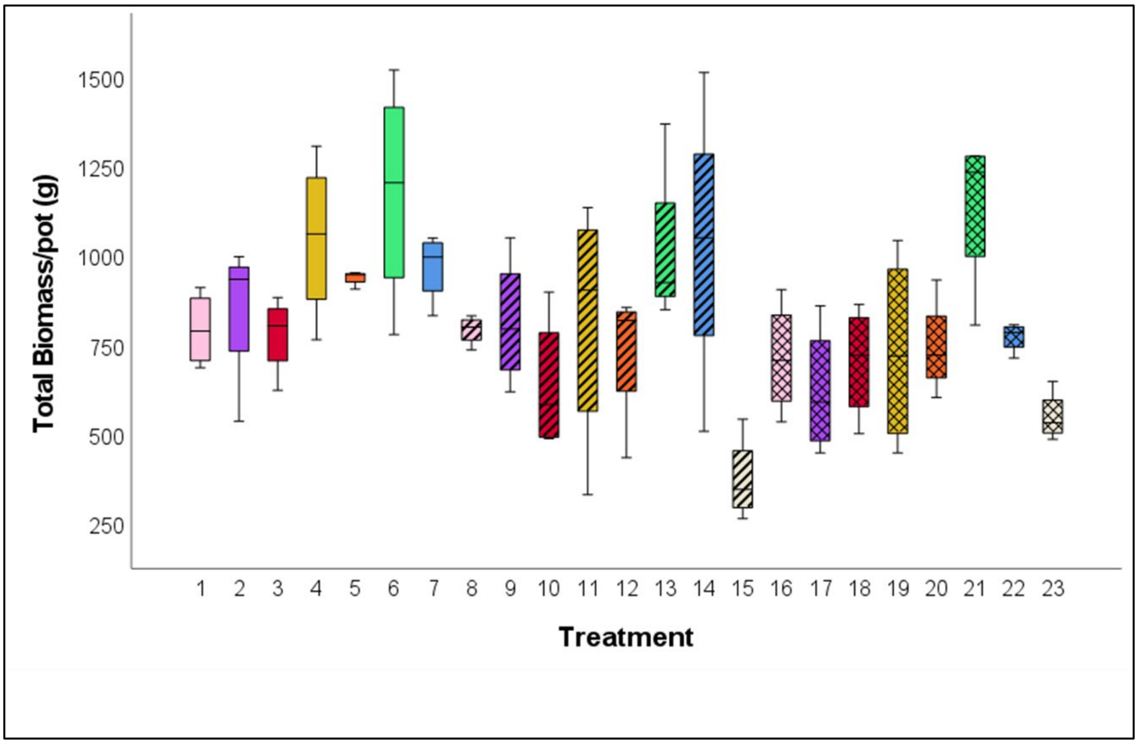

Figure 5 Total pot biomass. Total biomass (crop + weed) per pot for each treatment, not including root biomass. Graph indicates biomass limits for each line is conserved across blackgrass densities. For treatment numbers see table 2, colour refers to crop variety, pattern refers to blackgrass density.

In contrast, when we look at crop biomass per pot only (figure 6) we see a definite reduction in biomass as black-grass density increases, especially from none to low density. For most lines there is a slight further decrease in biomass from low to high density. If we look at percentage decrease for each crop line (Table 3)

