

Studentship Project: Annual Progress Report October/2020 to September/2021

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

Aims & objectives*:

The original aim of this project was to identify genetic loci in wheat that increase 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. To screen elite and diverse germplasm for enhanced competitiveness against black-grass
3. To identify genetic loci that regulate competitiveness against black-grass
4. To test the growth of competitive germplasm against black-grass in outside conditions

2. Key messages emerging from the project

*We believe that the data from the project suggests that initially agreed aims and objectives are no longer useful or tenable.

- Due to the very slow growth of black-grass, we are unable to observe useful direct competition between black-grass and wheat under any growth conditions, making it impracticable to screen for wheat lines that are more or less competitive.
- However, the data we have collected suggest a clear hypothesis for the competitiveness of black-grass against winter wheat under field conditions.
- We believe that, given sufficient time over winter, black-grass eventually out-competes wheat because its roots grow to much higher density, allowing it to dominate resource-capture in spring.
- We believe that the inherent differences in root growth between barley and wheat (as a result of the trajectory of wheat breeding in the last 40 years) explains why wheat is susceptible to black-grass, but barley is not.
- Screening for root growth in wheat lines may allow for the identification of more/less competitive wheat lines, without the need to observe direct wheat/black-grass competition.
- We have also identified differential below-ground chemical signalling between wheat and black-grass, that may play an important role in their interactions in the field.

The results described in this summary report are interim and relate to one year. In all cases, the reports refer to projects that extend over a number of years.









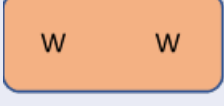


While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law, the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document. Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended, nor is any criticism implied of other alternative, but unnamed, products.

3. Summary of results from the reporting year

Understanding the effects of neighbour presence in wheat – black-grass competition

To identify the general mechanisms of competition between winter wheat and black-grass, I performed an experiment in which spring wheat (Mulika) and black-grass were grown together in a 1-litre rectangular pots under standard glasshouse conditions (16h day length at 22°C). Spring wheat was initially used due to ease of experimentation. To distinguish between root and shoot competition, the plants were either separated by an impermeable 'hytex' root barrier (preventing any root competition) (BHW) (Table 1), a permeable 'cutex' root barrier (allowing chemical communication but not direct root competition) (BCW) or no barrier (allowing complete competition) (BW).

Table 1 Experiment set-ups showing the treatment, the barrier type, the species of plants A and B in each pot and the schematic layout of each pot.

Treatment	Barrier type	Plant A	Plant B	Schematic – Rectangular pot, with combinations of black-grass (B) and wheat (W) and barrier types (none/cutex/hytex).
BHN	Impermeable 	Black-grass	-	
BB	None	Black-grass	Black-grass	
BW	None	Black-grass	Wheat	
BCW	Permeable 	Black-grass	Wheat	
BHW	Impermeable 	Black-grass	Wheat	
WW	None	Wheat	Wheat	
WHN	Impermeable 	Wheat	-	

As controls, we included plants grown in free competition with the same species (BB and WW) (Table 1), and plants grown with no competing plant, but with a hytex barrier preventing them accessing the other half of the soil volume (BHN and WHN) (Table 1). This experiment was designed to determine the effects of shoot competition (i.e. BHW), chemical root communication (i.e. BCW) and full root competition (i.e. BW) on the overall competition between wheat and black-grass. For the barrier experiment, data was first tested for normality using the Shapiro-Wilk test. Normally distributed data was tested for significance using an ANOVA before comparing each different barrier test through Tukey's HSD post-hoc test for identification of significantly different data.

There were three main findings from this experiment:

1. There are below-ground interactions occurring between wheat and black-grass.
2. Intraspecific competition affects wheat and black-grass differently.
3. Black-grass loses in competition to wheat in this set-up.

The following results (sections 1, 2 and 3) are all from this single experiment, with the data presented in multiple figures to clearly show the main findings. Section 4 presents results of subsequent experiments, carried out to further our understanding.

1. Below-ground interactions

Below-ground interactions between plants are not well understood, it was theorised that these interactions could consist of physical root interactions, the production of chemical root exudates or a combination of both. We cannot rule out other factors also playing a role in plant-plant interactions.

Firstly, we aim to understand to what extent chemical interactions are affecting wheat-black-grass competition. To test this, we compared black-grass and wheat growth in competition separated by a permeable cutex barrier (BCW) to their growth without competition (BHN and WHN) (Figure 1). In the BCW treatment, where wheat and black-grass are in open competition, both the black-grass plants and the wheat plants were smaller (significantly so, in the case of wheat) than the BHN and WHN controls, suggesting that wheat and black-grass can mutually inhibit each other through chemical exchange (Figure 1). This indicates bi-directional chemical inhibition between wheat and black-grass. The nature of this chemical inhibition requires further study.

Secondly, we aim to understand to what extent physical root interactions are affecting wheat-black-grass competition. To test this, we compared black-grass and wheat growth in open competition (BW) to their growth without competition (BHN and WHN) (Figure 2). In the BW treatment, I observed that the black-grass plants were smaller than the BHN controls, while the wheat plants were the same size as the WHN controls (Figure 2). Thus, in this scenario, wheat was able to outcompete black-grass, presumably by occupying the 'root space' of the black-grass plants.

