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Student Report 63

Wheat germplasm for enhanced competition against black-grass

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Figure / Table	**Data collection carried out by:**	**Notes**
Figure 2.1		Adapted from Wheeldon., (2023) with permission.
Figure 3.13	Cara Wheeldon	Panels E, F based on data collected by Cara Wheeldon.
Table 3.1	Cara Wheeldon	Barley calculations based on values collected by Cara Wheeldon.
Figure 5.1	Fay Walsh	Adapted with permission from figure provided by Fay Walsh (Unpublished).
Figures 3.2, 3.3, 6.2, 6.4	Jed Clark, Lynn Tatnell and ADAS Boxworth field team.	Container and field trial plant assessments.

Abstract

Black-grass (*Alopecurus myosuroides*) is the major weed species affecting crops of winter wheat (*Triticum aestivum)* in the UK. Changes in arable cropping and tilling systems have led to black-grass becoming an increasing issue in modern crops. A lack of understanding of crop-weed interactions, particularly below-ground, contributes to the failure to combat black-grass infestations. Control of black-grass relies on herbicide application and integrated weed management (IWM), but more needs to be done to protect winter wheat harvests. Competitive cultivars have shown promise in black-grass management however uptake by farmers has been poor due to variation in results at the individual field level. There is therefore a requirement for a quick, easy screening approach by which to assess cultivar competitiveness prior to sowing in the field. This thesis aims to determine how and when black-grass gains its competitive advantages, to assess and screen crop varieties for possible traits (including root growth) associated with conveying competition, determine the competitive ability of crop varieties against black-grass and to determine any correlations between traits and competitive ability. The main hypothesis being tested in this thesis was that crop cultivars would vary in competitive ability due to differences in root growth. It was observed that black-grass relies on increased root growth, faster winter growth and the longer winter growing season to accrue competitive advantages over winter wheat. However, barleys (*Hordeum vulgare*) and landrace wheats with larger root systems showed increased competition against black-grass compared to elite wheats indicating root growth may impact. Variation in both competitive ability and root growth was observed in a range of crop varieties. Container and field trials confirmed controlled screen competition results are transferable to real conditions. Overall, correlations were seen between root growth and competitive ability between crop cultivars, with increased root growth resulting in increased competition. However, within elite wheats this relationship was less clear. Clear correlations were observed between shoot and root production in winter conditions. This novel competitive screening approach will allow for further testing of competitive cultivars. Understanding black-grass growth will allow for further work to identify the best approach for crop breeding to increase crop competitiveness. Further analysis of winter growth rate and early vigour may allow for the indirect selection of higher rooting varieties with increased competitiveness against black-grass through increasing the ability to withstand biotic and abiotic stresses.

List of abbreviations

µg / mg / g /Kg	micro- / milli- / kilo/ gram
µL / mL / L	micro- / milli- / litre
µmol / µM	Micromolar
1/pot	1 plant per pot
2D	2 dimensional
3D	3 dimensional
4/pot	4 plants per pot
ACCase	Acetyl CoA carboxylase
AHDB	Agriculture and horticulture development Board
ALS	Acetolactate synthase
AMF	Arbuscular mycorrhizae fungi
Annual ryegrass	*Lolium rigidum Gaud*
ANOVA	Analysis of variance
Arabidopsis	*Arabidopsis thaliana*
ATS	*Arabidopsis thaliana* salts
Barley	*Hordeum vulgare*
Barnyardgrass	*Echinochloa crus-galli*
BG	Black-grass
Black-grass	*Alopecurus myosuroides*
C	Cutex
Common chickweed	*Stellaria media*
Common poppy	*Papaver rhoeas*
d	Day(s)
DIMBOA	2,4-dihydroxy-7-methoxy-1,4-benzoaxazin

	-3-one
Fava bean	*Vicia faba*
GS	Growth stage
H	Hytex
Ha	Hectare
Horseweed	*Conzya canadensis*
hrs	Hours
HWB	Hybrid winter barley
IPM	Integrated pest management
Italian ryegrass	*Lolium multiflorum*
IWM	Integrated weed management
Jointed goatgrass	*Aegilops cylindrica*
Lug-leaved speedwell	*Veronica hederifolia*
M	Mole
mm/ cm/ m	milli- / centi- / metre
n	Number of samples
N	None
^{o}C	Degrees celcius
Pigweed	*Amaranthus spp.*
QTL	Quantitative trait locus
Rice	*Oryza sativa*
RL	Recommended list
RSA	Root system architecture
s	Second(s)
Scentless mayweed	*Tripleurospernum inodorum*
SL(s)	Strigolactone(s)
SW	Spring wheat

SY	Syngenta
t	Tonne
UK	United Kingdom
VOC’s	Volatile organic compounds
W	Wheat
WB	Winter barley
Wheat	*Triticum aestivum*
Wild oats	*Avena spp.*
WW	Winter wheat

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Chapter 1 General introduction

1.1 Weeds in agriculture

A weed is defined as ‘a plant, or population of plants, in a specific time and place, causing significant harm, either immediately or in the longer term, based on a holistic analysis of both their positive and negative attributes’ (Merfield, 2022). Therefore, categorization as a ‘weed’ is primarily due to human perception of their negative impacts, by occurring in unwanted places. Agriculture has also been defined as ‘the management of terrestrial ecosystems to divert their productive capacity to serve human needs’ (Millennium Ecosystem Assessment, 2005). Humans are therefore in a constant battle to prevent nature returning to its natural state. Work is underway to distinguish whether ‘weediness’ has a genetic basis (MacGregor, 2020), and whether plants that become weeds have certain genetic advantages over other plants. However, currently the aforementioned definition remains the best description of what a weed is, with no definite answer to the question ‘What makes a weed a weed?’ (Bourgeois et al., 2019).

The intensification of crop management has decreased the diversity and abundance of arable weeds (Sutcliffe and Kay, 2000). Although as humans we view weeds in a negative light, they remain of vital importance to the sustainability and biodiversity of ecosystems (Storkey and Westbury, 2007). Weeds may act as primary producers, providing food for birds in the form of seeds while also hosting food in the form of invertebrate (Hawes et al., 2003). The loss of arable weeds has left a hole in the food chain that cannot be filled by monoculture crops, the knock-on effects have included the loss in invertebrate presence and diversity and the resultant decline in farmland bird populations (Chamberlain et al., 2000). There is therefore a constant struggle between maintaining biodiversity for a healthy ecosystem and reducing weed presence for increased crop yields.

In 1994 it was estimated that competitive effects of weeds are responsible for a 10% reduction in agricultural production worldwide (Oerke et al., 1994). There are three types of weeds, annuals, biennials and perennials grouped according to lifespan (Talaka and Rajab., 2013). In each group weeds can be either broadleaf or grasses. Annual weeds have short lifecycles of less than a year. However, they make up for this through their ability to produce a vast number of seeds which can be very difficult to eradicate (Talaka and Rajab., 2013). Biennials live for two years, often remaining vegetative in the first year, utilising the tap root as a food-storage organ, before flowering and producing seed in the second year after which it dies (Talak and Rajab., 2013). Perennials have the capacity to reproduce through seed production like annuals but also through vegetative propagation (Håkansson., 2003). Root structures of perennials such as rhizomes, stolons and creeping roots allow the storage of nutrients allowing perennials to be persistent and durable with them being able to survive for over 2 years (Håkansson., 2003; Favrelière et al., 2020) even with the loss of above ground structures. Common agricultural weeds affecting arable crops in the UK include Alopecurus myosuroides (black-grass), Lolium multiflorum (Italian rye-grass), Avena spp. (wild-oats), Papaver rhoeas (common poppy), Stellaria media (common chickweed) and Tripleurospermum inodorum (scentless mayweed), all of which are good at developing herbicide resistance (Hull et al., 2014).

Agricultural weeds occur specifically in areas of land reserved for crop growth, where crop yields can be massively reduced by weed pressures through competition for space, nutrition, light, and water (Bastiaans et al., 2000). The presence of weeds in agriculture results in significant agronomic and economic damage (Moss, 2019). Weeds compete with the crop; therefore, it is essential to understand crop-weed interactions in order to reduce crop yield losses.

Bourgeois et al., (2019) carried out a study to determine the key functional attributes of arable weeds compared to non-weed plants, these attributes and favourable conditions will not be true for all weed species but in general they found weeds to be therophytes, annual plants with quick growth with high specific leaf area. Weeds tended to have earlier and longer flowering whilst having a high affinity for nutrient rich soils, high light availability and dry conditions. Weeds are also able to produce a large number of seeds that are able to stay dormant in the seed bank for a long time through harsh conditions until the conditions are favourable for germination (Norris, 2007).

1.1.1 Reduction in crop fitness increases susceptibility to weeds

Dense monoculture crop growth has provided the perfect conditions for weed proliferation (Cusworth and Lorimer, 2024). To maximise field level yields, crop plants within a field are required to be as similar as possible in size. This makes harvest easier, and this way each individual plant will be taking a similar share of space and resources, allowing all plants to grow efficiently. To maximise crop resource capture and to ensure equal sharing of resources, it is theorised that breeding has minimised competition between crop plants, and maximised population growth, by a reduction in individual plant fitness (Weiner et al., 2010; Weiner, 2017). If individual crop plants were fitter and better at competing it would lead to some plants outcompeting others. This would produce a heterogeneous field containing some outcompeted plants, likely smaller and lower in yield than their competitors thus reducing overall field level yields. This selection for field level yields and thus varieties with lower competitiveness very likely contributes to the susceptibility of crops to competitive, fast-growing plants such as weeds. Weeds may have a significant competitive advantage over the less competitive crop plants, increasing resource capture by the weeds, resulting in quick drastic losses in crop yields (Weiner, 2017).

1.2 Black-grass

Black-grass (*Alopecurus myosuroides Huds.*) is an annual agricultural grass weed species propagated solely by seeds (Moss, 1983). Black-grass is endemic to Britain, France, Italy, Germany, Denmark and Holland (Cusworth and Lorimer, 2024) having been first noted for its negative impact on UK agriculture nearly 200 years ago (Sinclair, 1838). Over recent years black-grass prevalence has increased dramatically and is now reportedly found in over 60 countries worldwide impacting arable crops mainly in Western Europe (CABI, 2008) whilst also appearing to increase in prevalence in Scandinavia and Eastern European countries including Poland (Moss, 2010). Black-grass prefers cool damp conditions, growing best on water retentive soils, making it more of an issue in heavy clay or silt soils, whilst it is less of an issue on lighter sandy ground (Moss, 2010).

1.2.1 Black-grass eradication is virtually impossible

Black-grass seed is small and numerous, ensuring plenty of seed returns to the soil and seedbank for subsequent seasons. Black-grass seed is typically shed in June/July, before the harvest of winter wheat (Moss, 1983). In crops of winter wheat, black-grass ear number per plant is highly variable, between 2-20, depending on the growing conditions. Each ear can produce approximately 100 seeds and with populations able to reach 500 ears/m^2, seed return can exceed 50,000 seed/m^2 (Moss, 2010). Black-grass seed however has low viability, with typically only around 50-60% of seeds containing a caryopsis when shed (Moss, 1999). The cross-pollinating nature of black-grass may explain the low and variable viability (Moss, 2010). Once the seed is in the soil, approximately 70-80% will become unviable, with only 1-3% remaining viable after three years (Moss, 2010). Only seed within the top 5cm of soil will successfully emerge (Moss, 2010). Reducing seed return to the seedbank is a key area of research for black-grass control (Bitarafan and Andreasen, 2020) however the sheer number of seed make it almost impossible to irradicate after infestation.

Geographically black-grass is likely spread due to the movement of seed between fields by agricultural transport and machinery such as grain harvesters and tractors (Walsh, Newman and Powles., 2013). Upon harvest and grain separation current equipment is unable to prevent the separation of black-grass seed from the chaff fraction resulting in its redistribution throughout the field (Broster et al., 2016; Glasner et al., 2018; Bitarafan and Andreasen, 2020). Although this increases black-grass prevalence, it doesn't explain the intensification of infestations within fields meaning other factors are also impacting how well black-grass is able to survive and compete in its environment.

1.2.2 Why is black-grass such a problem?

Black-grass is one of the most problematic weed species in the UK and Northwest Europe affecting autumn sown crops (Riches, 2022). Black-grass populations if left uncontrolled, can increase 30-fold each year (Moss, 2010). Herbicide resistance and changing cropping practices may go some way to explain the dramatic increase in black-grass prevalence over the last 40 years. Black-grass is able to dramatically decrease crop yield, particular winter wheat. It does this through competition for space and resources, in particular nitrogen (Moss, 2010). At black-grass population densities between 12-25 plants/m^2 winter wheat sees yield losses of between 0.4-0.8 t/ha whereas at much higher black-grass densities of 100 plants/m ^{2} yield losses can reach 2 t/ha (Moss, 2010). This loss in yield often has a huge economic impact on growers highlighting the importance of further research into black-grass management.

1.2.3 Herbicide resistance in black-grass

Herbicides were introduced in the 1940s and have allowed for agricultural intensification and increased control of weeds. They remain the main control method for agricultural weeds. A 5% crop yield loss due to weed presence is the threshold to justify the use of herbicides against weeds (Moss, 2010). A 5% reduction in winter wheat can be caused by a black-grass density of 12 plants/m^2 (Moss, 2010) but in extreme cases herbicide application may be justified at lower black-grass densities of below 1 plant/m^2 (Moss, 2010). In cereals, pre-emergence herbicides, (herbicides that are sprayed onto field prior to crop sowing to prevent germinated weed seedlings from becoming established), can give good control over black-grass, killing seedlings that have already germinated, reducing levels by 50-80% (Moss, 2010) whereas post-emergence herbicides that can be used on established weeds, have the potential to reduce black-grass by more than 95% (Moss, 2010).

However, black-grass has gained notoriety, mainly due its acquisition of herbicide resistance to a wide range of herbicides with multiple modes of action. Black-grass has been able to gain target site resistance to both acetolactate synthase (ALS) and acetyl CoA carboxylase (ACCase) classes of herbicides (Hull et al., 2014).

ALS herbicides are a class of herbicides introduced in the 1980s that inhibit the enzyme acetolactate synthase (ALS). This enzyme is important in the biosynthesis of branch chain amino acids valine, leucine and isoleucine in plants, bacteria, fungi, yeasts and algae (Whitcomb, 1999). In plants ALS herbicides can have negative effects on both plant growth (Blair and Martin, 1988) and reproduction (Fletcher et al., 1993). Resistance to ALS inhibitors has been seen in over 140 weed species, more than any other class of herbicides (Heap, 2014) likely because ALS herbicides have only one mode of action (Whitcomb, 1999). In black-grass target site resistance caused by mutations in the target genes Pro-197-Thr and Trp-574-Leu ALS was seen in 7% of plants treated with Atlantis WG (Bayer CropScience) herbicide (Moss et al., 2014) with a further 20% of plants surviving the herbicide treatment indicating both target site and non-target site resistance can occur including cross resistance to other herbicides (Hull et al., 2014; Alwarnaidu Vijayarajan et al., 2021).

ACCase herbicides are a class of herbicides first introduced in 1978 (Kaundun, 2014) that inhibit acetyl CoA carboxylase (ACCase) in grass weeds. This enzyme is important for the biosynthesis of fatty acids (Harwood, 1988). Inhibition of this enzyme blocks fatty acid biosynthesis, preventing the formation of lipids and secondary metabolites. This results in a loss of cell membrane integrity, causing metabolite leakage and cell death (Délye, 2005; Kaundun, 2014). In black-grass, resistance to ACCase herbicides is predominately due to mutations at the Ile-1781 codon (Délye, 2010) however many more mutations at different codons have also been identified (Hull et al., 2014).

Black-grass has also gained non-target site resistance. Resistance occurring in a different location to the herbicide mode of action target. Non target site resistance, including enhanced metabolism affects both ALS and ACCase herbicides amongst others, and is the most common form of herbicide resistance in UK grassweeds (Moss, 2017). Non-target site resistance can occur through multiple mechanisms (reviewed in Jugulam and Shyam, 2019) however in simple terms, weeds are able to detoxify herbicides, and as a result become more tolerant, irrespective of herbicide chemistry or modes of action.

As of 2020 there were 253 herbicide resistant weed species worldwide (Varah et al., 2020), with the number of resistant species steadily increasing (Heap, 2023). Herbicide resistance is now considered widespread in black-grass, having been first detected in 1982 in the UK (Moss and Cussans, 1985) with resistant populations having now been found in at least 15 countries (Heap, 2023). In the UK in 2014, herbicide resistant black-grass was found in 35 counties out of 48 (Hull et al., 2014). Also In the UK, it was found that of the 20,000 farms that regularly spray herbicides for black-grass control, black-grass remained on at least 80% of them (Moss et al., 2011). In 2013, 122 non-random black-grass samples were tested for resistance with three different herbicides, it was observed that 98% of black-grass samples showed resistance to at least one herbicide whereas 46% showed resistance to all three (Hull et al., 2014) with some studies going as far as suggesting herbicide resistance occurs in pretty much all UK black-grass populations (Hicks et al., 2018). In 2014 in the UK, it was predicted that due to herbicide resistance, black-grass was responsible for the loss of 0.8 million tonnes of wheat yield annually resulting in an estimated profit loss of £0.4 billion (Varah et al., 2020). Despite the problems facing herbicide resistance, thankfully the complete loss of herbicide control against black-grass is extremely rare (Moss, 2010).

1.2.4 Black-grass takes advantage of changing cropping practices

Black-grass is able to germinate in both spring and autumn plantings however it much favours the autumn with approximately 80% germination during this time (Moss, 2010). Black-grass germination is much reduced in spring plantings, decreasing by between 78-96% compared to winter plantings (Lutman et al., 2013). For UK farmers this is a particular problem due to the high percentage of autumn sown crops (Storkey and Westbury, 2007), a problem now mirrored in places such as Denmark where during the last 30 years the frequency of autumn sown crops has increased by 70% at the expense of spring crops (Andreasen and Streibig., 2011). This shift to autumn sowing from spring sowing favours the growth and proliferation of annual winter weeds such as black-grass (Bitarafan and Andreasen, 2020) as weed community composition shifts from broadleaf to grass-weeds (Storkey and Westbury, 2007).

Since 1980 the sowing date of autumn sown crops has become earlier and earlier (Moss, 2017) as to increase the time available for crop growth prior to winter. It has been suggested that increased growth and vigour earlier in growth results in crops better suited to withstand weed pressure and that early height, not necessarily mature height was key in gaining a competitive advantage (Andrew et al., 2015). Earlier sowing has therefore led to unforeseen advantages for black-grass. In later sown autumn crops the largest percentage of black-grass seedlings would generally emerge before sowing of the crop (Colbach et al., 2006), these could then be sprayed away with non-selective herbicides prior to sowing (Bastiaans, Paolini and Baumann, 2008; Cook et al., 2023). Delayed sowing however reduces the growth period for wheat and takes place at a less easy period for cultivation (Lutman et al., 2013) therefore farmers have brought forward the date of sowing. Unfortunately, earlier crop plantings result in a larger proportion of black-grass emerging at the same time as the crop (Cook et al., 2023) preventing the use of non-selective herbicides for black-grass control and increasing crop – weed competition.

Changes to growing practices have resulted in farmers becoming over-reliant on selective herbicides, this in turn increases the chance of developing mutations and resistance in black-grass populations with UK black-grass showing resistance to over 80% of the herbicide active ingredients developed in the last 60 years (Moss, 2017). It is estimated that complete herbicide resistance in black-grass would result in a wheat yield loss annually of 3.4 million tonnes at a cost of £1 billion (Varah et al., 2020), it is therefore essential for continued but reduced use of herbicides for black-grass control.

1.3 Plant-plant interactions and crop-weed interactions

Nature is stressful. Plants, including crops and weeds, are not passive in their environments. They are actively sensing and adapting to their surroundings to aid their chances of survival and reproduction (Bilas et al., 2021). One such environmental stress is competition from neighbouring plants. Harper, (1977) defined competition as “a series of interrelated physiological and morphological changes that occur as a result of both resource-dependant and resource-independent processes. Due to plants being sessile, the presence of neighbouring plants can have a huge impact on their growth and reproductive success. Plants therefore require mechanisms to ensure survival whilst enduring neighbour presence. In plants, resource dependant competition comes down to the ability to capture resources and monopolize space better than neighbouring plants (Aschehoug et al., 2016). Plant traits are defined as any characteristic whether morphological, physiological or phenological which can be used to predict the fitness of an individual in its environment (Andrew, 2016). In terms of competition, traits described as competitive will increase a plants fitness through its ability to compete with neighbouring plants. The presence or absence of morphological and physiological traits in plants that promote these abilities can determine the plants overall competitive ability when resources and/or space is limiting (Swanton et al., 2015).

1.3.1 Plant-plant interaction strategies

Interactions between neighbouring plants can have negative, neutral or positive effects on plant growth depending on the identity of the two plants, their competitive strategies and resource availability. These interactions have been termed as either competitive, cooperative, or facilitative (Bilas et al., 2021).

1.3.2 Competition

In competitive plant interactions, if two plants compete and one is more competitive it will be able to outcompete the other for resources, increasing the fitness of the ‘winner’ and decreasing the fitness of the ‘loser’. However, if a plant sets out to be competitive and to compete with its neighbour only to find out that its neighbour happens itself to be more competitive, then the plant will have spent vital resources with little or no benefit to show for it. In this case either one or both plants may see reduced fitness. (West et al., 2007). Competition doesn’t guarantee victory it is therefore important that plants choose the correct strategy based on the identity of their neighbours.

1.3.3 Cooperation

Cooperation is defined as ‘a behaviour which provides a benefit to another individual, and which is selected for because of its beneficial effect’ (West et al., 2007). In cooperative plant interactions, either one or both plants can benefit from neighbour interactions. When both plants benefit from cooperation it is referred to as ‘mutualism’ (West et al., 2007), this increases the fitness of both plants ensuring a share of resources, if both plants avoid competition, then they will waste less resources. When the cooperative relationships are costly to one plant but beneficial to the other it is referred to as ‘altruism’ (West et al., 2007), here one plant gains a benefit whilst the other sees either no change or a decrease in fitness over its lifetime. An example of altruism would be in kin selection, one plant reduces its competitive ability to aid the growth of kin neighbours (Murphy et al., 2017).

1.3.4 Facilitation

Facilitation is defined as ‘The positive effect on the environment by one species that improves the fitness of another’ (Bertness and Callaway 1992; Bronstein 2009). In facilitative plant interactions, there will be negligible cost or benefit to one plant but there will be a benefit for the other. An example of facilitation can be seen in intercropping systems. Li et al., (2016) observed facilitative phosphate uptake in cereal/legume intercrops particularly in low phosphate soil. They found that intercropping of wheat and fava bean (*Vicia faba*) led to rhizosphere acidification, increasing the concentrations of malate, citrate and phosphatase in the wheat rhizosphere. This process mobilizes phosphate for fava bean whilst also facilitating phosphate for wheat. Overall intercropping improves phosphate uptake by wheat.

1.4 Neighbour detection mechanisms

The response to neighbouring plants will vary depending on the identity of both plants and the mechanisms by which the two plants are able to detect one another. Neighbour detection was traditionally thought to occur by resource depletion, with plants passively detecting one another through changes in resource availability (Pierik et al., 2013). As resource levels change, plants can adjust their growth strategy in an attempt to improve resource uptake and survivability. Competition for light, water and soil nutrients are three resource-dependent ways plants are able to detect neighbours. However, while for a long time, passive mechanisms relying on detecting changes in resource availability were thought to be the only way plants could detect and respond to the presence of neighbouring plants, it is clear now that plants are active in their environment and are able to detect the presence of neighbours through a greater range of stimuli, including, volatile organic compounds (VOCs), root exudates and touch (Bilas et al., 2021).

1.4.1 Light signalling

In the pre-emption of light availability plants can grow taller, faster and earlier than neighbours to ensure the positioning of their leaves closest to the light source, limiting shading of its leaves by neighbours (Aschehoug et al, 2016). The larger the plant is, it will receive a disproportionately greater share of the available light (Hautier, Niklaus and Hector., 2009) therefore traits that promote quick early growth can provide long-lasting benefits over smaller neighbours. The selection of these traits may give a competitive advantage over neighbours when competing for light however can come with negative trade-offs in the plants ability to gather other resources. For example, a plant with increased height and leaf area; traits important in light capture, will likely have reduced net carbon gain. This may impact the plants ability to gather water and nutrients from the soil, overall, this may reduce the plants competitive ability (Aschehoug et al., 2016).

Despite this, shaded plants are not left in the dark. Pierik et al., (2013) determined the presence of a shade avoidance syndrome in plants, initiated when plants become shaded. Chlorophyll absorbs red and blue wavelengths, resulting in the relative increase in abundance of far-red light in the presence of neighbouring plants. In the presence of other plants (even when there is no direct shading between neighbours), the ratio of red to far red light is reduced (Franklin., 2008). It was found that, in the presence of neighbours, leaves can detect changes in incoming light (red: far red ratio) which is signalled through phytochrome photoreceptors initiating a response in the plant in which growth promoting genes are expressed (Leivar and Quail., 2011). This response includes cell expansion and division resulting in the elongation of hypocotyls, internodes and petioles. These physical changes allow the plant to position its shaded leaves into areas of increased light quality, reducing stress caused by shading. The movement of leaves will likely have knock on effects for neighbouring plants who will in turn alter their own positioning creating a continuous cycle of competition. These adaptions to light presence are further backed up by Roig-Villanova and Martínez-García., (2016) who identified that plants are able to adapt their growth due to changes in light availability, even if changes in light availability are due to neighbouring plants or other factors that are not plants.

1.4.2 VOCs

The production of VOCs by plants has been linked with defensive priming when under attack from herbivorous insects (Ninkovic et al., 2013). When plants detect predation, they release VOCs which in turn attract predators of these herbivorous insects, reducing damage to the plant (Ninkovic et al., 2013). Neighbouring plants in the vicinity of the predated plant can detect these VOCs being released by the predated plant and can respond by preparing their immune system for attack (Baldwin et al., 2006). There is however scope for these same VOCs to be involved in the detection, identification and response to the presence of neighbouring plants (Bilas et al., 2021).

Over 1700 VOCs ranging in size from small molecules such as ethylene to larger sesquiterpenes can be produced by plants and can be released both above-ground as VOCs or below ground as chemical root exudates (Ninkovic et al., 2019). It is possible that plants can emit different combinations of VOCs which may identify not only the physiological status of the plant but also the species (Dicke, 2015).

The size of these VOCs can vary with their weight and volatility determining the distance they can diffuse, with heavier less volatile compounds able to travel further (Baldwin et al., 2006). The proximity of neighbouring plants is therefore key in determining the type and concentration of VOCs they are likely to receive. In denser stands where competition is likely to be greater, the concentration of VOCs will also likely increase (Karban et al., 2003). Due to differences in diffusion rates, it may be possible for the plant to determine the proximity of neighbours and due to the type of VOCs detected, the plant may also discern the identity of those neighbours, however more work is required to understand if neighbour identity is indeed possible.

Plants have been shown to respond to VOC presence with changes in growth, Ninkovic, (2003) showed that when one barley variety was exposed to VOCs released from a different barley genotype, it increased biomass allocation to the roots by 20-40% compared to when there was no VOCs present or when the VOCs were from the focal plant. It is therefore possible that VOCs could be used to alter the growth of neighbour plants and may even vary depending on neighbour identity. Research into this area is however lacking and much more evidence it required to determine the main function of VOC production; whether the main target is in response to herbivorous insects or whether the main reason is for self-signalling or signalling between neighbouring plants. The mechanisms involved in VOC detection, identification and response between plants also require further study (Bilas et al., 2021) however it is clear they are able to play some role in plant-plant signalling and therefore there's the possibility of them having a role in plant-plant competition.

1.4.3 Root exudates

Research into root exudates is limited, therefore the roles they play in the rhizosphere and surrounding soil are often speculative. Root exudates are released by a plant into the soil, these chemicals can play many roles in the soil such as altering soil pH and altering soils adhesive properties (Vives-Peris et al., 2020) along with possibly many other currently unknown functions. It is possible one function that these exudates may have is in plant-plant signalling and therefore plant – plant competition. Plants are known to release exudates to affect their environments, such exudates may act allelopathically against competitors – this has been described in VOCs above-ground, some may suppress pathogens protecting from disease, and some may result in beneficial microbial symbiosis leading to greater nutrient uptake, all of which can aid the plants survival (Rolfe et al., 2019; Ehlers et al., 2020). It is therefore likely that exudate functions may include plant neighbour detection and response. There is some evidence for this, with plants showing changes in root growth and architecture in the presence of exudates from other plants (Yang et al., 2018). Dependant on the concentration and combination of exudates produced it may allow plants to determine the proximity and identity of neighbouring plants (Bilas et al., 2021) resulting in changes in root growth to avoid neighbours (Fang et al., 2013). Recently plants have been shown to use exudates to alter shoot growth as a result of neighbour presence and soil volume availability (Wheeldon et al., 2023). Not enough is known about different exudates and their functions as well as their detection methods in plants. Due to their complexity, it has been suggested they may play a role in neighbour detection, distinguishing between self/non-self and kin/non-kin neighbours (reviewed in Bilas et al., 2021). If plants can distinguish exudates of different neighbours, then exudates could play key roles in plant-plant competition. Particularly so if root exudate detection and response differ between species. If plants are using exudates to detect neighbours but exudate production and detection strategies differ between species this could lead to unbalanced responses between species leading to one species gaining a competitive advantage. For example, if one plant was ‘blind’ to exudates then it may continue to grow despite of neighbour presence, whereas plants that are able to detect the exudates may respond by altering their growth. Work is required to determine if chemical competition; if occurring, has an impact on plant growth, before attempting to identify possible exudate candidates for testing.

1.4.4 Touch

Plants can respond to ‘touch’ signals from their environment allowing them to detect and respond to stimuli such as wind, insects and obstacles such as other plants. Plants are very sensitive to touch from neighbouring plants, Arabidopsis thaliana has been shown to be able to detect neighbours through their root tips and leaf trichomes (Massa and Gilroy., 2003; Zhou et al., 2017). These detection mechanisms allow plants to determine the presence of competitors in their immediate vicinity (Markovic et al., 2016). Plants may then respond to these stimuli by increasing growth away from its competitors or may change growth strategy producing structures to improve its competitive ability. For example, touch stimuli from other plants have been shown to alter the production of root exudates and VOCs (Elhakeem et al., 2018; Markovic et al., 2019) which in turn can have their own competitive effects on neighbours. There is however no evidence to show that plants can identify the type of neighbour through touch (Bilas et al., 2021).

1.5 Crop-weed interactions

Crop – weed interactions form an agriculturally-important subset of plant – plant interactions. Weeds and crops occupy the same environmental niche and are often very similar in terms of biology and ecology (Tian et al., 2020). Due to these similarities, there is often high levels of competition between crop and weed for shared space and resources. Crop and weed will therefore gradually evolve certain traits in order to gain a competitive advantage. Certain traits have been described as increasing the competitive ability of crops against weeds. The vast majority of current research to date has focussed on above ground plant growth with little investigation of below-ground competition.

1.5.1 Above-ground competition

If a crop is taller with a larger canopy, it is expected to have greater access to light whilst also increasing the shading of any smaller weeds (Huel and Hucl., 1996; Lemerle et al., 1996). Indeed, typically this is true, with taller cultivars being better tolerators and suppressors of weeds than shorter semi-dwarf varieties (Chaudhary et al., 2020). More recently height has been linked with the increased competitiveness seen in hybrid barleys compared to elite wheats (Cook and Roche., 2018). Height however doesn't always indicate increased competitiveness with shorter crop varieties of wheat having been shown to have increased competitiveness than taller varieties against a mixture of annual weeds (Wicks et al., 2004). There must therefore be other factors influencing competitive ability, not just one trait. Both crop and weed growth strategies need to be taken into account for example some weed species such as Ivy-leaved speedwell (*Veronica hederifolia)* are shade tolerant, therefore growing larger in cultivation with taller crop lines than shorter ones (Gooding et al., 1993). It has been suggested that tall height at early growth stages is more important than height at maturity (Ogg and Seefeldt., 1999) this would indicate that getting a head start over any weeds is vital to a crop's competitiveness given that the larger the plant, the greater amount of resources it can acquire. Early vigour therefore whether it be through increased height, biomass, leaf area or allelopathy may increase a crop's competitive ability (Huel and Hucl., 1996; Rebetzke and Richards., 1999; Bertholdsson., 2005). This has been shown previously by Christensen., (1995) who determined that barley varieties that develop quicker had increased weed suppression. Early vigour may be particularly important for winter crops in order for them to gain a competitive advantage over weeds prior to winter.

Tiller production in grasses is a sign of plant fitness and will reduce under increased competition from both inter- and intra-specific neighbours (Huel and Hucl., 1996). The role of tiller production in competitive ability has been disputed with some studies indicating increased tiller number can reduce seed production in weeds (Challaiah et al., 1986) whereas other studies have shown no correlation (Moss, 1985). It is possible tiller number is only a response to resource availability and competition rather than a driving force behind it. Increased early vigour may increase early tiller number in turn then increasing light and nutrient acquisition and as a result suppressive ability. How tiller production and root production correlate are also up for discussion. Are they positively correlated so as one increases in size so does the other? This would make sense as a greater shoot system will require greater nutrients from a larger root system. Or is there a negative correlation between shoot and root system as a result of a trade-off, where the plant favours one over the other depending on its current requirements.

One example of crop-weed competition is rice-barnyard grass competition. The most common weed species affecting rice (*Oryza sativa*) paddies is barnyard-grass (*Echinochloa crus-galli*). Barnyard grass is able to inhibit rice growth and reduces overall grain yields (Zhang et al., 2021a). Barnyard-grass is native to Asia however can now be found throughout the world where it prefers to grow on wet soils with high nitrogen availability and high light availability (Chin., 2001). It is a particular problem due to its biological and ecological similarities to rice (Tian et al., 2020; Rahaman et al., 2021). It is able to outcompete rice for nutrients such as nitrogen in the vegetative period and potassium in the reproductive period, vastly depleting nutrient levels in the soil leading to large losses in crop yields (da Rosa Ulguim et al., 2020). Furthermore, each plant is able to produce between 2000 - 40,000 seeds making it extremely difficult to exterminate once established (Galinato., 1999). As with most weed species the duration and timing of weed emergence, the weed density and crop sowing approach can affect the overall yield losses of the crop, in rice, barnyard grass can result in yield losses between 21-79% (Zhang et al., 2021a).

In rice, taller, leafy genotypes; genotypes that prevent light reaching the weeds, are typically considered to have increased competitiveness compared to shorter varieties (Suzuki et al., 2002). However, studies have also shown that root growth and root competition is also vital to the overall competitive outcome of the different rice genotypes (Gibson and Foin., 1999). Zhang et al., (2021a) found that when rice was grown in competition with three different barnyard grass genotypes that in each case rice grain yield was significantly reduced compared to the crop only control. Crop shoot traits were dramatically affected by barnyard grass competition including dry shoot weight and leaf photosynthetic rate as well as root traits such as dry root weight, root length density and root surface area. They also showed that rice shoot growth was correlated with its root growth indicating that both shoot and root growth reductions contribute to losses in crop yield when in competition with barnyard grass.

1.5.2 Below-ground traits – root growth and allelopathy (root exudates)

Below-ground traits have been far less studied than above-ground traits when it comes to crop-weed interactions. This is mostly due to difficulties working with roots in the soil, and the lack of techniques available to assess root growth accurately. Plant root system structure is shaped by an endogenous genetic program as well as in response to external biotic and abiotic factors (Hochholdinger et al., 2004). It is therefore difficult to determine exact causes in changes in root growth. There are two main ways plants may compete below-ground, either through physical root production for increased resource acquisition or through the production of root exudates with allelopathic potential.

1.5.3 Physical root production

Certain physical root traits have been linked with increased competitiveness in crops including root length density, number, total length and elongation rate (Fargione and Tilman., 2006; Stevanato et al., 2011). Increased root growth in crops increases anchorage as well as water and nutrient uptake compared to neighbouring weeds (Finch et al., 2017). The presence of black-grass has been shown to decrease root length in wheat however this effect is not unique to black-grass, with other grasses showing the same results (Finch et al., 2017). Due to the lack of research into black-grass root growth it is impossible to determine whether root growth is an important factor in black-grass competitiveness. Root growth has been linked with increasing black-grass fitness in certain conditions. Research has suggested that black-grass roots play an important role in its ability to withstand waterlogged conditions. Black-grass has comparatively large aerenchyma-like structures reducing hypoxia stress allowing them to produce larger aerial tissue biomass compared to wheat through increased gas exchange (Harrison et al., 2024). It is possible that black-grass' patchy distribution in fields is due to increased water presence in these black-grass infested areas (Metcalfe et al., 2018; Harrison et al., 2024). Roots are therefore used by black-grass to give it an advantage in certain environmental conditions, it may therefore be plausible it utilises root growth to gain a competitive advantage against neighbouring plants. Elite wheats on the other hand have seen vastly reduced root growth compared to historic varieties (Fradgley et al., 2020), increased root number is thought to increase competitive ability against weeds (Richards., 2007) therefore reduced root growth and reduced competitive ability in elite wheats is possibly making them more susceptible to environmental stresses such as competition from competitive weeds with high root growth. Further work is required to determine the role of root growth in the competitive ability of crop lines against weeds such as black-grass.

1.5.4 Allelopathy

Another method by which plants may compete with one another below-ground is via allelopathy. Allelopathy involves either the inhibitory or stimulatory effects of one plant on another through the production and release of chemicals into the environment (Zhang et al., 2021b). The aim of the exuding plant is to influence the growth, survival, development, and reproduction of neighbouring plants as to increase its own fitness (Cheng and Cheng., 2015). Allelochemicals can affect plant development through its whole lifecycle affecting many different physiological and biochemical processes including cell division, photosynthesis and water and nutrient uptake amongst others (Rahaman et al., 2021). Allelopathy is regulated by resource availability such as light, water and nutrients and environmental conditions such as temperature and humidity (Sowiński et al., 2020).

Competition between plants can trigger allelopathic responses, however allelochemicals are still released in non-competitive scenarios in order to influence other organisms (Gfeller et al., 2018). Inhibitory allelopathy involves chemical interactions between plants with the aim of impacting neighbouring plants to decrease their ability to compete for resources (Gioria and Osborne., 2014). Stimulatory allelopathy on the other-hand will have a positive effect on its target whether the target be another plant, microorganism or symbiont whose presence in turn may aid the focal plant. The positive effects on neighbouring plants may include inducing stress tolerance and stimulating growth (Agathokleous and Calabrese., 2019; Stebbing., 2003). Allelopathy can be involved in plant defence mechanisms, resource allocation and ecosystem regulation, chemicals released can affect the physiological function, seed germination and root growth of nearby plants (Mushtaq et al., 2024). It is also possible that allelochemicals will have auto-toxic effects on the exuding plant, however more research is needed to determine if and how plants distinguish between self/non-self-exudates (Rice, 2012).

Allelochemicals consist mostly of secondary metabolites such as alkaloids, phenolics and flavonoids etc (Kato-Noguchi and Peters., 2013). Allelochemicals are released into the environment in different ways, either into the air through volatilisation as VOCs (see earlier section), deposited in pollen, or into the soil or rhizosphere via root exudation, plant residue decomposition, or leaching caused by rain (Kong et al., 2018; Kong et al., 2004; Roshchina et al., 2009; Rice., 2012). The presence of weeds has been shown to induce allelopathy in different plants including wheat, sorghum, rice and buckwheat (Gfeller et al., 2018; Uesugi et al., 2019) with allelopathy being shown to be induced in wheat by the presence of root exudates from competing weeds (Delory et al., 2016), such root exudates include jasmonic acid, methyl jasmonate and (-)-loliolide (Kong et al., 2018).

Concentrations of allelochemicals such as benzoxazinoids released into the soil are generally small and released slowly, not persisting for longer than a day in the soil meaning that they are unlikely to cause damage to weeds (Rice et al., 2012). Thus, despite their potential phytotoxicity, it remains unclear as to whether allelochemicals actually accumulate to high enough quantities in natural conditions to negatively impact neighbouring plants (Zeng., 2014). It might be that the main function of allelochemicals is currently unknown, with phytotoxicity being a side effect of allelochemical production. However, it remains possible that competition is the main role of allelochemicals, with other mechanisms in place to counteract side-effects such as auto-toxicity (Bilas et al., 2021). There remain questions to be answered regarding how plants detect allelochemicals in the soil and how these are then taken up by the plant and how they then result in physical changes (Mushtaq et al., 2024).

1.5.5 Allelopathy in agriculture

Allelopathy can be used in agricultural management, having positive effects playing roles in crop protection, weed control, crop re-establishment, growth regulators, herbicides and insecticides (Cheng and Cheng., 2015). Allelochemicals can inhibit weed growth and reduce the requirement for herbicides (Zhang et al., 2016). Allelopathic crops cultivars can be particularly effective against herbicide resistant weeds by inhibiting root growth (Yang et al., 2017). Allelopathy however can also have negative effects for agriculture, such as leading to biological invasion, soil sickness and autotoxicity (Cheng and Cheng., 2015). It is therefore important to attempt to control allelopathy to prevent these negative side effects and promote crop positive interactions. Below are two examples of allelochemicals being used for weed control in crops.

1.5.6 Allelopathy in rice

Selecting rice cultivars with increased allelopathy can be used as an ecological control against weeds such as barnyard grass. In rice, allelopathy has been seen to negatively impact the growth of the weed barnyard grass during co-cultivation. It was found that the presence of allelochemicals negatively impacted barnyard grass shoot and root growth (Ayub et al., 2012) most likely due to decreasing nitrogen intake (Karim et al, 2012). Rice allelochemical production was also seen to decrease barnyard grass germination (Alam et al., 2018). Momilactone B has been described as the main allelochemical in rice plants, where it is excreted from the roots in order to suppress weeds such as barnyard grass (Minh and Xuan., 2024). Reverse genetics showed that the knockout of genes responsible for momilactone biosynthesis in rice resulted in a significant decrease in the ability of two rice cultivars to inhibit barnyard grass (Estiati., 2019). In co-cultivation the release of momilactone B by rice increased 6.9-fold compared to rice only cultivations

(Kato-Noguchi., 2011). However, barnyardgrass can also act allelopathically towards rice. When grown together both rice and barnyardgrass seedlings were suppressed, with both species showing increased allelopathic activity (Kato-Noguchi and Ino., 2013). It has therefore been suggested that rice is able to recognise the presence of barnyardgrass allelochemicals, resulting in increases in momilactone B production. These findings indicate both species have developed chemical cross-talk mechanisms to detect and respond to signals from neighbouring plants to aid in their defence (Kato-Noguchi and Ino., 2013; Khanh et al., 2018).

1.5.7 Allelopathy in rye

Rye has long been noted as a potential cover crop due to its allelopathic potential (Mushtaq et al., 2024). Benzoxazinoids are a type of allelochemical released from the roots of certain grasses (Hussain et al., 2022) with the purpose of plant protection from pests and diseases as well as the inhibition of neighbouring plants (Hu et al., 2018). In rye allelopathic benzoxazinoids have been shown to have potential for weed suppression, successfully suppressing the emergence of weed species *Amaranthus* spp, (pigweed) *Echinochloa crus-galli* (barnyardgrass) and *Conzya canadensis* (horseweed) (Barnes and Putnam., 1987; Przepiorkowski and Gorski., 1994). More recently rye has been shown to significantly increase its production of benzoxazinoids in response to pigweed age-dependently, resulting in a significant decrease in pigweed growth (Mushtaq et al., 2024).

1.5.8 Allelopathy in wheat – black-grass competition

In terms of black-grass – wheat competition, determining if allelopathy is occurring will be important not only for understanding how the two plants recognise each other's presence but also in how they may compete with one another. Literature is scarce on allelopathy occurring between wheat and black-grass however it has been reported that allelopathic wheat may be a method of suppressing herbicide resistant black-grass (Bertholdsson., 2011). How black-grass responds to allelopathic wheat and how they chemically communicate below-ground is still poorly understood (Yang et al., 2020). Recent work looking at whether allelopathy can impact herbicide resistant black-grass found that allelopathic wheat was able to inhibit both roots and shoots of both herbicide resistant and susceptible black-grass significantly, shaping black-grass root behaviour through the production of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) a of benzoxazinoid compound (Yang et al., 2020). DIMBOA was found to be released by allelopathic wheat in response to at least 100 plant species and has therefore been considered as a potentially effective allelochemical against weeds (Kong et al., 2018).

The presence of black-grass resulted in increased allelochemical production from wheat, with root exudates from black-grass inducing wheat allelochemical production (Yang et al., 2020). These findings are backed up by a recent thesis by Hickman., (2022) who found crude wheat and rye root exudates to be inhibitory towards black-grass in soil, however it was no possible to identify the particular allelochemicals responsible. Allelopathic crops therefore, in combination with cultural control methods, may provide better black-grass control than crops with lower allelopathic potential and should be considered as a cultural control approach for black-grass.

1.6 Traits linked with crop – weed competition

In crops in general, cultivars have been shown to vary in their ability to deal with weed pressures, whether it be in their ability to suppress weed growth or in their ability to tolerate weed presence (Christensen, 1995; Mohler, 2001). Certain traits have been suggested to contribute to a cultivars competitive ability against weeds such as crop leaf angle, leaf area index, crop stature, canopy duration, relative growth rate and allelopathic potential (Callaway, 1992; Olofsdotter et al., 2002). Crop varieties with specific traits that allow for a higher capacity for resource capture will reduce resources available to weeds in turn suppressing weed growth (MacLaren et al., 2020). This will also promote weeds that require different resources to the crop, reducing overall weed pressure on the crop (MacLaren et al., 2020). Increasing sowing density and ensuring prime sowing patterns and inter-row spacing will make sure that the crop is able to capture as much resources as possible in order to reduce resources available for weed growth (Mohler 1996; Colbach et al., 2014).