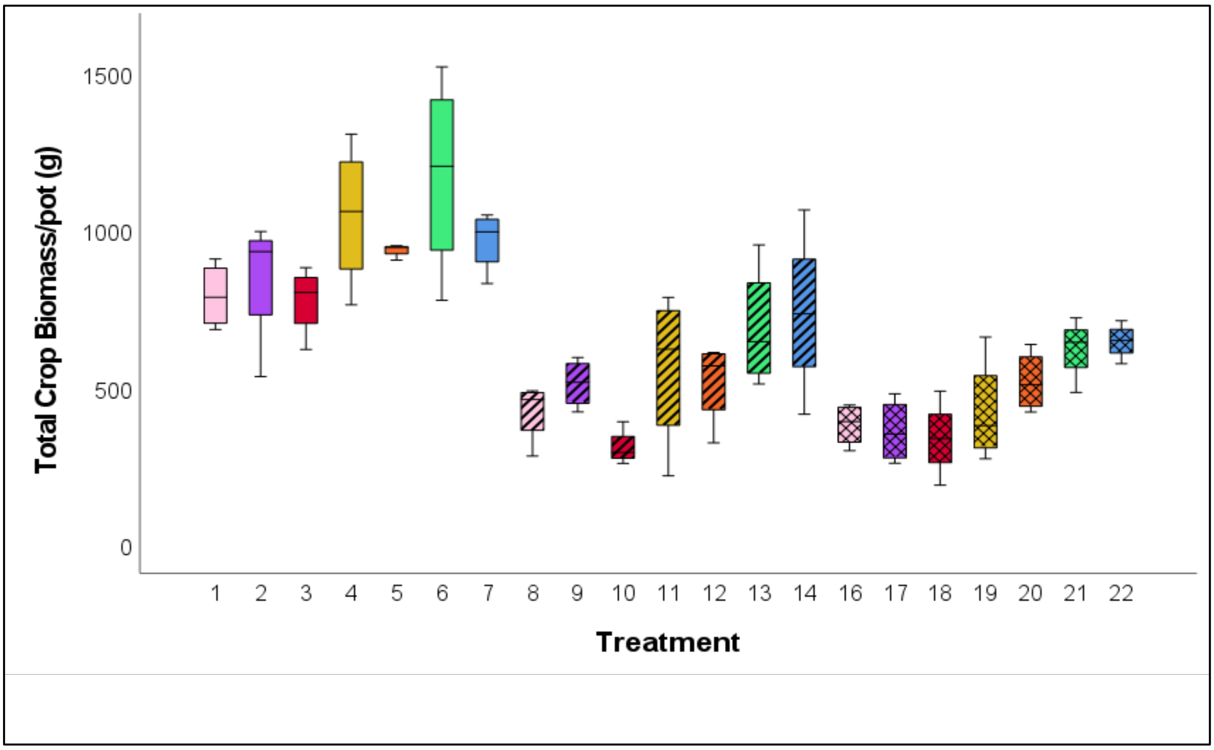

Figure 2 Total crop biomass. Total crop biomass per pot for each treatment, not including root biomass. Clear reduction in crop biomass across the board from no-low blackgrass density. For treatment numbers see table 2, colour refers to crop variety, pattern refers to blackgrass density.

we can see that the smallest decrease occurs in the HWB, followed by one of our landraces and the winter barley, the other landrace and the elite winter wheats have the largest decrease in biomass. These findings are again promising in regard to our root growth hypothesis. The ability of one of our landraces to deal with black-grass competition is particularly promising, especially if this is echoed in root traits determined in other ongoing experiments.

Table 3 Percentage decrease in total crop biomass at low and high blackgrass density compared to crop growth without blackgrass

Crop Variety	% decrease in crop biomass	
	Low BG	High BG
Kerrin	46.4	51.7
Elation	39.5	57.4
Barrel	60.1	56.4
Turkey	46.2	59.6
USSR	44.5	44.4
Bordeaux	41.4	46.9
SY Kingsbarn	23.8	33.0

When comparing total black-grass biomass per pot across densities (figure 7) total black-grass biomass per pot doesn't increase dramatically with density, even though the number of black-grass plants has increased. Bordeaux (Treatments 13, 21) would be the exception to this which shows a large increase in black-grass biomass at high black-grass density. Interestingly our HWB at high black-grass density results in a much lower black-grass biomass than all other treatments.