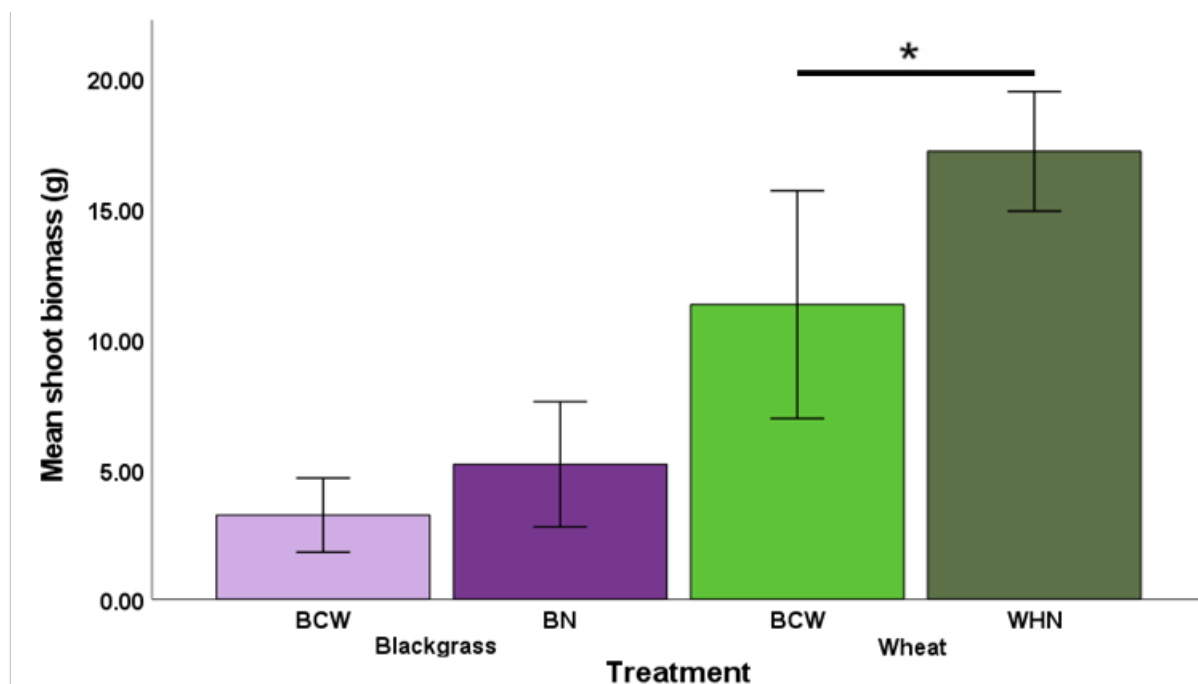


Figure 1 Mutual chemical inhibition between wheat and blackgrass. Blackgrass in the presence of wheat separated by a cutex barrier (BCW – Blackgrass)(N=7) has a slightly smaller mean biomass ($3.22 \pm 1.42\text{g}$) than blackgrass grown by itself (BHN)($5.17 \pm 2.40\text{g}$)(N=4). Wheat in the presence of blackgrass with a cutex barrier (BCW – Wheat)(N=7) has a significantly (*) ($p < 0.05$) smaller biomass ($11.30 \pm 4.37\text{g}$), than wheat grown by itself (WHN)(N=4) ($17.18 \pm 2.29\text{g}$). The presence of these differences with a cutex barrier indicates bi-directional chemical inhibition. Error bars show standard deviation.

We can, therefore, conclude that it is likely that both physical and chemical interactions are playing a role in wheat-black-grass competition. It is, therefore, important to understand to what degree each is responsible for black-grass winning in competition against wheat when in the field.