1.7 Integrated weed management

Integrated weed management (IWM) is a component of integrated pest management (IPM). Over the past 50 years the aims of IPM have been to reduce the reliance on pesticide usage and to promote non-chemical alternatives (Ehler, 2006). Specifically, for weed control, IWM aims to increase management options available to farmers through the use of biological, cultural, genetic and mechanical methods by which to control weed presence and reduce the impact of weeds on crops (Swanton and Weise, 1991). IWM is to be used combined with the continued but reduced use of chemical herbicides (Shaner, 2014).

1.7.1 Non-chemical approaches to weed control

Weed control can be split into three objectives, the first objective is to reduce weed density prior to sowing through disturbance of the soil, the second is to reduce the damage caused by surviving weeds by increasing competition against the weeds, and the third is to increase diversity in control approaches to prevent increases in weed presence for future plantings (Melander et al., 2017). There are many non-chemical approaches to IWM available for use by farmers that can cover these three objectives, these include longer crop rotations, rotational ploughing, stale seed beds, delaying autumn sowing, increase crop seed rates, spring cropping, spraying off weed infested crop areas, hand rouging, grass breaks, fallowing, cover cropping and the use of competitive crop varieties (Moss, 2018). Although the control provided by each method may be low, multiple approaches in combination with herbicides should improve overall control (Moss and Lutman, 2013).

1.7.2 IWM uptake by farmers

Despite lots of research into IWM methods, the uptake of these practices by farmers has been poor, with what uptake that has occurred being due to farmers attempting to reduce the negative impact of reduced herbicide efficacy rather than as an herbicide alternative (Moss, 2018). Ultimately the application of herbicides is easier and cheaper than experimenting with alternative approaches which may or may not provide an overall benefit (Moss, 2018). The continued use of herbicides therefore remains the main control approach for most weeds including black-grass (Moss, 2018), even despite of the known consequences of black-grass herbicide resistance. Moss, (2018) puts forward a seven-point action plan to improve the uptake of IWM approaches. To truly increase uptake of IWM approaches a better understanding is required amongst famers of the long-term benefits of uptake, and amongst researchers of the individual farmer’s needs, attitudes and perspectives (Moss, 2019). Due to the financial pressures placed on farmers, they cannot risk spending time and money without a strong guarantee that they will benefit. IWM approaches are not always successful and are often costly and time-consuming (Melander et al., 2017). Alternative approaches to IWM that address farmers needs are therefore required to further increase IWM uptake and our control of black-grass.

1.7.3 Competitive cultivars for black-grass suppression

Competitive ability of crop against weed has been shown to differ between genotype in many crop species including Wheat, Barley and Rice (Cook and Roche., 2018; Mahajan et al., 2014). The use of competitive crop cultivars has been highlighted as a possible cultural approach to IWM (Andrew et al., 2015), aiming to strengthen crop growth and suppressive ability against weeds whilst reducing the requirement for herbicides (Harwood, 1990; Melander et al., 2017). This method would utilise crop cultivars with natural increased competitive ability against black-grass whether the competitive ability be suppressive (reducing black-grass) or tolerant (maintaining crop biomass) (Andrews, 2016). Variation in competitive ability has been seen in wheat varieties, with more competitive varieties being shown to reduce black-grass mean head counts by between 22-30% compared to less competitive varieties (Lutman et al., 2013) thus reducing black-grass seed return. Moss and Lutman, (2013) reviewed the use of competitive cultivars and found the percentage control of black-grass ranged from 8-45% indicating that the use of competitive cultivars can have positive black-grass control albeit marginal in some cases. A simple substitution of one non-competitive variety for another more competitive variety is all that is required when it comes to competitive cultivars. Therefore, competitive cultivars have the advantage of reduced time and costs of labour that are negatively associated with many other IWM approaches.

1.7.4 Need for a recommended list in wheat for black-grass competition

The use of competitive cultivars against black-grass is currently in use by some farmers, however there is little underlying evidence supporting the farmers' choice of cultivars. For a farmer to select a new crop variety to sow they need convincing that the new variety is going to provide an overall net benefit or advantage over their current line (Evenson and Gollin, 2003). To aid farmers in their choices, and to ensure cultivars chosen are providing a benefit, work is required to test the status of competitive ability of current elite crop lines against black-grass. The uptake of competitive cultivars by farmers has been limited due to highly variable results at the individual field level (Lutman et al., 2013). Just because one cultivar shows promise in one set of conditions does not mean that it will perform well in others. Due to the lack of testing of varieties at field level scale in a range of different field types, there is currently no recommended list available for farmers to tell them which crop varieties are best at suppressing black-grass or tolerating black-grass competition. Assessing crop cultivars is required to ensure farmers can choose crop lines, supported by evidence, that are going to be more competitive against black-grass.

1.7.5 How to test for competitive cultivars

Currently there is no quick and easy method of assessing crop lines for their competitiveness against black-grass (Andrews, 2016). An approach to test and identify competitive cultivars is therefore required, ideally an approach that is not reliant on timely and laborious full length field trials.

Phenotypic assessment

Identification of competitive lines may be achieved by phenotypic assessment of specific traits linked with increased competitiveness. Screening for specific traits can highlight variation within a certain trait but doesn't necessarily prove that the trait in question is directly involved in competition. Direct testing, growing crop varieties against black-grass in a competitive scenario and assessing final growth of both crop and black-grass can identify which lines are more competitive but not necessarily the traits enhancing crop competition. A combination of both approaches would be a good starting to point to infer any correlations between differences in competitive ability and differences in traits.

Genetic assessment

Ideally, once a certain trait had been identified, genetic mutant lines in which genes controlling one specific trait have been altered to result in an altered phenotype, could be utilised to directly assess the role of that specific trait on competitive ability. If an altered phenotype in a specific trait result in a change in competitive ability, then it is clear that the trait is vital in controlling competitive ability. There has however been little progress in weed molecular biology, with little research into the regulation of genes and proteins that allow weeds to successfully compete with modern crop varieties (MacGregor, 2020). Due to limited information on traits and a lack of mutants for these traits, it is not currently possible to identify specific genes in wheat that are responsible for competitiveness against black-grass. Any screening approach therefore will be required to be solely phenotypic until further advances have been made. In the future with greater phenotypic data available it may be possible to undertake qualitative trait locus (QTL) analysis to determine the genetic basis of competition in wheat.

1.8 Wheat – black-grass competition

In order to develop a screen to test competitive ability we first require an understanding of how wheat – black-grass competition is occurring.

1.8.1 Traits in wheat for increased black-grass competition

Certain traits have been described as increasing the competitive ability of wheat cultivars against black-grass either through black-grass suppression or tolerance. Such traits include increased crop height (Wicks et al. 1986; Huel & Hucl 1996; Lemerle et al. 1996), aspects of canopy architecture (Champion et al., 1998) and prostrate growth habit (Andrews, 2016). Increased tiller (Grass shoots) production has been shown to increase competitive ability in some studies (Lemerle et al. 1996; Hucl 1998; Korres & Froud-Williams, 2002), however other studies see contrasting results indicating only weak correlations (Wicks et al. 1986; Champion et al. 1998). Although there seems to be a link between crop height and competitive ability it has been highlighted that there must be other factors that are also responsible for competitive ability against black-grass in wheat (Mason, Goonewardene and Spaner, 2008). It has been suggested in annual systems that crop lines with greater more rapid resource capture are more suppressive against weeds (MacLaren et al., 2020) with these lines tending to be taller, higher rooting, with high early vigour (Andrew et al., 2015) and with large seeds (Mohler, 1996).

1.8.2 Barley shows increased competitiveness against black-grass

Barley can also be utilised for competitive trait detection. Barley is reported as being more competitive than wheat against black-grass, with black-grass seed return decreasing in winter barley relative to winter wheats (Cook and Roche, 2018). Companies such as Syngenta suggest the planting of their hybrid barley line SY Kingsbarn for use against grass weeds such as black-grass (Syngenta, 2024). Hybrid barley has been shown to decrease black-grass seed return to a greater extent than standard winter barley (Cook and Roche, 2018). Why barley is better able to compete than wheat is a current area of interest for research. Given winter barley is grown at the same time as winter wheat, there must be some physiological reasons as to why winter barley is better at withstanding black-grass competition than winter wheat. Studies have suggested that barley is more competitive than wheat due to its increased height and due to wheat having a more open canopy (Cook and Roche, 2018). Hybrid barley may also use height to its advantage, standing around 30cm taller than standard barley (AHDB, 2024), this increased height allowing it to stand taller than black-grass throughout the growing season (Cook and Roche, 2018). However, there may also be other factors at play. If we can understand what phenotypic traits makes barley more competitive than maybe these same traits could be bred for in wheat to also increase the competitiveness of wheat.

1.8.3 Below-ground competition has been overlooked

Despite the above-ground aspects of growth that may be impacting competitive ability, it is possible that researchers are overlooking what lies beneath the surface. Below-ground, plants can interact with organisms in their immediate environment to aid their survival, from fungal symbiosis for nitrogen fixation (Akiyama et al.,2010), to detecting and adapting growth to chemical signals from neighbouring plants (Cook et al., 1966). Wheeldon, (2023) found there to be a link in wheat between what occurs below-ground and what is produced above ground, with root density being a good predictor of shoot system size. It is therefore likely that root growth plays a key role in competition with neighbouring plants and the overall productivity of the plant.

1.8.4 Root growth

One possible mode of below-ground competition comes from root growth. The vigorous production of roots can allow plants to dominate space and resources, allowing them to gain a competitive advantage over neighbouring plants including weeds (Andrew et al., 2015). However, investigating root systems is challenging, particularly in field conditions, roots are inaccessible, they have high phenotypic plasticity and there is currently no high-throughput screening approach (Manschadi et al., 2006) therefore any phenotyping work is slow and laborious with high levels of variation (Gregory et al., 2009). Assessment of crop root growth has therefore relied on controlled lab experiments that assess root phenotype over the first couple weeks of growth (Atkinson et al., 2015) however these experiments do not reflect real field conditions with results often inconsistent between methods (Wojciechowski et al., 2009) and are on the most part pointless, due to short term root growth not being reflective of field scale root growth (Bai et al., 2019). Due to these difficulties, no studies have assessed the potential of below-ground traits as an explanation for black-grass outcompeting wheat. Due to the lack of clear evidence regarding crop root traits plant breeders have been hesitant in directly selecting for wheat root traits (Fradgley et al., 2020). This lack of intentional selection may have in itself led to decreased root growth amongst elite wheat lines. Due to the same difficulties seen in below-ground crop assessments, little is also known regarding black-grass root growth. Work is therefore required to determine the root growth characteristics of black-grass to assess whether root growth is a trait prioritised by the weed. If black-grass has low levels of root growth it will be unlikely that they will provide it with a competitive advantage, however if black-grass has a large degree of root growth then this may be a method by which it is able to outcompete wheat.

1.8.5 Chemical root exudation

One possible mode of below-ground competition is the production and exudation of chemicals from the roots. Plants are able to release organic molecules from their roots into the surrounding soil in the form of root exudates, these exudates are able to manipulate the rhizosphere (Bais et al., 2003) which could theoretically be detected by neighbouring plants (reviewed in Clark and Bennett, 2024).

Allelopathy, discussed earlier, is one candidate as to how wheat and black-grass compete below-ground. The release of allelochemicals by wheat has been shown to be induced by the presence of black-grass, negatively impacting black-grass growth (Delory et al., 2016). It is possible that different wheat cultivars may have different levels of allelopathy and therefore may have greater potential in black-grass suppression.

Plants may use chemical root exudates to sense their surroundings and adapt their growth dependant on neighbour presence (Biedrzycki et al., 2010; Wang et al., 2021) and soil volume availability (reviewed in Clark and Bennett, 2024). One such chemical root exudate are Strigolactones (SLs). Wheeldon et al. (2022) and Yoneyama et al, (2022) both show in rice and pea that the uptake of SLs exuded by neighbouring plants results in down-regulation of SL synthesis and exudation proportionally to neighbour density (reviewed in Clark and Bennett, 2024). These responses to neighbour presence attempt to increase survivability of the focal plant in the presence of neighbour stress. In the case of interspecific competition, if plants differ in their response to SLs, then the downregulation may not occur in both plants, this may lead to one outcompeting the other. In wheat – black-grass interactions it may be that there are differing levels of exudation and detection between crop and weed to chemical exudate presence in the rhizosphere which may explain why black-grass is able to outcompete wheat. It may also be that different wheat varieties differ in their levels of chemical exudation which in turn may affect how they compete.

1.8.6 Changes in wheat root growth due to breeding

Modern high yielding varieties of crops such as rice and wheat have been developed since the green revolution in the 1950s. Intense selection pressures over this time has led to increased harvest index, increasing the grain yield capacity of those crops. The incorporation of dwarfing genes allowed the development of shorter stiffer straw varieties that focussed energy into grain production whilst limiting straw and leaf production (Evenson and Gollin, 2003). The increased selection for harvest index has also been shown to coincide with changes in root growth architecture and with decreased root growth in both Chinese wheat varieties and UK wheat lines (Zhu et al., 2019; Fradgley et al., 2020). Further adaptation of crop lines for control of flowering time has also been shown to decrease crop root growth (Voss-Fels et al., 2017). However, if there are increased selection pressures on wheat for certain traits (such as increased harvest index) it is likely resulting in a trade-off in growth of other traits (such as root growth). The lower levels of selection in barleys during the green revolution may have resulted in less competitive trade-offs allowing it to continue to be better at competing against black-grass than wheat which saw greater selection (Evenson and Gollin, 2003).

Landrace crop varieties are older varieties that have not undergone the same level of intense selection as modern-day elite varieties. If traits that aid in competitive ability have been lost from elite varieties, then these older varieties may have retained some of these traits. This may show that older varieties are better adapted to environmental stresses such as weed competition. Root growth has been highlighted as a trait that differs between modern elite and older landrace varieties, with modern varieties having fewer roots per plant (Fradgley et al., 2020). Assessing differences in growth of elite and landrace varieties could highlight traits linked with increased competitive ability which could then inform breeders for trait selection against weeds. It is possible that with the selection of large rooting wheat varieties and breeding for improved root system architecture (RSA) that the negative impact of black-grass competition through natural crop suppression could be reversed.

Certain elite wheat varieties will have greater levels of root growth than others. Bai et al., (2019) showed that wheat varieties varied in both shallow and deep root production, with those lines showing the deepest root production also having the greatest number of shallow roots. It is hypothesised that this comparatively higher root growth will increase their ability to compete with black-grass. It may be that increased prevalence in black-grass is due to both its high root growth and the reductions seen in wheat root growth amongst elite lines.

1.9 Aims

Given the requirement for new approaches to IWM, the main aim of this project was to assess the use of different wheat varieties as competitive cultivars and to determine the mechanisms of wheat – black-grass competition through phenotypic assessment of traits that may convey competitiveness. Due to the lack of knowledge in both areas the main goals of this project are:

- Identification of traits and mechanisms involved in wheat – black-grass competition, when and how does black-grass outcompete wheat?
- Develop screening methods for the testing of competitiveness amongst crop varieties.
- Determine whether certain traits can be used to predict wheat competitiveness against black-grass.

- Provide a recommended list of winter wheat varieties/traits for use in black-grass competition.

Chapter 2 Methods and materials

2.1. Biological material

2.1.1 Wheat (*Triticum aestivum*)

Wheat lines, both winter and spring, were used for plant growth experiments. These lines consisted of current elite varieties as well as landrace varieties. All lines were obtained either from current university stocks or from breeders (Table 2.1). Unless otherwise stated experiments in winter conditions used winter wheat variety Claire whilst experiments in spring conditions used spring wheat Mulika.

2.1.2 Black-grass (*Alopecurus myosuroides*)

Seed from a black-grass population was obtained from ADAS, Boxworth field C (Table 2.1). Seed from the same population was used for all black-grass experiments.

2.1.3 Barley (*Hordeum vulgare*)

Barley lines used in experiments included spring, winter, and hybrid barleys. All lines were obtained either from current university stocks or from breeders (Table 2.1). Unless stated otherwise, experiments in winter conditions used winter barley variety Bordeaux whilst experiments in spring conditions used spring barley Bowman.

Table 2.1 Plant lines used in experiments

Variety	**Type**	**Source**
Black-grass	Grass weed	ADAS Boxworth Field C
Elation	Winter wheat	Elsoms
Bowman	Spring barley	Franckowiak et al., 1985
Feeris	Winter barley	KWS
Tardis	Winter barley	KWS
Kerrin	Winter wheat	KWS
Barrel	Winter wheat	KWS
Zyatt	Winter wheat	KWS
Guium	Winter wheat	KWS
Dawsum	Winter wheat	KWS
Extase	Winter wheat	KWS
Brium	Winter wheat	KWS
Cranium	Winter wheat	KWS
Palladium	Winter wheat	KWS
Spotlight	Winter wheat	Limagrain

Skyscraper	Winter wheat	Limagrain
Prince	Winter wheat	Limagrain
Astronomer	Winter wheat	Limagrain
Crusoe	Winter wheat	Limagrain
Redwald	Winter wheat	Limagrain
Typhoon	Winter wheat	Limagrain
Illuminate	Winter wheat	Limagrain
Claire	Winter wheat	Limagrain
Dickens	Winter wheat	Secobra
Bordeaux	Winter barley	Senova
Mulika	Spring wheat	Senova
SY Kingsbarn	Hybrid barley	Syngenta Seeds
Graham	Winter wheat	Syngenta Seeds
W1190488	Landrace winter wheat	Watkins collection
W1190637	Landrace winter wheat	Watkins collection

2.2 Chemical materials

2.2.1 ATS solution

Arabidopsis thaliana salts (ATS) (Wilson et al., 1990) was used for the supply of nutrients to plants in the hydroponic system. ATS consisted of KNO_3 (5 mM), KH_2PO_4 (2.5 mM), $MgSO_4$ (2 mM), Ca $(NO_3)_2$ (2 mM), Fe-EDTA (50 µM) and the following micronutrients: H_3BO_3 (70 µM), $MnCl_2$ (14 µM), $CuSO_4$ (0.5 µM), $ZnSO_4$ (1 µM), $NaMoO_4$ (0.2 µM), NaCl (10 µM) and $CoCl_2$ (0.01 µM). The volume of ATS made up, was dependant on the number of hydroponic pots being used at any one time (See hydroponics methods).

2.3 Plant Growth conditions

2.3.1 Glasshouse conditions

Plants referred to as being grown in ‘glasshouse’ or ‘spring’ conditions were grown in controlled temperature glasshouses with a temperature of 22^{o}C, day length of 16 hours, night length of 8 hours and a light intensity of 250 $\mu mol/m^{-2}$ s^{-1} (Attis 7, Phytolux LED lighting).

2.3.2 Winter chamber conditions

Plants referred to as being grown under ‘winter’ conditions were grown in Weiss growth cabinets at a temperature of 10°C, with supplemental LED lighting with an average intensity of 198 μmol/m^{-2}s^{-1} on an 8-hour day, 16-hour night cycle.

2.3.3 Walk-in cabinet conditions

For germination and early growth assessments plants were grown in a Walk-In cabinet at 20°C, with supplemental LED lighting with an average light intensity of 310 μmol/m^{-2}s^{-1} on 16-hour day, 8-hour night cycle.

2.3.4 Seed germination conditions

For general seed germination, room temperature conditions were used with seeds placed on a windowsill with access to natural light. Pregerminated seed were grown on damp filter paper within a petri-dish placed for up to a week prior to sowing (species dependant). Unless stated otherwise, all black-grass, wheat and barley seed was pre-germinated.

2.4 Growth substrates

2.4.1 Soil based experiments

Experiments with a soil substrate used Petersfield No.2 compost. When potting up it was ensured that all pots/containers were full to their true volume after watering and gentle compaction.

2.4.2 Sand/perlite hydroponic preparation

In preparation for growth in hydroponics, plants required first to be germinated in 100ml pots containing a 50:50 mix of sand and perlite. In preparation sand and perlite were combined in equal quantities until thoroughly mixed. The mixture was then placed into 100ml pots and then lightly watered. A small hole 1-2cm in depth was made in the centre of each pot. A single seed was sown into each pot, the surrounding substrate was then pinched around the seed to ensure the seed is covered. Pots were then watered from the base by adding water into a tray in which the pots were standing.

2.4.3 Water based hydroponic experiments

Plants were grown in 2L hydroponic pots. Within the hydroponic pot 15ml of ATS solution was added, the remaining volume of the pot was then filled with water. The hydroponate was disposed of every 2-weeks, replaced by fresh ATS and water. This was repeated throughout the length of the experiment.

2.5 Experimental set-ups

2.5.1 Assessing black-grass seed viability

The black-grass population received had an estimated seed viability of 70%. To ensure the viability of seed used in the experiments, all black-grass seed was pre-germinated prior to sowing (except for seed used in 2.5.2). Seed was pre-germinated in petri-dishes on filter-paper saturated with water and grown at room temperature conditions for a maximum of 7 days. Once germinated black-grass seedlings of a similar size and age were selected for sowing into experiments. To ensure that enough germinated seed was available for each experiment, double the required number of seed was sown for any experiment.

2.5.2 Determining germination characteristics of Black-grass and elite winter wheats

Ungerminated seed of three elite wheats (Kerrin, Elation and Barrel) and black-grass was sown into petri-dishes each containing filter paper saturated with water and placed within the Walk-In cabinet set at 20^{o}C. Seeds were assessed for germination (defined as the first emergence of either the root or coleoptile from the seed) every 24 hours. Once roots/coleoptile were visible their length was recorded using a ruler every 24 hours up to 14 days post germination.

2.5.3 Determining short term growth rate of black-grass and elite winter wheats

Seed of three elite wheats and black-grass were pre-germinated at room temperature in petri-dishes each containing filter paper saturated with water. Once germinated each seed was transferred to a 100ml pot containing soil. The pots were placed into the glasshouse for 2 (2-week seedling biomass) or 4 (4-week shoot biomass) weeks respectively dependant on the desired plant age. After 2 or 4 weeks respectively, the shoots were harvested.

2.5.4 Rhizoboxes for root assessments

Rhizoboxes were made from modified Corning 245mm X 245mm square plates (Figure 2.1A). The base of the plates was modified in two ways, firstly, 6 small holes were created on the base edge of the plate by melting the plastic using a hot metal skewer, this is to allow water access to the soil from below. Secondly, a rectangular hole 5x2cm was cut into the top edge of the plate using a hacksaw, this was to allow shoots to grow vertically out of the rhizobox. Soil (Petersfield Potting Supreme No. 2 compost) was sieved to remove any large clumps, before being compacted into the base of the plates and watered. A singular seed was then sown into the soil approximately 1-2 cm down from the rectangular hole in the top edge of the plate. The lid was then attached to the plate before being covered with an opaque plastic cover (also sporting a hole for the shoots) that prevented light access to the roots. The rhizobox was then placed within a custom-made stainless-steel rack (built by High Peak Sheet Metal LTD) (Figure 2.1B). The rack holds the rhizoboxes at a 45^{o} angle so the roots would grow against the base of the rhizobox, allowing for the best possible viewing of the roots (Bontpart et al., 2020). The rack was then placed within a tray which was filled with water to the point at which the base of the plates was just underwater. The water level was maintained just above the base of the rhizobox throughout growth. The rhizoboxes were placed in glasshouse conditions and grown for 10 weeks. The base of the rhizoboxes were scanned weekly using an Epson Perfection v850 scanner to produce a 2D image of root growth which could then be analysed. At the end of the experiment the top of the plates were also scanned, this is due to some root growth also occurring on the top face of the plate.

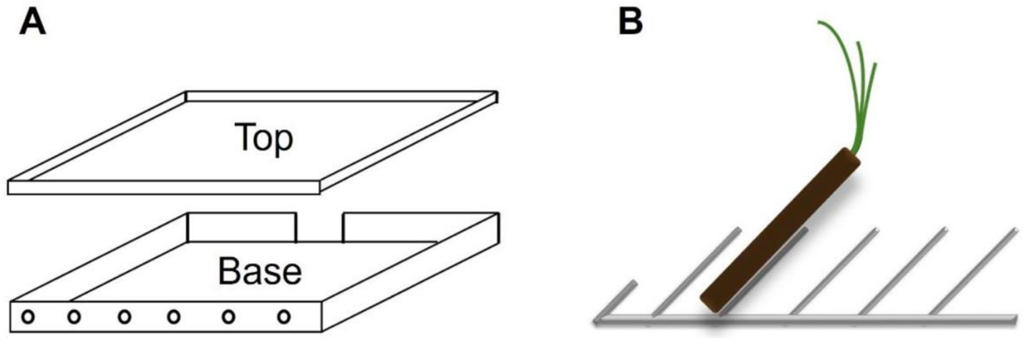

Figure 2.1 Rhizobox schematics

Schematics of rhizobox system used for root system analysis, panel (A) shows the base and lid of the rhizobox plates prior to the addition of soil and seed. Circles represent holes in the base for water transport to the soil, gap on the opposite side allows for shoots to exit. Once soil is added and seed is sown the top is attached to the base. Panel (B) shows a complete rhizobox with emerging germinated plant, placed at 45^{o} angle within a stainless-steel rack. Schematics used with permission from Wheeldon., (2023).

2.5.5 Hydroponics for root assessments

Seeds require a root system before entering the hydroponic system to aid growth after germination. The seeds were therefore grown in sand and perlite in glasshouse conditions for 14 days prior to transfer to the hydroponic system (See 2.4.2). Twice the required number of seeds were sown into sand: perlite for each experiment to ensure enough plants germinate and that similar sized plants could be selected for transfer. Upon transfer the whole plant was carefully removed from the sand: perlite mixture, ensuring the root system remains intact. The roots were then carefully washed in water to remove any sand or perlite remaining attached to the roots. Once the roots were clean, a foam bung was placed around the root-shoot junction of the plant. The plant and bung were then placed within a modified, open-ended falcon tube (that had been cut to be 4-5cm in length) so that the shoots were protruding from one end and the roots from the other. The foam bung and falcon tube would hold the plant in place within the hydroponic lid. Hydroponic pots were placed into either winter growth cabinets or the glasshouse (Specified in results). An electronic pipette and 25ml stripette was used to add 15 ml of ATS (See 2.2.1) to each 2L hydroponic pot. The remainder of the pot was then filled with water. An air pump (All Pond Solutions, AP-12-Kit pump) supplied air and agitation to each pot via tubing and an air-stone. An air-stone was placed under the water within each pot. Splitters on the tubing allowed for multiple air stones to be used per air pump. The valves on the splitters were adjusted to ensure equal air flow to each pot. A circular hole, the same diameter of the modified falcon tubes, was cut into the centre of the hydroponic lids, the lid was then placed on the hydroponic pot, leaving a slight gap for the air tube. Once set-up of the hydroponic pot is complete the falcon tube containing the plant can be placed in the hydroponic lid with the roots entering the hydroponic pot through the hole in its lid and the shoots pointing upwards out of the pot. The water within the hydroponic pots was topped up as required every couple of days. Every 2 weeks the whole pot was drained and fresh water and ATS were added.

2.5.6 Split-pot root barrier experiments

For root barrier experiments, three different pot set-ups were utilised. 500g rectangular pots (formerly grape boxes) with holes in the base, had either no root barrier attached, or one of two commercially available root barriers attached (Geofabrics). The two root barriers were a permeable Cutex root barrier (Figure 2.2A) or an impermeable Hytex root barrier (Figure 2.2B). The barriers were attached across the narrow width of the pot across the centre using sealant. The sealant was used to secure the barrier to the pot, prevent the barrier from moving and to prevent any water or roots traversing around the barrier. The pot was then filled with soil (Figure 2.2C). Depending on the experiment, pre-germinated seeds were then sown into the soil at depths of 1-2cm at either side of the root barrier ensuring equal distances are maintained between the barrier and between plants (Specific plant layout described in results) (Figure 2.2D). Plants were placed in spring glasshouse conditions and grown for 3 months. After which, shoots were harvested.

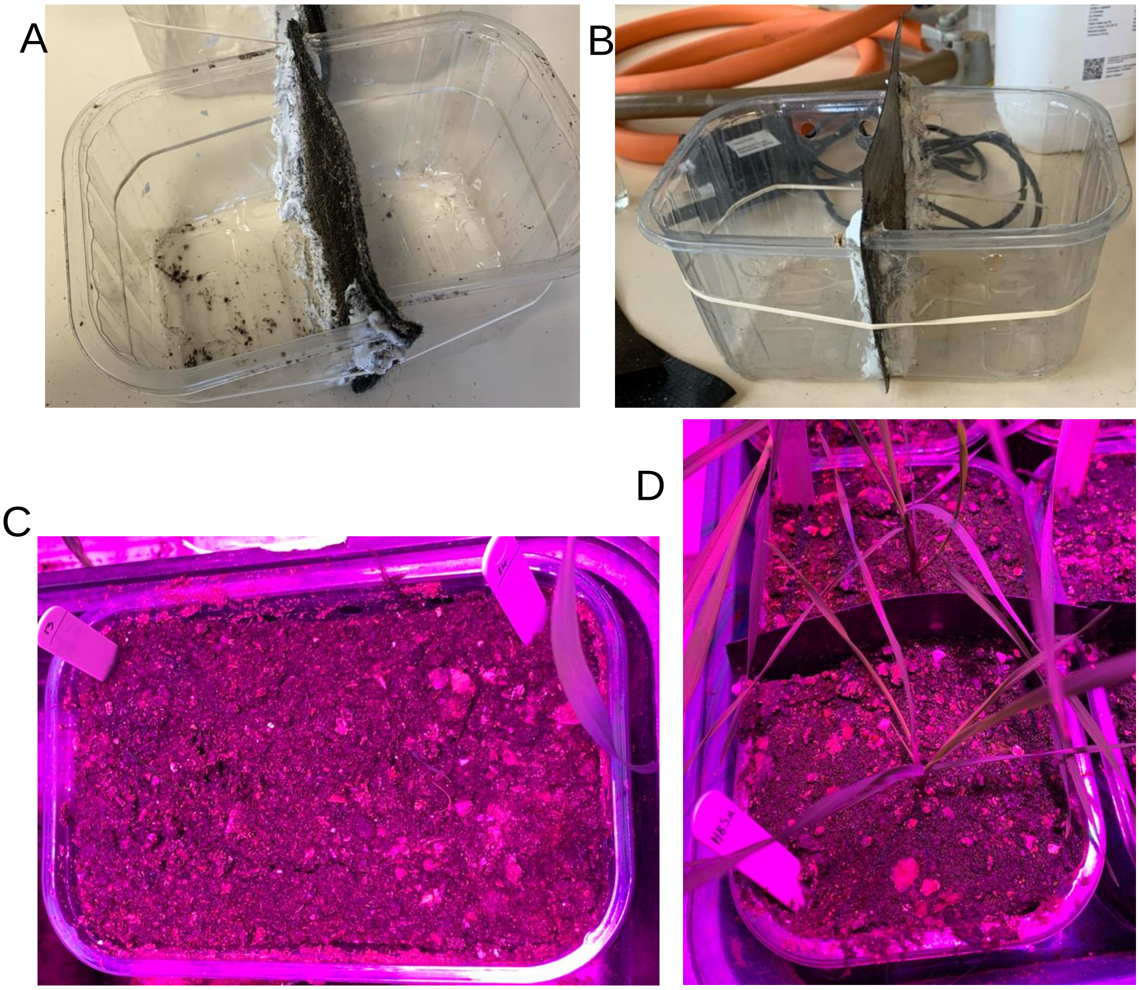

Figure 2.2 Root barrier set-up

Images of pots used in root barrier experiments prior and during experiments. Image (A) shows a Cutex root barrier and image (B) a Hytex root barrier attached to the plastic plant pots prior to the addition of soil. Image (C) shows a pot without a barrier containing soil, and image (D) a pot containing a Hytex barrier with soil and established plants.

2.5.7 Controlled conditions crop competition screen

Spring conditions were utilised for the controlled crop screen. 2L pots were filled with soil and watered. For crop only controls, a single pre-germinated crop seed was sown at 1-2cm depth in the centre of the pot. For the competitive setup (black-grass: wheat, 6: 1), a single pre-germinated crop seed was sown at 1-2cm depth in the centre of the pot, six pre-germinated black-grass seed were sown at 1-2cm depth at equidistance in a hexagonal formation around the centre crop. Crop tiller number was recorded every 7 days. After 3 months crop and black-grass shoots were harvested at the soil surface. For black-grass, the biomass of all black-grass plants within the same pot was weighed together as one. For winter experiments the method was the same except plants were placed into the Weiss cabinet and harvested after 6 months.

2.5.8 Container trial and crop assessments

Container trials were carried out to determine if different varieties of winter wheat and barley respond differently to the presence of black-grass competition and as a test to determine whether the controlled screen results are transferable to different environments. Container trials took place from October 2021 to July 2022 at ADAS's Boxworth site in Cambridge (Figure 2.3). The trials consisted of 23 different treatments (Table 2.2) each with 4 replicates giving a total of 92 pots organised in a fully random split block design. Pots used were 20L circular pots, filled with sterilised loam mix (Rothamsted 'weed mix' - sterilised kettering loam and lime free grit 3-6mm in a 4:1 ratio plus 2kg/m3 Osmacote) to a depth of 2 cm below the rim. The pots were placed on an uncovered hard-standing area and watered well using an overhead watering system. The area was fenced to prevent predation from ground animals (rabbits etc). In all treatments except BG only treatments, pots were sown with 10 crop seed of corresponding variety, at a depth of 1-2cm. Seed was positioned evenly across the pot surface avoiding sowing within 15mm of the pot edge. Crop seed was thinned to 6 plants per pot after emergence (4 weeks post-sowing) whilst at the leaf stage of emergence (GS11-12), ensuring equal spread of remaining crop plants. In treatments 8-23 upon sowing, an uncontrolled number of black-grass seed was scattered onto the pot surface then covered with a fine layer of soil to an even depth of 1cm. These pots would later be thinned after emergence (4 weeks post sowing) to achieve the desired black-grass densities (10 or 20 plants per pot), ensuring BG remaining after thinning were equally spread across the pot. In treatments 8-15 the pots were thinned to 10 BG plants per pot, treatments 16-23 were thinned to 20 BG plants per pot. Thinning was done to achieve as even a spread of BG throughout the pot as possible. Thinning consisted of removing as much of the plant as possible including shoots and seed, some roots may have remained. The pots were left outside throughout the winter growing season. Pots were watered as required from above, in March pots were stood in trays and watered from below. No chemical or biological treatments were given to the plants. Pots were checked for other weeds such as annual meadow grass and weeded if necessary. First plant assessments were carried out in March 2022. These assessments consisted of tiller counts on both crop and BG. The tiller number of each crop plant and each BG plant in every pot was recorded. Further assessments were carried out in May 2022. This consisted of counting the total number of BG ears present in each pot. In July 2022 the pots were harvested.

Table 2.2 Container trial treatment list, treatment number, crop variety and planting density

Treatment number	**Treatment Crop/variety + weed**	**Planting density - plants/container (crop/BG)**
1	WW - Kerrin	6
2	WW - Elation	6
3	WW - Barrel	6
4	WW - W1190637	6
5	WW - W1190488	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 - 1190637 + BG	6 + 10
12	WW - 1190488 + 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 - 1190637 + BG	6 + 20
20	WW - 1190488 + BG	6 + 20
21	WB - Bordeaux + BG	6 + 20
22	HWB - SY Kingsbarn + BG	6 + 20
23	BG alone	20

Unfortunately, the plots were subject to predation by birds, the barley seed was heavily predated therefore recorded biomass of barley (Bordeaux and SY Kingsbarn) will be lower than the maximum they achieved when in full seed prior to predation. All wheat varieties and BG showed no visible signs of predation. Small amounts of biomass may have been lost at harvest and transport as well as human error in separating crop from BG biomass.

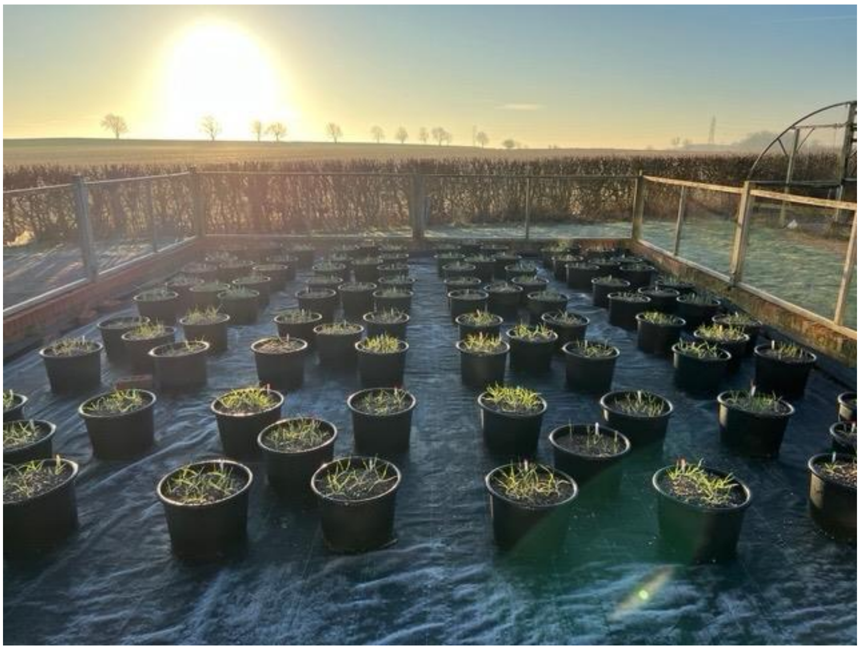

Figure 2.3 Container trial set-up

Image showing the random arrangement of container pots on a hard standing area during the winter.

2.5.9 Field trial and crop assessments

Field trials were carried out to assess the competitive effects of different varieties of winter wheat and barley on black-grass growth and development and as a test to determine whether the controlled screen results are transferable to different environments. The trials took place from October 2022 to July 2023 at ADAS's Boxworth site in Cambridge (Figure 2.4). A field with an existing BG population was selected. The trial consisted of 8 different treatments, 7 crop treatments and one black-grass only control (Table 2.3).

Table 2.3 Field trial lines. The seven crop lines used for experiments including the variety and source

Type	**Category**	**Variety**	**Source**
WW	Elite	Kerrin	ADAS
WW	Elite	Elation	ADAS
WW	Elite	Barrel	ADAS
WW	Landrace	W1190637	University of Leeds - Watkins collection
WW	Landrace	W1190488	University of Leeds - Watkins collection
WB	Elite	Bordeaux	ADAS
HWB	Elite	SY Kingsbarn	Syngenta seeds
BG	Weed	Black-grass	Field C, Adas Boxworth 2021

Each treatment had 4 replicates giving a total of 32 plots. One treatment (W1190637) only had enough seed for three replicates therefore the fourth plot was covered with Extase. Plots were 5m x 2m and crop seed were sown at seed rates of 325seed/m2 using an Oyjord drill at a depth of 4cm. Black-grass was also hand sown to all plots to ensure complete coverage over the whole trial area. Crop plots were randomised within the trial area, black-grass only plots were placed at the end of the trial area to ease drilling. 4 days post drilling, glyphosate (Samurai 4l/ha) pre-emergence herbicide was applied to the trial area.

Figure 2.4 Field trial plots

Image showing the field trial plots in Boxworth, Cambridge. Image taken in April.

First assessments were taken in April 2023 at crop GS30-31 (Figure 2.5). In each plot, Black-grass plants were counted within a 0.5m2 quadrat which was placed randomly within the plot avoiding the outermost rows. Average crop height (3 plants) and crop tiller number (3 plant) per plot was recorded for each plot. Further assessments were carried out in June 2023. In each plot black-grass ears were counted within a 0.5m2 quadrat which was placed randomly within the plot avoiding the outermost rows, this was done by holding the quadrat above the plants and counting the number of ears that were within the quadrat square. Crop height (3 plants) was also recorded for each plot. In July 2023 the plots were harvested.

2.6. Phenotypic assessments

2.6.1 Tiller counts

Shoot number (tillers in grasses) was counted and recorded throughout all experiments. In black-grass, it was not possible to accurately count tiller number due to the vast number of small tillers.

2.6.2 Biomass recording and harvesting

In soil-based experiments shoots were harvested at the base of the shoot as close as possible to the soil surface. Roots were not harvested from soil-based experiments. For hydroponics, shoots were harvested close to their base in line with the top of the foam bung. Roots were harvested being cut as high as possible at the base of the foam bung. Upon harvest all shoots were placed into individual paper seed bags. Roots were squeezed as to remove any excess water then also placed into paper seed bags. Both roots and shoots were placed in a drying oven at 60^{o}C for seven days. Once dry, plant material was removed from the seed bags and weighed using a balance.

For container and field experiments wet shoot biomass was recorded therefore the drying stage was omitted. For container trials in each pot the plant shoots were cut at soil level and all above ground biomass was collected and transferred to the on-site lab. Crop and BG biomass was separated by hand, before being weighed using a balance, final wet shoot weight was recorded for both crop and BG for each pot. For field trials two grab samples were taken from each plot. The grab samples were collected as follows; two 0.5m2 quadrats were randomly placed in each plot. All plants that have their shoots originating within that quadrat were cut at soil level, bundled together, and collected. The two grab samples from each plot, were then combined to form a single biomass sample for each plot and transported to the on-site lab. Crop and BG biomass were then separated, as was any biomass from any other weed species present, this unwanted biomass was discarded. Once separated, crop and BG wet shoot biomass was recorded using a balance. Primary crop tiller number (tillers with ears) was also recorded.

2.6.3 Rhizobox root analysis

To produce root images, the top and base of the rhizobox plates were periodically scanned. Images were then assessed using ImageJ software. Percentage root cover was recorded, this assumed that roots are white, and soil is black. Percentage root cover was calculated through white pixel assessment. Using ImageJ, each pixel within an image was graded for intensity on a scale of 0-255, where 255 = completely white and 0 = completely black with the numbers in-between being the range from white to black. The mean pixel intensity can therefore calculate the amount of white in the whole image, this value will be relative on a scale of 0-255, which can be converted to a percentage. This gives a value for percentage white pixel cover and therefore a value for percentage root cover. For the images, the polygon tool was used to draw around the desired part of the image, ensuring to avoid areas of the plate not consisting solely of soil or root, as this would affect the result. White pixel cover was then recorded within the selected area. For these experiments the image contrast was set at 25/299 and was kept constant across all images.

2.7. Experimental design and statistical analysis

2.7.1. Sample size and replication

Sample size will be stated in the figure legends. Replication was not always possible for larger scale experiments due to the length of time each experiment took and the limited growth space availability.

2.7.2. Statistical analysis

Data was tested for normality prior to testing groups for significant differences using the appropriate parametric or non-parametric tests. If significant differences were found further post-hoc tests were then carried out to determine the nature of these differences. Statistical tests used for each experiment will be identified in the corresponding results text and figure legends.

Chapter 3 Understanding wheat – black-grass competition

3.1 Introduction

Little is known about the mechanisms involved in wheat – black-grass competition. Black-grass is highly effective at reducing yields of winter wheat (Moss, 2010) however how it manages to do this is not well understood. Black-grass is known to be highly competitive against wheat, but only in autumn plantings (Moss, 2010). In plantings of spring wheat, the effects of black-grass are much reduced (Moss., 2018). It is therefore highly likely that the seasons and environmental conditions play a role in aiding black-grass competition. Barley is also known to be better at competing with black-grass than wheat (Cook and Roche., 2018). Given winter barley is grown at the same time as winter wheat, there must be some physiological reasons as to why winter barley is better at withstanding black-grass competition than winter wheat, but these are currently unclear.

Several studies have highlighted above ground traits such as increased height and early vigour within barley that make it more competitive than wheat against black-grass (Christensen., 1995; Cook and Roche., 2018). However, many plant-plant interactions occur below-ground, with the role they may play in competition long overlooked (Andrew., 2016). In wheat it is suggested that the growth of both shoot and root systems are connected (Wheeldon., 2023) indicating a link between what occurs below-ground and what is produced above ground. Ensuring below-ground fitness may therefore be just as important to plant survival as the competitive interactions known to be occurring above-ground.

Plants are constantly adapting their growth based on feedback mechanisms originating in signals detected in the soil by the roots whether these be resource dependant (nutrient limiting) or resource independent (allelopathy) (Aschehoug et al., 2016). It is therefore possible that wheat and black-grass are competing unseen below the soil surface, and therefore if that is so, it is possible that a competitive advantage may be being gained by black-grass below the surface.

Two possible below-ground mechanisms that may be deployed by a plant to aid itself in competition are the use of chemical root exudates and the production of roots (Finch et al., 2017; Yang et al., 2018). The use of both were assessed in this thesis. Plants may use chemical exudates to sense their surroundings and adapt their growth dependant on neighbour presence and soil volume availability (Wheeldon et al., 2023) or to inhibit the growth of neighbouring plants (allelopathy) (Zhang et al., 2021b). The presence of allelopathy in wheat – black-grass competition has been recognised (Delory et al., 2016) however to what degree allelopathy is able to impact competition in real conditions is unknown due to limited knowledge on plant detection and response to these chemicals as well as the short lifetime of allelochemicals in the soil (Rice et al., 2012). Conversely, root growth can allow plants to dominate space and resources, allowing them to gain a competitive advantage over neighbouring plants through monopolisation (Finch et al., 2017). It is hypothesised that under-ground interactions are key to understanding wheat-blackgrass competition.

3.2 Aims

The main aims of this chapter were to:

1. Determine when black-grass gains a competitive advantage over wheat.
2. Determine whether there is wheat – black-grass competition occurring below-ground.
3. Determine to what extent different modes of below-ground interaction contribute to wheat – black-grass competition.

3.3 Black-grass is slow to establish and weedy early in growth

To explain the competitive advantage of black-grass, it was firstly hypothesised that black-grass would be quicker to establish in fields than wheat plants grown at the same time, i.e. that it gains a ‘head-start’ on wheat. To test this idea, multiple comparisons were made between 3 elite wheat varieties and black-grass, in terms of germination and early growth (collectively constituting establishment). Specifically, the following parameters were measured:

1. Time to germination.
2. Time to produce a root 4cm in length.
3. Time to produce a coleoptile 2cm in length.
4. Shoot biomass 2 weeks post germination.
5. Shoot biomass 4 weeks post germination.