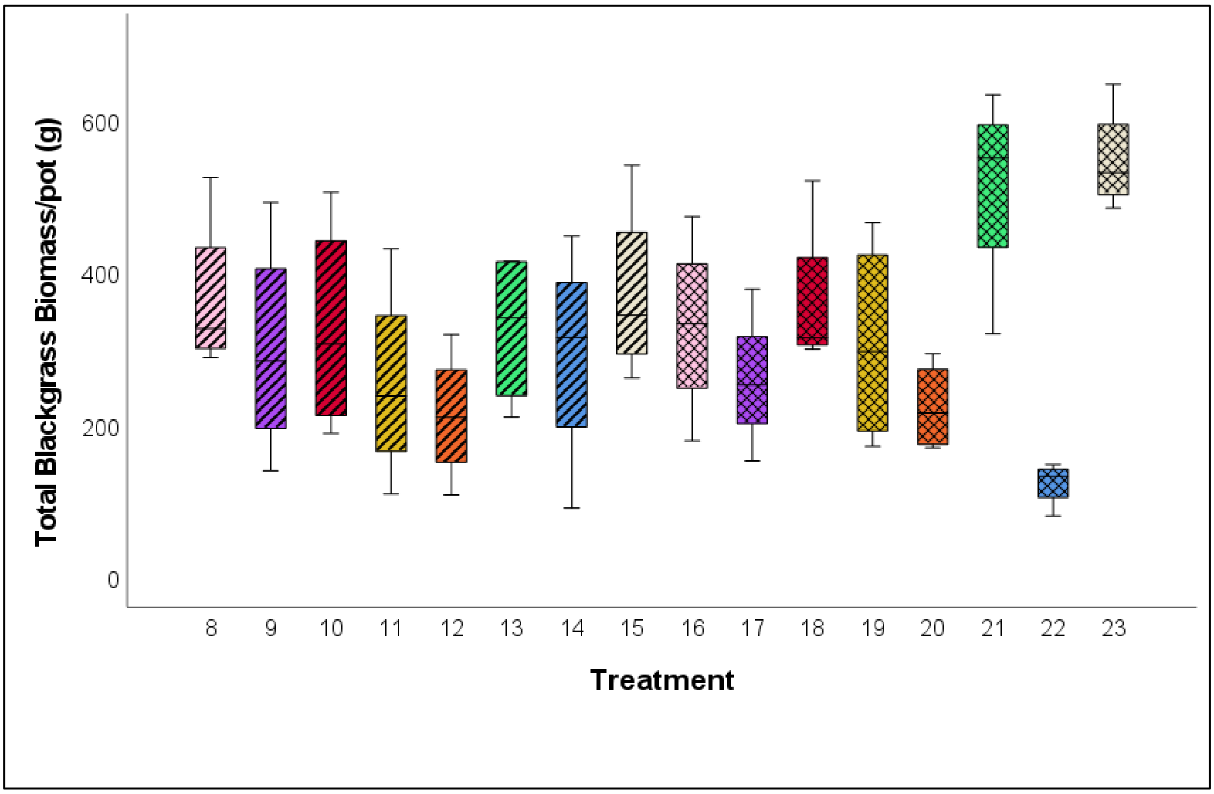

Figure 7 Blackgrass biomass per pot. Total blackgrass biomass per pot not including roots, total biomass per pot conserved despite changing density. For treatment numbers see table, colour refers to crop variety, pattern refers to blackgrass density.

Given that black-grass biomass is generally similar despite planting density, we would have to conclude that each plant is therefore smaller when at higher density, and this is exactly what we found when we look at the black-grass biomass per plant (figure 8). We can clearly see that black-grass is smaller under high density treatments than low density treatments across all crop lines, with black-grass being particularly small in the presence of HWB and 216 USSR.