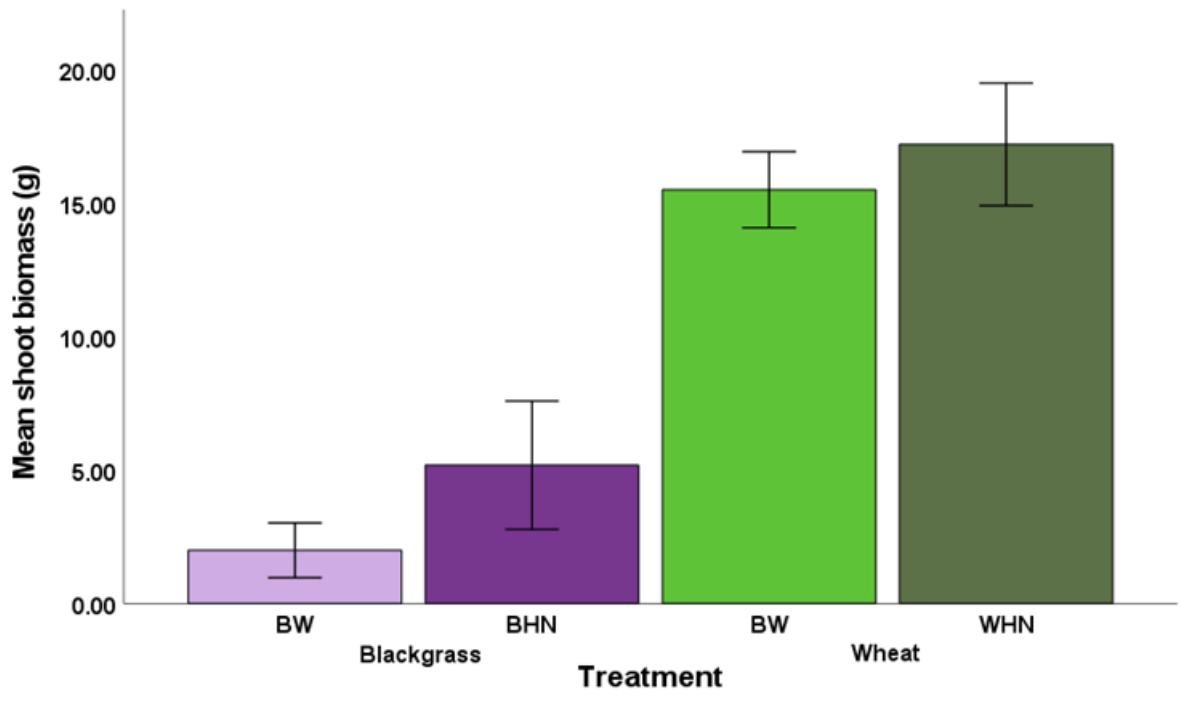


Figure 2 Possible evidence of physical root interactions. Bar chart showing blackgrass and wheat growth in open competition (BW)(N=4) compared to when they are grown by themselves (BHN and WHN)(N=4). No significant difference ($p>0.05$) was seen between BW (wheat)($15.49 \pm 1.42\text{g}$) and WHN ($17.18 \pm 2.29\text{g}$). Also, no significant difference ($p>0.05$) was seen between BW (Blackgrass)($1.99 \pm 1.03\text{g}$) and BHN ($5.17 \pm 2.40\text{g}$) however the mean of BHN is much greater than that of BW (Blackgrass). This indicates that wheat may be gaining an advantage by occupying the 'root space' of blackgrass and that root growth may be an important factor in competition. Error bars show standard deviation.

2. Intraspecific competition

Initially, intraspecific competition was not a factor that we were looking to investigate, however, the results we have found may have important ramifications for wheat-black-grass competition and could aid in our understanding in how black-grass outcompetes wheat.

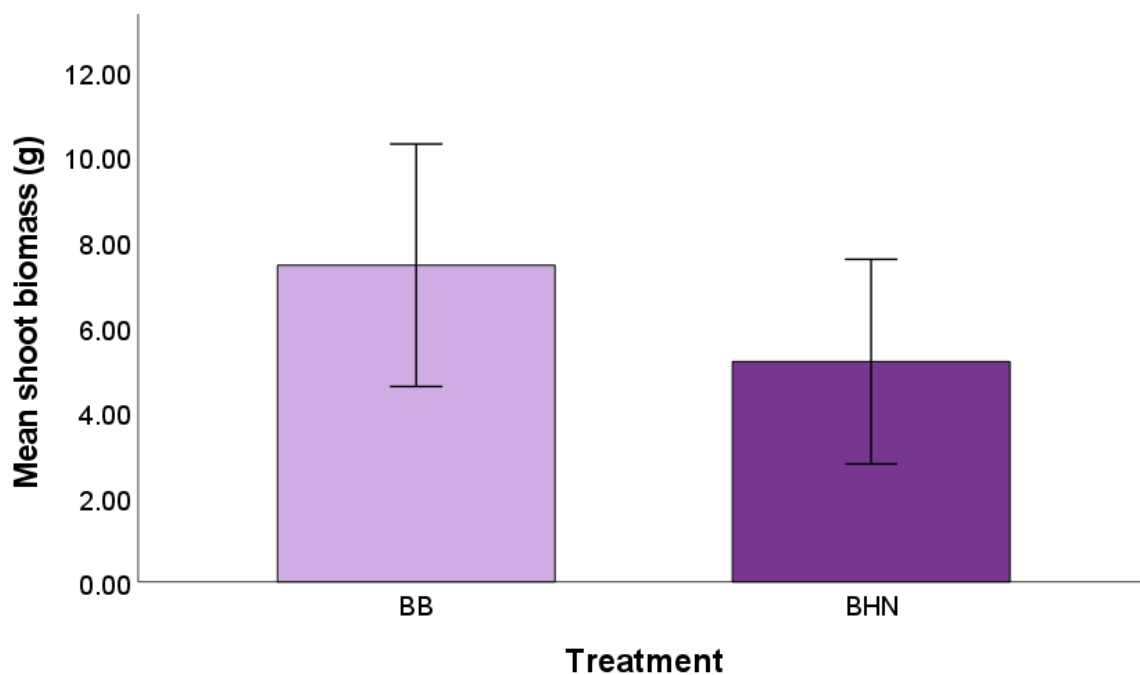


Figure 3 Intraspecific competition in black-grass results in possible up-regulation. Bar chart showing mean shoot biomass of black-grass in intraspecific competition (BB)(N=16) and black-grass grown by itself out of competition (BHN)(N=4). Although not significant ($p>0.05$), BB had a slightly larger mean shoot biomass ($7.43 \pm 2.85\text{g}$) than BHN ($5.17 \pm 2.40\text{g}$). Error bars show standard deviation.

In order to test the effect of intraspecific competition on wheat and black-grass, we compared plant shoot biomass in intraspecific competition (BB and WW) to when plants were grown by themselves without competition (BHN and WHN) (Figures 3 & 4). The BHN and WHN treatments provided a clear reference point for plants grown in the absence of close competition. Interestingly, when black-grass was grown in open competition with itself (BB), the plants were slightly larger than the BHN controls (Figure 3). Conversely, when wheat was grown in open competition with itself (WW), the plants were significantly smaller than the WHN controls (even though the plants had access to the same average soil volume as the WN controls) (Figure 4). This may reflect the fact that wheat has been extensively bred to not compete with other wheat plants in the field (Wiener et al, 2017), and suggests that wheat may actively downregulate its growth in the presence of neighbouring plants.