Germination was defined as the time from seed imbibition to first emergence of either the root or coleoptile (whichever occurred first). It was observed that black-grass took significantly longer to germinate than the three elite wheat varieties (Kruskal-Wallis test, Dunn’s, $P<0.001$) (Figure 3.1A), black-grass taking on average 8 days to germinate whereas all wheat lines germinated after an average of 3 days. The time taken to produce a root of 4cm in length was significantly longer in black-grass than in Barrel (Kruskal-Wallis test, Dunn’s, $P<0.001$) (Figure 3.1B) with black-grass taking on average 13 days compared to 5 days for barrel. Elation and Kerrin also took far less time (~ 6 days) to produce a 4cm root however this was not statistically significant due to experimental variability. It was observed that black-grass took significantly longer to produce a coleoptile of 2cm in length than the three wheat varieties (Kruskal-Wallis test, Dunn's, P<0.001) (Figure 3.1C), black-grass taking on average 14 days whereas all wheat lines achieved a 2cm coleoptile after 6 days.

Shoot biomass 2-weeks post-germination was significantly smaller for black-grass than all three wheat varieties (ANOVA, Tukey HSD test, P<0.001) (Figure 3.1D), black-grass had an average biomass of 0.0095g in comparison to Barrel (0.055g), Elation (0.059g) and Kerrin (0.042g). After 4 weeks of growth post-germination, it was observed that shoot biomass remained significantly smaller in black-grass than in the three wheats (Kruskal-Wallis test, Dunn's, P<0.01) (Figure 3.1E), black-grass having an average shoot biomass of 0.17g compared to Barrel (0.36g), Elation (0.32g) and Kerrin (0.4g).

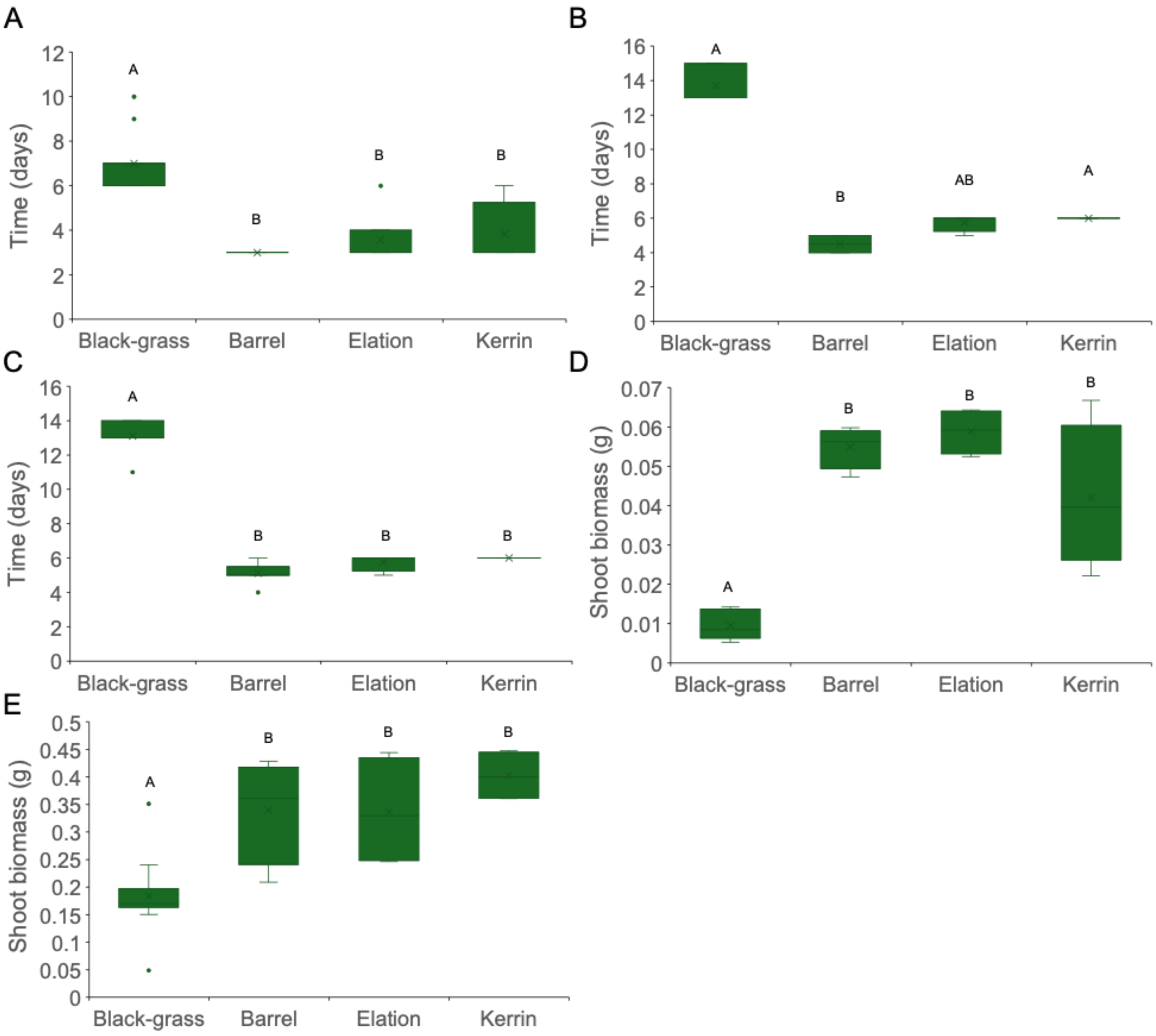

Figure 3.1 Black-grass is slow to establish in comparison to wheat

Boxplots comparing the early growth of black-grass and three elite wheats (Barrel, Elation and Kerrin). Boxes indicate interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values, the X within the box is the mean.

Circles represent outliers. Different letters above the boxes indicate significant statistical differences between the groups, calculated for panels A, B, C and E by (Kruskal-Wallis, Dunn's adjusted with Bonferroni correction, P<0.05) and for panel D by (ANOVA, Tukey's HSD test, P<0.05). Panels show (A) time taken to germinate (n=6-11), (B) the time taken to produce a root of 4cm in length (n=4-8), (C) the time taken to produce a coleoptile of 2cm in length (n=4-11), (D) seedling shoot biomass 2 weeks post germination (n=4-11) and (E) seedling shoot biomass 4 weeks post germination (n=4-12).

These findings disagree with the hypothesis that black-grass would be quicker than wheat to establish and larger than wheat earlier in growth. The comparatively slow growth of black-grass early on in establishment indicates that black-grass would be unlikely to gain a competitive advantage over wheat via root or shoot production within these very early stages of growth. If any competition occurs this early in growth it is likely that the larger plant, in this case the wheat, would outcompete the smaller black-grass plant. Obviously, there are many other environmental factors in the field that may be affecting competition however the data suggest that for black-grass to have the negative impact on wheat yields that are seen in the field, it must gain a competitive advantage later in growth.

3.4 The impacts of wheat – black-grass competition cannot be seen until after winter

A series of 'container' and field trials were carried out, growing black-grass in competition with wheat and barley lines, which are fully detailed in Chapter 6. Although not the primary reason for conducting them, data gathered during these trials helps to determine when black-grass gains a competitive advantage over wheat. Due to the previous results showing slow black-grass establishment, and the knowledge that black-grass is mainly an issue in winter plants (Moss., 2010), it was hypothesised that black-grass requires an extended period over the winter, in which to gain a competitive advantage over wheat. To test this, seven crop lines were grown in competition with black-grass in both container and field trials. These trials were sown in the autumn, grown over winter then harvested the following summer. Crop and black-grass assessments were taken throughout the season.

The container trials consisted of seven crop lines, grown as either 'crop only' at a crop density of 6 plants/pot, or as a 'competition' set-up, where the 6 crop plants were sown with 20 black-grass plants. To determine when black-grass impacts wheat growth, plant assessments were taken two times prior to harvest, once in March and then again in

May. The first plant assessments, which took place in March, consisted of crop and black-grass tiller counts. It was observed that there were minimal differences in crop tiller number with and without black-grass competition at this time point (Figure 3.2A, B), with tiller number being slightly lower in competition for the wheats. Without black-grass competition (Figure 3.2A) tiller number of both barleys is higher than that of all wheats, significantly so for Bordeaux (Kruskal-Wallis, Dunn's, $P<0.001$). In black-grass competition, the difference between barleys and wheats is greater with both Bordeaux and SY Kingsbarn having significantly greater tiller number than all wheats (Kruskal-Wallis, Dunn's, $P<0.001$) (Figure 3.2B) indicating barleys may already have gained some competitive advantage compared to wheats. For both treatments there are no significant differences in tiller number between wheat varieties indicating that wheat varieties more susceptible to black-grass cannot be identified at this stage of growth. Black-grass tiller number was also recorded in March with a significant difference in tiller number seen between crop lines SY Kingsbarn and Elation (Kruskal-Wallis, Dunn's, $P<0.05$), however no clear differences were observed between any other lines likely due to the small range in tillers produced at this stage of growth (Figure 3.2C). This would indicate little competitive effect of wheat on black-grass at this stage of growth, or not enough of a competitive effect as to distinguish differences in the competitive ability of the crop lines. This is further supported by the tiller number of the black-grass only control plants, which are not significantly different from any crop treatment (Kruskal-Wallis, Dunn's, $P>0.05$) (Figure 3.2C) indicating no competitive effect on the shoot growth of black-grass at this stage.

Further assessments were carried out in May, when black-grass ear count was recorded for each crop line (Figure 3.2D). It was observed that there was now greater variation in black-grass presence between crop treatments, with black-grass ear count differing significantly (ANOVA, Tukey HSD test, $P<0.001$) depending on the crop it was competing with. Black-grass had the greatest ear count when it was not in crop competition. This indicates that all crop varieties suppress black-grass growth to some extent. Kerrin, Elation and Bordeaux have similarly high levels of black-grass. SY Kingsbarn, Barrel and the two landraces have the smallest number of black-grass ears present in their pots, each having a significantly smaller number of black-grass ears than the black-grass only pots (ANOVA, Tukey HSD test, $P<0.001$). The clear difference in black-grass ear count between crop varieties in May suggests that competition is initiated, or at least the effects of competition are first seen, at some point between crop assessments conducted in March and May, possibly coinciding with stem elongation at growth stage 31.

A two-way ANOVA was conducted that examined the effect of cultivar and black-grass competition on crop tiller number. There was a statistically significant interaction between the effects of cultivar choice and black-grass competition presence on crop tiller number (2-way ANOVA, F (6,322) = 2.58, p=0.019).

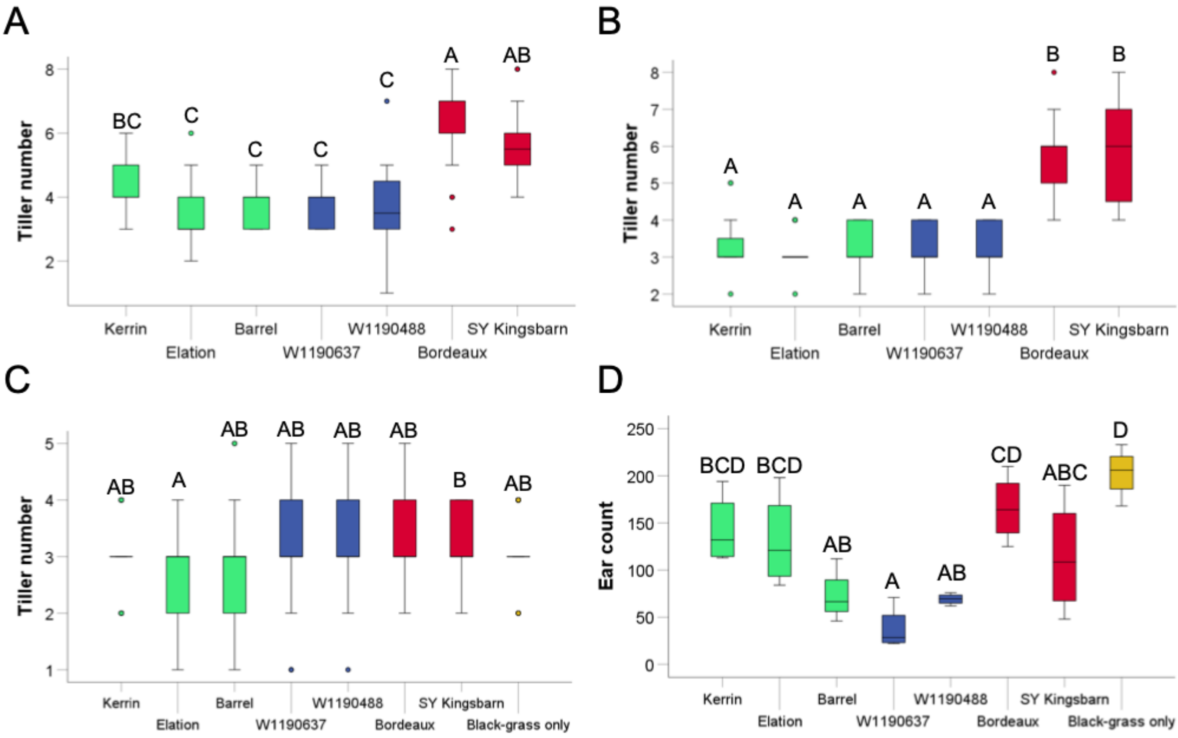

Figure 3.2 Competition delayed in container trials

Panels (A and B) show boxplots showing the crop tiller number of three elite wheat lines (Kerrin, Elation and Barrel) two landrace winter wheats (W1190637 and W1190488) a winter barley (Bordeaux) and a Hybrid barley (SY Kingsbarn) 5 months after sowing without (A) and with (B) black-grass competition. Panels (C and D) show black-grass tiller number (C) five months after sowing and Ear number (D) seven months after sowing when in competition with each crop variety. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Circles represent outliers. Different letters above the boxes indicate significant statistical differences between the groups calculated for (A, B and C) by (Kruskal-Wallis, Dunn's adjusted with Bonferroni correction, P<0.001), n=24 and for (D) by (ANOVA, Tukey's HSD test, P<0.001), n=4.

The field trials consisted of the same seven crop varieties in competition with black-grass. This time a black-grass only control was used to determine differences in black-grass levels between crop treatments. The first field assessments were carried out in

April, when black-grass counts within a 0.5m^2 quadrat were carried out in each plot (Figure 3.3A). It was observed that there was a significantly greater number of black-grass plants in the black-grass only plot than in all crop plots (ANOVA, Tukey HSD test, P<0.001) (Figure 3.3A). All crop plots had similarly low levels of black-grass, with no significant differences seen between them (Figure 3.3A). Crop tiller count was also recorded in April, it was observed that there was a significant difference (Kruskal-Wallis, Dunn's, P<0.05) (Figure 3.3B) between tiller number of W1190488 and Bordeaux, however there was no significant difference between any other lines. Given black-grass counts were consistent across crop treatments, it is likely that these slight differences seen in crop tiller number are due to natural differences in development rate rather than due to any impact of black-grass competition.

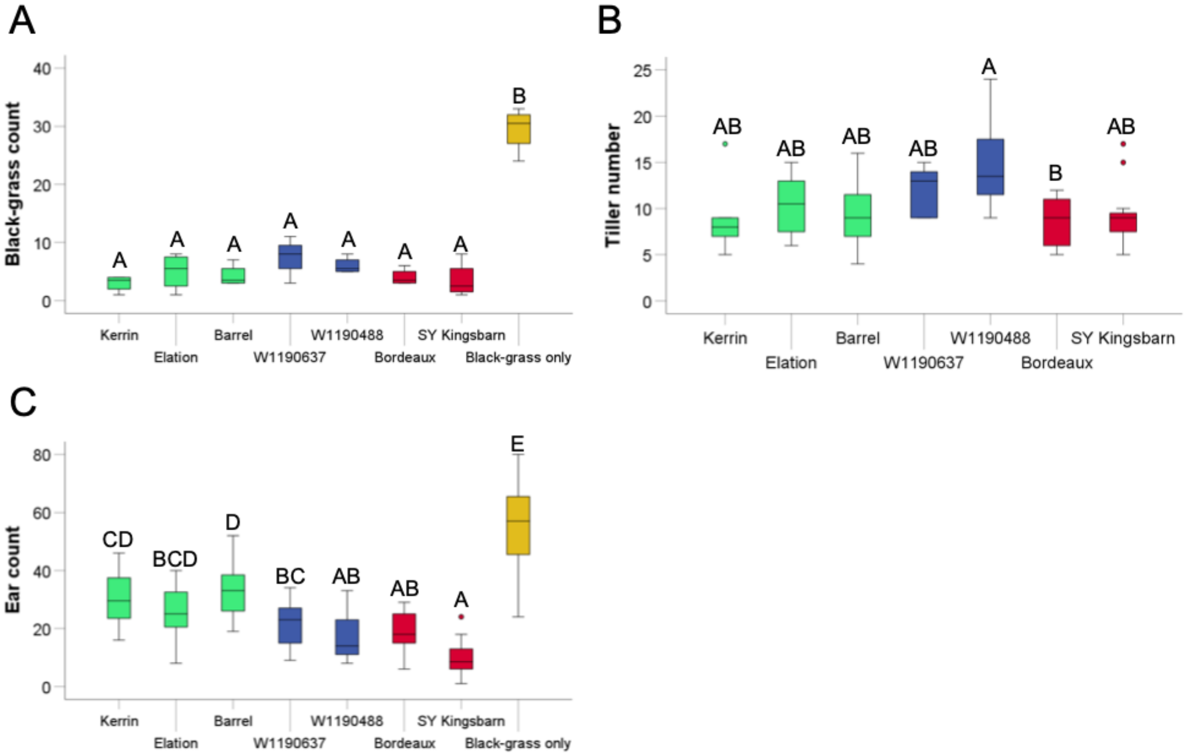

Figure 3.3 Competition delayed in field trials

Seven crop lines including three elite wheat lines (Kerrin, Elation and Barrel) two landrace winter wheats (W1190637 and W1190488) a winter barley (Bordeaux) and a Hybrid barley (SY Kingsbarn) were assessed for their growth in black-grass competition in field conditions. Boxplots show (A) black-grass plant counts in different crop treatments 6 months post sowing (n=4) (B) crop tiller number 6 months post sowing and (n=9-12) (C) black-grass ear counts 8 months post sowing (n=15-20). The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Different letters above the boxes indicate significant statistical *differences between the groups calculated for (A and C) by. (ANOVA, Tukey's HSD test, P<0.001) and for (B) by (Kruskal-Wallis, Dunn's adjusted with Bonferroni correction, P<0.001).*

Further field assessments were carried out in June, this time black-grass ears within a 0.5m^2 quadrat was recorded for each plot (Figure 3.3C). It was observed that there was a significant difference in black-grass ear count depending on the crop variety present in the plot (ANOVA, Tukey HSD test, P<0.001) (Figure 3.3C). Black-grass only plots continue to have the highest number of black-grass ears, significantly greater than all crop treatments (ANOVA, Tukey HSD test, P<0.001) (Figure 3.3C), this again shows that all crop varieties suppress black-grass growth to some extent. The three elite wheat varieties Barrel, Kerrin and Elation have the highest black-grass ear count of all crop treatments. The two landrace varieties W1190637 and W1190488, along with the winter barley Bordeaux all have similar numbers of black-grass ears, slightly lower than that of the elite wheats. SY Kingsbarn, the hybrid winter barley, has the lowest number of black-grass ears, significantly smaller than all elite wheat lines tested (ANOVA, Tukey HSD test, P<0.001*)* (Figure 3.3C).

The findings from both the container and field trials support the hypothesis, showing that little to no impact of wheat-black-grass competition is visible on either crop or black-grass in March and April. This indicates that throughout the winter that the competition between wheat and black-grass is yet to reach a point that affects their above-ground growth. In May and June however, differences start to be seen in black-grass presence dependant on the crop treatment. This indicates that the effects of competition are now visible between crop and weed. How and when the competition that results in these effects occurs remains to be answered however. Are wheat and black-grass only competing after April, at which point they enter a frantic battle? Or has competition already occurred during the winter, resulting in the later effects on wheat growth, which is only seen visibly in shoot growth at stem elongation?

3.5 Wheat lags behind winter barley and black-grass in both root and shoot growth in winter conditions

It was hypothesised that, even though it is not necessarily visible in terms of competitive outcome, black-grass gains its competitive advantage over wheat during winter, and not in the spring. Specifically, it was hypothesised that black-grass would have a greater root system than wheat in winter conditions. Furthermore, it was hypothesised that this would not be the case in barley, and hence explain why barley is more competitive against black-grass. To test this, winter wheat, winter barley and black-grass were grown in a hydroponic system in winter conditions. Final dry shoot and root biomass was recorded. Shoot to root biomass ratio was also calculated for each species.

For shoot biomass, it was observed that both barley and black-grass had a significantly larger shoot biomass than wheat (ANOVA, Tukey HSD test, $P<0.001$) (Figure 3.4A), wheat having an average biomass of 5.8g compared to 11.43g for black-grass and 14.6g for barley. For root biomass, it was similarly observed that both barley and black-grass produced a significantly larger root system than wheat (ANOVA, Tukey HSD test, $P<0.001$) (Figure 3.4B), again barley had the largest average root biomass of 8.72g compared to 7.41g for black-grass and 2.82g for wheat. The shoot to root ratio of biomass was not significantly different between species (Kruskal-Wallis test, $P>0.05$) (Figure 3.4C) however of the three, wheat allocated a greater proportion of its biomass to its shoots, whereas black-grass allocated a greater proportion into root production, compared to wheat and barley. Thus, in winter conditions, wheat grows much more slowly than both black-grass and barley, not only in roots but in general.

To determine the impact of the growth conditions, black-grass and wheat were also grown in hydroponics in spring conditions. It was hypothesised that wheat would grow faster than black-grass in spring conditions, contributing to the lack of competitive advantage black-grass has with spring wheat plantings. To test this, black-grass and wheat were grown in the same hydroponic set-up in spring conditions. It was observed that wheat was able to produce a much larger shoot system than black-grass, however it had a significantly smaller root system (Independent samples t-test, $P<0.05$). Wheat also had a significantly larger shoot: root ratio than black-grass (Independent samples t-test, $P<0.05$). Thus, in spring conditions, wheat has much faster overall growth than black-grass, even if root growth is still less than black-grass.

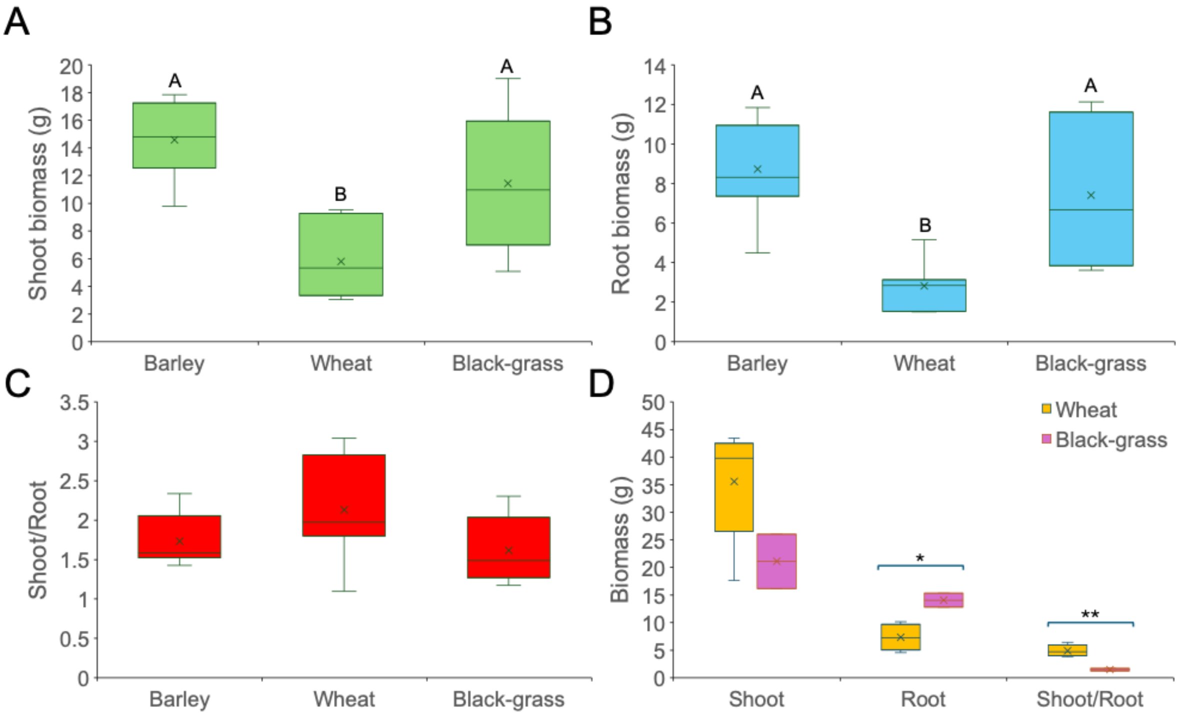

Figure 3.4 Barley and black-grass are both larger than wheat in root and shoot production

*Boxplots (A, B and C) showing the growth of Barley, Wheat, and Black-grass in a hydroponic system in winter conditions with (A) showing the final dry shoot biomass, (B) the final dry root biomass and (C) the final dry shoot to root biomass ratio. Boxplot (D) comparing wheat and black-grass growth in spring conditions. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values, the X within the box is the mean. Different letters above the boxes indicate significant statistical differences between the groups calculated for (A and B) by (ANOVA, Tukey HSD test, P<0.001), n=6-8. For (D) Asterisks denote significant statistical differences between the groups (Independent samples t-test *=P<0.05, **=P<0.01), n=2-6.*

Root growth over time (Growth rate) was determined for Barley, Black-grass and Wheat when grown in hydroponics (Figure 3.5). Root growth here was measured via water displacement. Root growth therefore is calculated as the weight of hydroponate without root presence – weight of the hydroponate with roots present. In figure 3.5, after approximately 2-months of growth root production of the three species is very similar. However, after approximately 75 days wheat root growth seems to lag that of barley and black-grass. After 125 days, black-grass root growth is approximately double that of wheat and 1.25x that of barley. From days 125-175 the difference in root growth continues to increase with black-grass able to produce roots 3-4x that of wheat and 2x that of barley.

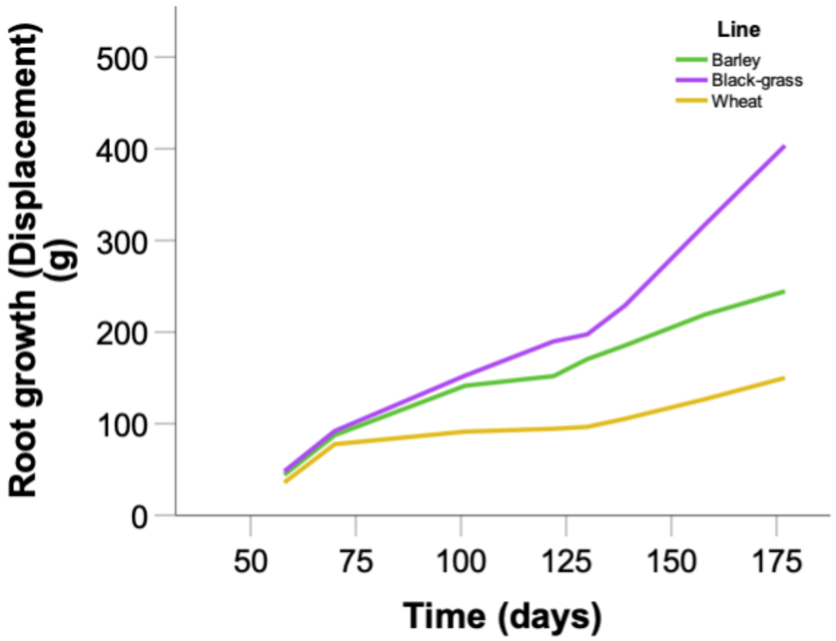

Figure 3.5 Variation in species root growth rate

Line graph showing root growth rate of Barley, Black-grass and Wheat when grown in hydroponics for 6 months. Root growth measured via water displacement, i.e. the space in the hydroponic pot that water can no longer occupy due to the presence of roots. Calculated as Weight of hydroponate in the absence of roots – weight of hydroponate in the presence of roots. Points are means (n=4-6).

These results support the hypothesis, showing that black-grass can produce a much larger root system than wheat in winter. Not only does black-grass produce more roots, but it focusses a greater proportion of biomass into its root system, this indicates that black-grass may prioritise root production to gain a competitive advantage. Black-grass also has a larger shoot biomass than wheat in winter conditions indicating that black-grass is generally better adapted to growing in these conditions, and a larger shoot system may give it a competitive advantage above-ground. Barley shows similar results to black-grass in terms of shoot and root biomass. The ability of barley to produce larger plants both above and below-ground supports the hypothesis and may indicate why it is better at competing against black-grass compared to the smaller wheat plants. The ability of black-grass and barley to produce much larger plants indicates the importance of biomass production in competition in winter conditions. Larger wheat plants therefore may be better adapted to compete with black-grass. The degree to which root production or shoot growth is the most important requires further work. The answer to this question would be vital to breeders for trait selection in wheat against black-grass pressures.

3.6 Below-ground competition occurs in wheat and black-grass competition

While the results show that differences in root growth rate might explain wheat – black-grass competition, it was also hypothesised that wheat and black-grass can compete below-ground through the production of chemical root exudates that inhibit the growth of the neighbouring plant ('chemical competition').

To test the role of chemical competition in wheat – black-grass interactions, multiple comparisons were made between wheat and black-grass plants grown in the same pot in the presence of different root barriers when grown in spring conditions. These root barriers allowed control of how neighbouring plants (plants in the same pot) can interact with each other. Three different root barriers were used (Figure 3.6):

1. None (N) – No root barrier was present, allowing both physical and chemical below-ground competition between neighbouring plants.
2. Cutex (C) – A permeable root barrier which allows chemical competition but prevents competition from physical root interactions.
3. *Hytex (H) – An impermeable root barrier which prevents all below-ground interactions.*

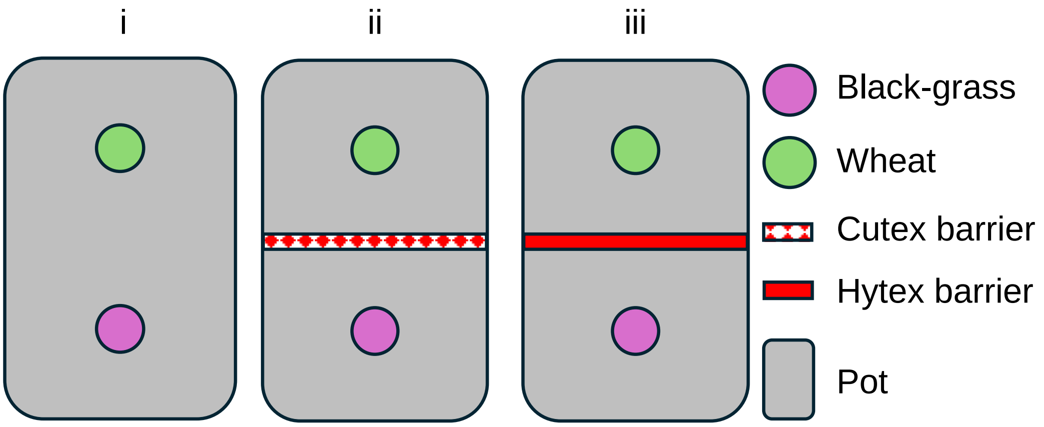

Figure 3.6 Root barrier experiment set-up

Schematic with key, showing the three different experimental set-ups used in the root barrier experiments. i) None, ii) Cutex and iii) Hytex. Barrier type determines the possible mechanisms by which competition can occur.

The different combinations of plants and barriers tested are as follows

BNB – Black-grass in competition with black-grass with no root barrier

BNW – Black-grass in competition with wheat with no root barrier

BCW – Black-grass in competition with wheat with a Cutex root barrier

BHW – Black-grass in competition with wheat with a Hytex root barrier

BHN – Black-grass not in competition in the presence of a Hytex root barrier

WHN – Wheat not in competition in the presence of a Hytex barrier

The plants were grown in rectangular pots, and each plant had theoretical access to the same soil volume (e.g. half the pot). In the test conditions, black-grass was grown in 1:1 competition with wheat, in the presence of one of the three barrier types. One key control condition was either wheat or blackgrass plant grown in a pot divided in two by an impermeable barrier, without any neighbouring plant in the other half of the pot (BHN, WHN). This condition helps define the ‘expected’ size of a wheat or blackgrass plant grown without competition in the half pot volume, in these growth conditions. An intraspecific control was also used for both species, without a barrier separating the plants (BNB, WNW). This provides a reference that helps to establish the effect of having a generic neighbour on plant growth relative to the no-competition control, and therefore whether the effects of interspecific competition are bigger than expected or not.

The mean biomass for black-grass in intraspecific ‘open’ competition with no barrier (BNB) was larger than for solitary black-grass plants (BHN). This is surprising as it would be expected that plants would grow larger without competition. However, the large range of biomasses observed in black-grass intraspecific competition (BNB) indicates high levels of competition are occurring between the two neighbouring plants, resulting in a ‘winning’ plant that dominates the shared pot compared to the ‘losing’ plant. This is consistent with highly competitive behaviour of black-grass in wheat fields. The ‘winning’ plant now has theoretical access to twice the soil volume of black-grass grown by itself (BHN) resulting in higher maximum biomass production in intraspecific competition (BNB).

For black-grass, it was observed that the biomass of black-grass when in open competition with another black-grass plant (BNB) (7.43g) was significantly larger than black-grass grown in open competition with wheat (WNB) (2.59g) (ANOVA, Tukey HSD test, P<0.001) (Figure 3.7A) indicating that wheat is more competitive against black-grass than black-grass is, under these growth conditions. The difference in the biomass of black-grass between black-grass only (BHN) and all interspecific set-ups (WHB, WCB, WNB) indicates that wheat is outcompeting black-grass, with black-grass consistently smaller in the presence of wheat.

Similarities in the biomass of black-grass when in open competition (WNB) and when in chemical competition (WCB) with wheat, indicate that chemical competition is important in the suppression of black-grass growth as black-grass suppression is still possible despite physical root interactions being prevented. Although not significantly different, the average biomass of black-grass in competition with wheat with an impermeable barrier (WHB) (4.54g) was greater than that of black-grass in chemical competition with wheat (WCB) (2.85g) (Figure 3.7A) showing a decrease in black-grass biomass when chemical competition can occur. This indicates that chemical interactions may have a role in wheat – black-grass competition and may be acting allelopathically to decrease the biomass of black-grass.

It was observed that wheat grown in open intraspecific competition (WNW) had a significantly smaller shoot biomass than wheat grown by itself (WHN) (ANOVA, Tukey HSD test, $P<0.01$) (Figure 3.7B). This indicates some form of mutual downregulation of growth between neighbouring wheat plants due to below-ground interactions. No significant difference was seen in the biomass of wheat when in competition with black-grass in the presence of a Hytex (WHB) or Cutex (WCB) root barrier, this indicates a limited impact of chemical competition from black-grass on wheat. This may indicate that chemical competition is not a method by which black-grass competes with wheat. However, the experiment design does not allow black-grass to successfully compete with wheat to its full ability, as seen in the field. Therefore, the real impact of chemical competition from black-grass may not have been observed. Chemical competition between crop and weed can therefore not be ruled out and requires further testing.

For wheat, it was observed that wheat grown in open intraspecific competition (WNW) had a significantly smaller shoot biomass than wheat grown in open competition with black-grass (WNB) (ANOVA, Tukey HSD test, $P<0.01$) (Figure 3.7B). The high biomass of wheat in open competition with black-grass (WNB) shows that wheat outcompetes black-grass in this set-up, this can equally be seen in the corresponding biomass of black-grass (WNB) in Figure 3.7A. The biomass of wheat is lower in intraspecific competition (WNW) than when in competition with black-grass (WNB), this indicates that wheat is more competitive intraspecifically in this set-up than black-grass is against wheat. The biomass of wheat in open competition with black-grass (WNB) is like that of wheat grown by itself (WHN) indicating very little impact of black-grass competition on wheat under these conditions.

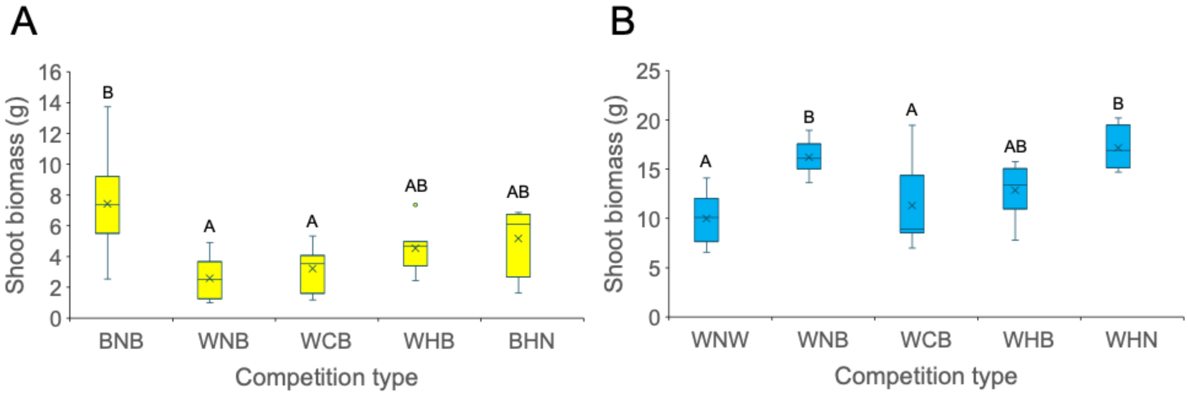

Figure 3.7 Evidence of below-ground competition

Boxplots showing (a) black-grass and (b) wheat shoot biomass in each root barrier set-up. B represents black-grass, W represents wheat, and N represents none referring to either barrier type or plant presence. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Circles represent outliers. Different letters above the boxes indicate significant statistical differences between the groups. (ANOVA, Tukey's HSD test, P<0.001), (a) n=4-16, (b) n=4-10.

Although there was a large biomass range of wheat in intraspecific competition (WNW), this was not the result of asymmetry in growth within each pot (see Figure 3.8A, B). This is consistent with wheat showing cooperative behaviour, and down-regulating its growth in the presence of neighbouring plants (Weiner et al., 2010). This appears to be an opposite response to black-grass when under intraspecific competition.

Figure 3.8 shows the biomass of both plants in each pot plotted against each other for each of the experimental set-ups. Interestingly there are clear groupings within the plotted area for plants in each barrier and experiment type. When wheat is grown in open competition with another wheat (WNW) (Figure 3.8A) the two plants in the pot are generally similar in size, with the data-points in the centre of the graph. In comparison, the data points for black-grass in open competition with another black-grass (BNB) (Figure 3.8B) are left of centre on the graph and more variable across the x-axis, the presence of small plants on one axis indicates that one of the plants is consistently larger than the other. These two graphs show that wheat is more likely to share the space and resources between neighbouring plants compared to black-grass which is more likely to compete for them.

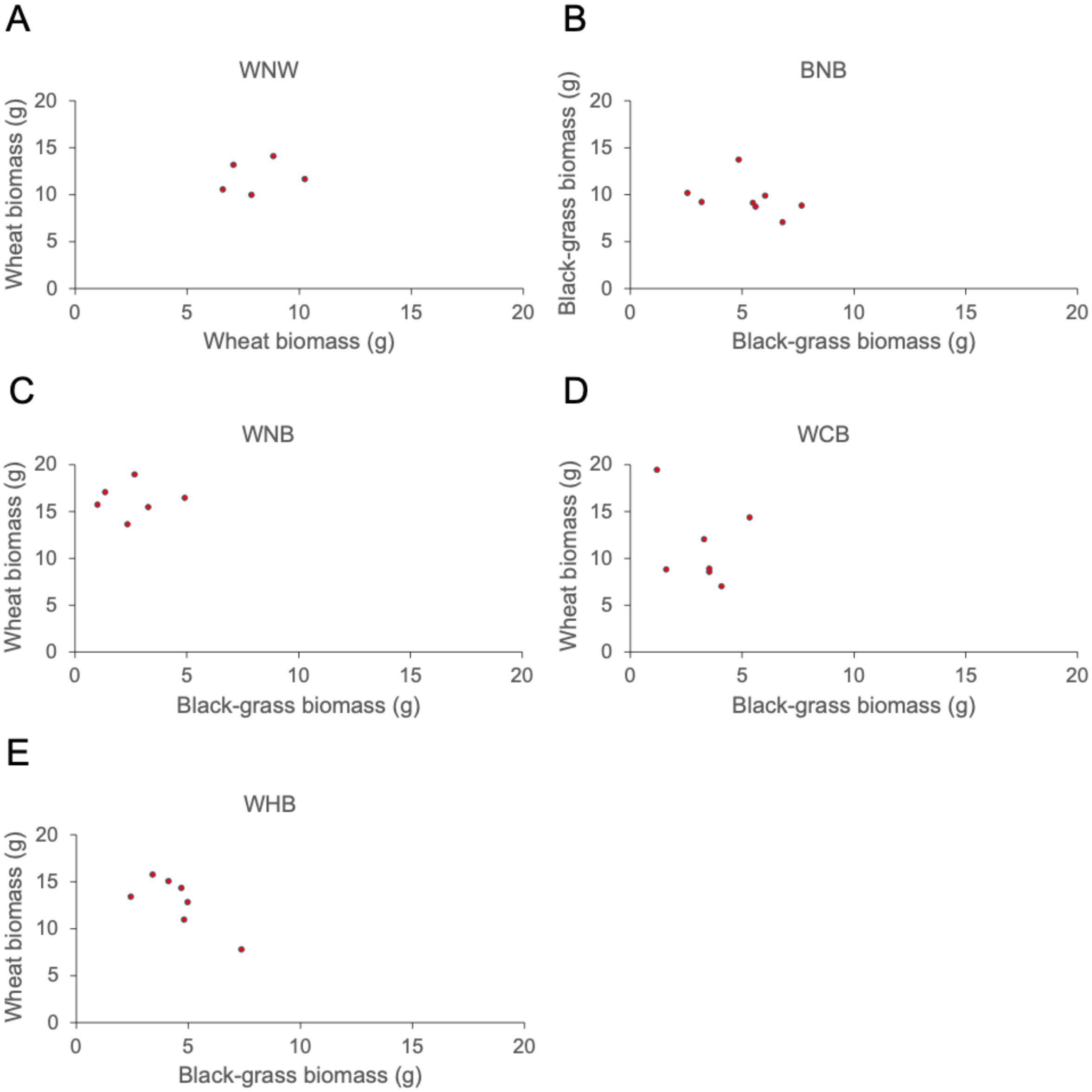

Figure 3.8 Biomass groupings vary with barrier type

Scatterplots comparing the shoot biomass of both ‘competing’ plants in each pot. Comparisons were made for each barrier set-up experiment (a) WNW (n=10), (b) BNB (n=16), (c) WNB (n=12), (d) WCB (n=14) and (e) WHB (n=14).

When wheat against black-grass in open competition (WNB) is plotted (Figure 3.8C) the data points cluster in the top left of the graph. This clearly shows that wheat plants are larger than black-grass and therefore wheat is outcompeting black-grass in this set-up. With the addition of a Cutex barrier (WCB) (Figure 3.8D) the data points are lower down the y-axis compared to WNB, indicating the biomass of wheat has reduced whilst the biomass of black-grass is relatively similar. The reduction in the biomass of wheat is very likely due to the reduction in available soil volume, due to the addition of the root barrier preventing the wheat from ‘stealing’ black-grass’s share of soil, however some part of the decrease may be attributed to chemical competition from black-grass. The same trend is seen when comparing WNB to WHB (Figure 3.8C, E). This would indicate some difference in the levels of competition occurring between the Cutex and Hytex barrier types. With wheat plants slightly smaller in Cutex pots than in Hytex pots, this would indicate that some level of chemical competition may be occurring between wheat and black-grass.

Interestingly the two species seem to have differing responses to intraspecific neighbour presence, wheat seems to down regulate its growth rather evenly between competing wheat plants, whereas with black-grass one plant seems to be taking a greater share of the overall pot biomass. Further analysis is required to clarify the nature of these interactions. Although identifying the mechanisms of competition require further work, it is clear that there are differences in plant growth in the presence of different root barriers supporting the hypothesis that below-ground interactions are important in wheat – black-grass competition and that such interactions may include chemical competition through root exudates.

3.7 Further investigation into wheat – black-grass mutual inhibition

The results have highlighted the possibility of chemical competition occurring in wheat-black-grass competition. To try to confirm this effect, the root barrier competition experiments seen in section **3.6** was simplified. This time, wheat was grown in interspecific competition with black-grass in the presence of either a Cutex (WCB) or Hytex (WHB) barrier, or no barrier (WNB). Final shoot biomass was recorded.

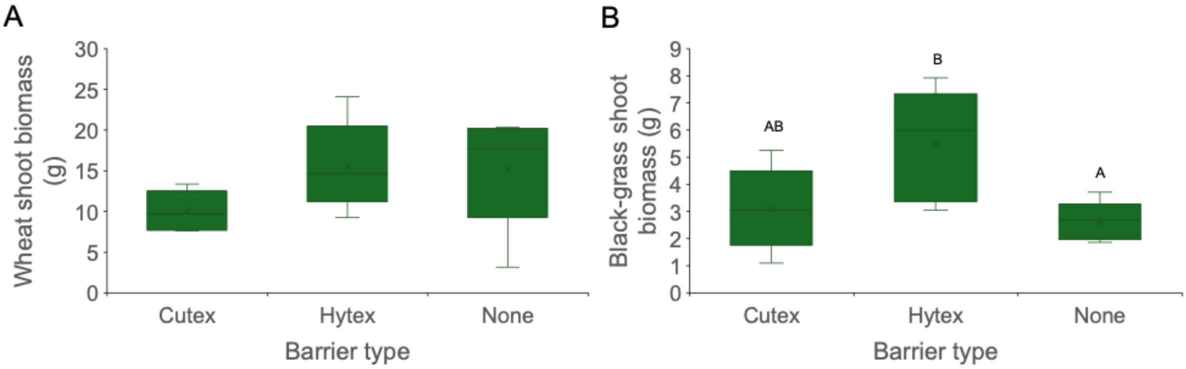

Figure 3.9 Black-grass more greatly affected by chemical competition than wheat

Boxplots showing the final shoot biomass of (a) wheat and (b) black-grass when grown in competition with one another in the presence of different root barrier conditions (Cutex, Hytex or None). The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Different letters above the boxes indicate significant statistical differences between the groups. (ANOVA, Tukey's HSD test, P<0.05), n=5-6.