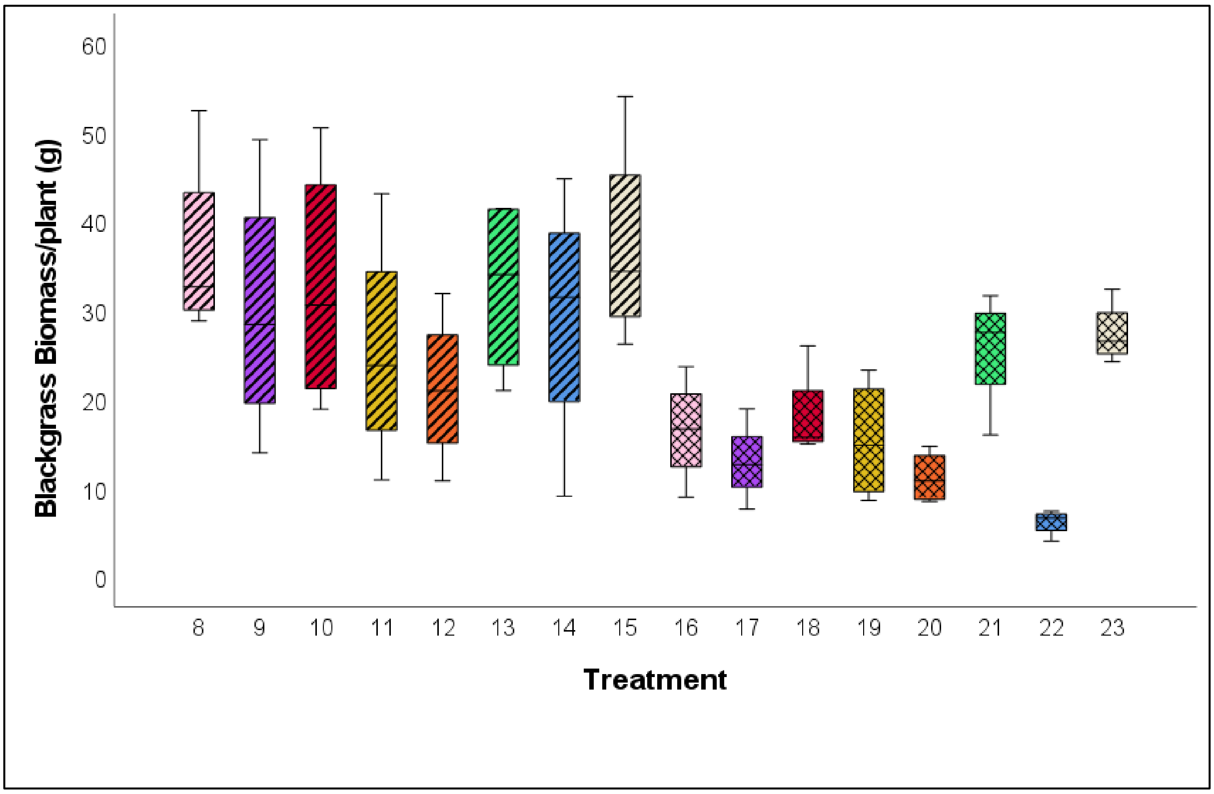

Figure 8 Blackgrass biomass per plant. Average blackgrass biomass per plant per treatment is smaller at higher blackgrass density compared to low density pots. For treatment numbers see table 2. Colour refers to crop variety, pattern refers to blackgrass density.

We have looked at total stem biomass, however the main interest for farmers lies in the ears and seed output, we therefore looked at how black-grass competition effect the ears of our different crop lines. We therefore recorded the number of crop ears per pot for each treatment. We can see clear differences between lines (figure 9) with 216 USSR producing the highest number of ears. HWB had the smallest number of ears at harvest, however this is due to almost complete bird predation, analysis of barley tiller number will show a better reflection of ear number for barley. We can see that each line shows a decrease in ear number as black-grass density increases from none to low, and again most lines show a further slight fall in ear number from low to high black-grass density. This would indicate that black-grass presence can decrease the ear number of our crops to different extents.

However, black-grass presence doesn't seem to affect ear biomass (figure 10), with biomass conserved across densities for all crop lines. Figures 9 and 10 therefore indicate that black-grass density can affect crop ear number but not individual ear biomass.