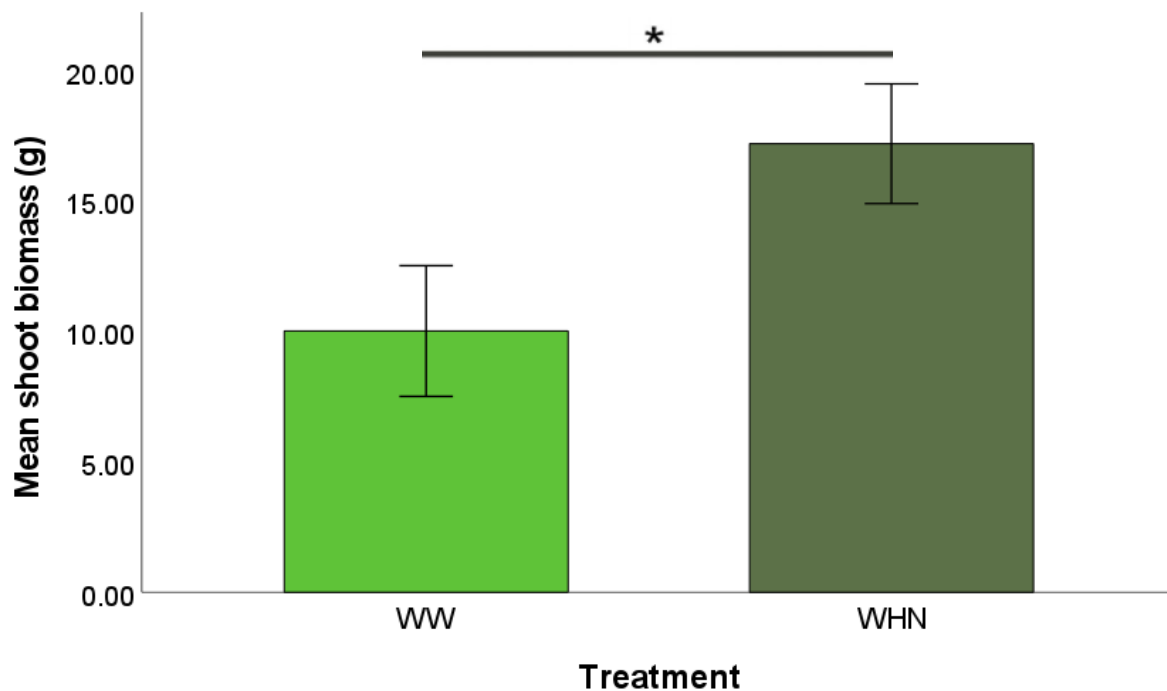


Figure 4 Intraspecific competition in wheat results in down-regulation. Bar chart showing mean shoot biomass of wheat in intraspecific competition (WW)(N=10) and wheat grown by itself out of competition (WHN)(N=4). WW had a significantly (*) ($p < 0.05$), smaller mean shoot biomass ($10.01 \pm 2.50g$) than WHN ($17.18 \pm 2.29g$). Error bars show standard deviation.

To further confirm the idea that wheat and black-grass have different growth responses to neighbour detection, we repeated part of this first neighbour presence experiment, growing wheat against wheat, and black-grass against black-grass, in the presence of a hytex root barrier (WHW and BHB) and absence of a hytex root barrier (WW and BB) (Figure 5) and compared shoot biomass between scenarios.

The WW and BB treatments provided a clear reference for plants grown in open intraspecific competition as would be seen in the field (Figure 5). Interestingly, when wheat was grown in the presence of a 'hytex' barrier (WHW) the plants were significantly larger than when the plants were in open competition (WW) (Figure 5). Furthermore, wheat grown in the presence of a 'hytex' barrier (WHW) had a slightly larger average weekly tiller number than those in open competition (WW) even though those in open competition (WW) theoretically had access to double the soil volume of those restricted to shoot competition (WHW).

For black-grass, we also observed the same as in the previous experiment; when black-grass was grown in the presence of a 'hytex' barrier (BHB) they were slightly smaller than black-grass grown in open competition (BB) (Figure 5). Furthermore, black-grass grown in the presence of a 'hytex' barrier (BHB) had a slightly smaller average weekly tiller number than those in open competition (BB).

The results of these experiment show how black-grass and wheat react differently to the presence of intraspecific neighbours; wheat actively downregulates its own growth due to below-ground neighbour detection, whereas black-grass indicates a neutral to positive effect of intraspecific neighbour presence. Since we do not see a negative response to neighbour presence in black-grass, this may indicate why black-grass is able to grow in such high densities on arable land.

In wheat, increasing planting density and decreasing soil volume has previously been reported to significantly decrease growth (Wheeldon et al, 2020). Black-grass can be found at high densities in arable crops indicating that it may be less sensitive to wheat in terms of intraspecific density pressures and soil volume availability. If so, this may contribute to the ultimate ability of black-grass to outcompete wheat for space and nutrient capture. We, therefore, hypothesise that black-grass and wheat will have unequal responses to changing neighbour density and soil volume, with black-grass showing lower sensitivity to increased neighbour density than wheat.