For wheat – black-grass competition, there was no significant difference in the biomass of wheat between barrier types when in competition with black-grass (Figure 3.9B), however, the biomass of wheat was smaller in the presence of a Cutex barrier (10.06g) than in open competition (15.08g) or in the presence of a Hytex barrier (15.52g). No difference between Hytex and None barrier type would indicate no below-ground competition, this is consistent with previous findings in figure 3.7, again this may be due to the relatively small size of the black-grass meaning it is unable to successfully compete with the wheat. However, differences between Cutex and Hytex show that chemical competition is occurring and negatively impacts wheat growth. It is therefore likely that wheat in competition with black-grass with no barrier is outcompeting black-grass so efficiently that it sees little negative impact of black-grass and therefore can take advantage of the greater soil volume available to it.

For black-grass in competition with wheat, a significant difference was observed, with black-grass having a significantly smaller biomass in open competition with wheat than when a Hytex barrier was present (ANOVA, Tukey HSD test, P<0.05) (Figure 3.9B). In open competition black-grass had an average biomass of 2.63g compared to 3.11g with a Cutex barrier and 5.48g in the presence of a Hytex barrier. These results are consistent with the previous results and indicate that chemical competition is playing a role in black-grass suppression. The difference in the biomass of black-grass between barrier types None and Hytex is the only significant difference, therefore it can be concluded that a combination of both physical and chemical competition is likely playing a role in wheat – black-grass competition.

These findings support the hypothesis that chemical root competition can and is occurring to some extent between wheat and black-grass. For black-grass, it is clear that it is outcompeted by wheat, and that non-chemical below-ground interactions also contribute to this, likely including greater root growth by wheat. Caution must be taken however when drawing conclusions, particularly as the growth of both plants in each pot rely on the growth of the other, if one plant fails to grow well, this can be perceived as a competitive effect from the other, whereas in reality it may not be. The lack of growth of one plant can allow the proliferation of the other which may be wrongly perceived as competition.

3.8 Black-grass and wheat respond differently to intraspecific competition

These experiments have highlighted that wheat and black-grass may respond differently to intraspecific competition. To try to confirm these previous findings, black-grass and wheat were grown in intraspecific competition with another plant of the same species in a 1:1 ratio. The plants either had their roots separated by a Hytex root barrier or no root barrier. Final shoot biomass was recorded, and the impact of below-ground competition could then be determined.

For black-grass, there was no significant difference in biomass between barrier types (Figure 3.10A), however black-grass in open competition (BNB) had a higher average biomass (12.49g) compared to competition in the presence of a Hytex barrier (BHB (9.88g). As seen previously in Figure 3.7, black-grass in open competition with another black-grass has a large biomass range. This large range indicates the presence of some very large and some very small plants whereas when below-ground competition is prevented (BHB) the range of biomass is reduced. This is consistent with previous findings.

For wheat it was observed that wheat was significantly larger in the presence of a Hytex barrier (WHW) then when in open competition (WNW) (Independent samples t-test, P<0.01) (Figure 3.10B). This is also consistent with the previously observed effect showing mutual growth inhibition through below-ground interactions.

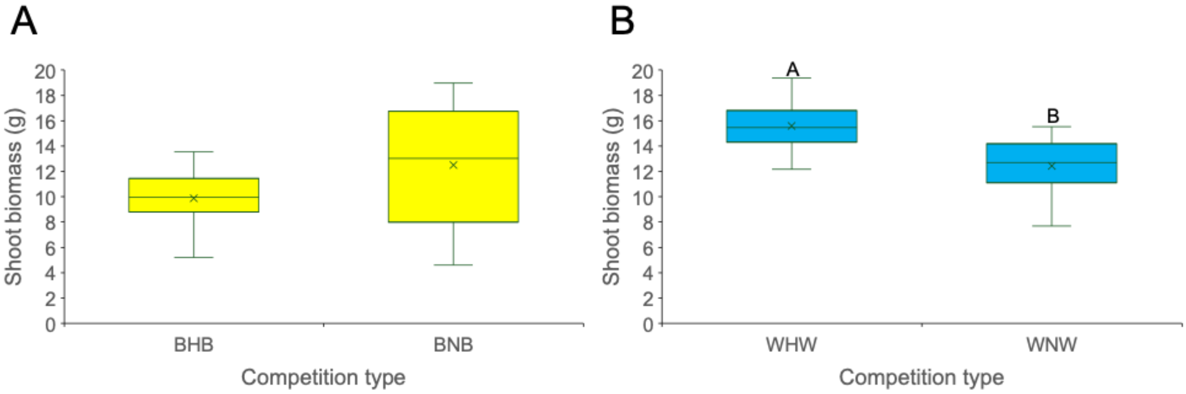

Figure 3.10 Black-grass and wheat have opposing responses to intraspecific competition

Boxplots showing final shoot biomass of (a) black-grass and (b) wheat when grown in intraspecific competition with different root barrier conditions (Hytex or None). The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X on the boxes represents the mean. Different letters above the boxes indicate significant statistical differences between the groups. (Independent samples t-test, P<0.05), n=12.

These findings support the hypothesis that wheat and black-grass have opposite responses to intraspecific competition. In wheat, below-ground intraspecific competition leads to mutual inhibition of growth. Elite wheats are bred for maximum field level yields resulting in reduced fitness and reduced competitive ability of individual plants. In a field without black-grass this is ideal, as all wheat grows uniformly. The data suggest that this cooperative behaviour is partly mediated by below-ground chemical effects. For black-grass, when grown with a Hytex barrier the plants appear uniform in size, when the barrier is removed the range of biomass achieved increases (Figure 3.11). Interestingly without a root barrier present the maximum biomass achieved increases beyond that seen in the presence of a Hytex barrier indicating that the absence of the root barrier allows for greater maximum growth. This is likely due to a greater soil volume being available to plants when there is no root barrier. However, the available soil volume is now competed for, so removing the barrier leads to increased levels of competition as one plant ‘wins’ and another ‘loses’ (Figure 3.11). In the presence of a Hytex root barrier the mean percentage split of biomass between ‘winning’ and ‘losing’ plants is 57% - 43%, with a maximum of 68% and a low of 32% (Figure 3.11A). In the absence of a root barrier this mean split increases to 65% - 35%, with a maximum of 78% and a low of 21% (Figure 3.11B). Thus, showing that black-grass competition increases when below-ground interactions are allowed to occur. These results support what is seen in crop fields, where elite wheats are uncompetitive, and black-grass is highly competitive.

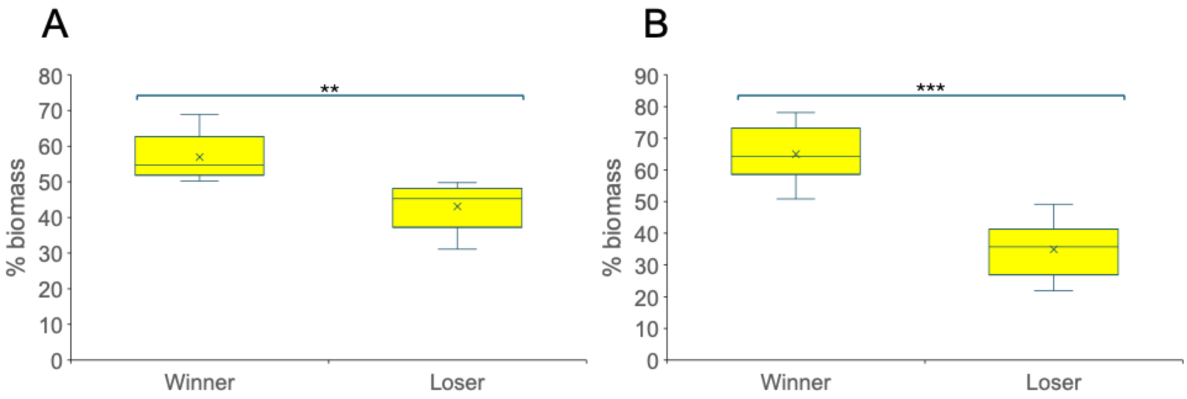

Figure 3.11 Black-grass intraspecific competition increases due to below-ground interactions

*Boxplots showing the percentage pot biomass attributed to the 'winning' and 'losing' black-grass plant in both (a) Hytex barrier experiments and (b) None barrier experiments. With 'winner' defined as the larger of the two plants in each pot and 'loser' as the smaller. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X on the boxes represents the mean. (Independent samples t-test, ** P<0.01, *** P<0.001), n=6.*

3.9 Wheat cooperation may increase the level of black-grass competition

If below-ground competition is occurring within wheat fields affected by blackgrass, it is possible that not only interspecific interactions between wheat and black-grass are impacting their competition, but that intraspecific interactions may also play a role, including the cooperative behaviour seen in elite wheats. This cooperation (reduced growth) may contribute to black-grass gaining a competitive advantage over wheat. It was hypothesised that wheat in the presence of other wheat plants will be more susceptible to black-grass competition. To test this, two wheat plants and eight black-grass plants were grown in the same pot either in open competition or with the pot separated by a Hytex barrier, preventing below-ground interactions between the two wheat plants (Figure 3.12A).

It was observed that there was no significant difference in the biomass of wheat between barrier types (Figure 3.12B), despite this, wheat in the presence of a root barrier had a greater biomass (12.44g) than wheat in open competition (8.01g). This would suggest that cooperation between wheat plants does reduce wheat size (as previously observed) and possibly competitive ability. Due to the wide range in the biomass of wheat in both barrier types, the percentage of the total pot biomass of wheat that was attributed to the largest individual wheat in each pot was calculated (Figure 3.12C) to determine if one wheat is outcompeting the other wheat to a greater or lesser extent due to the barrier presence. It was observed that there was no significant difference between the percentage biomass attributed to the larger wheat plant in both barrier types. The mean biomass of black-grass was also higher in the open set up, although not significantly (Figure 3.12D), which is consistent with the hypothesis.

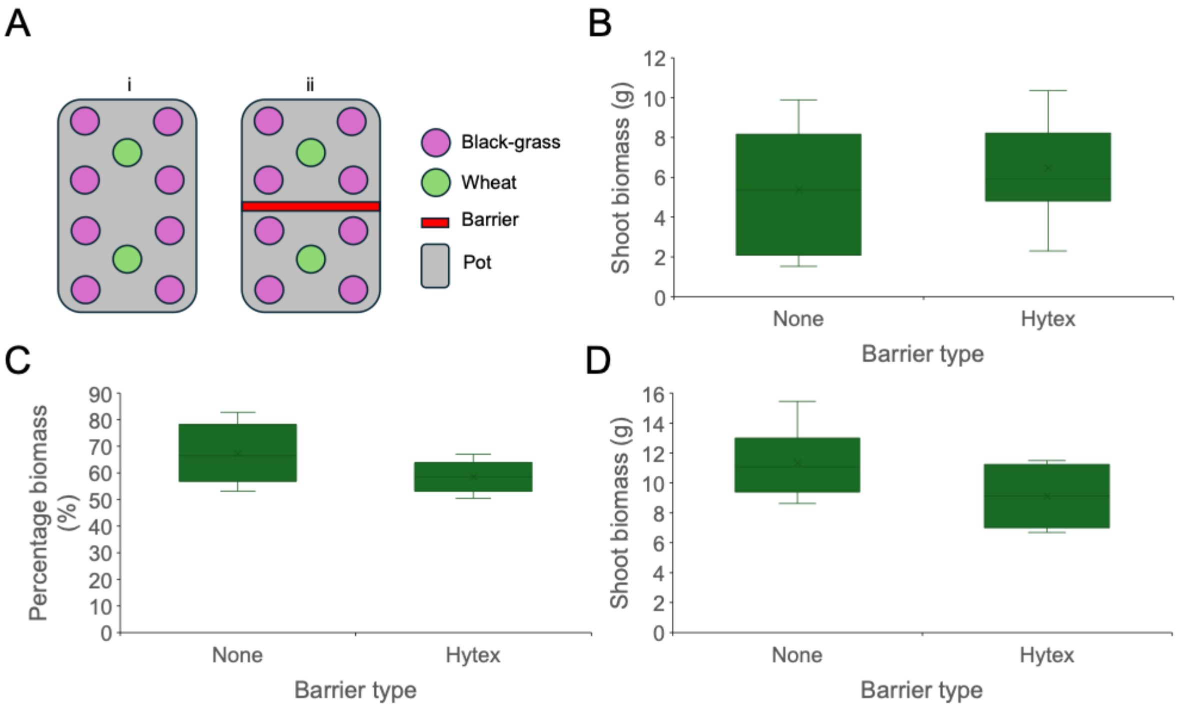

Figure 3.12 Mutual inhibition of wheat has no impact on wheat – black-grass competition

(a) Schematic and key of experimental set-up i) without Hytex root barrier, ii) with Hytex root barrier. (b-d) Boxplots showing (b) wheat shoot biomass (c) Percentage pot biomass attributed to the larger wheat plant in each pot and (d) black-grass shoot biomass. In each case values are given for both None and Hytex root barrier types. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X on the boxes represents the mean, n=6.

The findings tentatively support the hypothesis. Although there is no statistically significant impact of wheat-wheat cooperation on the ability of wheat to compete with black-grass in this experiment, black-grass tend to be larger and wheat to be smaller when wheat-wheat cooperation can occur. Despite this, it cannot be ruled out that the differences in the biomass of wheat could be due to differences in competitive ability from the black-grass rather than any effect of wheat cooperation.

3.10 Black-grass and Barley are better than wheat at taking advantage of the available soil volume

Chemical root exudation has been linked with soil volume and neighbour density sensing (Wheeldon et al., 2023). Given the previous results showing that chemical root exudation may be playing a role in competition and that wheat and black-grass may have opposing responses to intraspecific competition it was decided to assess whether black-grass and wheat also have opposing responses to a) decreasing soil volume availability and b) increasing intraspecific neighbour density. It was hypothesised that wheat would grow better at high planting densities and lower soil volumes compared to barley and black-grass due to the intensive breeding selection for co-operative growth in wheat. Whereas it was hypothesised that barley and black-grass would grow better at lower planting density and higher soil volumes because they are more competitive than wheat and are more able to take advantage of the available soil volume.

To test this hypothesis, wheat and black-grass were grown at a 1/pot or a 4/pot planting density in both 100ml and 500ml pots (Figure 3.13) using methodology from Wheeldon., (2023). Final dry shoot biomass was recorded for each plant (Figure 3.14A, C) and summed for each pot (Figure 3.14B, D). Fold changes in biomass was measured between soil volumes and planting densities (Table 1).

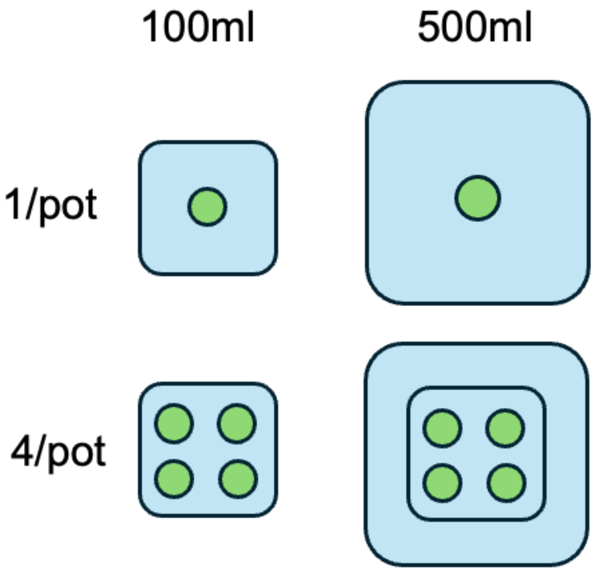

Figure 3.13 Schematic of planting density and soil volume experiment

Figure shows the four pot designs. Circles represent individual plants. Rounded squares represent pots. The two 100ml pots at planting densities of 1 plant/pot and 4 plants/pot *and the two 500ml pots also with planting densities of 1 plant/pot and 4 plants/pot. At 4plants/pot densities the planting distance between plants was maintained across pot sizes, the inner pot shown on 4/pot 500ml is to indicate this, there is no actual inner pot present in the experiments.*

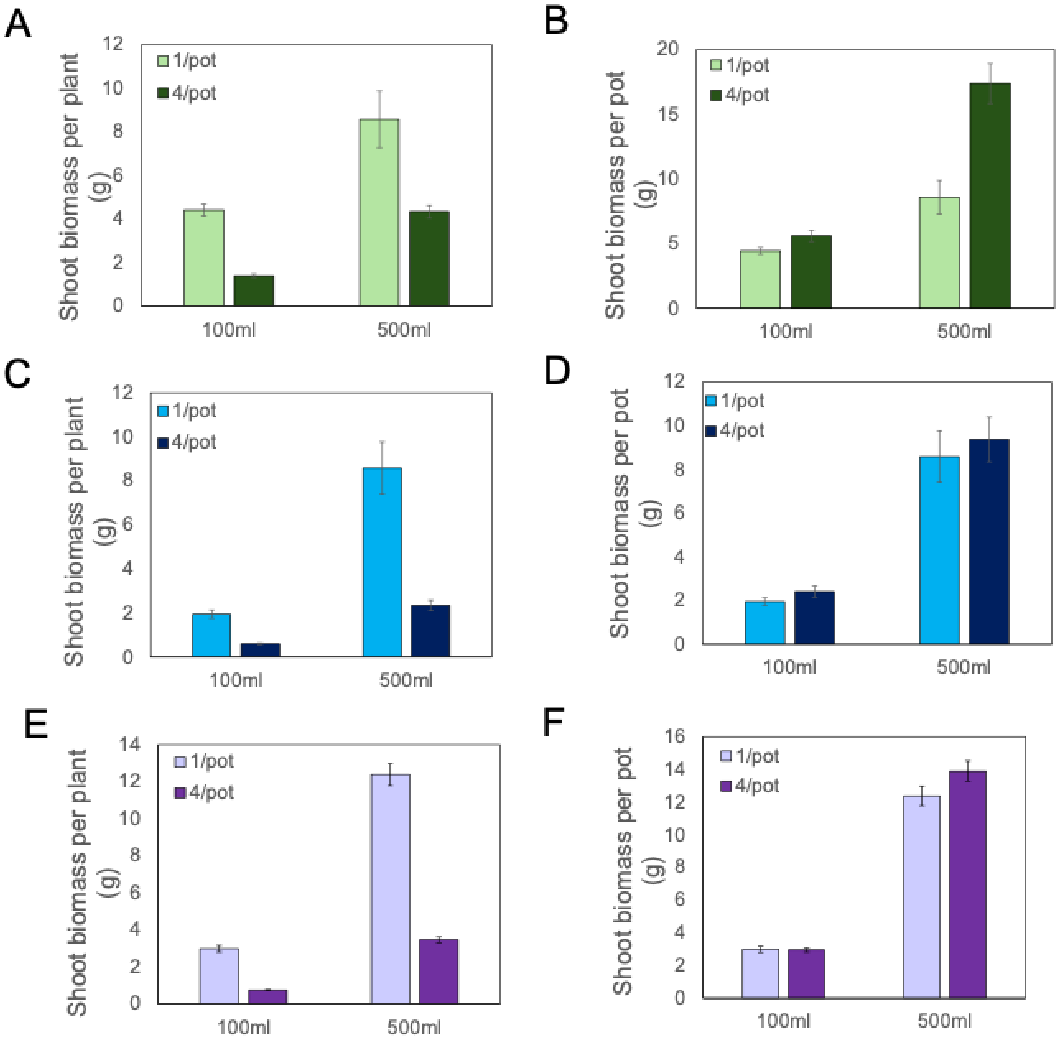

Figure 3.14 Responses to soil volume and planting density changes in wheat, black-grass, and barley

Bar charts showing the final dry shoot biomass of wheat (Green), black-grass (Blue) and Barley (Purple) (a, c, e) per plant and (b, d, f) per pot, when grown in 100ml and 500ml pots at 1/pot and 4/pot planting densities. Error bars are standard error, (a-d) n=10-12, (e and f) n=9-11. Work in Panels E and F carried out by Cara Wheeldon with figures taken from Wheeldon et al., 2023 with permission.

Table 3.1 Wheat, barley, and black-grass fold changes in biomass per pot and per plant at increasing soil volume and increasing planting densities

4/pot for each condition		Fold change		
		Wheat	Barley	Black-grass
per pot	500/100	3.11	4.70	3.88
per plant	500/100	3.11	4.62	3.88

1/pot for each condition		Fold change		
		Wheat	Barley	Black-grass
per pot	500/100	1.94	4.14	4.40
per plant	500/100	1.94	4.14	4.40

4/pot / 1/pot for each volume		Fold change		
		Wheat	Barley	Black-grass
per pot	100ml	1.26	0.99	1.24
	500ml	2.03	1.12	1.09
per plant	100ml	0.32	0.25	0.31
	500ml	0.51	0.28	0.27

4/pot 500ml / 1/pot 100ml		Fold change		
		Wheat	Barley	Black-grass
per pot		3.93	4.65	4.80
per plant		0.98	1.16	1.20

Focussing initially on individual plant biomass, increasing the soil volume from 100ml to 500ml at 1/pot planting density, resulted in a fold change of 4.40 for black-grass and 4.14-fold change for barley, but only resulted in a 1.94-fold change in wheat (Figure 3.13A, C, E; Table 3.1). This shows at 1/pot densities wheat is less able to take advantage of increasing soil volumes than barley and black-grass.

For the 4/pot treatment, the same increase in volume resulted in a 3.88-fold change for black-grass, a 4.70-fold change for barley, and a 3.11-fold change in wheat (Figure 3.14 A, C, E; Table 3.1). Again, wheat is less able to take advantage of increasing soil volumes than barley and black-grass however the difference between species was much lower at the 4/pot planting density. This indicates at higher planting densities wheat is better able to take advantage of the full available soil volume whereas barley and black-grass see less dramatic changes between planting densities. This difference in response may be due to wheat’s cooperative nature and the more competitive nature of barley and black-grass. Limited soil volume availability is much more restrictive in barley and black-grass than in wheat due to wheat’s lower competitiveness.

Per plant, increasing planting density from 1/pot to 4/pot in 100ml pots showed similar fold changes between species, with individual plant size decreasing as density increases, for black-grass there is a fold change of 0.31 per plant, for barley 0.25 and for wheat the fold change is 0.32. However, the response to increasing planting density from 1/pot to 4/pot at 500ml differed between wheat, barley, and black-grass. For black-grass individual plant size decreased by a fold change of 0.27, and for barley by a fold-change of 0.28, but whereas in wheat each plant only decreased by a fold change of 0.51. This likely reflects the fact that wheat does not efficiently utilise the large soil volume at 1/pot. This can be seen clearly when looking at shoot biomass per pot (Figure 3.14 B, D, F) in which wheat has a much-reduced shoot biomass in 1/pot 500ml compared to its 4/pot 500ml, a fold change of 2.03. Wheat at 1/pot planting density is unable to achieve the same biomass as when 4 plants are sharing the same soil volume. Black-grass and barley both have almost identical total pot shoot biomass at 500ml for both planting densities with fold changes being 1.09 and 1.12 respectively as planting density increases. This indicates at 1/pot densities black-grass and barley plants can take advantage of the full pot volume. As a result, at higher planting density individual plants are smaller due to competition.

Per pot, increasing planting density from 1/pot to 4/pot in 100ml pots saw a fold change of 1.26 in wheat, 1.24 in black-grass and 0.99 in barley. This indicates at low soil volume all species can utilise the whole soil volume at 1/pot densities, providing further evidence that soil volume responses differ between species. Generally, barley shows soil volume and planting density responses more like that of black-grass than wheat. Both show results that would indicate high competitiveness in comparison to wheat.

When the effects of increased planting density and increased soil volume are combined, going from 1/pot 100ml to 4/pot 500ml the overall fold changes per pot of black-grass is 4.8, barley 4.65 and wheat 3.93, and per plant fold changes are black-grass 1.2, barley 1.16 and wheat 0.98. For both biomass per pot and biomass per plant barley and black-grass can increase their biomass to a greater extent than wheat when soil volume and planting density is increased.

Analysis using 2-way ANOVAs was carried out to test the significance of pot size, planting density and pot size x planting density on plant shoot biomass. Analysis was carried out on shoot biomass per plant and per pot for each of the three species tested.

For wheat, shoot biomass per plant was found to be significantly affected by pot size and planting density (ANOVA, P<0.001). However, there was not a statistically significant interaction between the two independent variables (2-way ANOVA, F (1, 102) = 2.14, P=0.147). Shoot biomass per pot was also found to be significantly affected by both pot size and planting density in wheat (ANOVA, P<0.001). Unlike shoot biomass per plant, shoot biomass per pot did see a significant interaction between pot size and planting density (2-way ANOVA, F (1, 39) = 17.39, P<0.001).

For Black-grass, shoot biomass per plant was found to be significantly affected by pot size and planting density (ANOVA, P<0.001). Unlike in Wheat, for shoot biomass per plant, black-grass did see a significant interaction in independent variables (2-way ANOVA, F (1, 86) = 49.48, P<0.001). Analysis of black-grass shoot biomass per pot found shoot biomass to differ significantly dependant on pot size (ANOVA, P<0.001) however not on planting density (ANOVA, P=0.396). There was no significant interaction between the two independent variables (2-way ANOVA, F (1, 35) = 0.051, P=0.823).

For Barley results are like that of black-grass, shoot biomass per plant was found to be significantly affected by pot size and planting density (ANOVA, P<0.001). Again, unlike in Wheat, for shoot biomass per plant, barley did see a significant interaction in independent variables (2-way ANOVA, F (1, 92) = 74.68, P<0.001). Analysis of barley shoot biomass per pot found shoot biomass to differ significantly dependant on pot size (ANOVA, P<0.001) however not on planting density (ANOVA, P=0.084). There was no significant interaction between the two independent variables (2-way ANOVA, F (1, 35) = 3.46, P=0.071).

In summary, the 2-way ANOVAs indicate that per plant, all species show significant changes in biomass due to changing pot size and planting density. However, only in Black-grass and Barley is there a significant interaction between the two independent variables, wheat does not. Per pot, all species differ in biomass die to changing pot size whereas only wheat biomass significantly differs due to changing planting density. As a result, only wheat shows a significant interaction between the two independent variables.

These results support the hypothesis showing that wheat is poorer at adapting its growth to increasing soil volumes than black-grass and barley. Planting density results indicate wheat is better able to grow at increased planting densities than barley and black-grass, agreeing with the hypothesis of co-operative growth in wheat. Wheat growth is particularly small at 1/pot at 500ml indicating that wheat is poor at taking advantage of the total available soil volume in these pots compared to barley and black-grass. This agrees with the hypothesis showing wheat to be less competitive than barley and black-grass, both of which are better able than wheat to take advantage of the available soil volume at lower planting densities. It is possible root growth of barley and black-grass allow greater access to the soil volume resulting in greater shoot biomass whereas smaller rooting wheat is unable to take advantage of the greater soil volume.

3.11 Discussion

Black-grass competitiveness depends on growth conditions

Black-grass is known to be highly competitive against winter wheat in the field (Moss., 2010) however initial experiments have shown the opposite, with wheat consistently outperforming black-grass when grown in competition at a 1:1 ratio (Figure 3.7). It is not unheard of for wheat to be more competitive towards black-grass than vice-versa, Wenda-Piesik et al., (2022) found wheat to be more competitive against black-grass in 25 out of 28 cases, confirming decreased levels of competition in conditions less favourable for black-grass, in this case the high competitive ability of wheat was put down to early wheat emergence. High wheat competitive ability in this thesis is due to experimental design. Black-grass is known to be much more of a problem in winter wheat than in spring wheat (Moss, 2010) whilst also being less of a problem in winter barley which can better reduce black-grass seed return than wheat (Cook and Roche., 2018). This indicates that both environmental conditions and physiological factors are important in competing with black-grass. In spring plantings, levels of black-grass germination are lower than in winter plantings (Moss., 2010), this will contribute to the decreased levels of black-grass competition during this period. Observations suggest it takes up to between March and May of the winter growing season to visualise an impact of black-grass competition on wheat. It is possible that the shorter spring season does not give black-grass the time to accumulate the same below-ground advantages it can in the longer winter growing season. However, the differences seen in competitive ability between winter wheat and winter barley cannot be attributed solely to differences in germination or environmental conditions such as temperature or day length. The physiological traits present in barley must in some way convey a competitive advantage compared to wheat, otherwise black-grass would be just as big a problem in winter barley. Another explanation for limited black-grass competition in this chapter may be due to the ratio at which wheat and black-grass were grown. Black-grass can grow at very high densities in the field, with crop losses increasing as black-grass density increases (Moss., 2010). Increasing black-grass density to recreate more accurately field competition, should increase the impact black-grass has on the wheat allowing greater visualisation of competition in the lab. Recreating winter growth conditions and imitating black-grass crowding may therefore be vital to visualising true black-grass competition.

The findings therefore indicate that to successfully visualise black-grass competition on wheat, changes to the experimental set-up are required to better emulate field conditions in which black-grass thrives. Only then can the true competitive nature of wheat cultivars be assessed.

The role of root growth in wheat - black-grass competition

The results presented here show that elite wheats have smaller root systems than black-grass and barley. In the field, elite wheats are co-operative, being less competitive than both black-grass and barley. It is possible that low root growth and low competitive ability are connected. A small root system prevents wheat from accessing the available soil and nutrients resulting in it being outcompeted by larger rooting black-grass. Many studies have looked at wheat root growth and its response to abiotic stresses (Aschehoug et al., 206; Robinson., 1999; Poorter et al., 2012) and have surmised reduced root growth as a factor in their susceptibility to weeds (Finch et al., 2017). However, little is known regarding the root growth of black-grass, with no studies attempting to specifically quantify root production and root system architecture in black-grass despite studies calling for increased understanding of below-ground competition (Wenda-Piesik et al., 2022). Recently it was determined that root traits are important in waterlogging resilience in black-grass (Harrison et al., 2024) it is therefore likely that the root system of black-grass can also provide other benefits to the plant. Although initial black-grass germination and growth was observed to be slow in comparison to wheat (Figure 3.1), it was observed from hydroponic experiments that black-grass had the capability to produce a much greater root system than that of wheat, particularly when grown in winter conditions (Figure 3.4). Previously black-grass was only thought to compete with wheat above-ground (Christensen., 1995) however, there is now increasing evidence for below-ground interactions between plants resulting in competitive responses, from root production (Andrew et al., 2015) to chemical root exudation (Delory et al., 2016). Despite there being no visible above-ground effects of black-grass competition on wheat for long periods during the winter (Figure 3.2, 3.3), the increased level of root growth in black-grass suggests black-grass has gained an unseen competitive advantage below-ground. Increased investment in the root system has given black-grass a competitive advantage over wheat by the time it comes to the spring. The larger root system giving black-grass a monopoly on space and resources during the critical growth stages for the crop.

Decreased availability of resources to the crop inevitably results in large losses in crop yields as can be seen during nitrogen starvation (Fargione and Tilman., 2006). In elite wheats reduced root growth has been seen in both Chinese and UK varieties linked with increased selection for control of flowering time and harvest index (Voss-Fels et al., 2017; Zhu et al., 2019; Fradgley et al., 2020). This may have unintentionally reduced root growth to a level by which elite wheats can no longer compete with competitive weeds such as black-grass. Comparative analysis of wheat and black-grass with barley has shown that both black-grass and barley are able to produce larger plants than wheat during the winter (Figure 3.4). This observation indicates that larger root and shoot systems may convey a competitive advantage to both these species, with the poorly competitive nature of wheat during the winter being a result of its relatively small size. Barley and black-grass also proportioned a greater amount of biomass into roots compared to wheat (Figure 3.4) indicating they have a greater focus on root growth than elite wheats which may further help to explain their increased competitiveness. Previously landrace wheats were found to have lower shoot: root biomass ratios than elite wheat varieties, akin to the black-grass results in this chapter, whilst also having larger root systems than those elite wheats (Chapagain et al., 2014). In barley, increased plant height has been linked with increased competitiveness against black-grass, with shorter wheat varieties showing comparatively low levels of competition (Cook et al., 2023). It is therefore possible that there is a link between root and shoot system size and the ability to compete with black-grass. Cultivars with large shoot growth may be expected to have a correspondingly high root growth to maintain the shoots, this is possible, however it is also possible that there is a trade-off in the partitioning of resources between above and below-ground traits (Andrew et al., 2015).

The evidence displayed here supports the hypothesis that below-ground competition between crop and weed may be occurring through root growth, with crop lines with increased root growth showing increased levels of competitive ability. Although the findings do not prove root growth is involved in competition with black-grass it does provide a candidate trait in which to screen for. Large scale root growth screening of different crop varieties will assess root growth variation whilst also allowing comparisons to be made to crop competitive ability to assess the role or root growth in crop-weed competition.

The role of chemical root exudates in wheat – black-grass competition

It is clear though that plants have a range of mechanisms that allow them to communicate with and manipulate the growth of neighbours. The use of chemical root exudates is one method plants may be adopting in actively competing with undesired neighbours. Initial experiments highlighted chemical interactions to be occurring between wheat and black-grass, with both plants seeing decreases in growth in the presence of the other when only chemical competition could occur (Figure 3.7). For both plants this would indicate either an allelopathic effect on growth from the neighbouring plant, or a cooperative downregulation of growth due to the detection of chemical signals from neighbours. Chemical competition was observed to occur intra-specifically between neighbouring wheat plants and neighbouring black-grass plants, interestingly with opposing effects. In the presence of possible chemical competition wheat saw a reduction in growth, whereas black-grass saw little change in growth. Differences in the response of black-grass to a wheat and black-grass neighbour would indicate that black-grass could detect and respond differently to signals dependant on the identity of the neighbour. More specifically dependant on the chemical root exudates each neighbour releases. The specific exudates released by plants may be allowing plants to distinguish between kin/non-kin plants allowing them to gain a competitive advantage, this possibility has previously been suggested (Bilas et al., 2021).

Unfortunately, the release of, detection and response to root exudates in the roots is poorly understood, therefore interactions involving chemical root exudates remain mostly theoretical, likely involving diffusion and controlled release through transporters and vesicles (Field et al., 2006; Neumann and Romheld., 2001). Although there is evidence for exudates impacting plant growth in controlled conditions, the short time exudates are present in the soil and the possible loss of biological activity due to chemical oxidation, microbial degradation or immobilisation by binding to soil particles (Bertin et al., 2003) raises the question as to whether they are indeed capable in their small concentrations to influence the growth of neighbouring plants in real conditions (Field et al., 2006).

Possible candidates for allelopathic root chemical exudates involved in crop – weed interactions include momilactone B, shown to act allelopathically in rice against barnyard-grass (reviewed in Kato-Noguchi and Peters., 2013), benzoxazinoids, shown to act allelopathically in wheat against black-grass (Yang et al., 2020) and sorgoleone, produced by sorghum roots (Czarnota et al., 2003) shown to inhibit germination of susceptible weeds (Nimbal et al., 1996). Another possible candidate is strigolactones. Strigolactones are plant phytohormones known to be released into the soil via the roots, which is known to act allelopathically in the stimulation of germination of parasitic plants and the stimulation of arbuscular mycorrhizae fungi (AMF) hyphae growth (Soto-Cruz et al., 2021). It is therefore possible they may play a role in crop – weed interactions. A use for strigolactones in agriculture has arisen, the ability of strigolactones to act allelopathically on parasitic weeds indicates their possible use in trap crops to initiate suicidal germination of those weeds (Soto-Cruz et al., 2021).

Currently it is difficult to determine a causative link between allelopathic compounds and their supposedly allopathic effects, with most conclusions based on correlative data (Field et al., 2006; Kato-Noguchi and Peters., 2013). It is possible that allelopathy is a secondary role of root exudates and possibly an unintended one, manifesting as side-effects of plant environmental sensing. For example, strigolactone also plays a large part in the regulation of shoot branching, whereas more recently, they have also been shown to impact plant growth dependant on soil volume availability and planting densities (Wheeldon., 2023). The wide range of abilities makes it difficult to pinpoint desired and undesired effects of strigolactone release/production, this may be equally true of other apparently allelopathic exudates.

Determining the genes and enzymes involved in the biosynthesis of potential allelopathic compounds is required to allow manipulation of the biosynthesis pathway to determine the exact effect of the production of these compounds on neighbouring plants. It is possible that altering the production of these compounds may increase the allelopathic effects of crops on weeds helping to reduce weed presence (Field et al., 2006). More evidence however is required before allelopathic crops are used in IWM.

3.12 Conclusion

It is clear below-ground interactions and growth conditions are important in wheat – black-grass competition. The possibility of root growth and root chemical exudate production indicate possible traits by which competitive cultivars could be selected for. The number of unknowns surrounding the allelopathic nature of chemical root exudates has meant the focus of the remaining thesis will be on physical root growth as a trait to convey competitiveness against black-grass. Determining a screening method for both the testing of competitive cultivars and their root growth is required to determine the role of root growth in wheat – black-grass competition.

Chapter 4 Development of competitive cultivar and root growth screens

4.1 Introduction

There are currently no quick and easy methods by which to assess the competitive ability of different wheat varieties against black-grass (Andrew., 2016). The uptake of competitive cultivars by farmers as a black-grass control method has been limited due to highly variable results at the individual field level (Lutman et al., 2013). Variation in field conditions result in differences in the performance of a crop variety meaning a cultivar seen to be competitive in one field may not be competitive in another. High variability in competitiveness at the individual field level is not surprising, previously cultivar selection has been based on competitive evidence limited by a small cultivar pool, in most studies, the competitive ability of only a few lines is compared, this may provide useful information for cultivar comparisons within that pool, however the wider value of this data is limited (Andrew et al., 2015).

The lack of a recommended list for competitive wheat varieties against black-grass competition is due to the absence of a clear cultivar screening approach, which in turn is absent due to the lack of understanding as to what makes a cultivar competitive. The production of a quicker, easier method of competitive cultivar identification is required. Rather than testing cultivars straight in the field, approaches that are predictive in selecting cultivars may be of greater benefit to crop breeding (Andrew et al., 2015), ensuring lines have some known level of competition prior to testing in the field. In this chapter, two methods by which to predict cultivar competitiveness are assessed. The two methods used to test for competitiveness are direct competition and trait assessment. There are positives and negatives of both methods however combined, may help identify both competitive cultivars and competitive traits.

The first method is direct testing, growing crop varieties against black-grass in a small-scale competitive scenario and assessing the final growth of both crop and black-grass. This approach shows clearly which lines are more competitive however does not necessarily identify why. Vast, time-consuming phenotypic assessments can be carried out throughout the growing period to identify traits conveying competitiveness however causation between trait and competitive ability is difficult to prove. This method is typical of what would be done in field trials. The second method is screening directly for traits thought to be involved in competition and assessing cultivar variation for those traits. Screening for specific traits can highlight variation within a certain trait between varieties, suggesting a difference in competitive ability. However once again this approach alone does not prove causality of competitive ability.

A combination of both screening approaches will highlight competitive varieties and whether they have the suspected competitive traits. Correlations comparing the range of competitive ability with the observed trait ranges can determine whether a trait is correlated with competitive ability, this will be assessed in chapter 7. Once again it is likely that causation will not be identified without testing specific trait mutants in both screening approaches, unfortunately such mutants are not yet available. The identification of competitive lines and traits at this scale would provide a good base from which to select cultivars for larger scale testing.

In order to screen for specific traits, candidate traits are required. Certain above ground traits such as early vigour, height and prostrate growth habit have been highlighted in the literature amongst others as possible above ground traits in wheat that can increase competitiveness against black-grass (Andrews., 2016). However, screening of below-ground traits has seen little study. Root growth has been shown to be vital in resource-dependant competition therefore it is possible root growth is a possible trait which could affect competitive ability in crops (Aschehoug et al., 2016). Initial experiments in chapter 3, have highlighted high root growth in black-grass and barley compared to wheat therefore root growth would be a clear candidate trait that may affect wheat competitive ability.

Little work has been done previously on root growth, mainly due to the difficulties in working with and assessing below-ground traits (Andrew., 2016). In the field methods of root analysis exist including soil cores however in the lab different approaches such as soil columns and rhizotrons need to be taken to study plant root growth in as natural environment as possible (Atkinson et al., 2014; White et al., 2015). These methods however are often time consuming and labour intensive (Atkinson et al., 2014). Other possible approaches can assess root growth using artificial media such as agarose gel chambers, paper germination pouches and hydroponic culture (Atkinson et al., 2014). The first of the two approaches of root assessment tested in this chapter is the use of rhizotrons, or as they shall be referred to here, rhizoboxes, the development of which is reviewed by Klepper and Kaspar (1994). Rhizoboxes can be used for taking non-destructive repeated observations of plant root traits (Klepper and Kaspar., 1994). Recent research has used these thin 3D plates to produce 2D images of root growth at different time points in the early growth stages of a plant (Silva and Beeson., 2011). Comparisons of different crop varieties using this method could show variation in crop root growth. The second root screening approach that will be assessed is the use of hydroponics. Hydroponics have previously been used in neighbour detection experiments (Wheeldon et al., 2021) and are commonly used as a form of indoor plant growth (Baiyin et al., 2021). Unlike rhizoboxes this approach is soil free providing full access to plant roots. Final measurement using hydroponics will therefore include root biomass, something not possible in rhizoboxes. Both approaches provide slightly different data therefore it would make sense to assess the use of both screening approaches. The screening of wheat lines for root growth using both approaches will determine which lines have high or low values for this possibly competitive trait.

Testing directly for competition requires a cheaper and quicker approach. As discussed in the previous chapter, a method is required that recreates winter field conditions to get a true visualisation of black-grass competition on wheat. Utilising winter conditions and increased weed density would hopefully allow not only visualisation of black-grass competition but also highlight variation in crop competitive ability against black-grass.

4.2 Aims

Initial investigations have highlighted physical root growth as a possible factor that may play a role in black-grass – wheat competition. Two things are required to determine whether increased root systems in wheat correspond to increased competitiveness; firstly, a way of screening the root growth of different wheat lines; secondly a way of screening the same wheat lines for their competitive ability. This data would allow for comparisons to be made between the two to determine if there is a correlation between root growth in wheat and the competitive ability of wheat against black-grass. The main aims of this chapter were:

1. To produce screening method/s to test root production of different crop lines.
2. To produce a screening method to test the competitive ability of different crop lines.

4.3 Developing root screening methods

Two different approaches were developed for the quantification of root growth. Firstly, the use of rhizoboxes was assessed, this method allowed for the production of a 2D root system image which could then be assessed for different root traits. The downside of this approach was that the roots remain in the soil, therefore no root biomass could be recorded. Due to this drawback, it was decided to take advantage of the previously used hydroponic system as a second method to measure root growth. This method has easily harvestable roots which could be assessed for their overall biomass. The use of two systems allowed comparisons to be made between the two screening methods.

Initial rhizobox experiments focussed on growing two plants within the same rhizobox at different planting distances from one another (Figure 4.1). The aim being that it would be possible to determine variation in the root growth of different lines when under different neighbour pressures. After carrying out a pilot experiment it was found that this approach was not suitable. This approach was very good at providing root images of neighbouring plants and visually highlighting differences in the growth of different species (Figure 4.1). However, the problem came when attempting to assess root growth. Although visually differences could be seen, physically assessing root images to determine which roots belonged to which plant was impractical, especially if run on a larger scale. ImageJ software allows the percentage cover of the rhizobox surface that was taken up by roots to be measured however there is no way to separate and distinguish the roots of each plant without crudely estimating the position of each plants root systems. It is therefore not possible to indicate how well each plant was able to produce roots compared to its neighbour, especially as root systems often overlapped one another, with some roots not visible due to the 3D nature of the soil and 2D nature of the resulting image.

Figure 4.1 Competitive rhizoboxes

Images showing root growth in Rhizoboxes of two plants in competition, (a) barley (left) and black-grass (right), (b) wheat (left)) and black-grass (right). Images collected by scanning the rhizobox surface. Ruler for scale.

Due to the difficulties associated with distinguishing neighbouring plant roots it was decided to grow the plants individually within the rhizoboxes rather than with multiple plants in competition (Figure 4.2). This should allow clear visualisation of the roots of the plant whilst allowing clear measurement of the root coverage which can be attributed entirely to the tested cultivar. Although this removes the competitive aspect of root growth and will not highlight how roots react to neighbouring plants, it will indicate which lines produce greater or smaller root systems, which can then be compared to competitive ability at a later stage.

Assessing multiple plants in one rhizobox was quickly dismissed due to the lack of methodology and technology for accurately separating and measuring the roots of both plants. Recent studies are still relying on estimates from images of spatial root distribution as a way of assessing root growth in competition (Gottlieb and Gruntman., 2024). Although this may be possible in the early stages of growth with low root production this becomes more difficult at later stages with denser root systems. Later stage root analysis is required as short-term root growth is not always predictive of adult root growth (Bai et al., 2019).