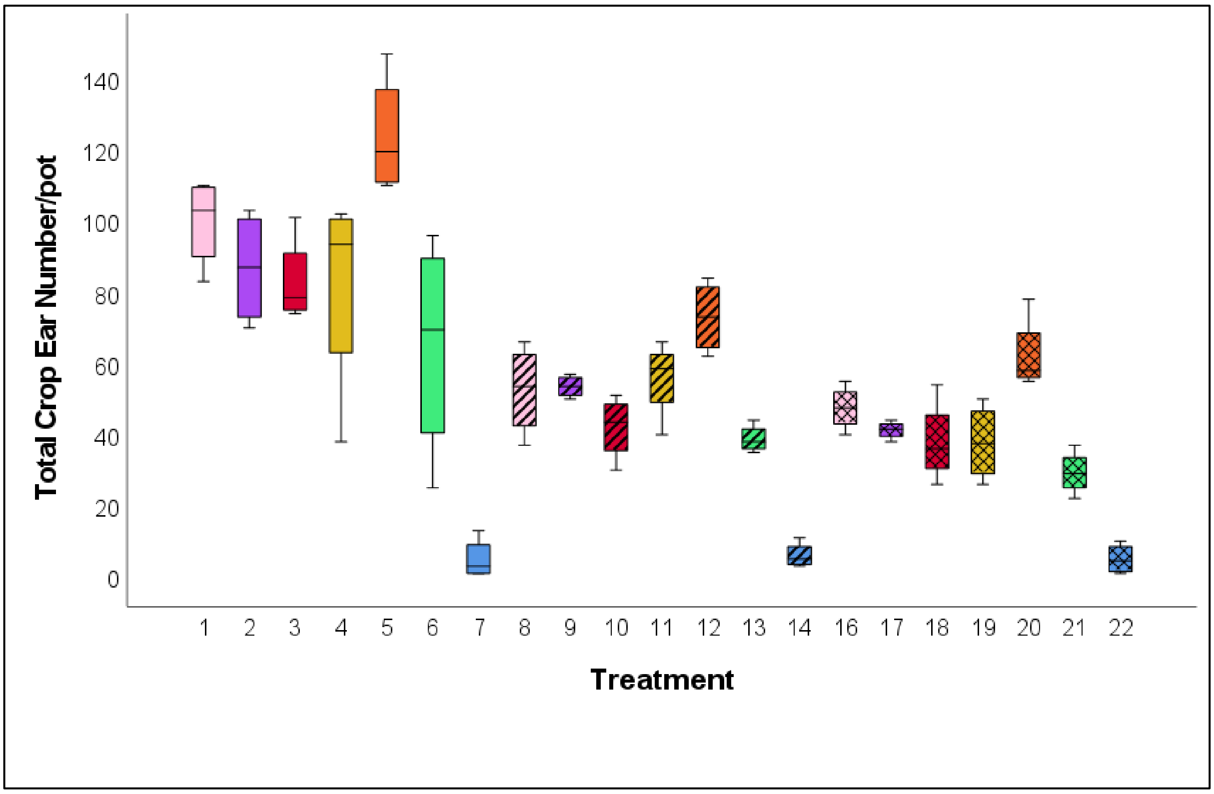

Figure 9 Crop ear number. Total crop ear number per pot per treatment. Clear decreases can be seen in ear number from no to low blackgrass densities. For treatment numbers see table 2. Colour refers to crop variety, pattern refers to blackgrass density.