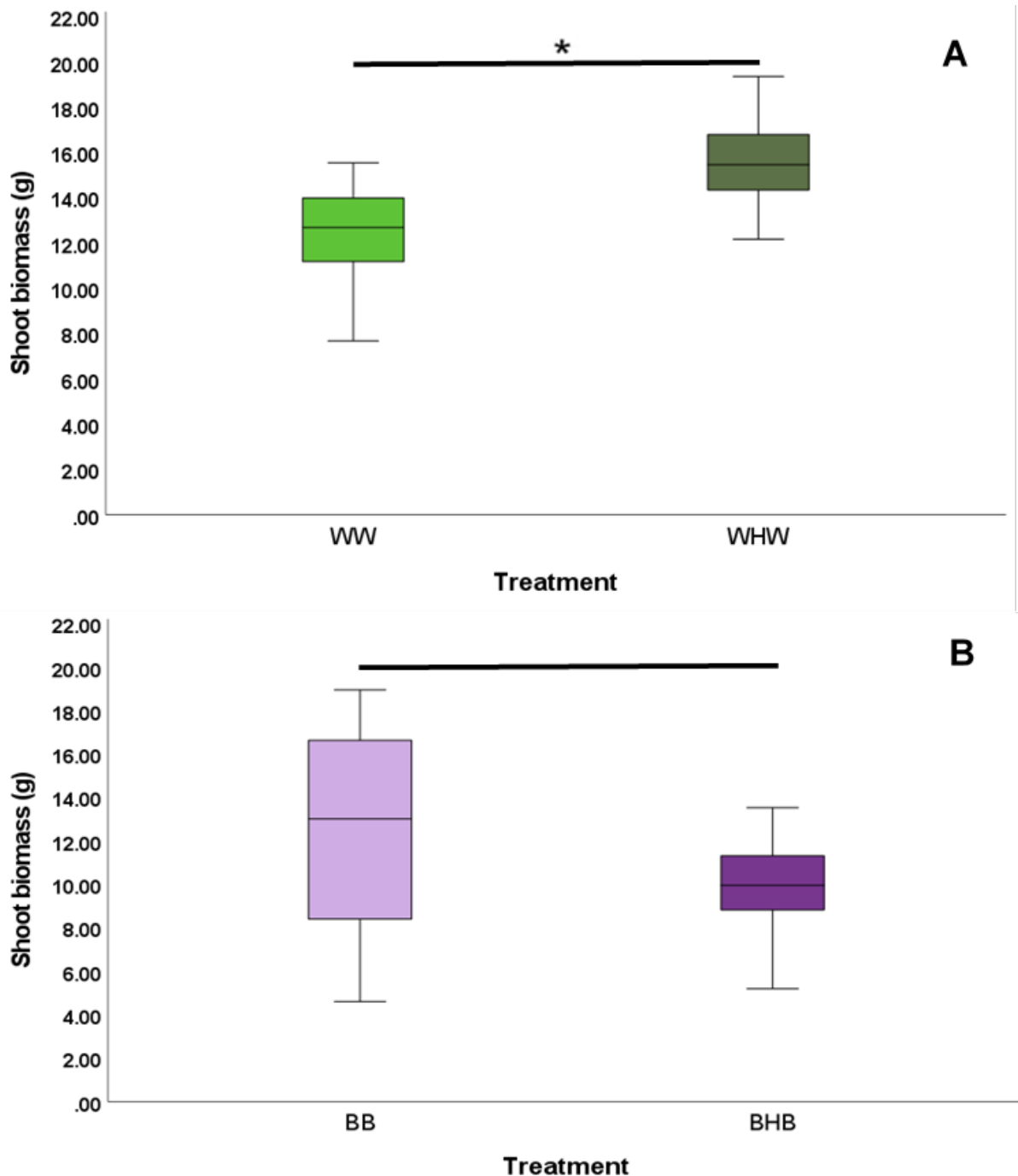


Figure 5: Wheat and blackgrass growth responds differently to barrier controlled intraspecific competition. Boxplots showing the dry shoot biomass (g) of (A) blackgrass and (B) spring wheat as a result of differing levels of intraspecific competition. WHW and BHB indicate the biomass of wheat and blackgrass with only shoot competition, separated by an impermeable Hytex root barrier. WW and BB indicate the biomass of wheat and blackgrass that are not separated by a root barrier, which are in open competition. * indicates a significant difference between samples (t-test, $n = 12$, $p < 0.001$). WHW was significantly larger ($15.59 \pm 1.91\text{g}$) than WW ($12.43 \pm 2.18\text{g}$) ($t(22)=3.785$, $p=0.001$). BHB ($9.88 \pm 2.11\text{g}$) were slightly smaller than BB ($12.49 \pm 4.87\text{g}$) although not significantly ($t(22)= - 1.707$, $p=0.102$).

3. Black-grass loses to wheat in this set-up

In this original experiment, we naively expected black-grass to be far more effective at competing with wheat than was observed. The reality is that under the conditions tested, black-grass is out-competed by wheat. This can be seen in Figure 2 where black-grass grown with wheat is smaller than black-grass grown by itself. We, therefore, required a different system that would better reflect the winter field conditions and, allow us to visualise the effects of black-grass competition on wheat.

Wheat – black-grass competition in winter conditions

I trialled a more realistic system to try and observe black-grass outcompeting wheat. I set up an experiment under winter conditions (8h day lengths at 10°C) to better recreate the field conditions in which wheat and black-grass grow. In this experiment, wheat and black-grass were grown together in a 2L pot. In order to increase the competitive effects of black-grass, six black-grass were planted at equal distance surrounding one centralised winter wheat plant. As a control, winter wheat was planted on its own without competition, also in a 2L pot. This experiment was designed to allow a better visualisation of the competitive effects of black-grass on wheat.

Wheat grown on its own (WN) (Figure 6) provides a clear reference point for viewing black-grass competition. For the first 4 months both sets of wheat plants gradually increased in their tiller number; there is no difference between the treatments during this period. From 4 months onwards, both treatments start to lose tillers as expected, and at this point significant (but small) differences arise between the treatments. Thus, any competitive effects of black-grass only appear late on in growth, after almost a full simulated winter. Even then, the effects are relatively small, given there are 6 more plants in the WB pots than the WN pots.