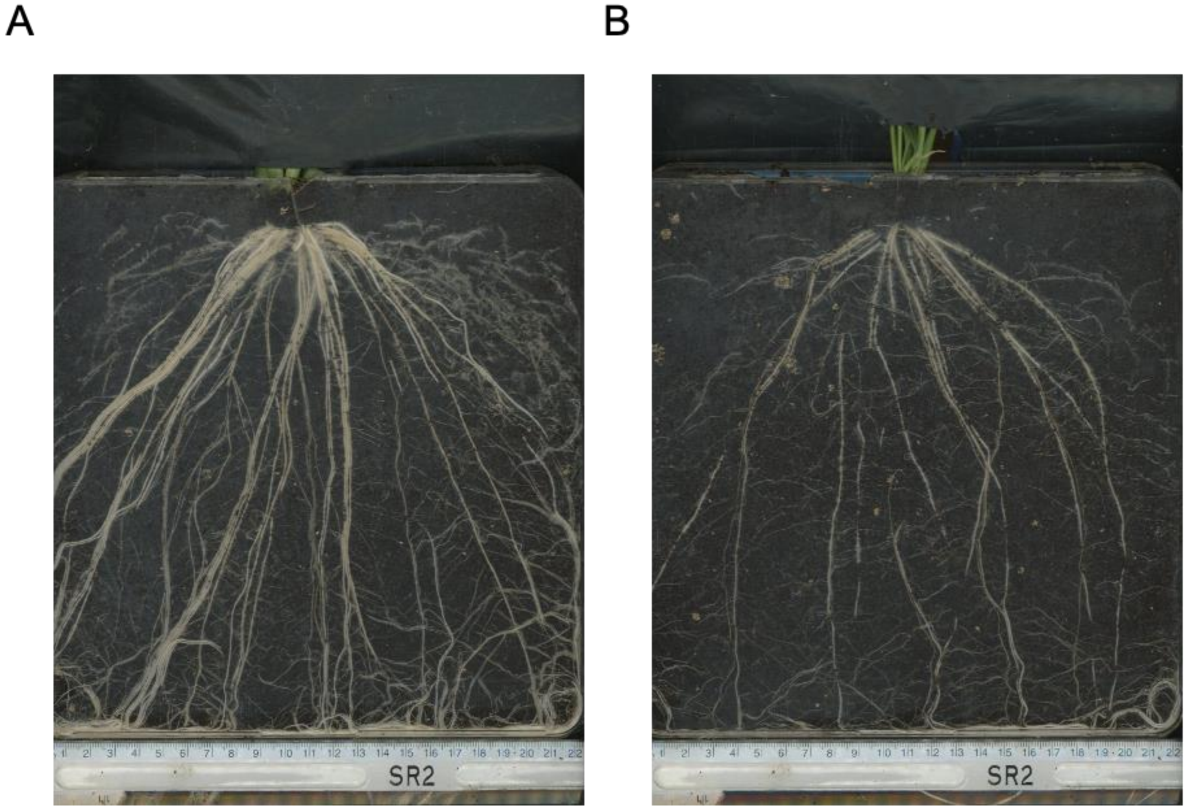

Figure 4.2 Individual Rhizoboxes

Images showing root growth in Rhizoboxes, one plant per rhizobox. (a) barley (Tardis), (b) wheat (Kerrin) images collected by scanning the rhizobox surface. Ruler for scale.

Despite working with winter varieties, the rhizoboxes were placed in spring conditions. Due to limited winter growth space, it would not be possible to run a full-scale root screen in winter conditions for the number of lines requiring testing, therefore it was decided to use spring conditions for this screen. Growth would also be quicker in spring conditions therefore it would allow for the screening of more lines in a shorter time. Although root growth may not be representative of root growth seen in winter conditions, all plants will receive the same conditions therefore root growth will still be comparable between lines.

Certain lines which showed differences in root growth using the spring rhizobox set-up were grown in winter conditions using the hydroponic system to determine whether root growth in spring conditions represents root growth in winter conditions (Figure 4.3). The hydroponic approach would see a single plant grown in water with the addition of a nutrient broth, root growth would be crudely measured overtime via water displacement, however final root biomass would be the main measurement.

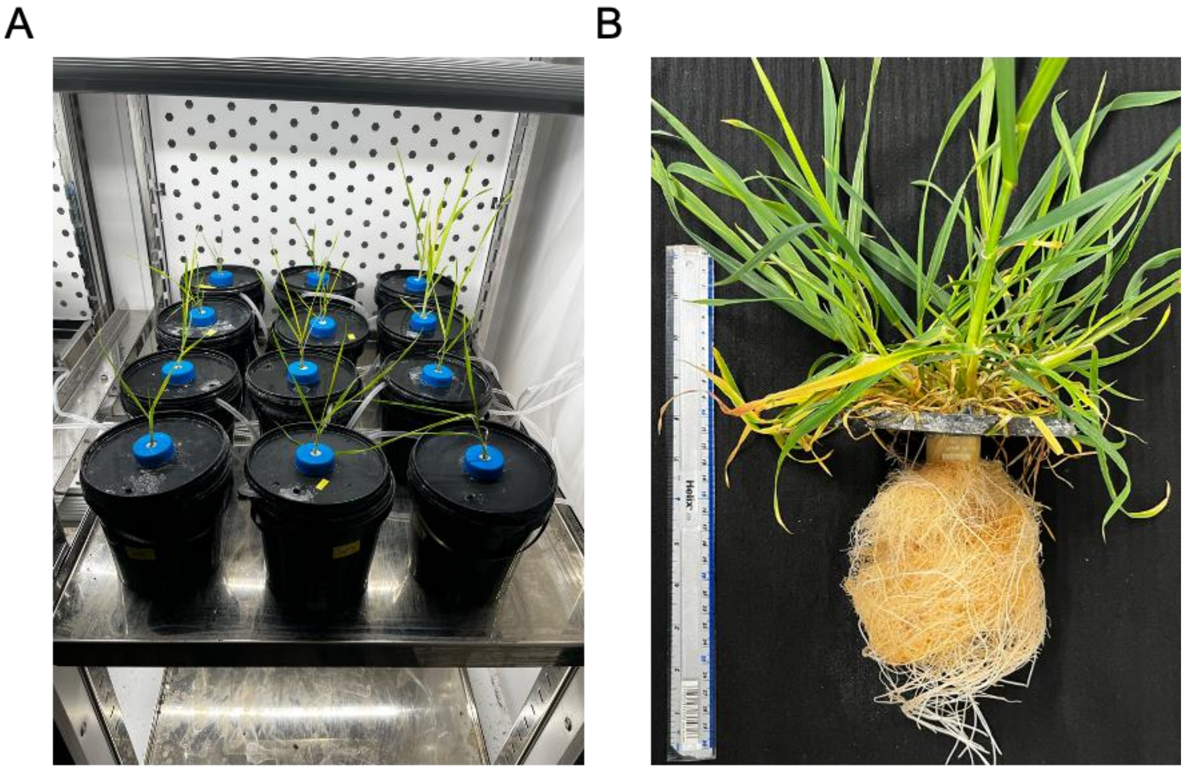

Figure 4.3 Hydroponic root screen

Images of hydroponic plant growth set-up (a) hydroponic pots within a winter growth cabinet (b) example of wheat root and shoot growth when removed from hydroponic pot. Ruler for scale.

It was then decided to test the two root screening methods. It was hypothesised that root growth would vary between crop lines, whilst variation in root growth in those lines would be consistent between the two screening methods. To test this, two wheat lines, Dickens and Graham were grown in both rhizoboxes and hydroponics root screens and were assessed for their root growth after three and six months respectively.

Firstly, in the rhizoboxes in spring conditions, after 10 weeks of growth it was observed that both lines had very similar levels of root growth at around 20% coverage, with Dickens having a slightly higher mean root coverage (Figure 4.4A). In winter conditions in the hydroponics, root biomass also did not differ significantly between the two lines, with Dickens once more having a slightly higher mean than Graham (Figure 4.4B). The range of results was much greater in the hydroponic system indicating that not all plants took well to growth in the winter conditions. In spring conditions, the range was much smaller indicating similar growth in all plants.

Both screening approaches therefore allow the visualisation of root growth and allow comparisons to be made between varieties. It was concluded that the two screening approaches can show similar results despite them both measuring different aspects of root growth. Due to the smaller variation, quicker growth, and available growing space, it was decided that the rhizobox system would be the primary method of assessing root growth. This test however only looked at two varieties, the full extent of the ability to compare the two screening methods will be discussed at a later point once all crop varieties have been tested in the rhizobox method and more lines have been tested in the hydroponic method.

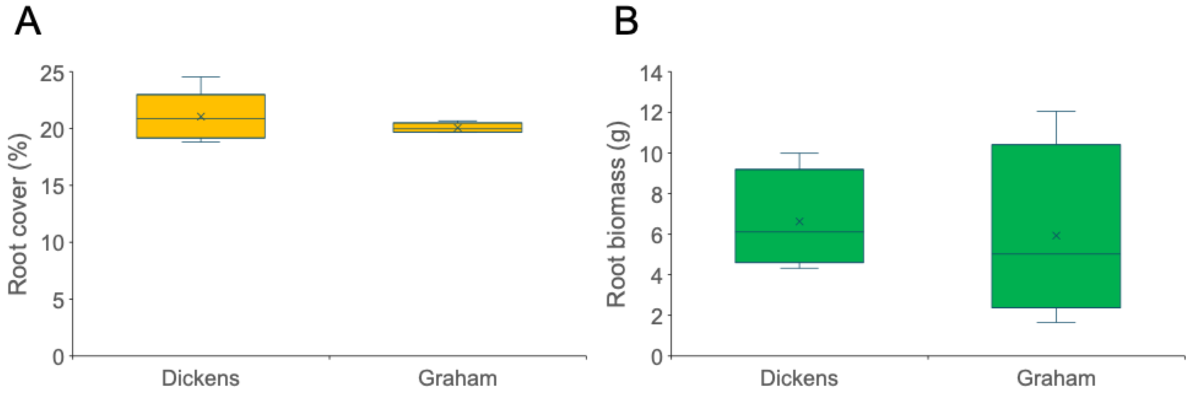

Figure 4.4 Screening methods show similar results

Boxplots showing root production of two wheat lines Dickens and Graham in (a) spring rhizobox screen (n=5) and (b) winter hydroponic screen (n=6). Rhizobox root growth is shown at 10 weeks and hydroponic root growth is shown after 3 months. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X denotes the mean.

4.4 Developing a screen to test for crop competitiveness against black-grass in winter conditions

Previous root barrier experiments discussed in chapter 3, in which the experimental set-up saw black-grass and wheat grown at a 1:1 ratio, saw black-grass consistently outcompeted by wheat. A screening method was therefore required in which the competitive ability that black-grass has in the field can be recreated in the lab. Given black-grass is much more competitive during the winter growing season it made sense to recreate those conditions as close as possible. The plants would therefore be grown at a lower temperature (10^{0}C) with shorter day lengths (8h) to better represent winter growing conditions. Black-grass is also known to grow at high densities therefore it was decided to increase black-grass density in lab experiments. The ratio of black-grass to wheat was therefore increased from 1:1 in the root barrier experiments to 6:1 in this new screening method. From the previous field and container experiments it was clear that black-grass took a long time to compete with the wheat (see chapter 3) therefore the plants were grown for approximately 6 months to allow time for a competitive effect to be seen. It was hypothesised that these conditions would allow the black-grass to outcompete the wheat. To test this, wheat was grown in the newly devised competition set-up, with wheat grown in competition with black-grass in winter conditions of 8h days and 10^{o}C at a 6:1 ratio of black-grass to wheat (Figure 4.5A). A crop only control was included to allow for quantification of black-grass competition (Figure 4.5B). Crop tiller number was recorded over-time and final shoot biomass was also recorded.

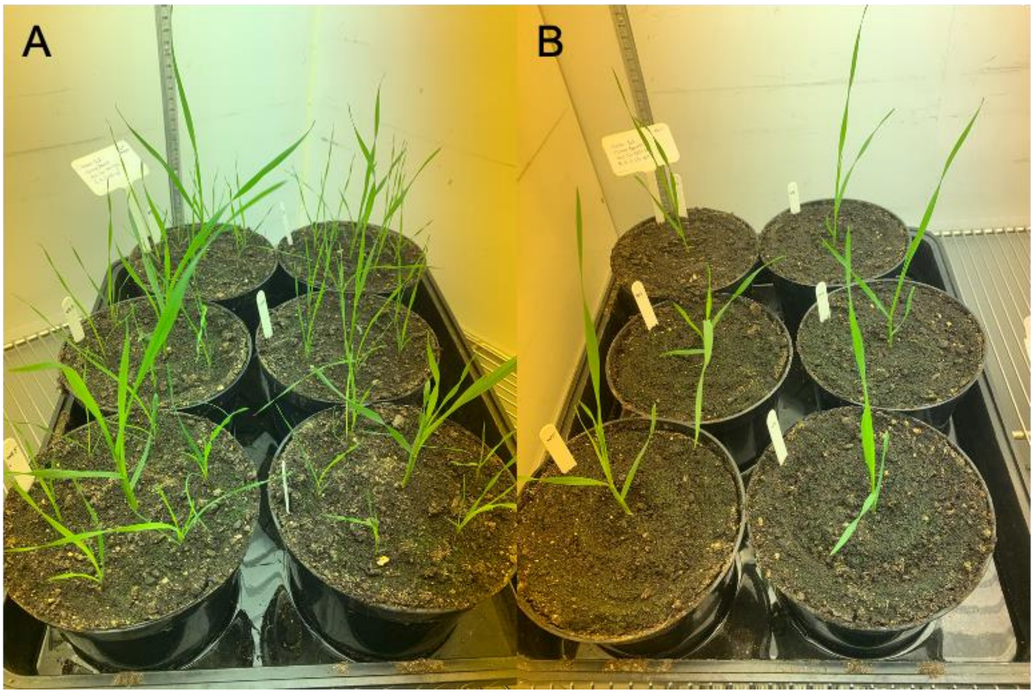

Figure 4.5 Crop competition screen

Images showing the system developed for the testing of competitive ability amongst wheat lines (a) Image of pots containing wheat in competition with black-grass in the at a black-grass to wheat ratio of 6:1 (b) Image of pots containing wheat at a 1/pot density in the 'crop only' control pots.

It was observed that wheat in black-grass competition had a significantly lower tiller number than wheat without black-grass competition however only after 118 days of growth (Independent samples t-test, $P<0.05$) (Figure 4.6A). Only between days 118 and 160 was wheat tiller number significantly lower in the presence of black-grass. Prior to this, wheat tiller number in the presence of black-grass was not significantly lower. Final shoot biomass of wheat was lower when in competition with black-grass than when the crop was grown by itself (Figure 4.6B) albeit not significantly. This successfully highlights the impact of black-grass competition under these growing conditions, supporting the hypothesis.

The impact is however lower than what may have been expected, with the loss in crop biomass not representative of that large crop yield losses seen in the field. The time taken for the effect of competition to be seen is long, however this is in line with the delay previously seen in container and field trials. These results suggest black-grass requires a long period of time in which to accumulate a competitive advantage. This may explain why black-grass is able to outcompete wheat during the longer winter growing season to a greater extent than during the shorter spring season. Due to the length of time taken to see a competitive effect of black-grass, it would not be feasible to carry out a large-scale wheat screen using these conditions.

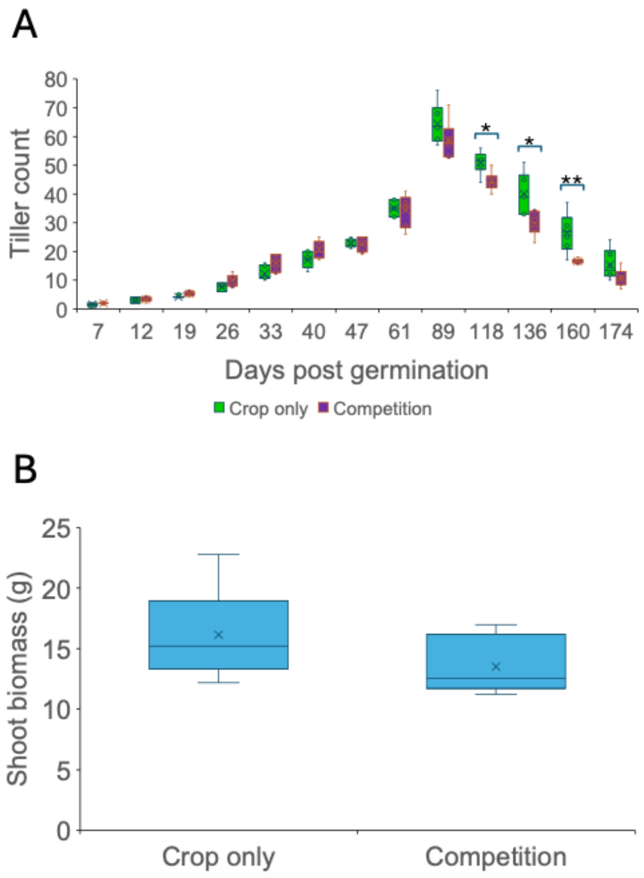

Figure 4.6 Competitive effect is delayed in winter conditions

Boxplots showing results of winter competition experiments (a) wheat tiller number over time with and without black-grass competition when grown in winter conditions (b) wheat final shoot biomass with and without black-grass competition. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X denotes the mean. Asterisks denote statistically significant differences between groups (Independent samples t-test/Mann-Whitney U-test, P<0.05) (<0.05, **<0.01), n=6.*

4.5 Wheat – black-grass competition in spring conditions

Temperature was key in the slow development of black-grass competition in winter conditions. Although black-grass is less competitive in the field in spring conditions, it may be possible to utilise these growing conditions to see a competitive impact in the lab in a much shorter timeframe. It was therefore hypothesised that black-grass competition could be visualised in a shorter timeframe in spring conditions compared to winter conditions. To test this, wheat was grown in competition with black-grass at a black-grass to wheat ratio of 6:1 as previously carried out in winter conditions in figure 4.6, however this time spring conditions were utilised. Again, a crop only control was also included. The plants had tiller number recorded over-time as well as final shoot biomass.

It was observed that wheat in competition with black-grass in spring conditions had a significantly lower tiller number than wheat without black-grass competition with the first significant difference being seen after 32 days (Independent samples t-test/Mann-Whitney U-test, P<0.05) (Figure 4.7A). This is much quicker than was observed in winter conditions. Final shoot biomass of wheat was significantly smaller when in competition with black-grass compared to the crop only control (Independent samples t-test, P<0.05) (Figure 4.7B). This difference was not significant in winter conditions. These results support the hypothesis, showing that spring conditions can be used to lessen the time taken to see a significant impact of black-grass competition on wheat.

Visualising the competitive ability of black-grass has proved difficult. Adapting the growing conditions to those seen in the field allowed the effects of black-grass competition to be visualised taking place after 4 months. Speeding up the process by growing the plants in spring conditions but keeping the same planting densities was successful showing clear differences in growth after one month. This provides a much more feasible timeframe by which to screen wheat lines.

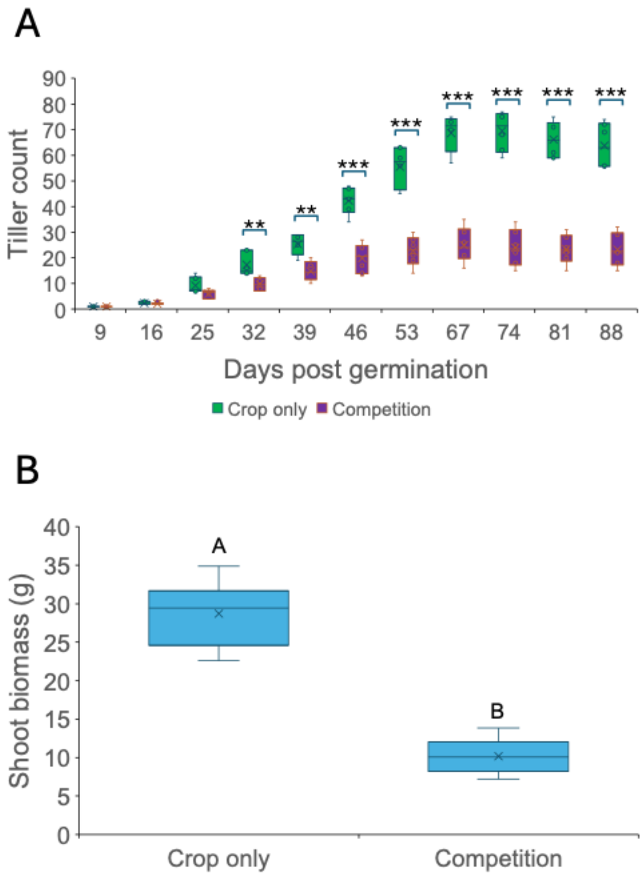

Figure 4.7 Competitive effect visible in shorter time frame in spring conditions

Boxplots showing results of spring competition experiments (a) wheat tiller number over time with and without black-grass competition when grown in winter conditions (b) wheat final shoot biomass with and without black-grass competition. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X denotes the mean. Asterisks denote statistically significant differences between groups (Independent samples t-test/Mann-Whitney U-test, P<0.05) (<0.05, **<0.01, ***<0.001), n=6. Different letters above the boxes indicate significant statistical differences between the groups. (Independent samples t-test, P<0.05), n=6*

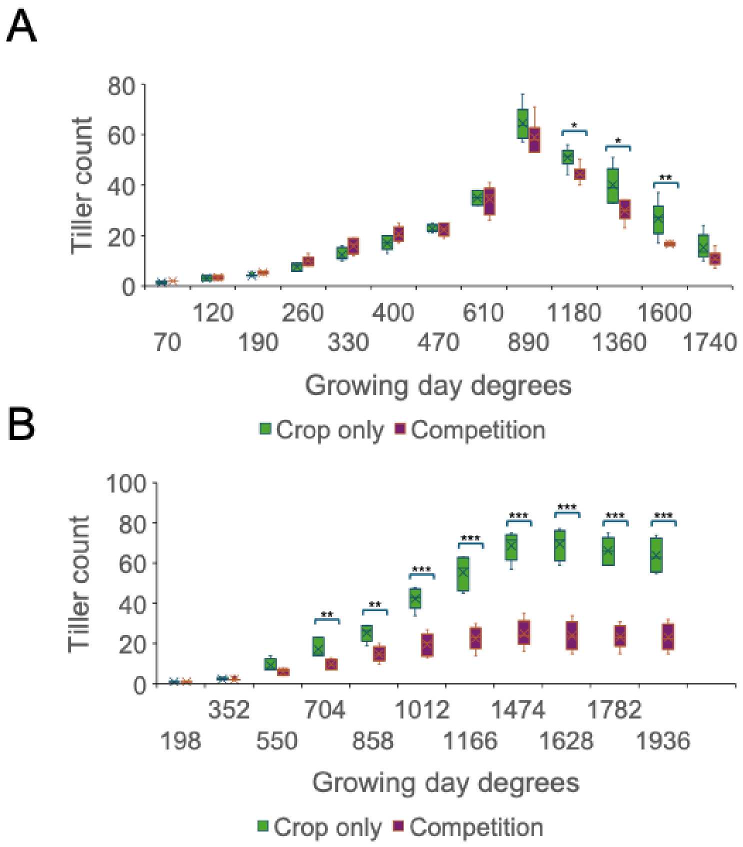

Figure 4.8 Growing day degrees indicate slow winter competition

Boxplots showing crop tiller number dependant on the number of growing day degrees with and without black-grass competition for both (a) winter and (b) spring growth conditions. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X denotes the mean. Asterisks denote statistically significant differences between groups (Independent samples t-test/Mann-Whitney U-test, P<0.05) (<0.05, **<0.01, ***<0.001), n=6.*

Converting days to Growing day degrees (GDD) allows a better comparison of growth in both winter and spring conditions by comparing ‘biological time’ rather than chronological days. GDD is calculated as the mean temperature minus a base temperature below which the plants don't grow. For the purposes of this analysis, 0°C can be used as the base temperature so GDD between two periods is just the sum of the mean temperatures which is 22°C for spring and 10°C for winter conditions. It remains clear from GDD analysis that the impact of black-grass on wheat takes far longer in winter conditions (1180 GDD) than I spring conditions (704 GDD) (Figure 4.8).

4.6 Nutrient availability impacts the competitive ability of black-grass

It is possible that changing the nutrient availability of the pots would impact the ability of black-grass to outcompete wheat, if so, different nutrient levels could be incorporated into the screening method. It was hypothesised that decreasing nutrient availability would increase the competitive ability of black-grass. To test this, wheat was grown in competition with black-grass at a ratio of black-grass to wheat of 6:1 in both high nutrient availability (100% soil) and low nutrient availability (75% sand, 25% soil). Crop only controls were included for both nutrient levels. Plants were grown in spring conditions for 3 months, at which point final dry shoot biomass was recorded.

In high nutrient conditions it was observed that the biomass of wheat in competition with black-grass was significantly smaller than the biomass of wheat without black-grass competition (Independent samples t-test, $P<0.001$) (Figure 4.9A). In low nutrient conditions it was also observed that the biomass of wheat in competition with black-grass was significantly smaller than the biomass of wheat without black-grass competition (Independent samples t-test, $P<0.001$) (Figure 4.9A). The presence of black-grass therefore clearly decreases the biomass of wheat in both nutrient conditions.

In the presence of black-grass competition the biomass of wheat was significantly greater in high nutrient conditions than in low nutrient conditions (Independent c=samples t-test, $P<0.001$) (Figure 4.9A). In the absence of black-grass competition the biomass of wheat was once again significantly greater in high nutrient conditions compared to low nutrient conditions (Independent samples t-test, $P<0.001$) (Figure 4.9A). This indicates that a decrease in nutrient availability results in smaller wheat plants both with and without black-grass competition. However, the level of competition occurring varies between nutrient conditions. In high nutrient conditions wheat lost 49.30% of its biomass due to black-grass competition. Whereas, in low nutrient conditions wheat lost 60.83% of its biomass due to black-grass competition. In the absence of black-grass competition the biomass of wheat decreased by 47.60% due to decreased nutrient levels. Whereas, in the presence of black-grass competition the biomass of wheat decreased by 59.52% due to decreased nutrient levels. It was also observed that the biomass of black-grass was significantly greater in high nutrient conditions than in low nutrient conditions (Independent samples t-test, P<0.01) (Figure 4.9B). As for black-grass, it lost 53.2% of its biomass going from high to low nutrient availability (Figure 4.9B). These results show that the loss of wheat biomass was greater in low nutrient conditions. They also show black-grass competition to be greater in low nutrient conditions.

A 2-way ANOVA was conducted that examined the effects of nutrient availability and blackgrass competition on crop shoot biomass. There was no significant interaction between the effects of nutrient availability and black-grass competition on crop shoot biomass (2-way ANOVA, F (1, 20) = 2.93, P=0.102).

These results agree with the hypothesis since black-grass presence in low nutrient availability results in a greater loss in crop biomass than in high nutrient conditions. Both wheat and black-grass prefer growing in high nutrient conditions. In the absence of nutrients, black-grass although itself smaller, has a greater competitive effect on the wheat, indicating that black-grass is better at acquiring the remaining nutrients when the wheat is also smaller. When there are more nutrients available, wheat can grow larger and better withstand black-grass.

For the wheat screen, the use of low-nutrient conditions would show a greater impact of black-grass competition on the wheat. However, in doing so would produce smaller, less healthy plants. It was therefore decided that the high nutrient conditions would be used for the competition screen going forward. Another benefit of using the high nutrient conditions is that using 100% soils in these experiments would allow for better comparisons with future container and field trials that will be soil-based.

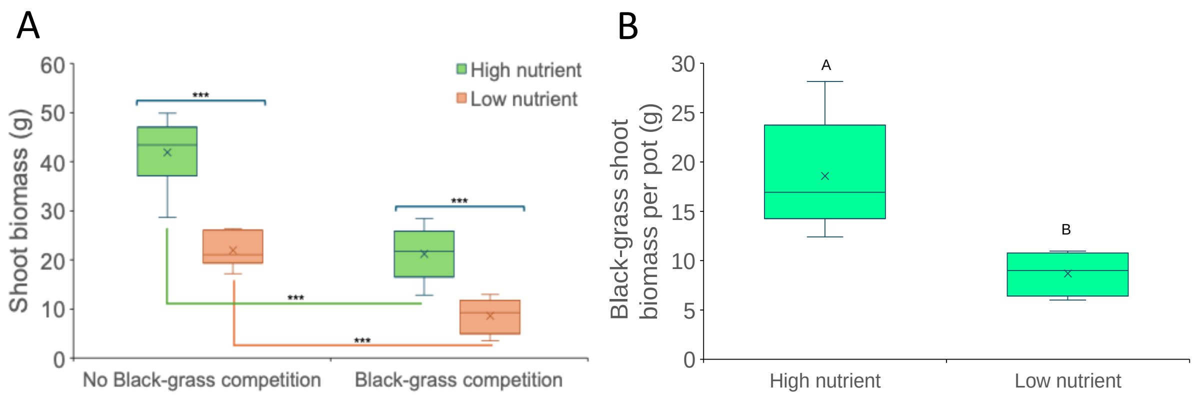

Figure 4.9 Nutrient availability reduces plant size but increases black-grass competitive ability

*Boxplots showing (a) shoot biomass of wheat in high and low nutrient availability with and without black-grass competition and (b) black-grass shoot biomass per pot (total 6 plants per pot) in high and low nutrient conditions in competition with wheat. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X denotes the mean. For (a) asterixis indicate significant statistical differences between the groups. (Independent samples t-test, *** P<0.001), n=6. For (b) different letters indicate statistical differences (Independent samples t-test, P<0.01), n=6*

4.7 Testing the competition screening method

Having developed a method of visualising wheat – black-grass competition that shows a negative impact on the wheat whilst maintaining healthy plants it was decided to test this screening approach. Two wheat varieties, Dickens and Graham, which had been used to pilot the root analysis methods, were grown in this competitive set-up. It was hypothesised that both lines would see reduced growth in the presence of black-grass and that the level of competition from black-grass would vary depending on crop variety. To test this, final shoot biomass was recorded and percentage loss in crop biomass in competition was recorded as well as percentage pot biomass that was black-grass.

It was observed that the biomass of wheat shoots was significantly smaller in black-grass competition than in the crop only controls for both wheat lines (Independent samples t-test, P<0.05) (Figure 4.10A). The average shoot biomass for Dickens in competition was 11.23g compared to 4.02g without competition, an average loss in crop biomass of 64.21%. For Graham, average shoot biomass in competition was 14.37g compared to 4.53g without competition, an average loss in crop biomass of 68.48%. The biomass of black-grass was not significantly different between wheat lines. Neither was the percentage of the total pot biomass that was black-grass (Figure 4.10B). In both wheat lines, black-grass took up most of the overall pot biomass indicating both crop lines were outcompeted by black-grass using this screening method.

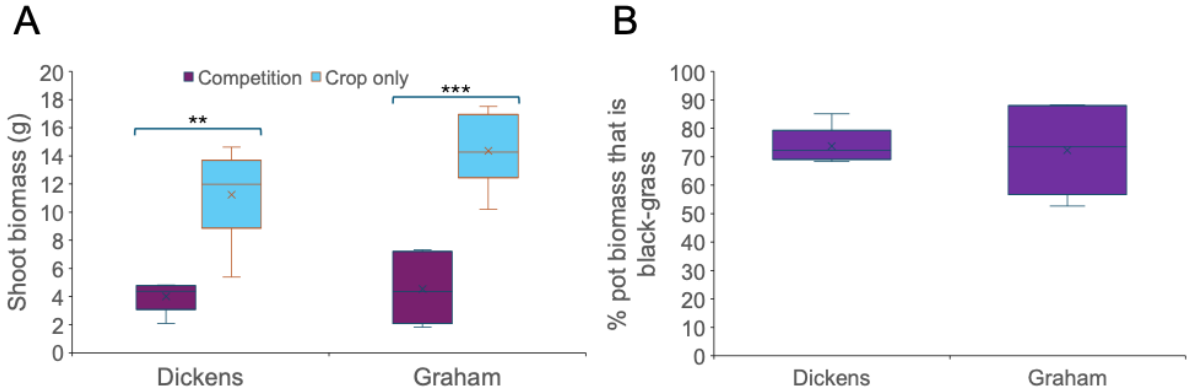

Figure 4.10 Testing wheat lines in the competition screen

Boxplots showing (a) crop shoot biomass of two wheat lines with and without black-grass competition (b) the percentage of the total pot biomass that is black-grass biomass in the 'competitive' pots for two wheat lines. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X denotes the mean. Asterisks above the boxes indicate significant statistical differences between the groups. (Independent samples t-test, P<0.001), n=6.

These results support the hypothesis, showing that this screening method can be used to successfully visualise the competitive effects of black-grass on the biomass of wheat. The amount of black-grass present in both lines was similar and although loss in crop biomass was different for both lines, the percentage lost was similar. Going forward repeating this approach with more crop varieties should highlight a greater range in competitive abilities and highlight those that are good and bad competitors against black-grass.

4.8 Discussion

Root screen development

Indirect screening for traits that may convey weed competitiveness in the absence of weeds is likely to be easier and less expensive than direct screening (Zhao et al., 2006). However, root assessments are difficult. Assessing roots in the field has its benefits as it will give values of root growth in real conditions, conditions that are not able to be replicated in the lab, however field assessments are often difficult and destructive. Direct screening in the field is possible via methods termed 'root crown phenotyping' or 'shovelomics' (York et al., 2018), by which plants are physically dug up, roots separated from soil and then assessed, or via soil coring were roots can be assessed to a depth of 1 metre by coring followed by washing and scanning of the roots (White et al., 2015).

Prior to the work in this chapter 'shovelomics' was attempted on wheat and black-grass plants in the fields of two farms, however due to the heavy clay nature of the soil, it was clear these methods and the non-destructive separation of roots from the soil would be impossible. It was therefore decided to utilise lab-based root screening methods for root assessments such as the use of Rhizoboxes and Hydroponics. Although not utilising field conditions these approaches still allow for comparative analysis of root growth under controlled conditions. This will allow for variation amongst varieties to be visualised. Further testing can then be done in field conditions to determine if lab-based findings are transferable.

The results of this chapter determined that the two screening approaches tested were able to give similar findings despite their differing methodologies. The slow growth in winter conditions indicates that a root screening approach in these conditions would not be feasible timewise. Given similar results were seen between winter and spring conditions it makes sense to utilise quicker spring growth for large scale crop root assessments with a smaller number of lines being tested in winter conditions to allow for continued comparisons to be made between root growth in the two growing conditions. Although this data only looked at two crop lines it provides a good basis for the assessment of more lines using both methods.

Despite seeing similar results in both root screening approaches, this does not prove that results in the lab will be representative of root growth in the field. There are many variable factors in the field that could affect crop root growth. These lab screening methods are somewhat crude however remain the best current option. The down-sides of rhizoboxes include, that they have limited volume, meaning only early growth can be assessed, they also only allow root growth to be visualised in two dimensions (Neumann, George and Plassard., 2009) meaning that not all roots are able to be seen as not all roots will grow towards the desired surface. Therefore, it is unknown whether the root data obtained is representative of the root growth of the plant (Nagel et al., 2012). In this chapter, root growth was found to both exit the rhizobox through the base water holes and a small amount of root growth was seen on the top side of the rhizobox, therefore the percentage coverage measurement of the base of the rhizobox will be an underestimate of total root growth with larger rooting plants likely being more greatly underestimated. In rhizoboxes it is assumed that reducing the growth to a 2D plane rather than a 3D plane does not affect plant root growth, however little work has been done to confirm this (Mašková and Klimeš., 2020). Maskova and Klimes., (2020) determined that crop growth in terms of both shoot and root biomass did not differ between cuboid and flat 2D rhizoboxes of the same volume, indicating that the thin nature of 2D rhizoboxes is not having an impact on plant root growth. This study however used perlite rather than soil as a substrate and assessed root biomass rather than percentage root coverage of the rhizobox surface. Therefore, although the shape of the rhizobox is not affecting overall root production, this study does not determine whether the amount of roots present on the rhizobox surface is representative of overall root growth. The rhizobox method of root assessment in a soil substrate is not perfect. This approach however still has benefits over using perlite when it comes to root harvest as roots can be lost and damaged by washing using this approach (Adu et al., 2017). Other rooting media has been used to assess root growth including agar, gels (Shi et al., 2013), paper pouches (Thomas et al., 2016). These substrates however do not mimic soil, factors such as water and nutrient dynamics as well as microbial interactions which are unique to soil will not be replicated in these other substrates (Adu et al., 2017).

One benefit of hydroponics compared to other soil-less substrates, is that it can be easily scaled to larger pots to allow plants to grow for longer. In hydroponics the major drawback is also its benefit, the lack of soil; removal of the natural growth medium means the plants are relying on additional nutrients being added (Conn et al., 2013), it is therefore impossible to perfectly mimic field conditions which will in turn affect the results. However, maintaining consistent conditions should allow for reliable comparisons of root traits to be made between crop lines. In terms of a screening approach for large scale use, the use of hydroponics in winter conditions would not be feasible due to the time and space required for the experiments to take place. Whereas the use of rhizoboxes in spring conditions provide quicker results, importantly these results are representative of those seen in winter conditions in the hydroponics in the pilot experiments. The rhizobox system is therefore the best candidate for root assessments with small scale hydroponics experiments as a comparison to ensure root growth trends remain similar between conditions.

Competition screen development

The identification of competitive cultivars through direct selection for weed competitiveness is currently a long and laborious process relying on full length field trials taking place over multiple years often with varying results due to varying environmental conditions (Zhao et al., 2006). The uptake of competitive cultivars as a cultural IWM approach has been limited due to the large field to field variation (Lutman et al., 2013) resulting in farmers selecting lines based on other factors other than weed presence. Identification of competitive varieties in controlled conditions is required for farmers to then make informed decisions based on other crop traits as to which line would be best for each individual field.

The results from the winter and spring competition experiments highlighted two important findings. Firstly, environmental conditions are important in black-grass competition, this would be expected as black-grass is known to impact winter wheat far more than spring wheat (Moss., 2010). In comparison to the barrier experiments in chapter 3, plant density increases the competitive ability of black-grass. These findings agree with the literature, which have shown that the higher the black-grass density the greater the loss in crop biomass in the field (Moss., 2010), indicating a greater level of competition.

Secondly, low temperatures in winter conditions dramatically decrease plant growth rate (Grace., 1988; Porter and Gawith., 1999) and as a result will affect the rate of competition. Decreasing temperature and day length will be more representative of winter field conditions, however results show it takes a long time to see any impact of competition. One requirement of a new screening method is to reduce the time taken to assess the competitiveness of cultivars (Andrews., 2016). Although less representative of real conditions, competition in spring conditions whilst maintaining high blackgrass density show a clear impact of black-grass competition in a much shorter time than in winter conditions. As a screening approach this would allow for relatively high throughput of many crop lines. Assessing a small number of lines in this lab screen method as well as in container and field trials will determine whether the screen is representative of field growth. Up to this point, it is possible to conclude that this spring-conditions screening method allows the visualisation of black-grass competition and therefore the comparison of the competitive ability of different crop lines.

Given competition is largely resource dependant and individual field conditions can vary dramatically, the role of decreased nutrient availability on competition was assessed. Nutrient management has been shown to improve the competitiveness of crops and decrease weed density (Conn., 2006), with correct fertiliser application and herbicide applications together showing decreases in weed density (Jiang et al., 2018). It has been suggested that the use of fertilisers is one factor benefiting weed presence in arable crops, with arable weeds being more responsive to high N levels than crops (Blackshaw and Brandt., 2008). In the absence of herbicides, N fertilisation significantly increased weed density and dry biomass (Jiang et al., 2018). The increased levels of nutrients providing ample resources for highly competitive weeds. It has been suggested that decreasing nutrient availability would decrease the competitive ability of black-grass (Finch et al., 2017). The findings presented in this chapter disagree with these findings, showing that under nutrient deficient conditions, black-grass was in fact more competitive. Both wheat and black-grass saw reductions in size in low nutrient conditions, a greater decrease in the biomass of wheat than in the biomass of black-grass would indicate that black-grass is better adapted than wheat to retrieve nutrients from the soil when they are limiting. This would back up the results of the volume density experiments in chapter 3 which show wheat was less able to take advantage of entire soil volume availability than black-grass.

Although the nutrient deficiency experiment showed increased competitiveness of black-grass in low nutrient conditions, for ease of experimentation and for better comparisons to future container and field trials, high nutrient level was maintained for use in the wheat screen. The high nutrient wheat screening method has allowed the competitive ability of black-grass to be visualised whilst allowing both crop and black-grass to still grow to a healthy size. A full screen of different crop lines using this approach would hopefully allow for the identification of lines that are more or less competitive against black-grass than others.

4.9 Conclusion

The work in this chapter has identified important factors impacting wheat – black-grass competition, including the slow nature of black-grass competition in winter conditions. A greater understanding of how and when black-grass competition occurs has allowed the optimisation of screening approaches to test for competitive ability at a small, quick, workable scale and to test for traits possibly attributed to increased competitiveness against black-grass. Both competitive ability and root screening methods will allow for correlations to be drawn between root growth and competitive ability. Above ground traits will be constantly recorded during the screens so that traits such as tiller number and shoot biomass can also being assessed any for correlations to competitive ability.

Chapter 5 Impact of genotype on root growth

5.1 Introduction

Wheat competitiveness against black-grass varies with crop genotype (Cook and Roche., 2018). Selection for competitive cultivars has therefore been highlighted as a possible method by which to better compete with blackgrass. However, identifying competitive cultivars remains difficult. A lack of knowledge surrounding what makes a cultivar competitive has hampered cultivar selection. It is poorly understood as to whether competitiveness against black-grass is dependent on below- or above-ground traits. Breeders have not directly considered root system architecture and weed competitiveness in their crop selection programmes mainly due to difficulties in observing root traits in the soil (Waines and Edhaie., 2007).

Root growth of wheat has dramatically decreased since the green revolution (Zhu et al., 2019). It is possible the selection for harvest index in wheat has resulted in negative trade-offs in other areas of wheat growth, including root growth (Evenson and Gollin., 2003). Breeding for harvest index has seen changes in root system architecture and root system size in both Chinese wheat varieties (Zhou et al., 2019) and UK wheat varieties (Fradgley et al., 2020). Breeding for other factors could also have negative connotations for wheat root growth, for example Voss-Fels et al., (2017) determined that breeding wheat varieties for control of flowering time resulted in decreased root biomass. Reduced height genes that are present in many elite wheat cultivars have also been reported to reduce root proliferation (Bai et al., 2013). In a similar period, black-grass has become increasingly prevalent, now holding the position as the UK's most problematic herbicide resistant weed species (Riches., 2022). There may therefore be a correlation between the planting of smaller rooting wheat varieties and the increasing prevalence of black-grass. Reducing root growth of wheat lines may therefore have resulted in elite wheat varieties more susceptible to environmental stresses such as competition from competitive weeds (Richards., 2007). It is possible that with the selection of larger rooting wheat varieties that the negative impact of black-grass competition could be reversed through greater natural suppression from the crop.

Older landrace wheat varieties that haven't been intensely bred for harvest index may have maintained traits that increase competitiveness against black-grass. Older wheat varieties have been seen to supress the weed Lolium rigidum Gaud (Annual ryegrass) greater than modern lines (Lemerle et al., 1996). Such traits may include increased root growth (Fradgley et al., 2020). Barley also has not seen the same level of intense selection as seen in wheat and therefore may also have maintained competitive traits such as root growth. Understanding how root growth varies amongst wheat lines but also between elite wheats, barley and landraces will allow for a better indication of the role of root growth in competing with black-grass.

5.2 Aims

Root growth could be a key factor in determining how competitive a wheat variety is against black-grass. The aim of this chapter was to quantify root growth for different crop lines. Screening approaches developed in chapter 4 are used to determine variation in root growth amongst crop lines. The main objectives were:

1. Determine whether seedling root growth corresponds to adult root growth.
2. Assess crop root growth in spring conditions using rhizoboxes.
3. Assess crop root growth in winter conditions using hydroponics.
4. Determine whether root growth is consistent between screening methods.

Firstly, an existing seedling root growth dataset was assessed to see if it could be used to predict root growth of adult plants, if so, this dataset could be used as a very quick way of screening root growth. Secondly, root growth of many crop lines, including elite wheats, landraces and barleys is assessed using two different set-ups (rhizoboxes and hydroponics). Finally, the ability of the two different screening approaches to produce root data will be compared and assessed to determine whether root growth in spring conditions is still indicative of root growth in winter conditions. The most appropriate data to use for correlation studies going forward will also be discussed.

5.3 Seedling root growth cannot be used to predict adult root growth

A pre-existing dataset was available to be assessed. This dataset was compiled and shared by Fay Walsh at the University of Leeds. The dataset consisted of 283 wheat lines including lines from the Watkins collection. The dataset assesses wheat root growth at 5 days old (Figure 5.1) when grown in germination pouches with Hoagland’s media. Roots were imaged and then analysed using RootNav (Pound et al., 2013). (All work on the pre-existing dataset was carried out by Fay Walsh). This data showed clear variation in root production across the 283 accessions with lines producing between 1 and 6 roots after 5 days growth.

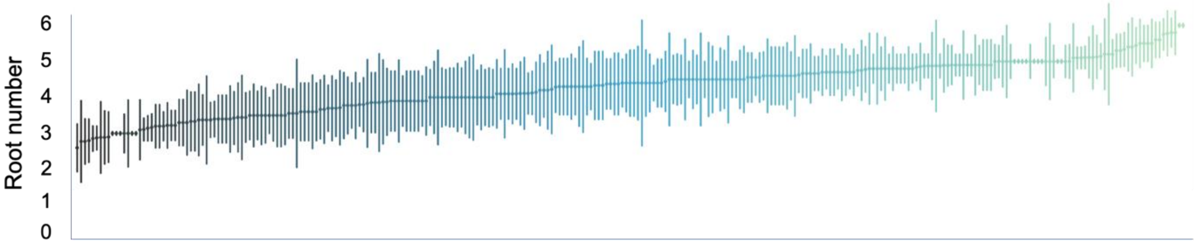

Figure 5.1 Variation in root number

Figure showing the seedling root growth of different wheat lines. Root growth of 283 crop lines ordered low to high by mean root number, error bars = standard deviation, n=3-10. (Graph edited with permission from original made by Fay Walsh).