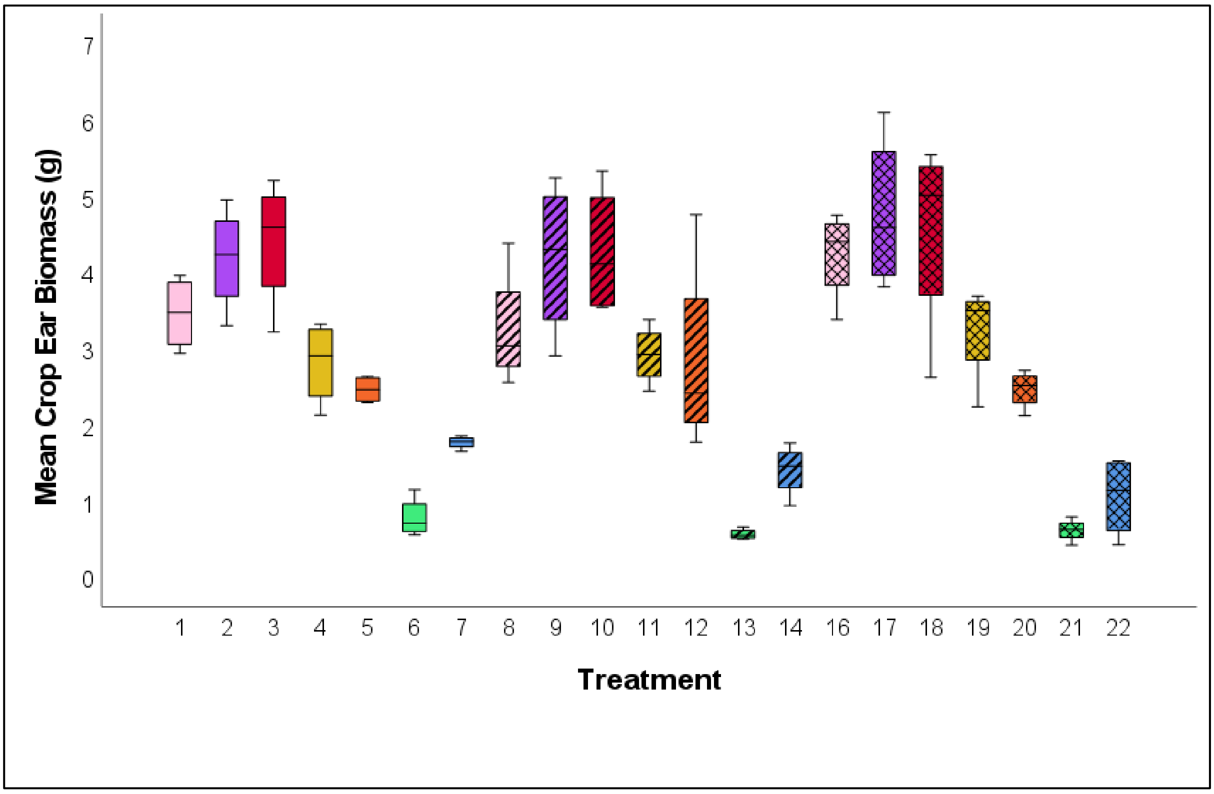

Figure 10 Crop ear biomass. Mean crop ear biomass per treatment (individual ear biomass). Crop ear biomass conserved despite blackgrass density. For treatment numbers see table 2. Colour refers to crop variety, pattern refers to blackgrass density.

Ongoing experiments

3.4 Root growth hypothesis - comparison of crop lines

b) Impact of black-grass competition on different crop lines – wheat screen

As detailed in 3.2, we have now developed a 6:1 system in which to assess wheat-black-grass competition under lab conditions. We are currently growing our 7 crop lines in this system, to assess whether they show the same pattern of competitiveness/un-competitiveness as in the container trials (see 3.3 above).

If this set-up mirrors the results of the container trial, then we can expand this to screen large numbers of current recommended list elite winter wheats for their ability to withstand black-grass competition. This hopefully will allow farmers to make an informed decision on which lines are best to grow in their black-grass laden fields.

c) Comparison of root growth traits between crop lines

To determine the root traits of the seven crop lines, each line was grown in our rhizobox system. A single seed was sown into a clear plastic 1000ml square plate (rhizobox) containing soil, the lid was placed on the plate and placed in water, standing at a 45-degree angle to promote downward root growth onto the base of the plate. Holes in the base of the plate allow water access to the soil within. The plates were then covered with plastic sheeting to prevent light hitting the plate, a hole was left at the top of the plate and cover for the shoot to grow from. Scans were taken regularly of both the base and top of the rhizobox. From these scans we determined, root length, root number, root type, root angle and quantity of roots (percentage surface coverage) over time for each line using ImageJ software.

Results – Images still under assessment therefore waiting until all have been completed before doing analysis.

d) Does chemical competition between black-grass and crop vary dependant on crop line?

Previous experiments (see 3.1) have highlighted mutual chemical inhibition occurring between wheat and black-grass. This was seen by comparing growth of wheat and black-grass in a single pot separated by either a permeable or impermeable root barrier. We therefore wanted to determine whether the level of this inhibition varies among our crop lines. In particular, do more competitive crop lines show increased chemical inhibition of black-grass? If so, chemical competition could be another factor alongside root growth that farmers could use to identify which lines to grow in their fields. We therefore will assess the level of chemical competition/inhibition occurring in our 7 crop lines using a split-pot root barrier system. Each pot will contain a single crop plant and a single black-grass plant separated by either a permeable (allows chemical competition) or impermeable (doesn't allow chemical competition) root barrier. Comparisons between growth of the crop and black-grass in the presence of the different barriers will indicate the amount of chemical competition occurring in each of the crop lines. If we see that there are differences between crop lines then further work will be required to identify which chemical root exudates are responsible for this competition and resulting differences in growth of both crop and weed.