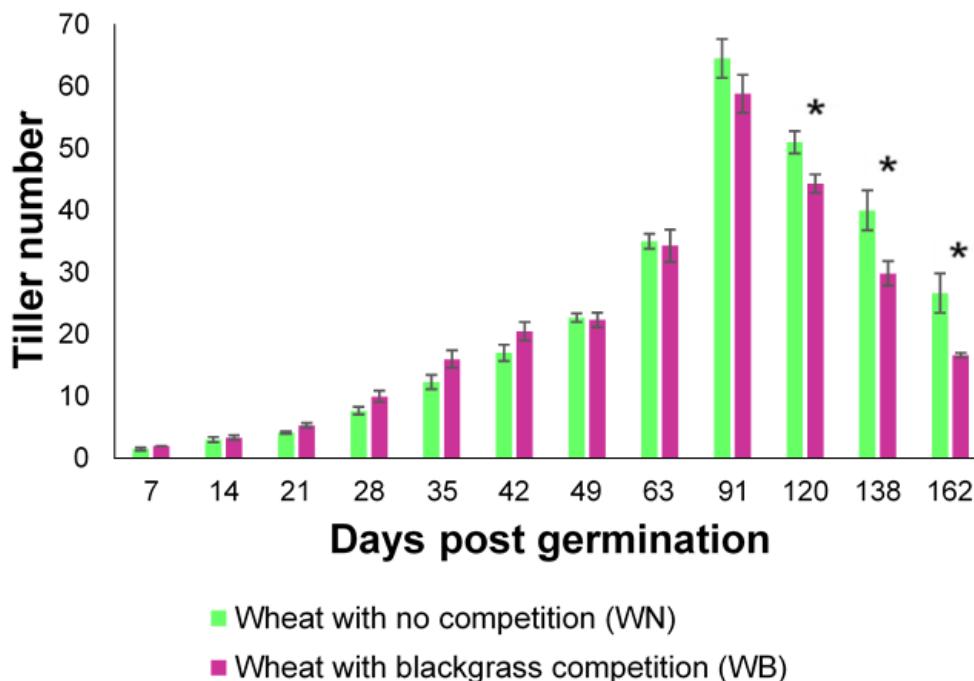


Figure 6 Blackgrass competition only decreases wheat tiller numbers late in growth. Mean tiller number over time for wheat grown on its own without competition (WN), and wheat grown with high density blackgrass competition (WB). T-tests were carried out at each time stage, (*) indicates significant difference ($n=6$, $p<0.05$) between wheat biomass in and out of blackgrass competition. Error bars show standard deviation.

The results of the experiment indicate the competitive ability of black-grass likely requires very extensive growth periods to emerge. This is consistent with the much poorer establishment of black-grass plants compared to wheat, and their initially slower growth. Effectively, it is a 'tortoise and hare' situation; black-grass may ultimately gain an advantage by growing slowly but consistently.

Regardless of the competitive outcome of this experiment it is clear that this set-up could not be used as a high-throughput screening method for competitive wheat lines, given the length of the experiments, and the difficulty creating these winter conditions. It, therefore, seems increasingly unlikely that a system testing directly for competition will be feasible using this approach.

4. Root growth hypothesis

From our initial experiment, there was evidence of below ground competition between wheat and black-grass, where the roots of the wheat seem to occupy the ‘root space’ of black-grass and thus, reduce black-grass growth (Figure 2). Given this finding, we wanted to gain a better understanding of black-grass and wheat root growth to determine whether there are any significant differences in the rate or amount of roots they produce. If so, this could indicate an important trait that could convey competitiveness.

To identify whether black-grass gains a competitive advantage over wheat during the winter due to differences in root growth, I performed two separate hydroponic experiments under different conditions in which winter wheat (Claire) and black-grass root growth was recorded over time by measuring water displacement caused by the roots (Figure 7). Black-grass and wheat were grown individually in 1L hydroponic pots in both standard (16h day length at 22°C) and winter conditions (8h day length at 10°C). This could allow both comparisons between species in each condition, and between conditions for each species. The standard condition experiment was stopped after 83 days due to no further increase in root displacement.