If root growth at this seedling stage corresponds to the root growth of the adult plant, then this dataset could be used to predict which of these lines will have greater adult root growth and as a result, may indicate which lines would be expected to be more or less competitive against black-grass if root growth does indeed correlate to competitiveness (Assessed in a later chapter). If root growth after 5 days does not correspond to adult root growth this will indicate that root assessments of older plants are required. It was hypothesised that root growth as a seedling would correlate to root growth as an adult. To test this, 4 wheat lines were selected from the 283 accessions in the YoGI dataset. The four lines selected were chosen based on their seedling root growth. Two lines with high root growth (recorded as number of roots) as seedlings (W1190349, P01C0202497) and two lines with low root growth as seedlings (W1190755, W1190127) were selected. These lines were then grown in a rhizobox system for 10 weeks to test root growth over time. Root growth was recorded as percentage coverage of the rhizobox surface.

It was observed that as adults there was a significant difference in max root coverage between lines (Kruskal-Wallis, Bonferroni correction, $P<0.05$) (Figure 5.2) with line P01C0202497 having significantly higher root production than line W1190349. There were no significant differences seen between other lines. Despite this difference, the range in percentage coverage for all lines was small; approximately 10%. Interestingly the only significant difference was seen between the two lines that showed high root growth as seedlings. The two lines with low root growth as seedlings intermediate adult root growth.

These results do not support the hypothesis. Although one of the two lines that had high root growth as a seedling had high root growth as an adult the other had the lowest adult root growth. The two low rooting seedling lines had middling adult root growth. This indicates that short term root growth is not a good predictor of long-term root growth. This dataset therefore should not be used to predict which lines will have greater adult root systems. Therefore, this dataset cannot be used to predict which lines would be more competitive against black-grass.

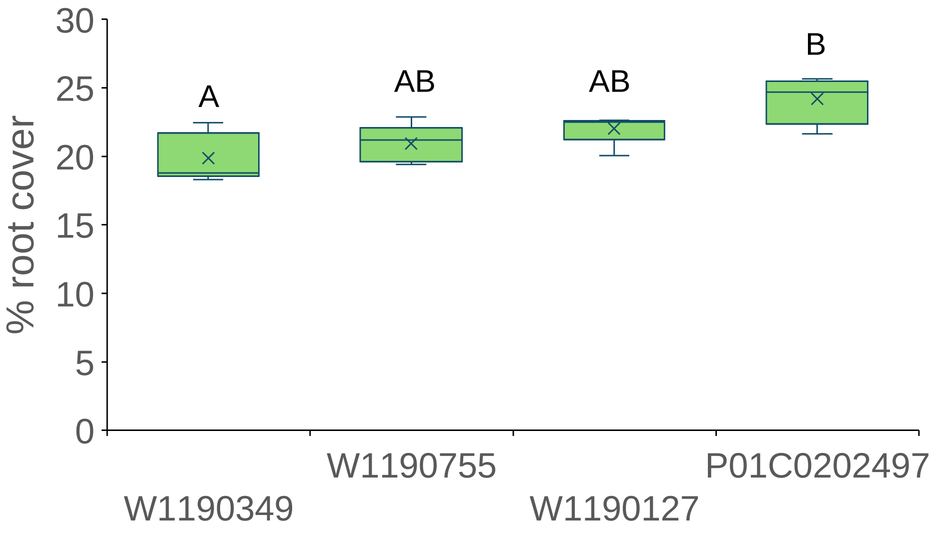

Figure 5.2 Short term root data does not predict adult root growth

Boxplot showing the percentage root cover in rhizoboxes of four landrace wheat lines with varying root growth as seedlings. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X denotes the mean. Different letters denote statistical differences between groups (Kruskal-Wallis test, adjusted with Bonferroni correction, P<0.05), n=5.

5.4 Rhizoboxes show root growth variation in crops in spring conditions

Rhizoboxes allow the visualisation of root growth in a 2D plane. From the resulting image of root growth, multiple components of root growth including total root production (assessed as % coverage of the image by roots) can be measured. It was hypothesised that crop varieties would vary in their root production using this experimental design. To test this, a single crop seed was grown in a rhizobox, the rhizobox was scanned weekly throughout growth. Maximum root coverage was recorded after 10 weeks of growth. A control line was repeated in each experimental batch. In total there were four batches.

The wheat line Kerrin was used as an internal control between batches. To determine whether batches are comparable, Kerrin growth was compared between batches. It was observed that there was no significant difference in Kerrin root growth between batches (Figure 5.3) with root cover lying consistently between 35-45%. This gives confidence that root growth of different lines can be compared directly between batches.

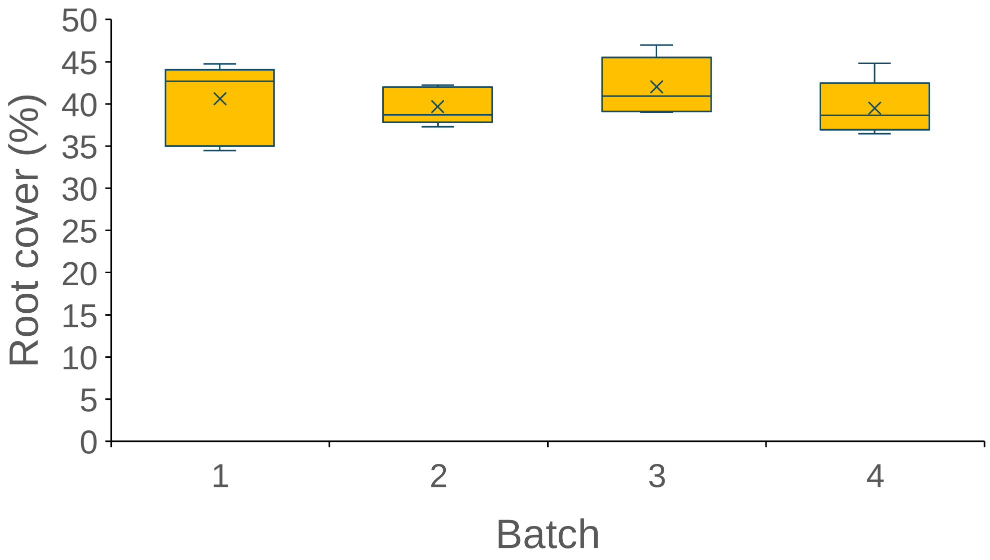

Figure 5.3 No batch-to-batch variation

Boxplot showing the percentage total root cover in Rhizoboxes of control line Kerrin in each batch of crop lines. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. X denotes the mean, n=5.

The root growth of all crop lines from all four batches was compared (Figure 5.4). It was observed that there was a significant difference in root growth between groups (Kruskal-Wallis, Dunn's with Bonferroni correction, $P<0.05$) (Figure 5.4). The two landrace varieties (Coloured in purple) had the lowest percentage root cover. Similarly to the landraces, black-grass (Coloured in yellow) also had low root growth, given the previous hypothesis about black-grass having increased root growth in winter conditions, this low level of growth is likely an effect of the growing conditions. Three of the five highest root cover lines were barleys (Coloured in red), with the other two being elite wheats. Barley showing high root growth is consistent with previous findings in chapter 3. The two high rooting elite wheat lines are Palladium and Zyatt, these are the only elite wheat lines that show a significantly higher root growth than any other elite wheat lines. Overall, there is little difference in root growth between most elite wheat lines with coverage ranging from 32-49%.

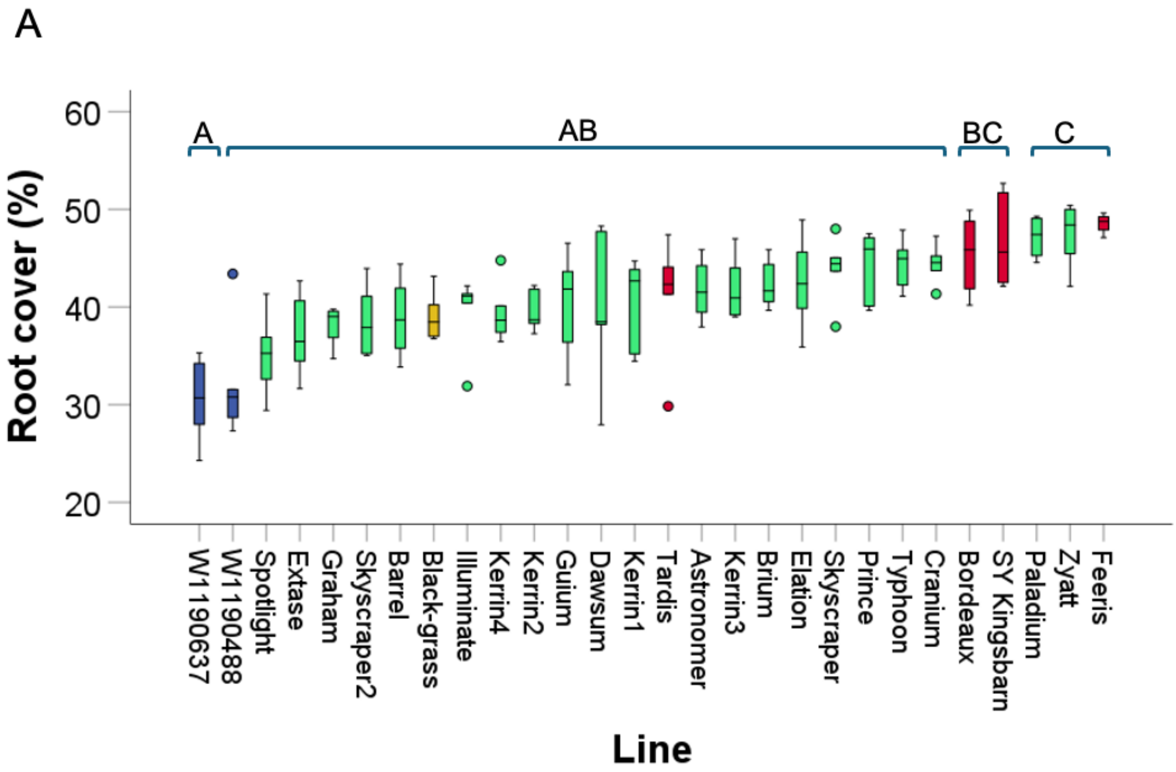

Figure 5.4 Root cover varies between lines

Boxplot showing the percentage root cover of the rhizobox surface for each crop variety. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Circles represent outliers. Different letters denote statistically significant differences between groups (Kruskal-Wallis, Bonferroni correction, P<0.05), n=5. Different colours represent different plant types, Yellow = black-grass, Blue = Landrace, Red = Barley, Green = Elite wheat.

This data agrees with the hypothesis, showing that rhizoboxes can show differences in root growth between crop varieties and between plant species. The screen has shown barleys to be high rooting, landraces to be low rooting and elite wheat lines to show a range of root growth under these conditions. This root growth data provides a basis for which to correlate to crop competitiveness. This system does have its limitations, these plants were grown for 10 weeks, with this graph showing maximum achieved root growth. The limited space for growth may impact plant growth particularly if certain lines have more vertical deeper rooting systems, it is therefore possible that root values are underestimated.

5.5 Hydroponic root growth analysis

The second method of assessing root growth was with hydroponics. Hydroponics allow direct measurement of root biomass which was not possible in the rhizoboxes due to the difficulties in non-destructively removing roots from soil. The hydroponics were placed in winter conditions, and it was hypothesised that these conditions would allow differences in root growth to be observed between crop lines. It was also hypothesised that results from the hydroponic screen would be similar to those in the rhizobox screen. To test this, 14 crop varieties were grown in two batches, with two lines being repeated in the second batch. Due to limited space in the winter growth cabinets, it was not possible to carry out all this work as a single batch experiment. The final shoot and root biomass was measured. The final shoot to root biomass ratio was also calculated for each crop line.

In batch 1, it was observed that shoot, root, and shoot: root ratio differed significantly between crop lines (Kruskal-Wallis/ANOVA, P<0.05) (Figure 5.5). Landrace W1190488 and SY Kingsbarn had a significantly greater crop biomass than Kerrin (Figure 5.5A). All other lines saw no significant differences with biomass typically ranging between 5-10g. For root biomass W1190488 and SY Kingsbarn again have the largest biomass, this time, significantly greater than three of the elite wheats tested (Figure 5.5B). Shoot: Root ratio differs greatly between lines, again W1190488 and SY Kingsbarn have similar results, both showing low shoot: root ratios indicating a greater proportioning of biomass to the roots in these lines. Of the elite wheats both Barrel and Elation also have a low shoot: root ratio, separating themselves from other elite lines, the majority of which have a significantly greater ratio. This indicates that amongst wheat lines there are those that attribute more biomass into root growth in winter conditions than others. If root growth ends up being important in competitive ability, then the selection of crop lines for this trait may be important in the identification of competitive cultivars.

In batch 2, lines Prince and Barrel were repeated along with four untested lines. Repeated lines were carried out as to allow comparisons to be made between the batches. It was however clear that the plants in the second batch were much more productive than the first batch, with plants achieving a much greater shoot and root biomass. Within batch 2 there was no significant differences in both shoot and root biomass between the different crop lines (Figure 5.6A, B). There was however a significant difference seen between groups in the shoot: root ratio (ANOVA, Tukey’s HSD test, P<0.05) (Figure 5.6C) with Barrel2 having a much lower shoot: root ratio than Graham. There were no significant differences seen between all other lines.

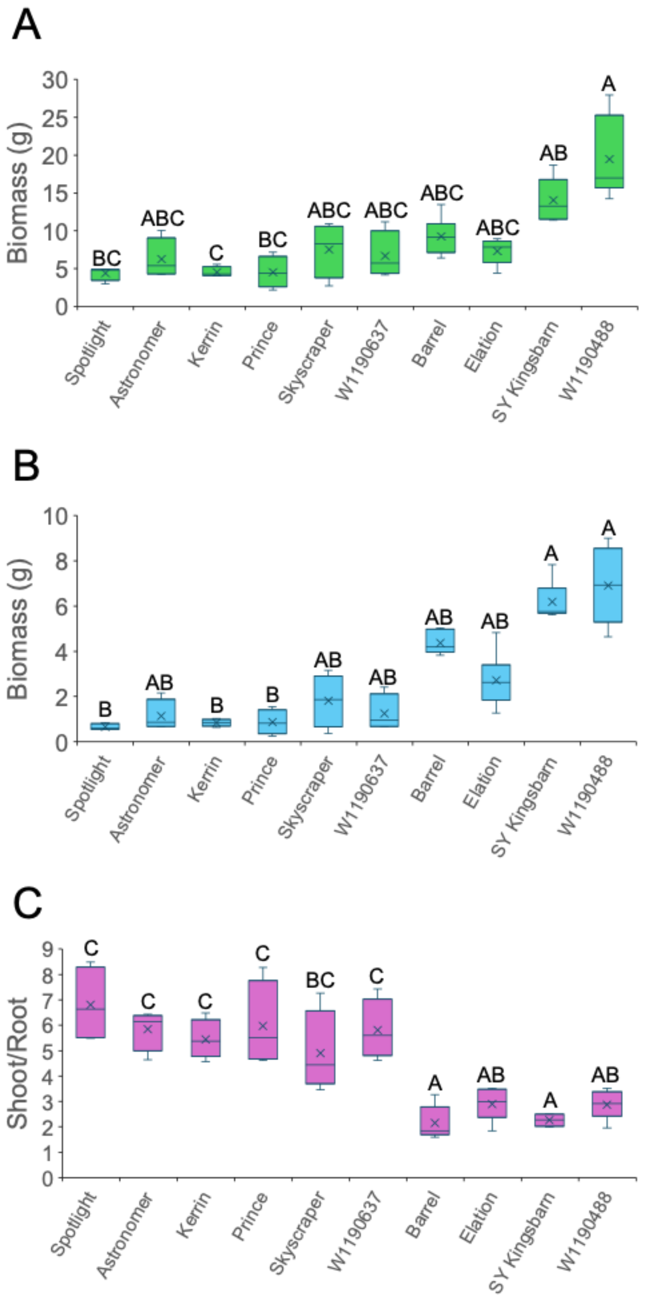

Figure 5.5 Crop growth in hydroponics (Batch 1)

Boxplots showing batch 1 hydroponic results for 10 different crop lines showing (a) crop shoot biomass. (b) crop root biomass and (c) crop shoot/root biomass ratio. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Different letters denote statistically significant differences between groups for (a and b) (Kruskal-Wallis test, Bonferroni correction P<0.05), n=4, for (c) (ANOVA, Tukey's HSD, P<0.05), n=4.

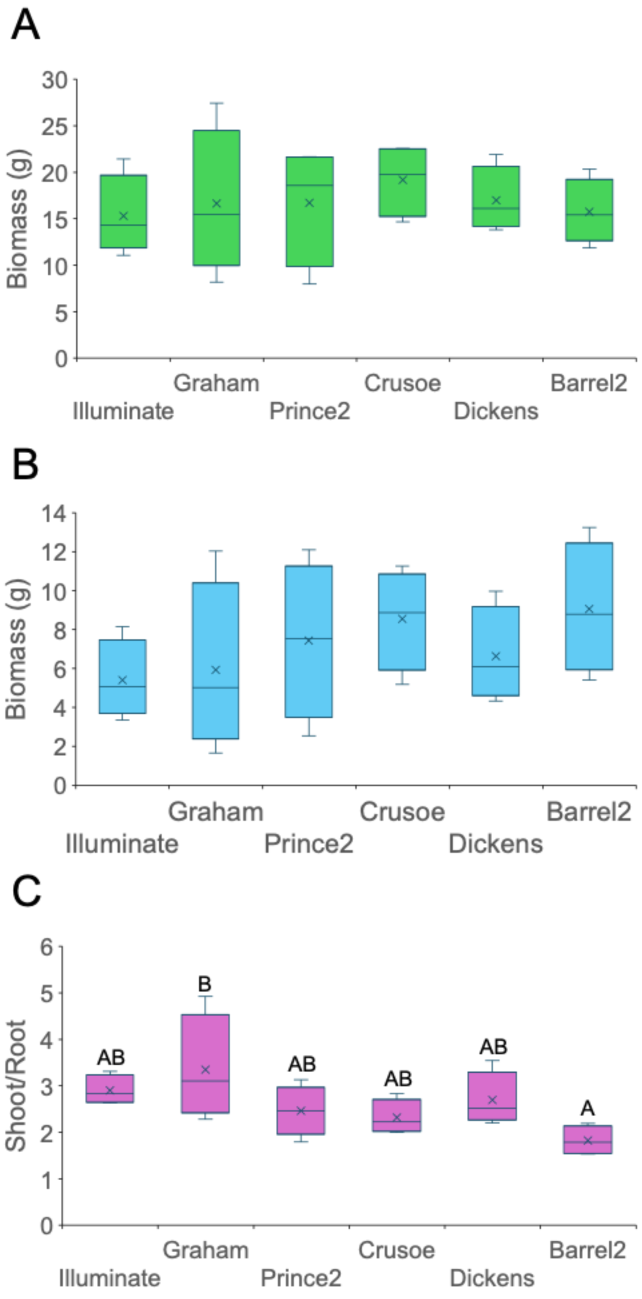

Figure 5.6 Crop growth in hydroponics (Batch 2)

Boxplots showing batch 2 hydroponic results for 6 different crop lines showing (a) crop shoot biomass. (b) crop root biomass and (c) crop shoot/root biomass ratio. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Different letters denote statistically significant differences between groups (ANOVA, Tukey's HSD, P<0.05), n=4.

Due to the differences in productivity of the two batches it was clear that a direct comparison of plants in both batches would not be possible as even the internal controls were vastly increased in the second batch. To attempt to compare the two batches, all data for each batch was normalised to the Barrel control within the corresponding batch, results of which can be seen in Figure 5.7. Both shoot and root biomass of lines in each batch show similar variation from the Barrel control, with a similar range of variation seen between batches. For shoot biomass there was a significant difference (Kruskal-Wallis, Bonferroni correction, $P<0.05$) (Figure 5.7A) between groups, with landrace W1190488 having a greater root growth than Spotlight, Kerrin, and Prince. Most lines saw no significant differences. Similar results were seen for root growth, a significant difference was again seen between groups (Kruskal-Wallis, Bonferroni correction, $P<0.05$) (Figure 5.7B) with W11900488 and SY Kingsbarn having a greater root growth than Spotlight. All other lines showed no significant differences. Normalising shoot: root ratio (Figure 5.7C) showed little difference between all lines however some lines had a large range of values.

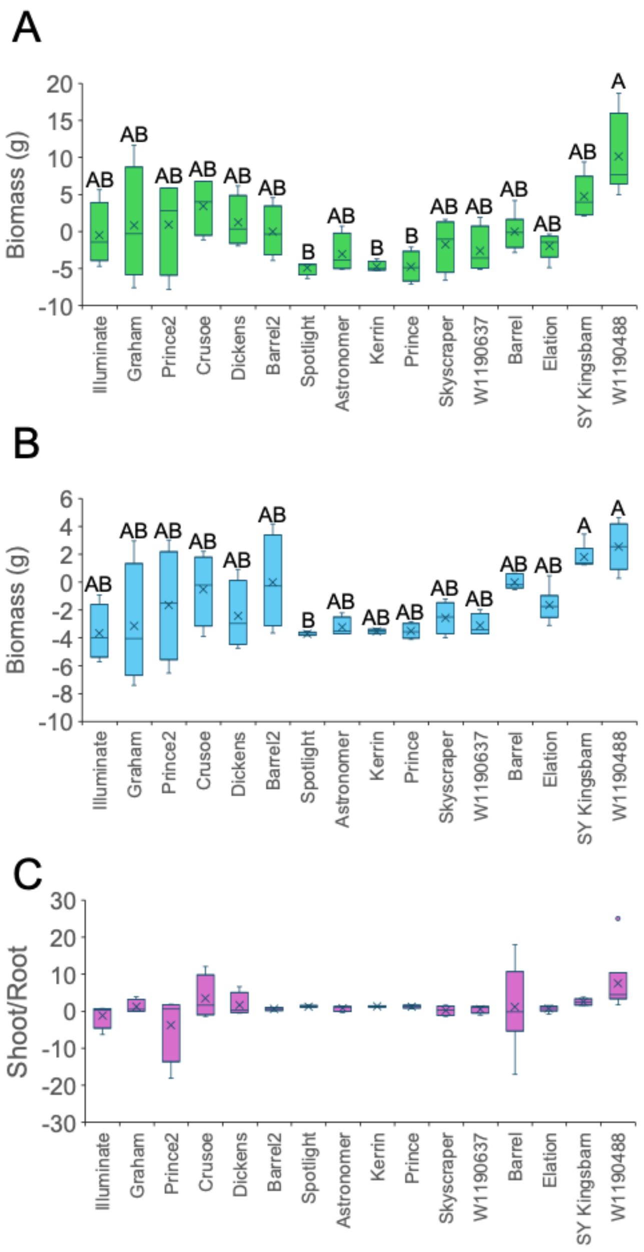

Figure 5.7 Normalised crop growth in hydroponics

Boxplots showing batch 1 and 2 hydroponic results normalized to the Barrel control within the corresponding batch. Boxplots show (a) crop shoot biomass. (b) crop root biomass and (c) crop shoot/root biomass ratio. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Different letters denote statistically significant differences between groups (ANOVA, Tukey's HSD, P<0.05), n=4.

Overall, the hydroponic experiments support the hypothesis highlighting that plant growth can vary between crop lines in winter conditions. The production of both shoot and roots was seen to vary between lines, whilst the ratio at which plants proportion biomass was also seen to differ dependant on cultivar. However, due to the large variability between batches it is not possible to accurately compare root growth between batches and the difference between batches throws doubt on both individual sets of results. Unfortunately, there is not enough time to further repeat the hydroponic experiments to allow for a clearer view of winter root growth.

5.6 Comparisons of root screening methods

Both root screening approaches measure root growth slightly differently therefore it is likely there will be some variation in the root growth of different varieties between screens. However, it was hypothesised that the relative root growth of crop varieties tested will be consistent between screening methods. To test this, crop varieties tested in both rhizobox and hydroponic screens were ranked from high to low depending on their mean root growth. This ranking was then compared for both screens.

The hybrid barley SY Kingsbarn has consistently high levels of root growth in both rhizobox and hydroponic screening methods. Elite wheat Prince also has shown the same consistency of high root growth. At the lower end of root growth, elite wheat Spotlight consistently ranks among the lowest rooting varieties in both screening methods. Elite wheat lines Illuminate and Barrel both have consistently middling levels of root growth relative to all other lines. These results indicate that both screening approaches are able to show consistent results for these lines.

However, despite these similarities it is clear that there are also some differences in cultivar root production between screens. The root growth of landrace varieties (particularly W1190488) was vastly different between screening methods, with high root growth in hydroponics but low root growth in rhizoboxes. Elite wheat lines Elation, Astronomer and Kerrin were among the highest rooting varieties when grown in rhizoboxes however in hydroponics they showed middling to low levels of root production. This variation indicates that the two screening approaches may not be directly comparable, meaning that results for root growth in spring conditions may not be used to predict crop root growth in winter conditions. It is possible; as seen in the landrace line W1190488, that some crop varieties are more sensitive to differences in growing conditions and are therefore unable to maintain levels of root growth in both spring and winter conditions.

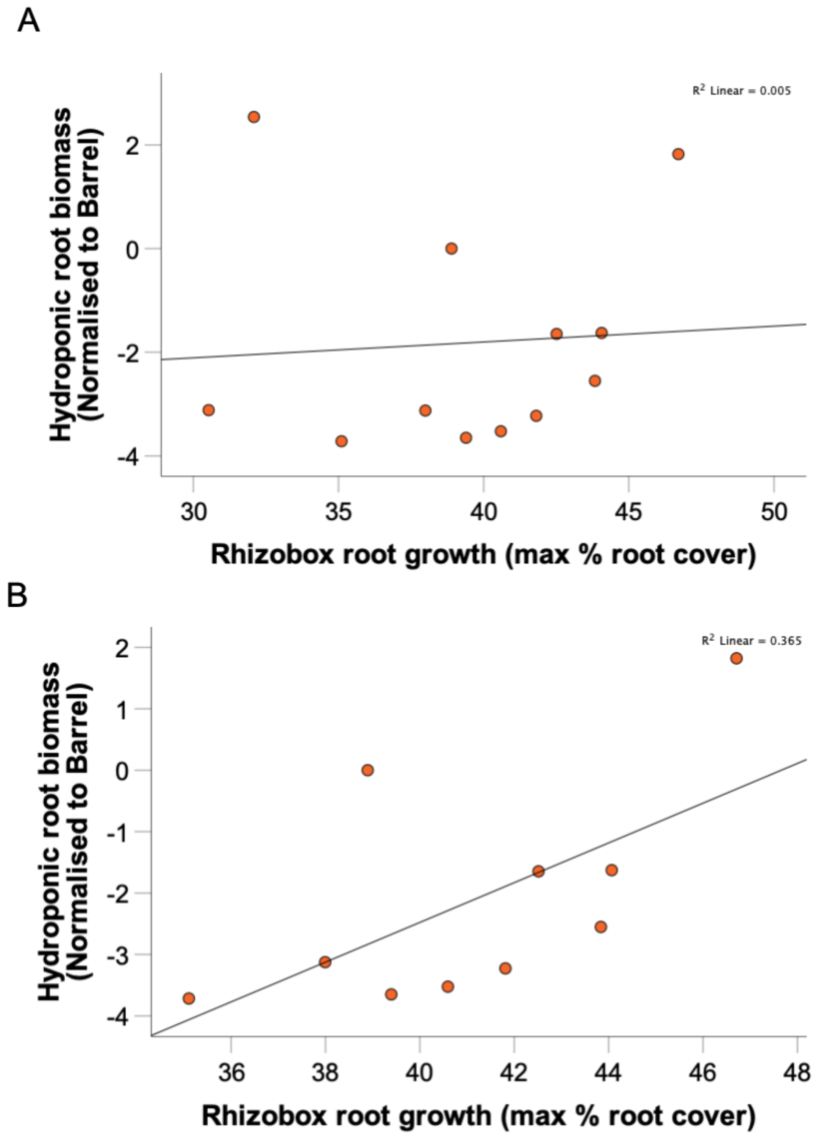

Figure 5.8 Screening methods are not comparable

Scatterplots showing the relationship between rhizobox and hydroponic root growth screening methods for (a) 12 elite crop lines including 2 landrace wheats (b) 10 elite crop lines excluding the landrace wheats. Trendlines represent linear lines-of-best-fit.

Spearman’s rho correlation was carried out to determine any relationship between the results of the two root screening approaches. Upon analysis of all lines examined in both screens (Figure 5.8A), it was observed that there was no significant correlation between the results of both the rhizobox and hydroponic screening approaches (Spearman’s rho, R_s (12) = 0.238, P=0.457). It was clear that at least one of the landrace varieties was vastly different in terms of root growth between the two screening methods. It was therefore decided to assess again the correlation between the two screening approaches without the landrace varieties to determine whether the result of no correlation is due to failings in the screening approaches or due to poor adaptability of different crop lines to changing growing conditions. Up reanalysis without the landraces it was again determined that there was no significant correlation between the two screening approaches (Spearman's rho, R_S (10) = 0.624, P=0.054) however the removal of these lines did vastly increase the similarities in root production between screening approaches.

Overall, these results reject the hypothesis, indicating that root growth was not consistent for all crop lines across screening methods. This result however may not be solely due to failings in the screening approaches, but rather also in part due to the poor adaptability of individual crop lines to changing growing conditions.

5.7 Discussion

Seedling root growth has often been used as a proxy for the level of root growth that would be achieved by a plant as an adult (Atkinson et al., 2015), with plants with greater root growth as a seedling being predicted to have a greater root system when mature. Some studies have suggested that seedling root growth cannot and should not be used as a predictor of adult root growth in wheat (Bai et al., 2019; Wojciechowski et al., 2009). Atkinson et al., (2014) indicated that limitations of their seedling root screening set-up where that the results may not correlate to results seen in the field in mature plants. With contrasting views in the literature, it was decided that it would be worth assessing the adult root growth of four lines with known varying seedling growth to determine whether root growth is comparable at different ages. The findings indicated that for the available dataset, seedling root growth cannot successfully predict root growth as an adult (Figure 5.2). As a result, this dataset cannot be used as a predictor of root growth. Work therefore focussed on both rhizobox and hydroponic approaches as they allow for root assessments in older plants which will be more representative of adult root growth. This dataset however could be useful in studies assessing early vigour as a competitive trait which has previously been highlighted as a possible trait that could increase competitiveness against black-grass (Bertholdsson., 2005).

Crop varieties are known to vary in their root growth dependant on genotype, species (Barley/wheat) and type (Elite/landrace) (Chapagain et al., 2014; Cook and Roche., 2018; Bai et al., 2019; Mahajan et al., 2014; Fradgley et al., 2020). The rhizobox screen successfully highlighted the generally observed differences in root growth between wheat and barley (Figure 5.4). Generally, barley was observed to have greater levels of root growth than the wheats. This finding agrees with Chapagain et al., (2014) who found elite barley varieties had greater root systems than elite wheats indicating possible trade-offs in wheat root growth in favour of harvest index, particularly as no difference was seen in root growth between elite and landrace barley lines. The rhizoboxes approach also successfully showed variation in root growth amongst elite wheat lines with some lines able to produce a significantly larger root system than others (Figure 5.4) this variation in root growth may explain variation in competitive ability, future chapters will explore this hypothesis further.

However, the rhizobox results indicated low root growth in landraces, this disagrees with what would be expected as landrace varieties have previously been described as having greater root systems than elite wheats (Bekas et al., 2016). Chapagain et al., (2014) determined that landrace wheats have longer, finer roots, greater root surface area, greater number of root tips and a higher branching angle than elite wheats, enabling landrace lines to have larger deeper root systems with better nutrient uptake efficiency and drought tolerance. This work by Chapagain et al., (2014) however was based on 10-day old seedlings which as previously discussed may not show root growth representative of adult plants. Given the rhizoboxes were grown in spring conditions it may be that due to their genetic backgrounds, landraces which are usually bred for growth in particular niche conditions, are less well adapted to the changing temperatures/growth conditions as modern elite varieties (Bekas et al., 2016). This theory is backed up by landrace root growth in the winter hydroponic experiments (Figure 5.5) which show landrace varieties (particularly landrace W1190488) to have amongst the highest levels of root growth of tested lines, this observation is more in line with what has previously been observed (Bekas et al., 2016; Chapagain et al., 2014). Thorup-Kristensen et al., (2009) observed winter and spring wheat varieties to produce roots at the same rate however due to the longer growing season, winter wheat was able to reach depths of 2.2m, double that of spring wheats, it may be that landraces in spring conditions haven’t had the time to show their full rooting capacity. Chapagain et al., (2014) also noted high variation in landrace root growth which may explain why one W1190488 has a larger root system than W1190637, again these differences are likely due to the history of the individual plants and the requirements for root growth in their individual niches. The considerable difference in root growth of these lines along with the other differences between screening methods described in section 5.6 indicates that the growth conditions are impacting plant root growth.

In winter conditions conclusions are more difficult to draw due to batch-to-batch variation affecting the results. This variation could be due to several factors including poor plant husbandry, unforeseen differences in growing conditions; winter growth chambers sometimes struggle to maintain the desired temperatures which may have affected growth or the presence of powdery mildew which was particularly bad for crop lines in batch 1. Despite inconsistencies between batches, comparisons can be made within each batch. As previously mentioned, landraces have equal or better root growth than elite wheats when grown in winter conditions (Figure 5.5). The hybrid barley SY Kingsbarn has a larger shoot and root biomass than all elite wheats, indicating that the size of both may play some role in increasing the competitiveness of barley against black-grass agreeing with findings by Cook et al., (2023). Batch 2 showed little variation in crop shoot and root growth however all lines tested where elite wheats therefore little variation may have been expected.

Due to the differences seen between hydroponic batches it was difficult to compare root growth between both screening methods. Normalising the hydroponic data allowed crude comparisons to be drawn between screens. Although some varieties showed consistency between screens there were others that showed clear inconsistencies. This would indicate that root growth in spring conditions could not be accurately used to predict root growth in winter conditions for all lines. Without the further time for repeated hydroponic experiments to reduce the uncertainty of batch-to-batch variation, the rhizobox root data alone would be used for future analysis of correlations between root growth and competitive ability. Although spring root growth will not be representative of growth in winter conditions it would allow direct comparison to the wheat competition screen which also took place in the same spring conditions. Correlations can therefore still be made between root growth and competitive ability of winter wheats without winter conditions. Further studies will be required to determine whether findings in spring conditions are consistent with findings in winter conditions.

5.8 Conclusions

This chapter has shown variation in root growth to exist amongst crop species but also amongst elite winter wheats. This information gained from the rhizobox screen will provide the basis by which to assess the role of root growth in wheat – black-grass competition.

Chapter 6 Impact of genotype on competitive ability

6.1 Introduction

Recommended lists (RL) are created by The Agricultural and Horticultural Development Board (AHDB) to provide farmers with up-to-date information about expected crop performance including grain yield and quality as well as agronomic features of the crop and the resistance of the crop to certain diseases. However, there is currently no recommended list for which crop varieties to plant should farmers have an infestation of weeds such as black-grass.

Breeding for competitiveness is currently not a priority for breeders, with breeders focussed on increasing yield to ensure their varieties remain on the recommended lists as to increase their cultivars profitability (Andrew., 2016). Traits conveying competitiveness have therefore been largely side-lined. It is seen that choosing a variety based on competitive ability may result in negative trade-offs in other desirable traits (Andrew., 2016) thus reducing profits for breeders.

The ability to select a crop variety based on weed competitiveness would allow for better control of weeds through natural crop suppression, resulting in reduced losses in crop yields (Lutman et al., 2013). The use of competitive cultivars has seen wide ranging black-grass control, being seen to reduce black-grass by between 8-45% depending on cultivar choice (Moss and Lutman., 2013). Limited useful research into weed competition has resulted in farmers selecting crop varieties for other properties for which recommended lists are available, choosing rather to rely on tried and proven herbicide application for their weed control. The choosing of a competitive cultivar relies largely on personal experience (Andrew., 2016) or the results of small-scale studies with results largely unbeneficial outside of the tested cultivar pool and specific environmental conditions (Andrew et al., 2015). The use of competitive cultivars has therefore been limited due to high individual field level variation (Lutman et al., 2013) and varying year to year results (Cosser et al., 1997). The unpredictability of competitive cultivars means farmers are unwilling to adopt them into their weed management strategies.

Certain crop lines such as the hybrid barley SY Kingsbarn have been suggested for use against grass-weeds such as black-grass (Syngenta., 2024), but for wheat varieties there remains a lack of a comprehensive, evidence-based, recommended list. In an attempt to build such a list, a method to test wheat genotypes for their competitiveness is required. Wheat competitive ability against black-grass has previously been seen to vary based on crop genotype (Hucl., 1998; Cook and Roche., 2018), this has resulted in a plea for increased research into the use of competitive cultivars as a cultural approach to integrated weed management (Andrew., 2016). Competitive cultivars have the capability of being an extremely cheap alternative to current integrated weed management approaches due to their being no requirements for extra physical inputs by the farmer. Currently the process of identifying competitive cultivars, through large scale trials is laborious and time-consuming (Brain et al., 1999). There is therefore a need for a quick and easy approach for assessing the competitive ability of different crop genotypes. A direct lab-based screening approach in competition with black-grass would allow for the identification of competitive cultivars in controlled conditions. Further testing of these lines in container and field trials would then confirm the suitability of the screening approach for predicting crop competitiveness in real conditions.

6.2 Aims

Competitive cultivars have the ability to withstand competitive pressures of black-grass through weed suppression and/or tolerance. Screening many different varieties using the screening method developed in chapter 4, will determine which varieties amongst current elite winter wheats, landrace wheats and barleys, are more competitive than others and therefore could be contenders for use as competitive cultivars in the field. The main aims of this chapter are:

- Using container and field trials determine to what extent the lab wheat competition screen results are representative of outdoor competition.
- Screen a wide variety of crop lines to test for competitive ability against black-grass.

In this chapter, comparisons will be made between the growth of seven crop lines in competition with black-grass in three different environmental conditions to determine whether the results of the controlled lab screen are representative of the results seen in real conditions. This screening approach will then be used to assess the competitiveness of a wide variety of elite wheats, landrace wheats and barleys to determine the variation in competitive ability amongst varieties and highlight possible competitive cultivar candidates in winter wheat.

6.3 Testing the wheat screening method

A crop screening method that allows visualisation and comparison of black-grass competition in different crop lines had previously been developed (see chapter 4). To ensure this is an effective method of comparing the competitiveness of different crop cultivars it is vital to ensure the results from the lab are representative of competition in outdoor conditions. To ensure results from the lab are transferable to the field, the growth of seven different crop lines were tested in relation to one another across three different environments. Firstly, they were grown in the controlled lab wheat screen, before also being grown in container trials and then finally field trials. It was hypothesised that the same trend in crop competitiveness will be seen for the selected crop varieties across environments. These experiments also allow comparisons of the competitive ability of different crop types (Barley/wheat) and lines with different genetic backgrounds (Elite/landrace) to see if more can be learned about what makes certain lines more competitive. Therefore, it was hypothesised that differences in competitive abilities will be seen between crop lines based on their crop type.

To test this, seven crop lines were selected (Table 6.1). These lines were selected due to their different backgrounds. Three elite wheats were chosen to represent the competitive ability of current crop lines. Due to the significant losses in elite winter wheat yields due to black-grass, it was expected that elite lines would be poor competitors. Two landrace wheat varieties were chosen, these lines have undergone far less intensive selection than the elite lines and therefore may have retained traits lost in elite varieties (Lazzaro et al., 2019). These retained traits may provide a competitive advantage against environmental pressures (Chapagain et al., 2014). It was therefore hypothesised that the landrace varieties would be better at competing with black-grass than the elite wheats. A winter barley was selected, barley is known to be more competitive against black-grass than wheat (Cook et al., 2023). Finally, a hybrid barley was selected, this line is recommended by Syngenta for use against black-grass (Syngenta., 2024). It was hypothesised that these different crop varieties would show a range of competitive ability.

Table 6.1 Seven crop varieties selected for container and field trials

Variety	**Type**	**Source**
Kerrin	Winter wheat	KWS
Elation	Winter wheat	Elsoms
Barrel	Winter wheat	KWS
W1190637	Landrace winter wheat	Watkins collection
W1190488	Landrace winter wheat	Watkins collection
Bordeaux	Winter barley	Senova
SY Kingsbarn	Hybrid barley	Syngenta Seeds

For each environment the experimental design differed (see methods) however the same data can be collected from each set-up. In the following section the hypotheses and results will be described in turn for each environment, before comparing the results from each. In each environment final crop biomass, black-grass biomass and the percentage of total biomass that is black-grass was calculated. For lab and container environments percentage loss of crop biomass when in competition was also calculated.

6.4 Lab wheat screen

Seven crop lines (Table 6.1) were grown in the controlled lab wheat screen. It was hypothesised that variation in competitive ability would be seen between crop varieties with barley being the most competitive followed by the landraces with elite wheat lines the least competitive. To test this, the crop lines were grown in competition with black-grass at a ratio of 6:1 (black-grass: wheat), alongside a crop only control. Crop biomass, black-grass biomass, % pot biomass that is black-grass and % loss in crop biomass in competition was recorded.

It was observed that the crop shoot biomass of SY Kingsbarn was significantly greater than that of all other crop lines (Kruskal-Wallis, Dunn's with Bonferroni correction, $P<0.05$) (Figure 6.1A). All other lines had a statistically similar shoot biomass. Black-grass biomass varied significantly between the different crop lines (Kruskal-Wallis, Dunn's with Bonferroni correction, $P<0.05$) (Figure 6.1B). The barleys had the lowest black-grass biomass, with Bordeaux having the overall lowest black-grass biomass. Elite wheat line Kerrin and the two landraces have a similar black-grass presence, one slightly higher than that of the barleys. Elite lines Barrel and Elation show the highest black-grass biomass of the lines tested. The percentage of the total pot biomass that was black-grass also significantly differed between crop variety (Kruskal-Wallis, Dunn's with Bonferroni correction, $P<0.05$) (Figure 6.1C). Once more the barleys had the lowest percentage black-grass. All elite lines and landraces show similar higher black-grass percentages. The average crop biomass was recorded for crops in and out of competition with black-grass, with the average crop biomass loss calculated (Figure 6.1D). This graph shows that SY Kingsbarn was able to maintain a high percentage of its biomass when in black-grass competition and was the best performing line of all seven tested lines. Across all panels of figure 6.2, SY Kingsbarn shows consistent high crop biomass and low black-grass presence indicating it is a good line at competing with black-grass. This would be expected as SY Kingsbarn is a variety currently being suggested for use against black-grass infestations (Syngenta., 2024). Bordeaux, despite having low black-grass presence, loses a large percentage of its crop biomass compared to the other barley SY Kingsbarn. This indicates that although it can suppress black-grass well, it is not as tolerant to black-grass as SY Kingsbarn. Elite wheat line Barrel shows the smallest loss in crop biomass compared to the other wheat lines; this is despite Barrel having high levels of black-grass. This indicates that Barrel may be a good tolerator of black-grass, however it is also a poor suppressor. The two landrace varieties do marginally better at maintaining crop biomass than elite wheat lines Kerrin and Elation despite them having similar percentage black-grass presence. This would indicate that these landrace lines are somewhat better tolerators than some elite lines.

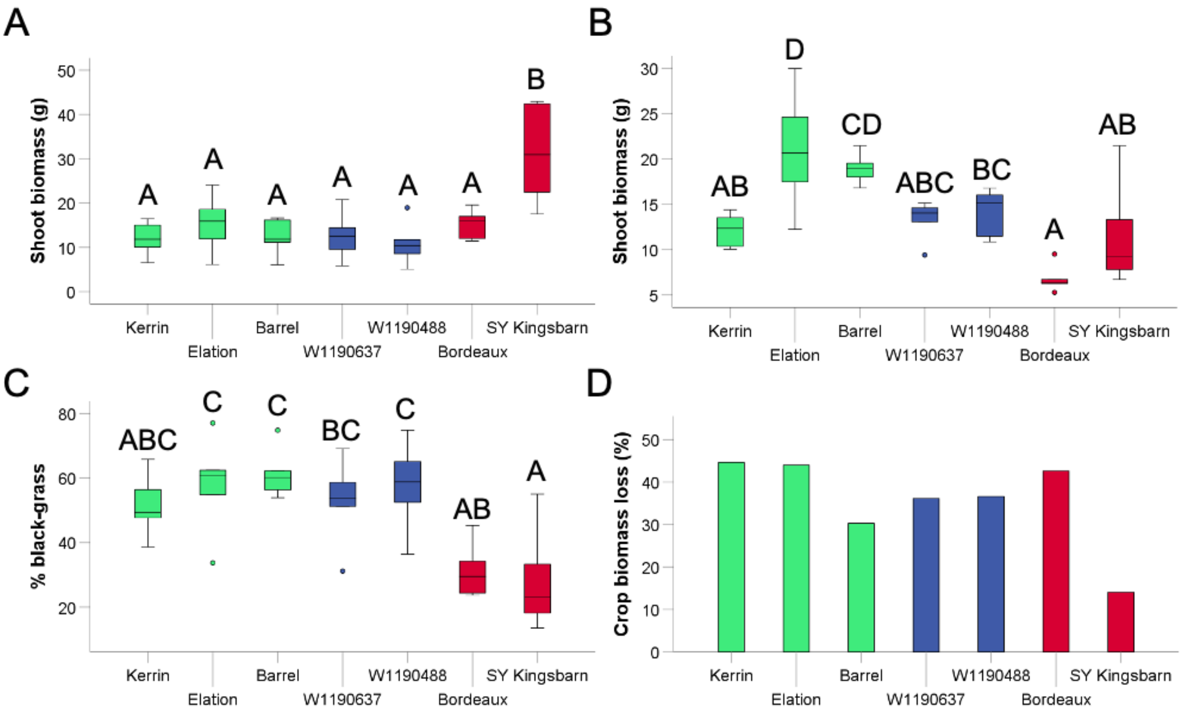

Figure 6.1 Selected crop lines show variation in competitive ability in controlled conditions

Crop assessments of competition screen under controlled conditions. (a, b, c) Boxplots showing (a) final shoot biomass of seven crop lines when grown in competition with black-grass at a black-grass: crop ratio of 6:1 (b) final shoot biomass of black-grass per pot (6 plants) when in competition with different crop lines (c) percentage of the total pot biomass that is black-grass biomass when in competition with each crop line The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values, the X within the box is the mean. Different letters denote significant statistical differences between the groups (Kruskal-Wallis, Dunn's with Bonferroni correction, P<0.05), n=6. (d) Bar chart showing the average crop biomass loss of each crop line when in competition with black-grass compared to the biomass of crop only controls, n=6. Colours represent crop type, Green = Elite wheat, Blue = Landrace wheat, Red = Barley

These lab results agree with the hypothesis indicating that there are differences in the competitive abilities of different crop lines against black-grass. As hypothesised the barleys were the best at competing with black-grass whilst the landraces performed slightly better than the elite wheats. This shows that the lab competition screen is successful in highlighting differences in competition between crop varieties, backing up what was previously seen in experiments with Graham and Dickens when developing the screening method.