Results – experiment ongoing

3.5) Hydroponic test of black-grass winter barley and winter wheat

We have previously seen using a hydroponic system that root growth of black-grass and wheat differs significantly over time and between spring/winter conditions, with black-grass producing a greater root system than wheat, especially under winter conditions. We have also seen in spring conditions differences between wheat and barley, with barley producing bigger plants in terms of both roots and shoots. We therefore would like to consolidate all these findings into a single experiment to support our previous findings. We therefore will grow black-grass, winter barley and winter wheat in hydroponics in winter conditions (10 degrees, 8 hour days) to compare root growth over time as well as tiller number and final root/shoot biomass.

Results – experiment ongoing

3.6) Bowman v VRS1 tillering mutant. Does reduced tillers result in reduced black-grass competitiveness in barley?

VRS1 is a tillering mutant of spring barley Bowman, producing less tillers at maturity than its WT. We have hypothesised that root production is essential in competition with black-grass however is this root production linked to tiller production. In other words: do plants with more tillers produce more roots, and are therefore more competitive against black-grass, compared to our VRS1 mutants with less tillers. To test this, we have run two experiments, using set-ups that I have previously described; 1) Rhizobox analysis of root growth, 2) Open competition with black-grass at 6:1, 0:1 densities. Together these will allow us to determine whether these lines differ in root growth and whether they have corresponding differences in competitiveness against black-grass.

Results – experiment ongoing

4. Key issues to be addressed in the next year

1. Field trial – investigating the ability of different crop lines to deal with black-grass competition in the field. This will be run in collaboration with ADAS over the winter growing season.

Here we will take our container trial a step further to full field conditions. We will grow our 7 crop lines that were used previously in the container trials, in a field containing a black-grass population. In contrast to the container trials only a single black-grass density will be used. Each crop will be grown in plots 7.5m in length at a sowing density of 375seeds/sqm, each line will have 4 replicates. All plots will undergo a full herbicide program to get a true reflection of crop competitive ability in field conditions. It may be possible that certain lines compete differently in field conditions compared to container and lab conditions, therefore this is an essential step required to test our findings to date.

2. Knowing which wheat cultivars are competitive against black-grass would be highly beneficial to farmers. Currently there is no recommended list of crop lines for competitive ability against black-grass. From our wheat screen experiments, in which we grow black-grass in competition with wheat at a density of 6:1 and 0:1, we hope to be able to rank wheat varieties; particularly current recommended list elite varieties, based on how well they compete with black-grass. We will also test certain barley varieties and landrace wheat varieties as comparisons. This information can then be used by farmers to decide which varieties to use in their fields.

3. Every year at ADAS Rosemaund in Herefordshire they host an open day showcasing a range of field trials. We have been invited to display our work at this open day, not only does this allow us to showcase our work and meet a range of farmers, agronomists and industrial professionals but it also provides an opportunity to carry out a second field trial at a smaller scale at no cost. The experimental set-up will consist of demo plots (size to be determined) for each of our 7 crop lines both with and without black-grass competition. This will allow us to see any difference in competitive ability against black-grass in our seven crop lines and will provide a good comparison to the field trial occurring at the same time.

4. **Outputs relating to the project**
   (events, press articles, conference posters or presentations, scientific papers):

Output	**Detail**
Article	**Arable farming – Research in action (Andrew Blake)**
Article	**AHDB agronomist conference article (Natalie Reed)**
Article	**University of Leeds – FBS news (Nick Robson)**
Conference	**Gave a talk at the AHDB agronomist conference 2021**
Conference	**Attended the 2021 BCPC weed review conference**
Conference	**Attended the Monogram 2022 conference**