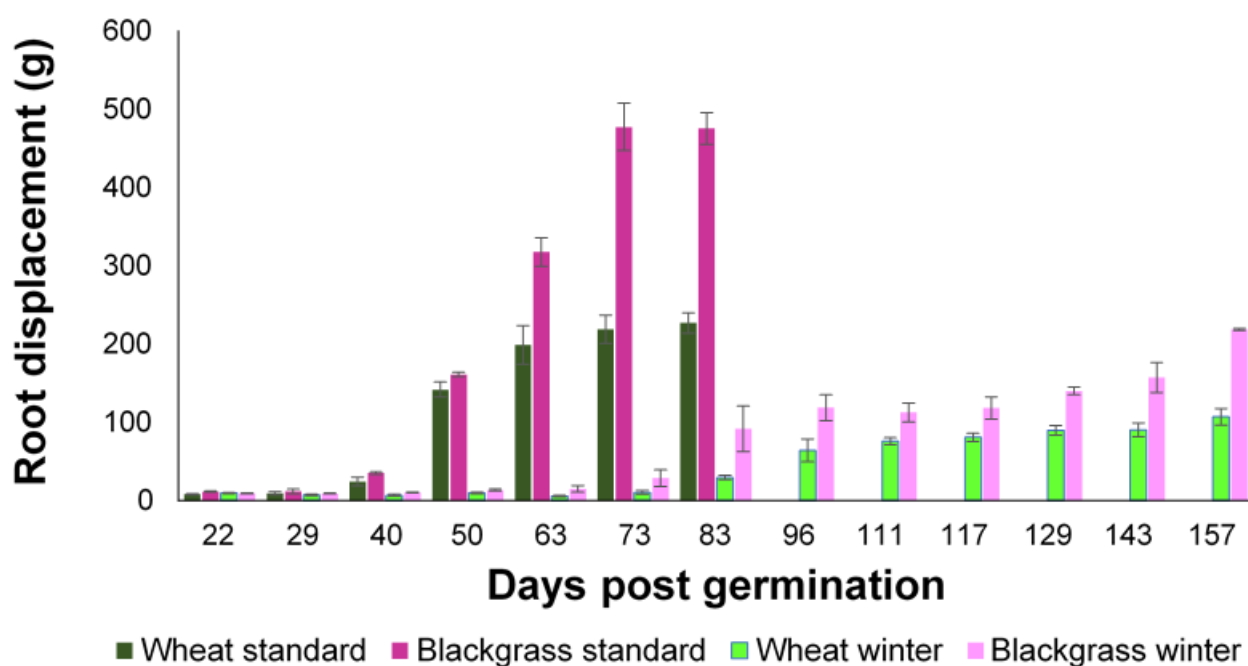


Figure 7 Root growth varies between species and conditions. Mean Root growth (g) measured through water displacement displayed over time after transfer to hydroponics for both blackgrass (N=2) and wheat (N=6) in winter and standard conditions. Error bars show the standard deviation.

Firstly, there is a clear difference between conditions; wheat and black-grass produce roots at a much greater rate under standard long-day spring conditions compared to short-day winter conditions (Figure 7). Within these spring conditions, we can see that black-grass manages to reach a much higher root density than wheat. The dry root mass taken at the end of the experiment shows the final root biomass of black-grass was almost twice that of wheat (Table 2), but the roots also formed a much larger proportion of the total plant biomass in black-grass, 40% compared to 17% in winter wheat. This demonstrates that black-grass prioritises root growth much more than wheat.

Table 2 Whole plant, shoot and root mass of Wheat and black-grass growth under standard conditions.

Species	Mean shoot mass (g)	Mean root mass (g)	Mean total mass (g)
Wheat	35.58	7.35	42.93
Black-grass	21.11	14.07	35.18

Under winter conditions, growth of both species is slow, but black-grass root growth is still approximately double that of wheat. This experiment is still on going, so it will be interesting to see if this difference between black-grass and wheat increases.

The results of this experiment show that root growth is dependent on the conditions and that under standard conditions, black-grass roots grow to a much higher density than wheat roots. The ability of black-grass to produce a larger root system indicates a resistance to density-induced growth inhibition. Root exudates have been shown to play a role in plant growth in response to density, this could therefore, indicate an alternate response in black-grass to these signals.

I hypothesize that the reason that black-grass ultimately outcompetes wheat under field conditions is that black-grass has much stronger root growth, which is also less density-sensitive than wheat root growth. During winter, black-grass builds a much larger and denser root system than wheat plants in the same field. This takes many months, and does not initially effect the growth of wheat plants when overall growth rates are slow anyway. However, in spring, when there is a demand for nutrient and water to fuel rapid growth, the much larger root system of black-grass then allows it to outcompete wheat plants, causing the familiar yield losses.

The time it takes for black-grass to build its 'hidden advantage' would then explain why spring wheat plantings are not affected by black-grass; there is not enough time for black-grass to gain the same advantage as it does over the winter, meaning spring wheat may be able to access a much larger 'root space' and therefore, better acquire resources. There may still be competition between spring wheat and black-grass, however, it is likely to be unsequential compared to wheat field level yields due to the much lower black-grass emergence during this time. The much more vigorous root growth of barley compared to wheat might also explain why black-grass is such a problem for wheat, but not barley.

5. Key issues to be addressed in the next year

Below I outline the main issues arising and how we plan on overcoming these hurdles as well as outlining possible directions for future research. Given the the findings so far, it is likely that our original plan for this project will require some revisions.

5.1) Developing a screen for wheat-black-grass competition

Our initial aim was to set up an unbiased (non-trait based) screening process to allow for the identification of more/less competitive wheat lines. The initial method tested under standard glasshouse conditions proved not to be effective due to the lack of competitive effect of black-grass. Using higher black-grass density and winter conditions, we expected to be able to see the competitive effects of black-grass. However, our findings have shown that despite this change, we are not seeing an effect from black-grass on wheat within the first four months of growth. Due to the length of time these experiments require, and the lack of suitable 'winter' growth space available, this set-up would clearly not be suitable for the unbiased screening of approximately 100+ wheat lines. It is unfeasible to directly screen for competition. We therefore, propose an alternate approach, in which we will screen for variation in root growth, which we think is the central area in which black-grass eventually gains a competitive advantage over wheat.

5.2) Winter root growth in wheat and black-grass

Despite these setbacks in screening for competition, a promising line of investigation relates to the higher rate of root growth observed in black-grass relative to wheat in our hydroponic experiments (Figure 7). We hypothesise that, in the field, black-grass outcompetes winter wheat because it has higher root growth during winter, and therefore, dominates resource capture in the spring (root-based hypothesis). To test whether black-grass root growth is indeed higher than winter wheat in the field, we will perform fieldwork in collaboration with our collaborators at ADAS. During the season 2021-2022, we will perform simple 'shovelomics' type assessments in fields which have a black-grass problem, which will involve the assessment of individual wheat and black-grass root growth plants a few weeks after drilling, in mid-winter, and then in the spring. This will tell us how root systems of the two species develop over time and whether by the end of growth, the black-grass root systems are larger than those of wheat.

5.3) Screening for wheat root growth

If our root-based hypothesis is correct, then wheat lines with great (winter) root growth may be the more competitive germplasm we are seeking. Thus, screening for wheat root growth properties may circumvent the issues with screening for direct wheat-black-grass competition. We already have seedling root growth data from 293 wheat landraces, but it is unclear whether greater seedling root vigour leads to long-term increases in root vigour.

a) We, therefore, plan on investigating the relationship between short- and long-term root investment in winter wheat landraces, to see if early differences in lines predict long-term root growth. We will pick 10 extreme lines (very low or very high seedling root growth) from the 293 landraces, and track their long-term root growth in hydroponics. If seedling root growth does predict adult root growth, and if our root-based hypothesis is correct, then we will already have sufficient data to select for more- and less- competitive germplasm.

b) We also plan to investigate adult root growth among current elite wheat cultivars in our hydroponic system. If our hypotheses are correct, this may directly identify current wheat varieties that are more competitive against black-grass.