6.5 Container trials

Repeating experiments in different environmental conditions will test the transferability of the lab screen data. In the case of crops, results produced in controlled lab conditions won’t necessarily transfer to outdoor conditions. It is therefore required to test how transferable the lab screening approach is from controlled to outdoor environments.

Container trials were grown throughout the winter growing season in Boxworth, Cambridge. Each crop variety was grown both in competition with black-grass at a ratio of Crop: Black-grass of 6:20 or as crop only controls at a crop: black-grass ratio of 6:0 (See methods for detailed methodology). The growth of the same seven crop lines that were grown in controlled conditions were grown in container experiments. It was hypothesised that differences would be seen in the competitive ability of crop varieties with barleys being the most competitive followed by landraces and then elite wheats being the least competitive.

Crop biomass, black-grass biomass, %pot biomass that is black-grass, and % loss in crop biomass was calculated. It is important to note due to high levels of predation by birds most barley ear biomass was lost, resulting in measurements that are lower than the actual amount of barley biomass produced. All other biomass recordings for wheat include ear weight in the biomass value.

Black-grass shoot biomass was seen to differ significantly between crop varieties (Kruskal-Wallis, Dunn’s adjusted with Bonferroni correction, $P<0.05$) (Figure 6.2A). The black-grass only pots had the highest black-grass biomass. SY Kingsbarn had the lowest black-grass biomass presence in its pots and was the only variety to have reduced black-grass biomass significantly from the black-grass only control. In contrast to SY Kingsbarn, winter barley variety Bordeaux had a very high black-grass biomass in its pots, higher than all the wheat lines. For Bordeaux the high levels of black-grass cannot be explained by the absence of crop as Bordeaux has the second highest crop biomass (Figure 6.2B). Either Bordeaux pots are just very productive or there has been some error in data collection. If these results are due to increased productivity, it would suggest a complete lack of competition between the crop and weed, particularly as black-grass levels in competition with Bordeaux are in some cases equal to the black-grass only controls (Figure 6.2A). Landrace variety W1190488 and Elation have slightly lower levels of black-grass than Kerrin, Barrel and landrace W1190637 however there was no significant differences between them due to the high variation between replicates.

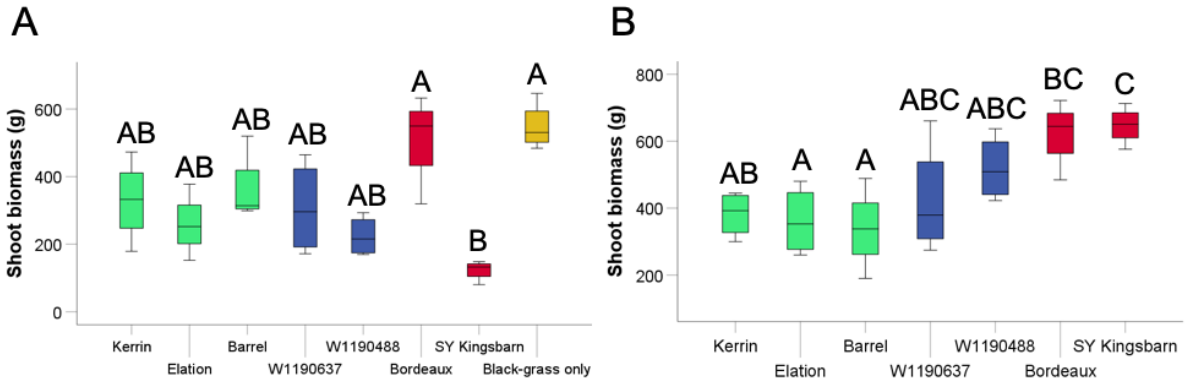

Figure 6.2 Variation in competitive ability in container trials

Boxplots showing container trial crop assessments (a) black-grass shoot biomass when in competition with six different crop lines and as a black-grass only control (b) crop shoot biomass of each line when in competition with black-grass. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Different letters denote significant statistical differences between the groups for (a) (ANOVA, Tukey's HSD test, P<0.01), n=4 for (b) (Kruskal-Wallis, Dunn's with Bonferroni correction, P<0.01), n=4. Colours represent crop type, Green = Elite wheat, Blue = Landrace wheat, Red = Barley, Yellow = black-grass only

As for crop shoot biomass it was observed that there were significant differences between crop varieties (ANOVA, Tukey's HSD, P<0.05) (Figure 6.2B). The barleys were able to produce the largest shoot systems, SY Kingsbarn producing a shoot biomass significantly greater than that of all elite wheat lines, and Bordeaux producing a shoot biomass significantly greater than both Elation and Barrel. Without the bird predation of the barleys, it is likely that these differences would have been even greater. The landrace wheat varieties had larger shoot biomass than the elite wheat lines but were smaller than the barleys. This would indicate that in the presence of black-grass, barleys are better able to produce shoot biomass than elite wheat lines, whilst landrace wheats, particularly W1190488, are also able to produce larger shoot systems.

Percentage loss in crop biomass when in black-grass competition was calculated for each variety, using the crop only controls as a baseline. At high black-grass density the elite wheats and landrace W1190637 all lose the highest amount of biomass (51.7-59.6%). The landrace W1190488 and barley Bordeaux show similar lower levels of biomass loss (44.4-46.9%), while SY Kingsbarn has the lowest crop biomass loss of all the lines (33%).

These results support the hypothesis, once again showing differences in the competitive ability of crop lines. In these container trials SY Kingsbarn has been seen to be the most competitive. The landraces and elite wheats see little differences in competitive ability; however, the landraces seem slightly more tolerant than elite lines to black-grass pressures. The results for Bordeaux - black-grass competition goes against the hypothesis of barleys being better suppressors of black-grass, however high Bordeaux shoot biomass would suggest Bordeaux maintains black-grass tolerance.

6.6 Field trials

The container trials are a good intermediate experiment between lab and field trials. There may however still be differences in how crop varieties grow between container and field trial conditions. Field trials will best show the true competitive ability of the crop lines. It was hypothesised that differences in competitive ability between crop lines would be visible in this set-up. To test this, the same seven crop lines that were grown in controlled and container experiments were grown in field conditions. They were sown in the autumn and grown through the winter growing season. The crops were sown into plots within a field that had an existing black-grass population however black-grass was also hand sown onto the field to ensure coverage of the total area. Black-grass only controls were included. Plants in field conditions received full chemical treatments (See methods for detailed methodology). All plots were harvested in July, due to the size of the plots and the limited time to carry out the work, two grab samples were taken from each plot and combined into one sample. All plant assessments were taken from those samples. Crop biomass, black-grass biomass and % plot that is black-grass was recorded.

It was observed that black-grass shoot biomass differed significantly between groups (Kruskal-Wallis test, Dunn’s with Bonferroni correction, $P<0.001$) (Figure 6.3A) with both barley varieties having a significantly smaller black-grass biomass in their plots than in the black-grass only control. Although black-grass biomass was clearly much reduced compared the black-grass only plots, no wheat lines statistically significantly reduced black-grass presence. There was also no significant difference between any of the crop varieties in their black-grass presence, however it can be seen that the barleys have a lower black-grass biomass than the two landraces, which in turn have a lower black-grass biomass than the three elite wheat lines. As for crop shoot biomass it was observed that there was a significant difference between groups (Kruskal-Wallis test, Dunn’s with Bonferroni correction, $P<0.05$) (Figure 6.3B) with Elation having a larger biomass than W1190488, on the whole however there was little difference seen in crop biomass across all varieties.

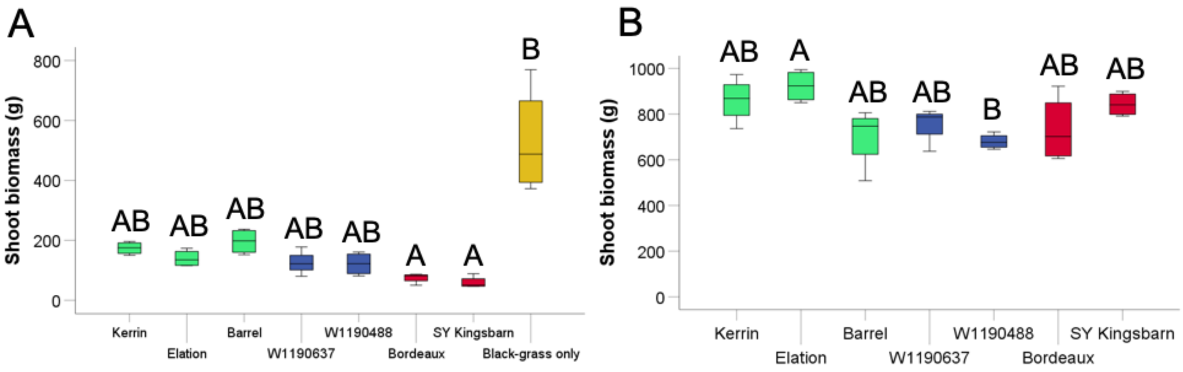

Figure 6.3 Variation in competitive ability in field trials

Boxplots showing crop assessments from field trials (a) showing black-grass shoot biomass when in competition with six different crop lines and as a black-grass only control (b) crop shoot biomass of each line when in competition with black-grass. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Different letters denote significant statistical differences between the groups for (a) (Kruskal-Wallis, Dunn's with Bonferroni correction, P<0.001), n=4, for (b) (ANOVA, Tukey's HSD test, P<0.05), n=4. Colours represent crop type, Green = Elite wheat, Blue = Landrace wheat, Red = Barley, Yellow = black-grass only.

The percentage of the overall plot biomass that was made up of black-grass biomass was calculated. It was observed that there was a significant difference in percentage plot biomass that is black-grass between groups (ANOVA, Tukey HSD test, P<0.001). A wide range of black-grass suppression was seen. Both barleys suppressed black-grass better than all wheats. SY Kingsbarn had the lowest percentage black-grass (6.5%) followed closely by Bordeaux (9.57%). W1190637, Elation, W1190488 and Kerrin all have slightly higher black-grass presence ranging from 12.83-16.96%. Barrel is the worst black-grass suppressor, having the highest percentage black-grass at 22.29%.

These results agree with the hypothesis, successfully showing variation in competition amongst the tested crop lines. They show that barleys are better competitors against black-grass than wheat whilst highlighting the differing ability of wheat lines to tolerate and suppress black-grass. It was also observed that landrace varieties are better than some elite wheat varieties at suppressing black-grass. This indicates that there may be traits within these landrace varieties that are giving them an advantage against black-grass, that have been lost in current elite varieties.

6.7 Competitive ability is consistent across environments

Comparing crop and black-grass shoot biomass between the three environmental set-ups will determine whether the controlled screening approach gives results for crop competitiveness that correspond to the level of competitiveness seen in field conditions. The results of the controlled lab, container and field trial are largely consistent (Figure 6.4). For black-grass growth (Figure 6.4A, B, C) high competitive ability is observed in barleys, followed by landrace wheats with average competitive ability and elite wheats with poor competitive ability. Exceptions to the trend include black-grass biomass in Kerrin pots in the lab environment (Figure 6.4A) and Bordeaux in the container trials (Figure 6.4B). Otherwise, the data supports the hypothesis showing that barleys are better at suppressing black-grass than wheats and that landrace varieties are also better suppressors than elite wheats. Crop biomass is more variable between set-ups, however despite the varying crop biomass, the impact on black-grass remains largely the same. It would be expected to see variation in biomass between set-ups due to differences in the ages of the plants and the space available to them. It is possible some crop lines are naturally better at growing in some conditions than others and that differences in crop biomass are not necessarily down to the presence of black-grass.

The fact that these are largely consistent across environments supports the hypothesis, showing that the controlled environment wheat screening method will give results representative of what would likely be seen in the field, conclusions further backed up by the intermediary container trials. Going forward, this indicates that the controlled screening approach would be suitable for testing a larger number of crop lines for their competitive ability and that the results gained from such a screen would have a good degree of confidence that they would be representative of competitive ability in field conditions.

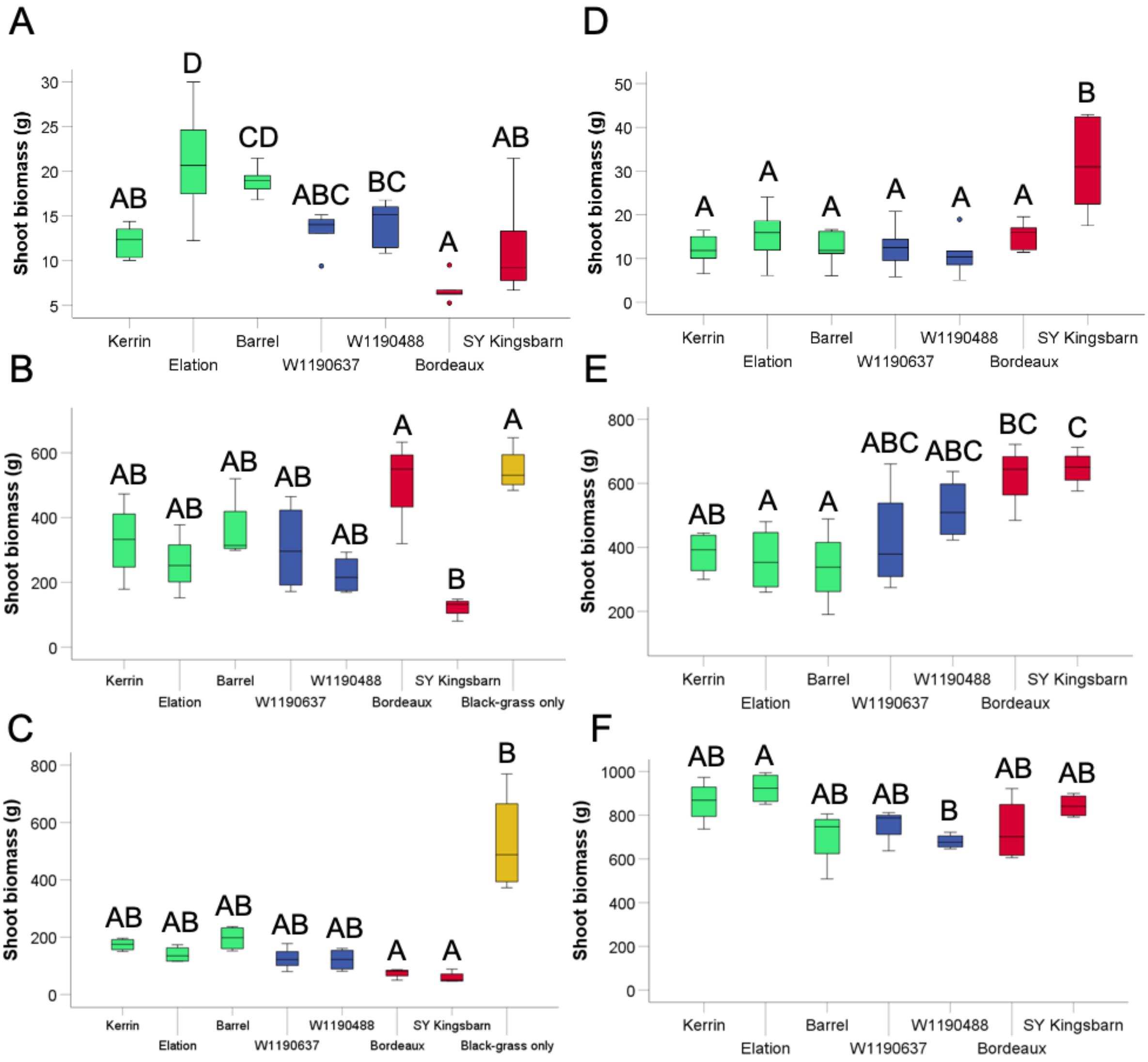

Figure 6.4 Competitive ability of crop lines is consistent across environments

Boxplots showing summary of competitive ability of seven crop lines across three different environments. Boxplots show (a, b, c) black-grass shoot biomass and (d, e, f) crop shoot biomass in (a, d) controlled lab conditions, (b, e) container trials and (c, f) field trials. Different letters denote significant statistical differences between groups (Kruskal-Wallis, Dunn's with Bonferroni correction, P<0.05) or (ANOVA, Tukey's HSD test, P<0.05) (See previous figures). Colours represent crop type, Green = Elite wheat, Blue = Landrace wheat, Red = Barley, Yellow = black-grass only.

6.8 Wheat screen - Crop varieties vary in competitiveness

With a successful screening approach, it is possible to directly screen a large number of crop varieties for their competitive ability. It was therefore hypothesized that crop varieties would show a range of competitive abilities against black-grass. It was also hypothesised that variation in competitive ability amongst current elite wheat lines would also be observed. To test this, current elite wheat varieties, along with two landrace wheats and a number of barley lines for comparison, were grown in the controlled environment wheat screen in the lab under spring conditions. These lines were grown as crop only controls and with black-grass competition at a ratio of black-grass: wheat of 6:1. Final dry shoot biomass of each wheat plant and the total pot black-grass biomass was recorded. From this data, for each crop line, the percentage loss in crop biomass when in competition with black-grass compared to the crop only control was calculated. This would highlight how well each variety can tolerate black-grass presence. The percentage of total pot biomass that was black-grass for each variety was also calculated. This will determine which crop lines are best at suppressing black-grass growth. The change in total pot productivity upon the introduction of black-grass was also calculated, this would determine crop growth strategy in response to black-grass. These three factors would provide a good indication of which lines are better competitors to black-grass along with information on the type of competition occurring, whether it be suppressive or tolerant.

Tolerance

The results show a wide range of tolerance amongst crop varieties to black-grass competition (Figure 6.5) with loss in crop biomass ranging from 12-80%. It is immediately clear that barleys (red bars) are generally more tolerant than most elite wheats (green bars), with the loss in barley biomass ranging from 12-60% compared to 30-80% in elite wheats. SY Kingsbarn, a hybrid barley, shows the greatest tolerance of all lines. Four elite wheat lines show promising tolerance, these lines are Barrel, Skyscraper, Elation and Kerrin. The majority of elite wheats however lose over 60% of their biomass indicating poor tolerance amongst elite varieties. The two landrace varieties show biomass losses of approximately 40%, outperforming most elite wheats and showing results comparative to and better than a number of barley lines. This indicates once more that landrace varieties may retain traits useful in black-grass tolerance.

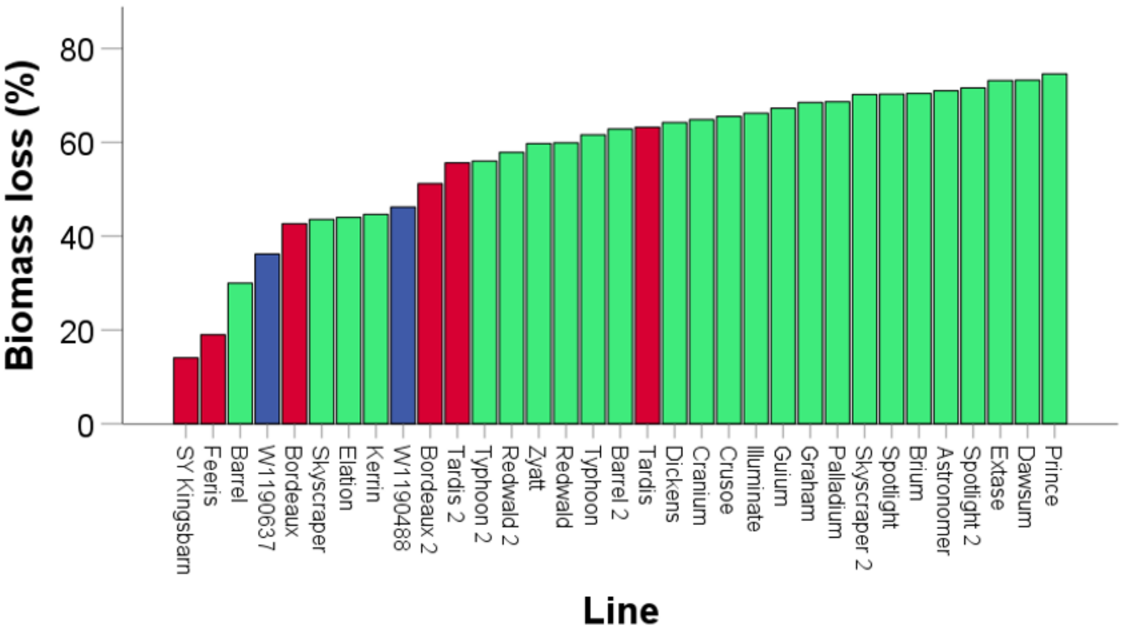

Figure 6.5 Crop lines vary in black-grass tolerance

Bar chart showing the average percentage loss in crop biomass of different crop lines when in competition with black-grass compared to the crop only control. Calculated by taking averages of the crop biomass in the crop only control pots and the average of the crop biomass in the black-grass competition pots. Percentage loss in biomass when in competition compared to the control was calculated from these two averages. Colours represent crop type, Green = Elite wheat, Blue = Landrace wheat, Red = Barley, Yellow = black-grass only.

Some lines were repeated in the wheat screen and are denoted by the number 2 after their variety name on the x-axis (Figure 6.5). For some lines, the results of the repeated experiments were very similar to that of the original screen, for example Redwald and Spotlight both lie very closely to their repeats (Figure 6.65. However, lines such as Skyscraper and Barrel show big differences between screens. It is important to note that conditions for growth although ‘controlled’, can fluctuate beyond our control. Glasshouse (spring) conditions can vary between glasshouses, and within glasshouses, plant position within a tray and plant distance from the lights may also affect how well a plant can grow. Furthermore, these values rely on plants growing well in two different set-ups, both in competition and on their own as the control, in order to then allow the calculation of these values. If one or both of the two set-ups is hampered in growth by external conditions, it could impact the calculated results presented in figure 6.5. In a perfect world, the wheat screen would have been repeated multiple times for every variety, due to time, space and financial limitations, this was unfortunately not feasible to do. Despite seeing some differences in results between repeats, it is promising that the majority of those lines repeated did show similar responses to black-grass competition, giving confidence that the results are representative of the crops ability to tolerate black-grass. Further repeats of lines Barrel and Skyscraper are underway however will not be completed in time for inclusion in this thesis.

Suppression

Crop tolerance (Figure 6.5) is useful for determining which lines can continue to grow in the presence of black-grass, however it says nothing of the crop’s ability to suppress black-grass. To compare crop suppressive ability, the percentage of the total pot biomass that is black-grass was calculated for each pot and thus each line (Figure 6.6).

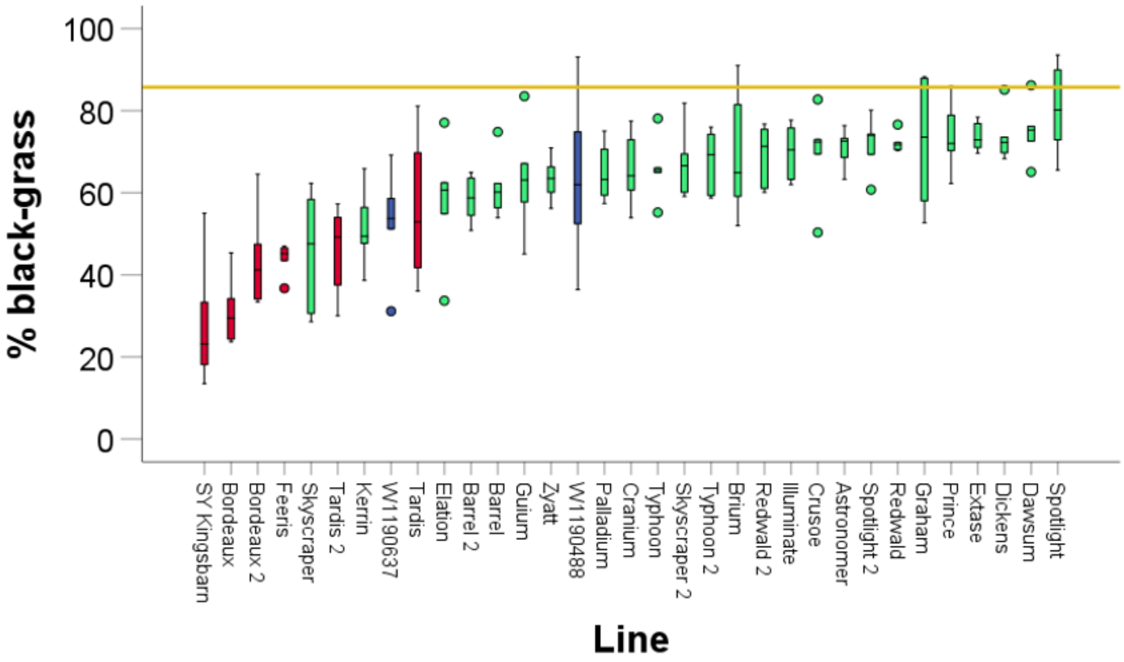

Figure 6.6 Black-grass suppression varies between crop lines

Boxplot showing the percentage of the total pot biomass that is black-grass for different crop lines all in competition with black-grass. The box indicates the interquartile range, the midline indicates the median, the whiskers are the minimum and maximum values. Circles represent outliers, n=6. Yellow line indicates the expected black-grass biomass in a pot if all plants grew to the same size and therefore the effect of competition being null. Bar colours represent crop type, Green = Elite wheat, Blue = Landrace wheat, Red = Barley.

It is clear from figure 6.6, that the suppression of black-grass varies dramatically between crop varieties, with percentage black-grass ranging from a low of 15% to a high of 90%. The yellow line, lying at 85.7% was calculated as (100/7) *6 with 7 being the number of plants in the pot, and 6 being the number of black-grass. This line shows the theoretical level at which the black-grass percentage would reach if no competition was occurring, and all plants grew to the same size. From the results it can be seen that no matter which crop line is planted with black-grass, black-grass will be suppressed to some extent. The distance below this yellow line indicates which lines are better/worse at suppressing black-grass, with those further away being better suppressors.

As seen previously in figure 6.5, again the barleys (red) dominate the left-hand side of figure 6.7. SY Kingsbarn has the lowest percentage black-grass and therefore is the best suppressor. Of the elite wheats, Skyscraper and Kerrin have the lowest percentage black-grass. The majority of elite wheats however have poor levels of suppression. The landrace varieties are better suppressors than the majority of elite wheats, however only W1190637 was able to show similar levels of suppression to that of the barleys with landrace W1190488 showing more variable results.

Suppression v tolerance

The data from Figure 6.5 and Figure 6.6 were plotted against each other (Figure 6.7). A clear trend can be seen, showing that as the percentage of black-grass present in the pot increases, the loss in crop biomass also increases (Figure 6.7), indicating lines with good suppressive ability also show good tolerance.

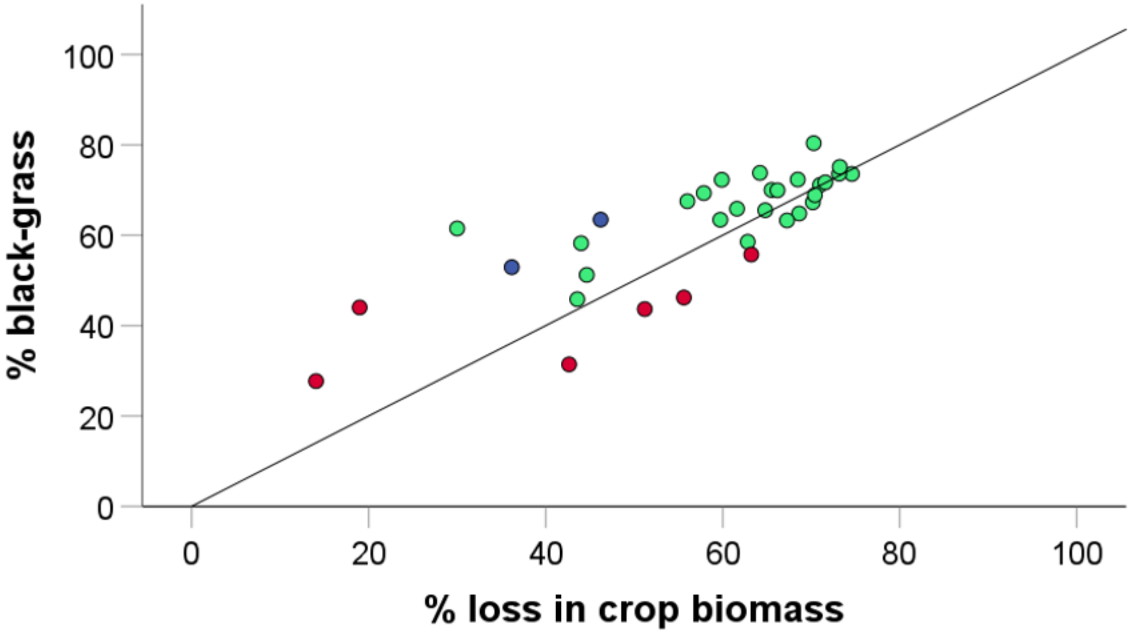

Figure 6.7 Variation in competitive ability

Scatterplot showing the relationship between percentage total pot biomass that is black-grass biomass and percentage loss in crop biomass in competition with black-grass compared to the crop only controls. Points are averages. Line = y=x, representing a zero-sum relationship. Red = Barley, Blue = Landrace wheat, Green = Elite wheat.

Clear clustering of barleys, landraces and elite wheats can be seen (Figure 6.7), barleys tend to have lower blackgrass presence than the wheats, showing good suppression, however some lines are poor tolerators, seeing large losses in biomass even at low black-grass presence. The two landraces are very similar, both showing good tolerance and mediocre suppression. Of the wheats, the landraces show more promise than the majority of elite lines. The elite lines are largely clustered to the top right of the graph (Figure 6.7) indicating poor tolerance and suppression. The line through the origin of the graph indicates a zero-sum relationship. A zero-sum relationship is where the losses of one equal the gains of another, in this case the loss in crop biomass would equal the gain in black-grass biomass. The majority of the wheat lines tested fall away from this line, indicating that wheat – black-grass competition is not a zero-sum game, and that in the majority of cases, the increase in black-grass is greater than the percentage crop biomass loss (Points lying above the line).

The variation in reaction to black-grass presence can be seen when the change in total pot productivity is calculated, comparing how total pot biomass changed with the introduction of black-grass (Figure 6.8).

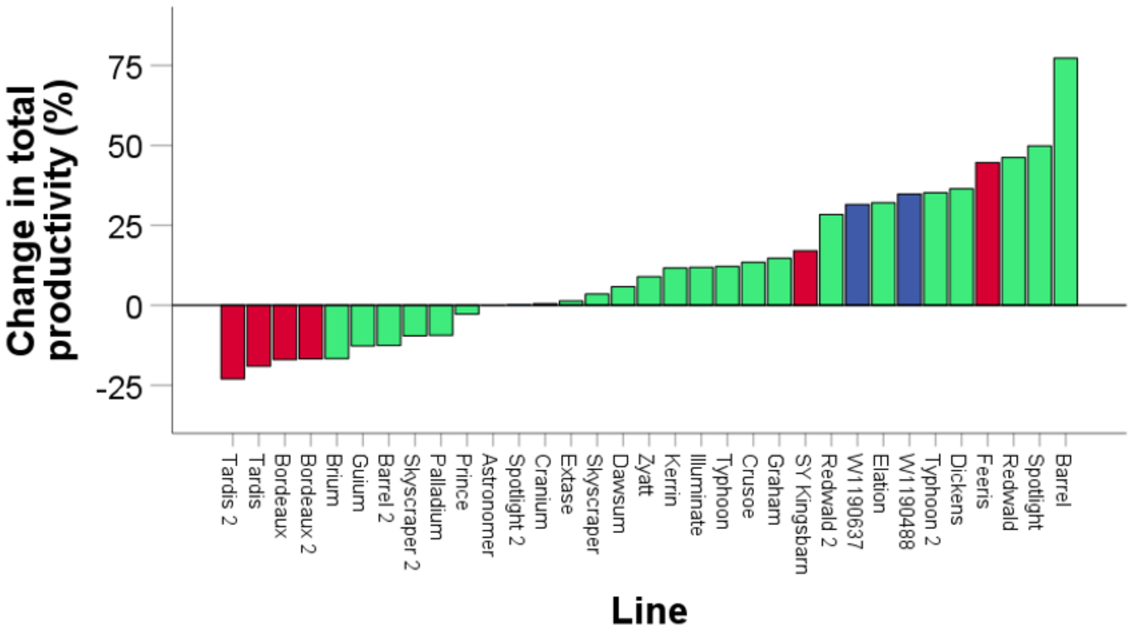

Figure 6.8 Change in pot productivity

Bar chart showing the average change in total pot biomass productivity from crop only pots to crop - black-grass competition pots. Zero-sum line denoted by Y=0. Colours represent different crop type, Red = Barley, Blue = Landrace wheat, Green = Elite wheat. (N=6).

A change in productivity of zero, indicates that the biomass of the crop only control pot is equal to the biomass of the competition pot (Figure 6.8). In these cases, there is a zero-sum relationship. This can be observed in both Astronomer and Spotlight 2, with a few other lines also coming close to a net zero change. A change in productivity of below zero, indicates that the biomass of the crop only control pot is greater than the competition pot. This would indicate some mutual suppression is occurring between crop and black-grass in these competition pots, not allowing for either crop or black-grass to prevail, resulting in an overall decrease in productivity. Amongst these lines are two barleys, both Tardis and Bordeaux with their repeats along with a minority of elite wheats. Two of these elite wheats are the Barrel and Skyscraper repeats which saw positive productivity changes in the original screen. The possible causes of these differences were discussed earlier. The majority of the lines see a positive change in productivity, this indicates that the biomass of the competition pot is greater than that of the crop only pot. This shows that both plants are able to grow in the presence of the other leading to a greater overall biomass. What is interesting though is the range of values seen, it is clear therefore crop varieties differ in their responses to black-grass competition, understanding how a variety responds to black-grass will therefore be vital in the selection of which variety to plant, particularly depending on the current black-grass status of a particular field.

Overall, it is possible to conclude that these results agree with the hypothesis that crop varieties vary in their competitive ability against black-grass, that elite wheat lines also vary in their competitive ability against black-grass with lines utilising different strategies either suppression or tolerance to counteract black-grass competition.

6.9 Discussion

Lab screening approach beneficial compared to current methods of cultivar identification

The results in this chapter have shown that the method developed here for screening crop varieties in a controlled environment is able to reproduce the results seen in both outdoor container and field trials.

The requirement for a quicker, cheaper, and easier approach for competitive cultivar selection has been made evident (Brain et al., 1999; Andrew., 2016). The development of such a system has been long delayed due to the unknown factors involved in wheat – black-grass competition. Trait based screening (discussed in a previous chapter) has the ability to determine cultivar variation in traits thought to convey a competitive advantage. The majority of experiments carried out in cultivar selection compare competitive ability of lines through shoot biomass or grain yield then make assumptions as to what has resulted in these differences based on trait differences between a small gene pool of cultivars. In the majority of cases root growth is ignored. Assessing crops directly for competitive ability against black-gras in controlled conditions clearly highlights lines that are more/less competitive than others, without the possibility of varying environmental conditions hampering the findings. Although the developed screening methods has its limitations, including the fact that winter plants are being grown in spring conditions, it is a method by which many cultivars can be simultaneously assessed and compared which has been shown to give results that are transferable to those seen in field conditions. The approach developed here has decreased the time required to identify competitive cultivars, whilst decreasing the cost and labour involved in full-scale field trials. Using this approach, lines that go on to be tested at larger scale will have already been shown to have some form of competitive advantage rather than varieties being tested based on anecdotal evidence passed from farmer to farmer (Andrew., 2016). It is possible that some genotypes are more susceptible to being grown in unnatural conditions than others and may have given misleading results using this approach, further testing is therefore needed of lines at field scale to continue to confirm the results of the controlled condition screen. Due to limited time and resources, it was not possible to test more lines across the three different environments.

If the controlled screening approach showed results that varied dramatically from those in the field, then farmers would be less likely to trust the findings and a new lab screening approach would be required. It was therefore vitally important that glasshouse results were validated in the field (Bektas et al., 2016). It is clear from the results that in the six crop lines tested across lab, container and filed scale trials, the trend in competitiveness of the six lines was in the most part highly consistent across environments. Of course, it is not possible to rule out the effects of environmental conditions on the container and field trials which if run over multiple seasons in different conditions/locations may have given different results, particularly as environmental conditions have been seen to have a greater impact on plant growth than differences seen between crop genotype (Hodgkinson et al., 2017). Despite the possible hinderances of the environment on container and field results, they remained consistent with lab findings. This data is highly promising showing that the lab screen is representative of field scale results giving good confidence that results gathered in the lab during the screen for competitive cultivars will be representative of how they would expect to behave in the field. This will allow farmers to make confident informed choices on which lines they select when selecting for greater black-grass competition. Continued testing to ensure competitive ability of current elite lines and landrace varieties is required prior to the eventual formation of a recommended list of winter wheats lines for black-grass suppression and tolerance.

Competitive cultivar screen

Analysis of competitive ability through direct competition with black grass has highlighted a wide range of competitiveness amongst crop lines. Work in this chapter has also highlighted the difference in response strategy of different crop lines to blackgrass presence indicating wheat - black-grass competition is a non-zero-sum-game.

The screen for competitive cultivars has successfully highlighted variation in competitive ability amongst species. Barley is known to be more competitive against black-grass than wheat (Cook et al., 2023) the results presented in this chapter agree with the findings of these studies. Consistently, the most competitive lines have been barleys, and the least competitive line have been elite wheats. However, the findings also show that barleys show variation in competitive ability against black-grass with the least competitive barleys being less competitive than the most competitive elite wheats. Suppressive ability of barley has previously been seen to vary between cultivars when in competition with volunteer oilseed rape (Christensen., 1995). It is therefore not as straight forward as assuming planting barley over wheat will increase black-grass control.

Interestingly landrace varieties show better tolerance and suppression than the majority of elite wheats. This would confirm suspicions that landrace varieties have maintained traits able to confer some level of increased competitiveness compared to some elite lines that have lost these traits due to intense selection for harvest index. Lazzaro et al., (2019) highlighted a trend of polymorphism loss between heritage and modern wheat germplasm, indicating lower molecular diversity in modern crop varieties, however many polymorphisms were unique to both heritage and modern varieties. It is therefore essential that both are targeted in order to increase traits not targeted by current breeding programmes such as competitiveness against weeds (Lazzaro et al., 2019). These results support previous findings that suggest landraces have the ability to show increased competitive ability and increased control of weeds (Lemerle et al., 1996). Due to the lack of selection for harvest index in landrace varieties farmers may be unlikely to select them as planting options due to the decrease yield potential and may be much more likely to choose to plant barley instead. The use of landraces may therefore be of more use to breeders who can use them to determine traits allowing them to gain a competitive advantage.

Variation in competitiveness amongst elite wheats indicates the presence/absence of certain traits that convey a competitive advantage to some and not others. Lines with high levels of competition would be good candidates as competitive cultivars. Lines such as Barrel, Skyscraper and Kerrin have shown good levels of black-grass suppression and tolerance compared to other elite lines in this study. However, variation between repeats of lines such as Skyscraper, mean that further testing is required to understand their true competitive ability. Repeats are underway however may not be completed in time for inclusion in this thesis.

It is clear that all crop lines are able to suppress black-grass growth to some extent (Figure 6.7). Variation in the suppressive ability of wheat has been noted in a number of weeds including *Lolium rigidum* (Lemerle et al., 1996) and *Aegilops cylindriea* (Ogg and Seefeldt., 1999). However, the data suggests that there are two different traits affecting competitive ability, the ability to suppress black-grass and the ability to tolerate black-grass. Tolerative ability has also previously been seen to vary in wheat (Challaiah et al., 1986) however tolerance variation has been seen to be lower than that of suppression and to be inconsistent between years and locations (Olesen et al., 2004). This suggests tolerance may be a stress resistant trait rather than a mechanism used to resist suppression from a neighbour. Wang et al., (2010) determined specific traits linked with suppressive ability in Poa pratensis and Achillea millefolium, however they found no evidence to suggest that tolerance was linked to any specific plant traits. They suggest that there is no single mechanism, nor set of traits that lead to enhanced competitive tolerance. Tolerance therefore, is rather a product of numerous plant traits occurring in many possible combinations and is highly variable dependant on the unique environmental conditions of each competitive scenario. The tolerative response will therefore differ dependant on the stress the plant is experiencing.

Generally, suppression and tolerance would seem to be correlated, with lines able to suppress black-grass also being good tolerators. Suppression and tolerance may be inter-related however the findings suggest they are not the same thing, as Barrel appears to have a high level of tolerance but relatively poor suppression, whereas Bordeaux seems to be a good suppressor but poor tolerator. Suppression and tolerance have previously been noted as two separate entities, with there being no clear consensus within the literature as to whether they are indeed linked (Andrew et a., 2015). It is encouraging that the findings presented in this screening method have been able to present the differences in black-grass response strategy of the different crop lines whether that be suppressive tolerative or both.

6.10 Conclusion

This screen tested here has successfully highlighted lines that are good candidates for competitive cultivars through their suppressive and tolerative abilities. Further analysis of these and other lines using this approach is required prior to the formation of a recommended list of crop varieties for black-grass competition. This data can be further used to correlate with root and shoot growth data to determine their roles in competitive ability (Chapter 7).

Chapter 7 Correlations between competitive ability and growth

7.1 Introduction

How wheat and black-grass compete with one another remains an important area of study. Research has looked in detail at the impact of above ground traits on black-grass competition and has highlighted certain traits thought to increase the crops competitive ability (Lemerle et al., 1996; Andrew., 2016). However, due to the many possible different factors affecting competition it remains difficult to prove a causation between single traits and competitive ability. Competitive ability is therefore a sum of a combination of traits, all of which contribute to the overall competitiveness of the plant. The contribution of root growth in wheat to overall competitive ability against black-grass is unknown.

The essential nature of root growth in water and nutrient acquisition would make it a prime candidate as a competitive trait. Vigorous root production in the pursuit of resources can lead to one plant gaining a competitive advantage over another (Andrew et al., 2015). In wheat, it has been suggested that there is a key link between shoot and root production (Wheeldon., 2023), assessing the role both roots and shoots play in competition and whether there is any correlation between the two will be key to understanding the growth response of wheat to black-grass competition.

Predicting competitive ability of crops has been shown to be difficult but possible, Hansen et al., (2008) developed criteria by which to rank the suppressive ability of spring barleys. They found traits that determined competitive ability included red-far red light reflectance (GS31), leaf area index (GS65), leaf angle and culm length. However, rankings were observed to differ between testing location reducing the strength of the predictions. Interestingly no below-ground traits were included in the ranking criteria, but given what has been found in this thesis, it is likely inclusion of below-ground traits would be vital in any future predictions. This is not to say above-ground traits should be ignored; indeed, the complex set of interactions between roots and shoots suggests dual involvement in competition.

7.2 Aims

Identifying traits that convey a competitive advantage against black-grass will make the process of identifying competitive cultivars much easier. Throughout this thesis, the focus has been on assessing crop root growth as a competitive trait. In this chapter data from the competitive wheat screen and root growth screens will be compared to determine whether there are any correlations between root growth and competitive ability in wheat. Furthermore, in each of the experiments in this thesis data looking at both shoot biomass and crop tiller number has been collected. Assessments therefore will also be made as to determine whether there is a correlation between above ground traits and competitive ability against black-grass. Finally, whether there is a correlation between shoot and root production in wheat is assessed to determine if large shoot production results in an equally large root system or whether there is a trade off in either trait as one increases. The main aims of this chapter were:

- To determine whether wheat root production correlates to its competitive ability against black-grass.
- Determine whether wheat shoot production correlates to its competitive ability against black-grass.
- Determine whether there is a correlation between root and shoot production in wheat.

7.3 High crop root growth correlates with black-grass suppression

To explain whether the competitive ability of crops is correlated to their root growth, data from the controlled competition screen was compared to root growth data from the rhizobox root screen. The reasons for using the rhizobox root data over the hydroponic root data has been discussed in a previous chapter. It was hypothesised that increased root growth in wheat would increase its competitiveness against black-grass.

Initially, analysis compared the loss in crop biomass when in competition with black-grass against the rhizobox root growth of the same cultivar (Figure 7.1A). It can be clearly seen that there is no correlation between the two. However, despite there being no correlation there seems to be clear groupings of barley and landrace lines. It was observed that these lines tend to behave differently to the elite wheats. For the landraces (Purple) there was a small loss in crop biomass whist root growth was far smaller than any elite line (Figure 7.1A). As for barley, three of the four lines tested show that barley lines with lower losses in crop biomass also have increased root growth (Figure 7.1A).

The story is similar when loss in crop biomass is replace by percentage black-grass biomass (Figure 7.1B). Here there seems to be a more negative correlation between root growth and black-grass suppression however the correlation is a weak one. Again, there are groupings of both landrace wheats and barleys similar to that in figure 7.1A. These results however are likely skewed by the presence of the landraces. The landraces show poor root growth in spring rhizoboxes whereas in winter hydroponics, which better resemble the real conditions landraces prefer to grow, root growth is much higher. Root growth of landraces in rhizoboxes is therefore most likely not representative of real root growth. When landraces are removed, a moderate negative correlation is seen between root growth and black-grass presence (Spearman's rho, R_s (22) = -0.402, P=0.064) (Figure 7.1C).

In barley, lines with increased root growth also have a lower percentage black-grass growth (Figure 7.1C). This indicates that one reason why barleys are more competitive than wheats against black-grass may be due to greater root growth. Shoot growth may also be playing a role in competitive ability of barley and should not be ruled out as a factor. It has previously been seen that barley is larger than wheat in growth both above and below ground. These results also show that there is variation in how well different barley lines can compete with black-grass (Figure 7.1C), therefore if barleys are being used in crop rotations, some may be more effective than others at depleting the black-grass seedbank.

Overall, these findings agree with the hypothesis showing that increased root growth is correlated to a crops suppressive ability against black-grass. However, this doesn't however prove a causation. It is possible that it is simply the case that the larger the plant is, the more competitive it is. Given the plausibility of above ground growth impacting competition the importance of root growth on competition can be neither accepted nor rejected.

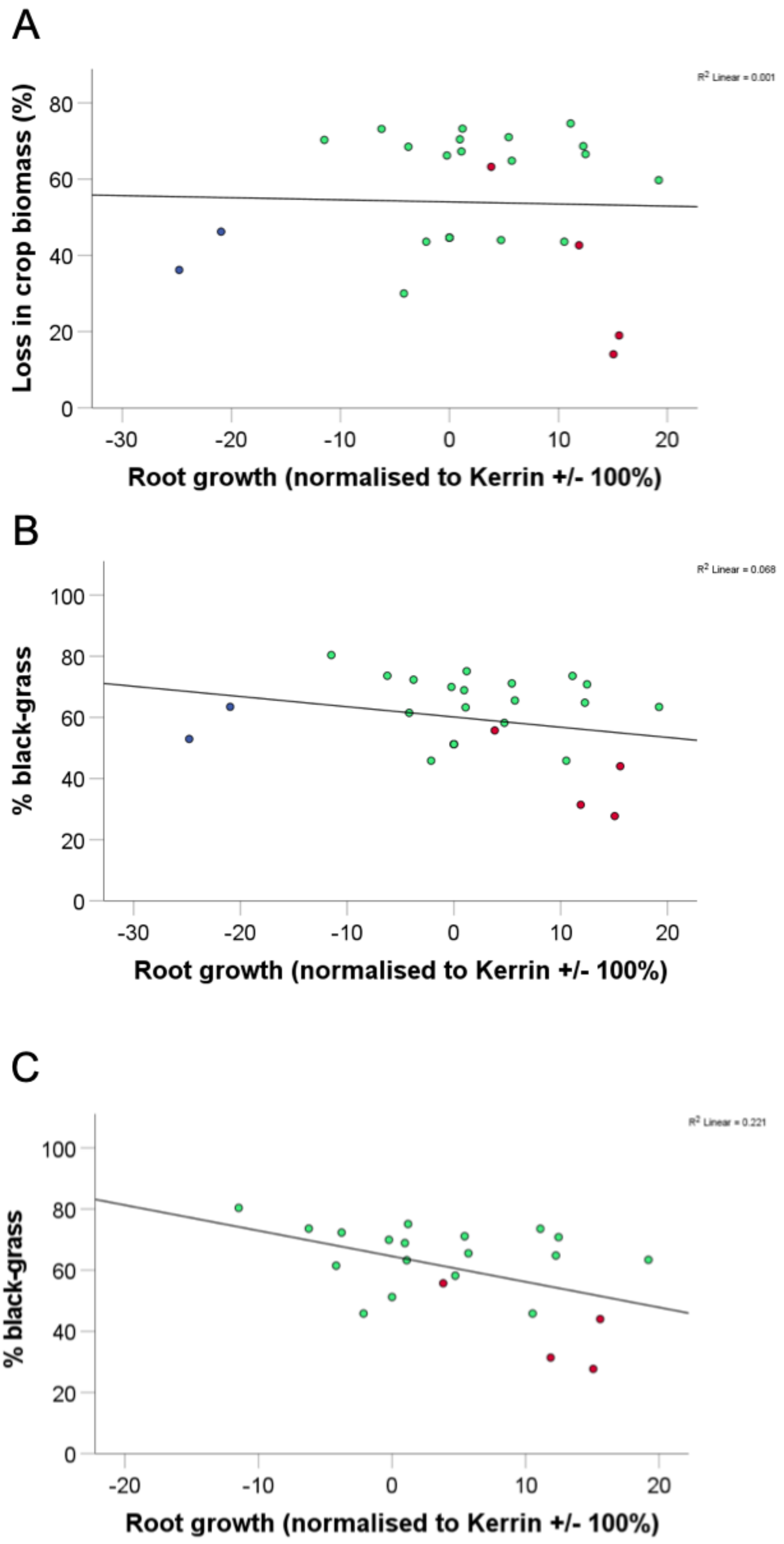

Figure 7.1 Barley and landraces show differences to elite lines

Scatterplots showing the relationship between competitive ability and root growth, root growth assessed by % coverage of the rhizobox surface (a) percentage loss in crop biomass in competition with black-grass, relative to crop only controls, plotted against *root growth in the rhizobox assay as a percentage of the internal Kerrin control sample (b) percentage of the total pot shoot biomass that is blackgrass, in crop/blackgrass competition samples, plotted against root growth in the rhizobox assay as a percentage of the internal Kerrin control sample (c) excluding winter wheat landraces, showing the relationship between the percentage of total pot biomass that is black-grass, plotted against root growth in the rhizobox assay as a percentage of the internal Kerrin control sample. Kerrin root growth =100%, represented as zero on the x-axis. Colours represent crop type, red = barley, blue = winter wheat landrace, green = elite winter wheat. Trendlines represent linear lines-of-best-fit.*

7.4 Shoot growth is not correlated to competitive ability

Previous results have shown that when grown in winter conditions barleys and black-grass have stronger root and shoot growth than wheat, resulting in the hypothesis that larger root systems increase competitive ability. It has been observed that for some wheat lines this may be true, however for most, root growth seems not to be the most important factor affecting competitive ability. It was therefore hypothesised that greater above-ground growth would increase competitive ability of crop lines against black-grass. To test this, crop biomass and maximum crop tiller number in the crop-only controls (as a measure of inherent shoot growth) without black-grass competition was plotted against the percentage loss in crop biomass when in competition with black-grass.

Interestingly, there was no correlation between crop biomass without competition (Crop only controls) and the loss of crop biomass in competition (Spearman’s rho, R_s (24) = -0.033, P=0.88) (Figure 7.2A). There was also no correlation between crop tiller number without competition (Tiller number of the crop only controls) and loss of crop biomass in competition (Spearman’s rho, R_s (24) = -0.039, P=0.855) (Figure 7.2B). There are many crop varieties with different crop biomass and tillering capacities which are poor tolerators of black-grass, losing around 70% of their biomass. Even lines with good tolerance show variation in crop biomass and tiller number. This indicates that neither crop biomass nor tiller number play a role in black-grass competition.

It can therefore be concluded that the results reject the hypothesis that the larger the inherent shoot growth of a crop the greater its black-grass tolerance. Crop biomass and tillering itself is a response to competition rather than a process used to effect competition. Plants which are capable of producing a larger biomass above-ground when in competition with black-grass due to factors such as enhanced winter growth rate or early vigour, may see cascaded advantages that in turn aid in competition. In this case shoot growth may be a method by which crops gain a competitive advantage through access to light.

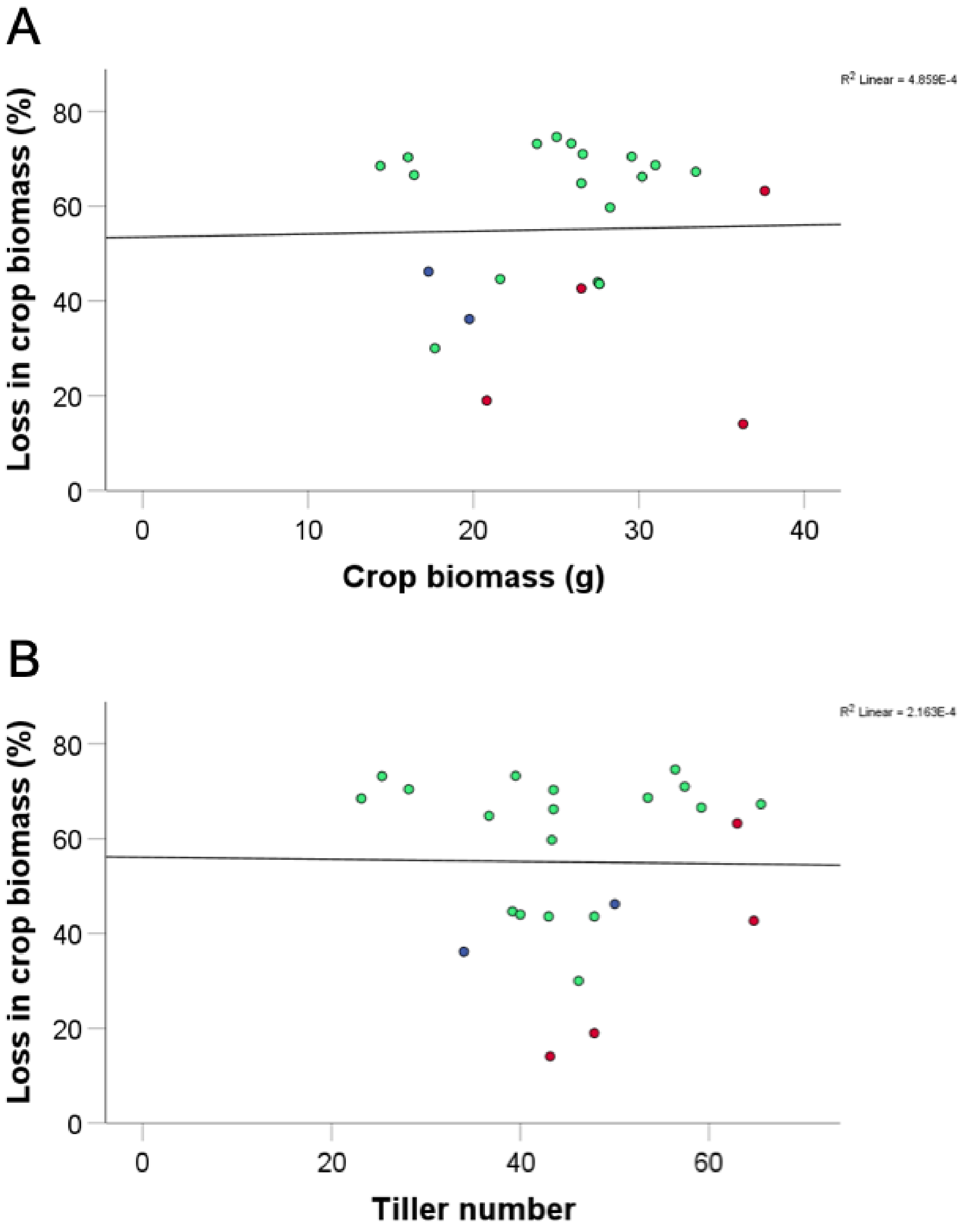

Figure 7.2 Loss in crop biomass not predicted by biomass without competition

Scatterplots showing the relationship between loss in crop biomass in competition and crop shoot growth without competition (a) loss in crop biomass in competition with black-grass plotted against final crop biomass without competition (b) loss in crop biomass in competition with black-grass against max crop tiller number without competition. Colours *represent crop type, red = barley, purple = winter wheat landrace, green = elite winter wheat. Trendlines represent linear lines-of-best-fit.*

7.5 Clear links between shoot and root growth in winter conditions

Prioritising one area of plant growth has often been seen to result in trade-offs in other areas of growth. In chapter 5, variation in root growth was seen between crop lines within both spring rhizobox and winter hydroponic conditions. Due to their different levels of root growth, it is possible that their shoot growth size is potentially altered as a trade-off for root production and *vice versa*. Previous findings in chapter 3 highlighted that both shoot and root production in barley and black-grass were larger than that of wheat when grown in winter conditions. This would indicate that root and shoot production are linked and that the production of a large shoot system doesn't necessarily result in a negative trade-off in root system size. It would make sense that a larger root system would be required to support a larger shoot system. It was therefore hypothesised that in both spring and winter conditions, crop varieties with larger shoot systems would also have greater root systems. To test this, shoot growth was compared to root growth in both spring rhizobox and winter hydroponic experiments.

In the rhizoboxes, it was observed that there was a non-significant weak positive correlation between shoot biomass and root growth (Spearman's rho, R_s (28) = 0.289, P= 0.136) (Figure 7.3A). Two of the four barley lines were observed to have both high shoot and root growth. One barley line had large shoot growth without large root growth and one barley had large root growth with low shoot growth. The landraces have an average shoot biomass but low root growth in spring conditions, as previously discussed the low root growth of these lines is likely anomalous given the high root growth seen in winter conditions. Black-grass has both low root growth and low shoot biomass in the rhizobox system. It is possible that black-grass acts similarly to the landraces and does not grow well in spring conditions and prefers the colder winter in which to grow roots. Of the elite wheats the majority vary very little in terms of root growth, however the lines with the highest shoot biomasses also have amongst the greatest root growth. Maximum tiller number achieved by each plant when grown in the rhizoboxes was also measured. It was observed that there was no correlation between tiller number and root growth (Spearman's rho, R_s (28) = -0.075, P= 0.704) (Figure 7.3B). For elite wheats, tiller number was generally consistent despite differing root growth. Three of the four barleys had a low tiller number despite the high root growth, these results suggest barleys make larger tillers rather than more. The landraces also had a low tiller number however their root growth was small. Black-grass however produced a lot more tillers than any crop line, however as can be seen from the biomass results, these tillers albeit numerous, were very small, with black-grass remaining weedy throughout the ten weeks of growth.

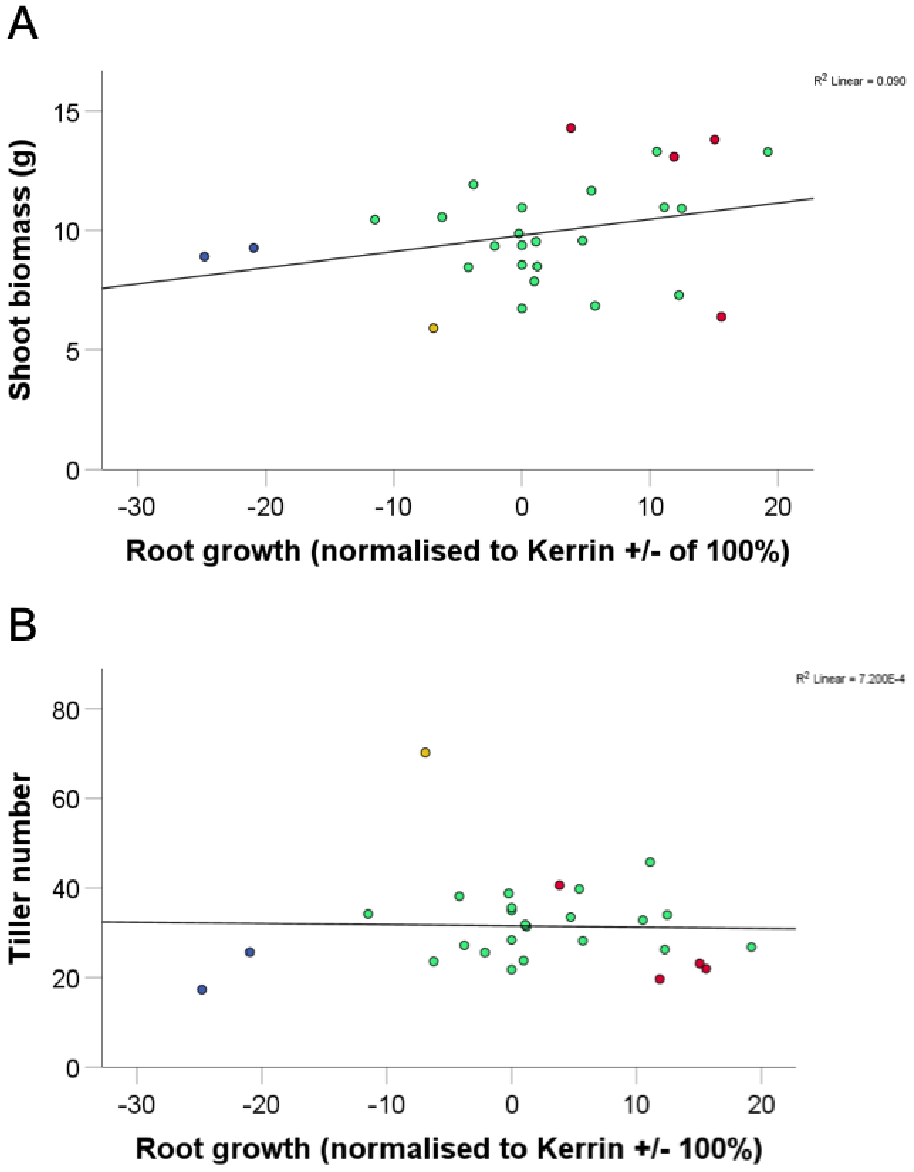

Figure 7.3 Rhizobox shows correlation between shoot and root growth

Scatterplots showing the relationship between crop shoot growth and crop root growth when grown in rhizoboxes in spring conditions (a) crop shoot biomass v crop root growth (b) crop tiller number v crop root growth. Colours represent crop type, red = barley, purple = winter wheat landrace, green = elite winter wheat. Trendlines represent linear lines-of-best-fit.

Correlations were more pronounced in the hydroponics. The hydroponics were placed in winter conditions, the relationship between shoot and root biomass and tiller number and root biomass was plotted for all lines tested. It was observed that there was a significant strong positive correlation between crop shoot biomass and crop root biomass (Spearman's rho, R_s (16) = 0.924, $P<0.001$) (Figure 7.4A). Although the specific positioning of correlation lines may be skewed due to the separation of the different batches, it is clear that as shoot biomass increases so does root biomass. Tiller number was also compared, it was observed that there was a significant strong positive correlation between crop tiller number and crop root biomass (Spearman's rho, R_s (16) = 0.7, $P<0.01$) (Figure 7.4B). Although the correlation is not as strong as shoot biomass it is still clear that larger tiller number coincides with increased root growth. Obviously, there are differences between the two root screening methods which may be affecting the results. The data shows a greater correlation between shoot and root growth in winter conditions than in spring conditions. The crop lines tested are all winter varieties which in real conditions would experience both spring and winter conditions, growing them solely in spring conditions will not be natural for them, as they will not have had the prior winter therefore this data does not take into account any differences in growth that may have accrued over winter, growth that may have affected later growth in the spring.

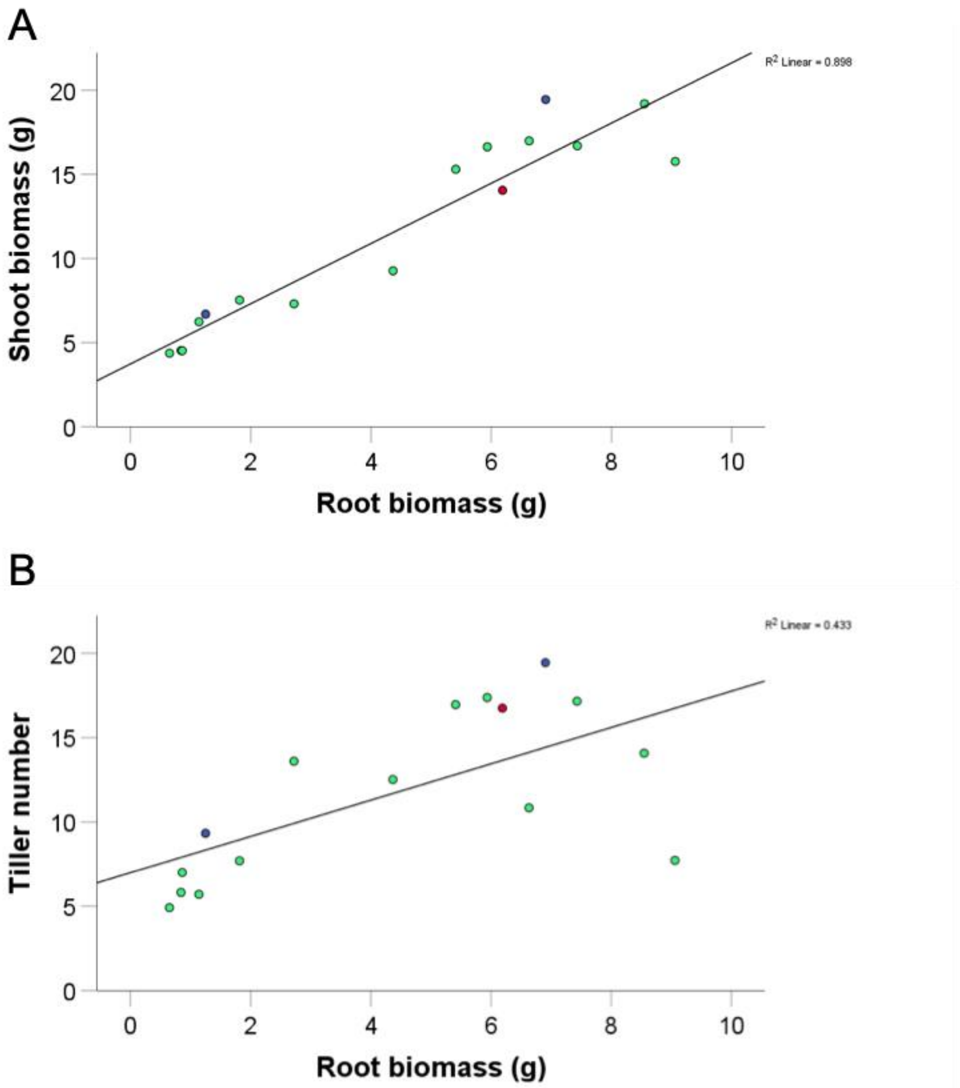

Figure 7.4 Hydroponics show correlation between shoot and root biomass

Scatterplots showing the relationship between crop shoot and root growth in hydroponics in winter conditions (a) crop shoot biomass v crop root biomass (b) crop tiller number v crop root biomass. Colours represent crop type, red = barley, purple = winter wheat landrace, green = elite winter wheat. Trendlines represent linear lines-of-best-fit.

7.6 Discussion

Impact of roots on competitive ability

The results of this chapter suggest root growth may be important for competition against black grass in some crop varieties (figure 7.1 C). However, it is clear that root growth is not a universal requirement of increased competition against blackgrass.

Root systems have long been known to provide benefits to plant survival. Vigorous early root growth has been shown to increase nitrogen uptake in young plants (Liao et al., 2004). Whilst adult plant drought tolerance has been linked with the angle of seminal root emergence at early growth stages, with this angle controlling root distribution over depth as the plant ages (Manschadi et al., 2008). Optimization of root system architecture has been highlighted as a possible method of enhancing nitrogen uptake efficiency this would reduce the requirements for nitrogen fertilisers leading to environmental and economic benefits for farmers (Foulkes et al., 2009). In rice root growth has long been linked with its competitive ability against weeds (Gibson and Fischer., 2001), rice cultivars with increased dry root weight have been seen to decrease the dry weight of the weed Watergrass. Due to these findings, it has been suggested that high yielding, weed-suppressive rice cultivars be selected based on root traits (Gibson et al., 2003). Despite the known benefits of increased root production wheat varieties released in the last 50 years have progressively seen reduced root biomass, length and density (Aziz et al., 2017) These reductions in wheat root systems have unintentionally been selected for in modern cultivars (Waines and Edhaie., 2007) due to the focus of breeders on increasing harvest index. In doing so negative trade-offs in other areas of plant growth have seen the development of more environmentally susceptible crop cultivars. It has been suggested that root biomass of elite wheats is less than 2/3 that of some landrace varieties (Waines and Edhaie., 2007). It would therefore seem that root growth would be vital in reversing the environmental susceptibility unintentionally bred into modern elite line. The high rooting ability of black grass suggests the use of roots in competition against crops. The large root growth of barley indicates a method by which to counteract the competitive nature of black grass.

One possible reason why correlations between root growth and competitive ability are limited is likely due to the experimental setup. The limited volume of the rhizoboxes maybe limiting crop root growth and therefore preventing the true range of root growth to be visualised. The results for wheat root growth lie within -10% and +20% that of the Kerrin control. Altering experimental setup to use larger riser boxes with greater soil volume that allow for deeper rooting roots would allow for more accurate visualisation of root growth of the crop lines and allow for clearer differences to be seen between cultivars. Due to limitations in assessing root growth it was not possible to use larger rhizoboxes. Detailed analysis of crop and black-grass root growth in competitive scenarios is required to fully understand roots interactions between the two. Detailed phenotypic analysis would then hopefully highlight any specific root traits in wheat that convey competitiveness. Utilisation of competitive barley lines as seen here, would allow for comparisons of root systems to aid in competitive trait identification.

It is likely that root growth is playing some role in competition with black grass whether that would be directly for resource or space capture below ground or indirectly through aiding shoot production. It is however clear that root growth alone is not universally important when it comes to competing with black grass.

Impact of shoots on competitive ability

The results of this chapter highlight that inherent crop shoot growth has no apparent role in black-grass tolerance (Figure 7.3). Crops do therefore not increase tiller count to compete with black-grass. Rather, tiller count is affected by black-grass, the ability to tolerate black-grass may result in higher tiller numbers when in competition.

Above-ground growth in wheat has long been suggested to aid in competition not only against weeds but neighbouring and competing plants. Above-ground biomass before stem elongation, tillering index, plant height and flag leaf morphology have all been linked with increased competitive ability of wheat against weeds (Andrew et al., 2015). Lemerle et al., (1996) determined highly competitive wheat cultivars had quick biomass accumulation early in growth, were able to produce a large number of tillers, were tall and also leafy. Tiller number and rate of tiller production have been shown to be morphologically plastic and density dependant, reducing in the presence of competition from both intra and interspecific neighbours in wheat, varying dependant on cultivar (Huel and Hucl., 1996). This reduction in tiller number has a knock-on effect reducing fertile head production and thus grain yield (Andrew et al., 2015). Although high tiller number may be a sign of increased tolerance, it is not necessarily the factor actually driving competitive ability. Other studies agree with the findings presented here and have seen no value of increased tillering on crop competitive ability (Moss., 1985). It may be that the benefit of increased tiller number is only seen in crop stands of low density where shading is low, high shading cultivars in these situations may be better adapted to compete if they are higher tillering. In dense stands, shading is automatically increased by plant numbers therefore individual plants tiller number is less important in weed suppression as stand tiller number is high (Hoad et al., 2006).

Andrew et al., (2015) suggest that tiller counts, and rate of tillering may give misleading results in regard to competitive ability against weeds and indicate tiller loss should be taken into consideration. Due to the experimental set-up used in this study, winter wheat was un-vernalised and grown in spring conditions for three months, admittedly these are not perfect growth conditions for these plants however this was the only set-up suitable for quick, large scale screening allowing visualisation of black-grass competition. Maximum tiller number rather than tiller loss was used as determination of tiller production as the plants did not go through their natural growth cycle. In hindsight the vernalisation of lines prior to placement in spring conditions may have been more suitable.

Shoot and root growth

Further comparisons between shoot and root production highlights clear positive correlations between root and shoot production in crops grown in winter conditions (figure 7.4). Correlations were less clear in spring conditions (figure 7.3) possibly due to plants not having firstly gone through winter growth. These findings suggest larger crop root systems result in larger shoot systems and vice versa.

Wheat cultivars with larger root systems have been shown to have increased above ground growth including leaf area and leaf biomass 25% greater than smaller rooting cultivars, it is likely that the higher photosynthetic capability of increased above ground growth, supplies increased carbon to be assimilated to the roots increasing root growth. In turn the increased root system allows for increased supply of water and nutrients to the larger shoot system (Figueroa-Bustos et al., 2018). Increased resource capture via larger root systems in comparison to smaller rooting neighbouring plants will give the greater rooting plant a competitive advantage.

The findings presented in this chapter highlight that larger root and shoot systems coincide with one another in winter crops rather than having a negative trade-off in one trait in order to increase the other. In figure 7.4 it is possible that some of the outlying cultivars are altering growth by either increasing tiller number or by increasing tiller size. It is clear one line in particular with high root growth has a small tiller number whilst maintaining high shoot biomass. Further work is required to determine plant strategies when it comes to choice of tiller size or number and if this has any correlation to root production. Wheeldon., (2023) highlighted that shoot and root production are linked in wheat, the findings presented here agree with this.

7.9 Conclusion

In summary, given that root growth is vitally important to plant survival, it is very likely that root growth does plays a role in competition with neighbouring plants including weeds. The data presented here fails to prove causation between crop root growth and competitive ability however provides enough evidence to suggest root growth can in some capacity impact the competitive ability of crops against black-grass. It has been suggested that breeding for root system architecture should form the basis of a second

‘Green Revolution’ aiming to increase the efficiency of crop root systems, to increase resource capture to further increase yield to keep up with the ever-increasing demands by an increasing population (Lynch., 2007).

Chapter 8 General discussion

Black-grass competition – A new perspective

The data presented in this thesis provides a new perspective on black-grass competition, and particularly as to how black-grass is a much more effective competitor in plantings of winter wheat then in plantings of spring wheat or barley. Low competitive ability of black-grass in spring wheat indicates environmental conditions and low levels of black-grass germination are impacting competition. Black-grass was seen to be very weedy, being very slow to germinate and establish in comparison to elite wheat lines (Figure 3.1). As a result, black-grass takes time to reach a sufficient size to compete with neighbouring wheat seedlings even at high black-grass density (Figures 3.2, 3.3). These findings are supported by Lutman et al., (2013) who found changing the planting density of crops had no impact on the presence of black-grass until the summer indicating a delay in black-grass competition. In shorter spring plantings black-grass is unable to accumulate a competitive advantage due to its lower growth rate in warmer conditions compared to wheat (Figure 3.4), while the same comparison in winter conditions shows black-grass to have faster growth.

The combination of increased growth rate and length of the winter growing season probably allows black-grass to gain a competitive advantage over winter wheat through increased root growth (Figure 3.4). High levels of root growth allow black-grass to dominate wheat underground, allowing black-grass to start outgrowing wheat above-ground by late spring. Root growth of winter wheat has been seen to reach soil depths twice that of spring wheat despite having the basic same root growth rate, so the increased time available allows winter wheats to produce larger root systems (Thorup-Kristensen et al., 2009). It is therefore likely that this same increase in time is vital for black-grass to produce a large root system. This may explain the sudden appearance of black-grass in the later spring in fields of winter wheat, that up to the spring had seemed relatively unaffected.

If winter conditions alone were enough for black-grass to gain a competitive advantage against winter wheat, then it should also be more competitive against winter barley. However, the different competitiveness of winter wheat and barley indicates some fundamental differences between the species impacting their competitive abilities. Winter barley has been seen to have a faster growth rate in winter conditions than winter wheat and is able to produce a larger root system (Figure 3.4). Previously, above ground traits have been linked with barleys increased level of competition (Cook and Roche, 2018) however below-ground comparisons had not been made. These findings would suggest that root growth is an important trait for competing with black-grass.

Further work is required to more extensively test this root growth theory. Utilising high/low root growth variants (or mutants) of the same wheat cultivar, differing only in root growth, would more clearly test if root growth determines competitive ability.

The second ‘Green revolution’ – Return of the roots

The ‘Green revolution’ led to a trend in wheat breeding to proportion a greater amount of a crop’s biomass into the seeds, increasing harvest indexes (Reynolds, Rajaram and Sayre., 1999). This has made modern elite lines very high yielding when grown in non-stressful conditions. This selection has resulted in trade-offs in other areas of growth which may have an overall negative effect on the plant, making it increasingly prone to losses under abiotic and biotic stress. Above-ground, trade-offs linked with selection for harvest index include a reduced biomass such as crop canopy size and reduced flag-leaf size (Reynolds, Rajaram and Sayre., 1999; Porker, Straight and Hunt., 2020; Hansen et al., 2005; Blake et al., 2007). Reduced canopy size and leaf area may increase susceptibility and effectiveness of pathogens. Trade-offs may also be occurring below-ground. The selection for harvest index has been linked with reduced root growth in both Chinese and UK wheat lines (Zhu et al., 2019; Fradgley et al., 2020). The adoption of high-input agricultural practices has reduced the requirement of roots for nutrient acquisition, allowing small rooting crop lines to be successfully grown. It has been suggested that modern elite lines have root growth less than two thirds that of older landrace varieties (Waines and Edhaie., 2007), resulting in crops susceptible to drought as their roots are unable to reach deeper water stores (Chapagain et al., 2014). The results presented here indicate that reductions in root growth have increased susceptibility of elite crops to environmental stress including from competitive weeds that monopolise space and resources due to their greater root systems (Figure 34.).

Breeding for competitiveness and breeding for root growth is currently not on the agenda for crop improvement. The findings presented here suggest that one method of increasing competitiveness of winter wheat against black-grass would be by restoring root system size to that of pre-green revolution cultivars. Limited variation in root growth was seen amongst the elite lines tested, possibly due to experimental design limiting root production. In barley and landrace wheats root growth and competitive ability are generally higher than elite wheats (Figures 5.7, 6.5, 6.6). In other species such as sugar beet, root growth has been shown to increase the crops competitiveness against weed species (Stevanato et al., 2011), with increased crop rooting linked with increased nitrogen uptake, resulting in greater weed suppression (Casper and Jackson., 1997; Tilman and Wedin., 1991). Reversing the general direction of wheat breeding to increase root growth would have other benefits for agriculture. Firstly, larger, denser root systems would be far better at nutrient acquisition in the form of soil residue or fertiliser, reducing the need for chemical input. Secondly, increased spates of drought and water-logging require plants with larger, deeper penetrating root systems to survive therefore increasing crop yields through ever more adverse weather conditions. Thirdly, increased root systems will aid in carbon storage within the soil (Dignac et al., 2017), increasing soil health and productivity (Chaperro et al., 2012). It has been suggested by Lynch., (2007) that root system architecture (RSA) should form the basis of a second ‘Green revolution’ to increase below-ground resource capture to increase yields to meet the needs of an increasing global population. Introducing greater genetic variation will aid in breaking current grain yield barriers (Bektas et al., 2016). Genetic diversity is key to achieve progress in breeding (Bektas et al., 2016), with it estimated that 75% of genetic diversity has been lost in the last 100 years (Pingali., 2012). The focus on root systems will make wheat more resilient and durable and would contribute towards combatting yield losses caused by black-grass infestations.

Identification of competitive cultivars – Attack of the crops

The possibility of competitive cultivar use for natural weed suppression has long been discussed but has been poorly implemented. Uptake of competitive cultivars by farmers is low due to high field variation in the success of these cultivars. The problem therefore lies with the lack of cultivar testing. Competitive cultivars have been shown to have potential, improving black grass control by 8-45% compared to less competitive varieties (Moss and Lutman., 2013). However, these studies are limited to, and only useful within the tested cultivar pool. For competitive cultivars to be successfully used in IWM, first a reliable, quick, easy, and cheap approach for testing and identifying such cultivars is required.

The screening system developed in this thesis allows for a relatively quick and easy approach for competitive cultivar identification. The findings are backed up by container and field trial data showing consistency in results across environments (Figure 6.4) indicating the results of the screen can be used to predict competitive ability in the field. Further field-scale trials are required to ensure these results for all lines. Different field conditions such as soil nutrient levels, water availability and soil type will alter the competitive ability of cultivars dependant on their individual preference for certain environmental conditions. Further work varying the controlled conditions for increased waterlogging, drought, and varying nutrient level conditions (As seen in Figure 4.8) would be useful to predict the best cultivars for each set of conditions.

Based on this data conclusions may be drawn on the best varieties to plant based not only on black-grass presence but on the environmental conditions at the individual field level. Such a thorough undertaking should provide enough data so that farmers will have confidence in variety selection based on the individual field conditions. This would not only help increase competitiveness against black-grass and improve winter wheat crop yields but also increase farmer confidence and uptake of competitive cultivars as a cultural alternative to IWM.

Shoot and root growth

Above-ground growth has long been implicated in increased competition against weeds in crops (Lemerle et al., 1996; Andrew et al., 2015). The majority of studies assessing phenotypic traits associated with competitive ability have focussed only on above-ground traits, ignoring roots due to the difficulties of root assessments in the soil (Gregory, 2007). There is a requirement for shoot and root systems to co-operate to ensure successful plant survival, ensuring the continued function of above-ground processes such as reproduction and photosynthesis and below-ground processes such as water uptake and nutrient acquisition (Puig et al., 2012). Crop improvements have long disregarded root traits due to a lack of understanding of root structure and function (Waines and Ehdaie., 2007). The findings presented here have shown correlations between shoot and root growth in wheat (Figure 7.4), with increasing root growth corresponding to increased shoot growth when grown in winter conditions in hydroponics. This finding agrees with other studies that have also seen positive correlations between shoot and root biomass in winter wheat (Bektas et al., 2016). It is therefore clear that selection for below-ground traits may also increase above-ground growth and indirectly impact competitive ability.

A better understanding of the role of hormone signalling in root and shoot development is required to optimise plant growth. Hormones such as Auxin, Cytokinin, Strigolactone and many others (reviewed in Saini et al., 2013) can affect shoot and root growth either directly or through inhibition of other hormones. How exactly these hormones control plant growth in response to environmental changes and nutrient acquisition is not yet fully understood (reviewed in Puig et al., 2012), there is a clear lack of detailed understanding of auxin biology in the ‘big three’ cereals (rice, maize and wheat) (Wakeman and Bennett., 2023) with further work required to understand their role in root and shoot production and the possible impacts they may have on plant-plant competition.

Wheat and black-grass – A non-zero-sum-game

The ability to compete with black grass has been described to occur in two ways, either through suppression of the weed by the crop or by tolerance of the weed by the crop (Andrew et al., 2015). Suppression allows increased crop growth through decreasing black grass growth. Tolerance maintains crop growth despite black grass presence. Different crop cultivars therefore have the capability to respond differently in response to blackgrass presence. The findings in this thesis back up this theory. Productivity assessments (Figure 6.8) highlighted the differences in response to black grass amongst cultivars. Increases or decreases in overall productivity was not found to be species dependent. It is therefore clear that wheat blackgrass relationship is a non-zero-sum-game relationship, indicating the loss of one plant (crop) does not equal the gain in the other plant (black-grass). Differences in levels of suppression and tolerance lead to some pots over-producing and some pots under-producing. Those lines seen to be better tolerators can be seen in figure 6.5 and those seen to be better suppressors can be seen in figure 6.6. These results therefore agree with previous findings in the literature (Andrew et al., 2015), that suppression and tolerance are two separate mechanisms, and although they may be interrelated it is likely they are controlled separately, particularly as some varieties have shown good tolerance but poor suppression and vice versa. Further work is required to understand the specific traits involved in supplying both tolerance and suppressive ability to the crops and to further determine the best combination of traits for selection in breeding to maximise crop yield and blackgrass control.

Other routes for black-grass control - IWM strikes back

The results of this thesis have shown that breeding for increased root system may increase the competitive ability of wheat. However, selection for root growth alone may not be the ideal target for breeding. Instead breeding and selection of winter wheat for increased growth rate in winter conditions may provide greater benefits both in above and below ground growth. The findings presented here have shown wheat to have a slow growth rate in winter conditions compared to the faster growth rate of both barley and black-grass (Figure 3.4). This would indicate that growth rates are not an environmental limitation, in fact winter wheat roots were found to be produced at the same rate as spring wheat roots (Thorup-Kristensen et al., 2009). It may therefore be physiologically possible to increase wheat winter growth rate to be closer to that of black grass. If this is possible it would prevent black grass accumulating such a large competitive advantage. The increased growth rates would increase shoot and root growth without necessarily altering the shoot to root ratio of the plant therefore successfully increasing root production without directly breeding for it. Early vigour of crops has also been highlighted as a possible factor that may increase wheat competitive ability against black-grass. Bastiaans, Paolini and Baumann., (2008) determined that crops that emerged earlier had improved access to resources than plants that emerged later. Therefore, it is possible that having early vigour, or a fast growth rate prior to winter, may aid crops in growth throughout winter and thus hinder black-grass during the period black-grass normally gains its competitive advantages. Again, it may be possible to increase root growth and competitive ability without directly breeding for root growth. The findings in chapter 7 indicate that shoot and root growth in crop lines grown in winter conditions are positively correlated indicating increases in shoot production result in increases in root production. It is therefore possible that the selection of above-ground vegetative growth may also result in an increase below-ground root production. Further work is therefore required to test these ideas to determine the best approach for competitive cultivar development.

The role of chemical exudate production in competition between wheat and black-grass was assessed (Chapter 3). Chemical exudates have the possibility of acting allelopathically towards neighbouring plants. Such allelopathy has previously been described to occur in crop-weed interactions. For example, the production of the allelochemical momilactone B is known to influence the outcome of rice-barnyard-grass competition (Kato-Noguchi and Peters., 2013). In wheat-black-grass competition no such similar interaction has yet been described. It may be possible that black-grass is able to inhibit wheat growth via allelochemical production or in fact ‘ignore’ allelopathic signals from neighbouring plants. It is also possible that allelochemical production by wheat may aid in black-grass control and that by increasing or optimising allelochemical production in wheat it may be possible to increase black-grass control. Despite the results presented here only theories as to the role of chemical root exudates in wheat-black-grass interactions can be presented; that is if indeed they play any role at all.

The use of competitive cultivars is only one of many possible IWM approaches for black-grass control. Moss., (2017) outlined five main areas for opportunities in which to increase black-grass control. These five areas are: controlled use of herbicides, increased knowledge of cultivations, increasing competition, preventing weed seed release and spread and optimising sowing date (Figure 8.1). Each IWM approach requires further research, testing and optimisation to determine the best strategy for use. The best combination of IWM approaches also requires further work and will likely vary field to field.

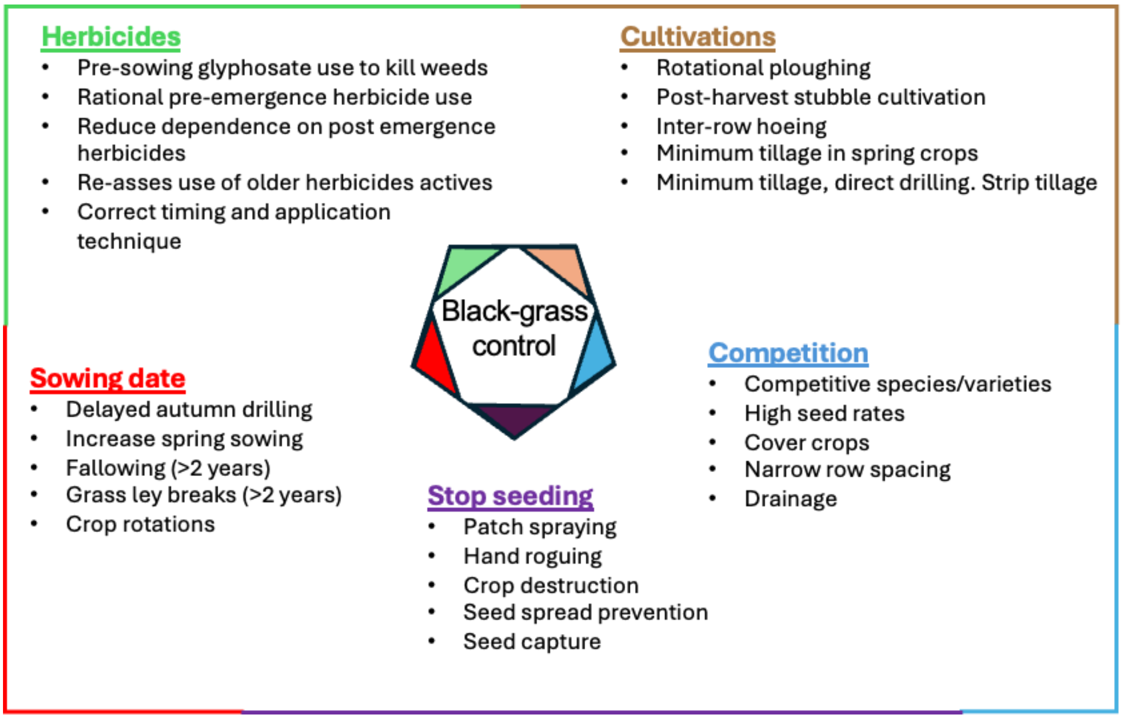

Figure 8.1 IWM approaches for black-grass control

Diagram showing the main areas for research focus on developing better more robust black-grass IWM approaches. Source: Adapted from Moss., (2017).

Concluding remarks

The work presented in this thesis provides further evidence towards the successful implication of competitive wheat cultivars as a cultural approach to reduce black-grass presence through natural crop competition. The screening approaches developed have highlighted variation in crop cultivars that may result in differences in competitive ability against black-grass. The findings have highlighted the importance of root growth and developmental timing in the success of black-grass. Counteracting these advantages through wheat breeding may allow for reductions in the advantage of black-grass over wheat. Adapting the direction of crop breeding to include traits for competitiveness will decrease crop susceptibility to environmental stresses. The use of competitive wheat cultivars with high root growth, high winter growth rate and early vigour prior to winter may provide the much-needed increase in black-grass control. These results should provide a basis for future testing and identification of competitive wheat cultivars for black-grass competition and the eventual successful formation of a recommended list for black-grass control. Overall, this research will hopefully reduce the negative impacts of black-grass on farmers and increase yields of winter wheat.

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