5.4) Directly testing the root growth hypothesis

To directly test whether high root growth confers increased competitiveness against black-grass, we will perform 'semi-field' experiments with our collaborators at ADAS. These 'container' experiments will allow for the competitive ability and related phenotypic traits of wheat to be assessed under semi-field conditions, as a step between glasshouse and field experiments. Wheat and black-grass or barley and black-grass, will be sown into large containers in set densities with one of three types of competition between plants; 1) shoot (roots separated by plastic dividers), 2) root (shoots separated using polyethene barriers) and 3) full (no separation of roots/shoots).

The crop lines selected will consist of 1) wheat landraces with vigorous root growth as seedlings and/or high tillering as adults (which would be expected to have increased rooting), 2) commercial lines, including a 'standard' winter wheat and winter barley as well as hybrid varieties that might have more vigorous rooting and 3) two wheat lines (Shamrock and RGT Wasabi) that have been identified as possibly having larger than average root systems. Containers will be placed outside on an uncovered hardstanding area from October-August. Growth of both wheat, barley and black-grass plants will be tracked monthly, with final wheat ear biomass and black-grass seed production also measured. We can then analyse the root growth of the different lines after the experiments have finished by sectioning the soil mass, and correlate that with the displayed degree of black-grass competition. The experiments may not prove conclusively that increased root growth causes competitiveness, but it may rule it out as a factor if there is no correlation between root growth and competitiveness.

Through these experiments, we aim to highlight whether root growth is a key factor in wheat competitiveness against black-grass. If so, we would be able to recommend high rooting cultivars as a way of suppressing black-grass growth.

Although root growth may be an important factor, it is important not to overlook the other complex interactions that may be occurring between wheat and black-grass. A second key aim will be to understand the nature of the signalling that occurs both inter- and intra- specifically between black-grass and wheat, and how this leads to changes in growth and development. These changes may be key in understanding how black-grass outcompetes wheat.

5.5) Understanding wheat-black-grass signalling

Our results indicate that interactions between the root systems of wheat and black-grass (and also between wheat-wheat and black-grass-black-grass) are critical in determining the outcome of competition between the species. Our experiments have suggested that it is likely that part of these interactions are chemical root exudate based. Further investigation using cutex permeable barriers will be used to more accurately determine to what level chemical root exudates are responsible for these intra- and interspecific below-ground interactions. If chemical root exudates are in-fact playing a role, identifying possible candidates from literature and isolating these certain exudates for testing with wheat and black-grass will allow us to see any effects they may have on growth.

5.6) Understanding black-grass density responses

Our results so far indicate that neighbouring black-grass plants may promote each other's growth (i.e. facilitation), whereas neighbouring wheat plants strongly inhibit each other's growth. In wheat, increasing planting density and decreasing soil volume has previously been reported to significantly decrease growth (Wheeldon et al, 2020). Black-grass can be found at high densities in arable crops indicating that it may be less sensitive to wheat in terms of intra-specific density pressures and soil volume availability. If so, this may ultimately give black-grass a significant competitive advantage over wheat for space and nutrient capture. We, hypothesise that black-grass and wheat will have unequal responses to changing neighbour density and

soil volume, with black-grass showing lower sensitivity to increased neighbour density than wheat. To test this idea, wheat and black-grass are currently being grown in 1/pot and 4/pot densities at both 100ml and 500ml soil volumes. This will allow us to measure the fold-change in growth of both species caused by increasing density or decreasing volume.

5.7) Multi-dimensional interactions

Our results indicate that the interaction between wheat and black-grass may be complex and multi-dimensional. We hypothesise that to see the full competitive effect of black-grass against wheat may require both the mutual interaction of wheat and black-grass, the self-promotive effect of black-grass on black-grass, and the self-inhibitive effect of wheat on wheat (Figure 2, Figure 3). We will first test the effect of removing mutual wheat inhibition, using a modified version of the set up described in Figure 3. Two wheat plants will be grown in a rectangular pot, either separated by no barrier (W2), or by an impermeable barrier (W1+1). Each wheat plant will be grown with 4 black-grass plants, placed in the corners of each half pot (i.e. W2/B8, W1+1/B4+4). Each rectangular pot will contain the same number of plants, each with the same mean soil volume available, and with the same spacing between shoot systems. However, the W1+1/B4+4 pots will have no root interaction between the two half-pots, removing W-W inhibition from the system. Conversely, the W2/B8 pots will have the full range of interactions available. Given the data in figure 3, we hypothesise that the W2/B8 wheat plants should be smaller than in the W1+1/B4+4, and therefore, may be more strongly outcompeted by the black-grass plants (i.e. more black-grass biomass in each half-pot). If this experiment is successful, we will look at ways of testing whether the positive black-grass-black-grass interaction is needed for competition, although this may will be more difficult, given the ratio of black-grass:wheat we are currently having to use.

6. Outputs relating to the project

(events, press articles, conference posters or presentations, scientific papers):

Output	Detail
Presentation	University of Leeds Faculty of Biological Sciences postgraduate symposium
Presentation	AHDB Agronomists' Conference 2021
Conference	Attended Monogram 2021

7. Partners (if applicable)

Scientific partners	
Industry partners	RSK ADAS Ltd.
Government sponsor	