MECHANISM OF WEED SUPPRESSION IN EARLY VIGOUR & WEED SUPPRESSIVE WHEAT GENOTYPES.

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Field trials were performed in 2014-15 as part of the UCS 00200 research project evaluating mechanisms of weed suppression in diverse wheat genotypes in both Wagga Wagga and Condobolin NSW. Crop and/or weed growth were monitored at 4 stages of growth at each location: early season (tillering), grain filling stages, crop maturity at harvest and also postharvest to the crop. Field trials have demonstrated significant differences between wheat cultivars in crop biomass, growth vigour, yield, leaf area index (LAI), weed suppression, weed count and biomass. It is likely that these cultivar competitive traits are clearly influenced by both genotype and environmental factors, as shown by differences in cultivar performance among the two locations. Cultivars that performed well in terms of crop biomass, yield and weed suppression in both locations included Espada, Condo and to a lesser extent, Janz. Additional experimentation using metabolomics to profile primary and secondary metabolite differences among cultivars, locations, plant part and timing of collection will be performed using LC-MS QToF for metabolite profiling. This will provide important information regarding crop physiological and biosynthetic differences that may impact crop competitive traits against common weed species.

INTRODUCTION

Herbicide-resistant weeds are on the rise across Australia, including an increasing number of cropping weeds experiencing resistance to multiple herbicides (Owen et al., 2013). For instance, glyphosate-resistant weeds across Australia now include annual ryegrass (Lolium rigidum), barnyard grass (Echinochloa oryzicola), liverseed grass (Urochloa panicoides), windmill grass (Chloris truncata), brome grass (Bromus inermis) and fleabane (Conyza bonariensis). As well, 103 populations of annual ryegrass resistant to glyphosate have been confirmed (Preston et al., 2010). In comparison with pests and diseases, weeds have the potential to incur the greatest yield loss, through competition with the crop and decreasing yield quality, and can therefore incur high costs of control (Oerke, 2006). In Australia alone, weeds cost about \$4 billion per annum in lost production, decreased quality and in control measures; weed control measures are estimated to be more than \$700 million in the wheat industry (Sinden et al., 2004).

Development of wheat cultivars (Triticum aestivum L.) with increased inherent competitiveness against herbicide-resistant weed species is a potential nonchemical alternative to chemical weed control. To date, limited success has been achieved in Australia breeding cultivars for enhanced competitive ability, mainly because complexity of weed suppression is influenced by many factors (Mokhtari et al., 2002; Zerner et al., 2008; Bertholdsson, 2010). Crop competitive ability can either be specified in terms of crop tolerance against weeds or growth inhibition of weeds by resource competition (Bertholdsson, 2010).

Crop tolerance through toleration of depleted resources and continuation of growth is measured by crop growth or dry matter accumulation whereas resource competition by suppressing weeds through rapidly depleting resources is measured by weed biomass or weed number. Competitive genotypes have the ability to better access light, nutrients, and water resources in limited space, thus suppressing the growth and reproduction of nearby weed species (Worthington et al., 2015).

Although cultivars with high competitive potential have been identified amongst cereal crops, competitiveness has not traditionally been considered a priority for breeding or farmer cultivar choice (Andrew et al., 2015). In Greece, the use of competitive cultivars alone has already been demonstrated to allow for a 50% reduction in total amounts of herbicides used for weed control in wheat (Travlos, 2012; Andrew et al., 2015). Thus, developing grain cultivars with superior competitive ability against weeds will complement cultural methods for weed control in maintaining acceptable yields and suppressing weed populations (Worthington & Reberg-Horton, 2013; Andrew et al., 2015).

To realise the potential of competitive crop cultivars as a tool in integrated weed management, a quick and simple-to-use protocol for assessing the competitive potential of new cultivars is required; it is likely that this will not be based on a single trait, but will need to capture the combined effect of multiple traits (Andrew et al., 2015). A recent study has reported that weed suppressive ability was correlated with competitive traits, including vigour and erect growth habit during tillering (Zadoks GS 29), high leaf area index (LAI) at stem extension (GS 31), plant height at tillering and stem extension (GS 29, 31), grain yield in weedy conditions, and grain yield tolerance (Worthington et al., 2015).

Therefore, based on both field and controlled environment studies, the objectives of this study are to 1) assess the competitive traits of selected superior Australian winter wheat genotypes which are well adapted for the southern faming region, 2) assess the impact of environmental factors such as moisture and temperature on weed suppressive ability of wheat, 3) assess and measure wheat metabolites involved in weed suppression and 4) measure weed suppression by wheat stubble postharvest.

MATERIALS AND METHODS

Field trials were sown on 6th and 20th of May 2014 at two different locations of low (Condobolin) and medium

(Wagga Wagga) rainfall respectively in replicated (6) and randomized trials. Eleven wheat cultivars representing 4 major genotypes of winter wheat typically grown in Australia, plus one cultivar of rye known to be weed suppressive, were established for further study. The wheat cultivars included short and long maturing varieties and 2 cultivars of grazing wheat. The cultivars grown included Condo, Corrack, Gregory, Espada, Janz Cl, Scout, Suntop, Livingston, Mace, Wedgetail, Whistler and Grazer rye.

At sowing, soil samples were taken from each replicate to determine the weed seedbank present at experimental initiation. Weed seedbanks were evaluated in the glasshouse over several months. At Condobolin site the crop was sown at standard 33cm spacing and at Wagga Wagga the crop spacing was a standard 25cm, suitable for these areas due to rainfall differences. No pre- or post- emergent herbicides were used at either site. Sites possessed weed infestations typical of each region for commercial production; that is weed numbers were not particularly high in each location and reflected commonly encountered species including annual ryegrass (Lolium rigidum), bromegrass (Bromus inermis L.), witchgrass (Panicum capillare), stonecrop (Crassula helmsii), capeweed (Arctotheca calendula), paterson's curse (Echium plantagineum), fleabane (Conyza bonariensis), mustard (Sisymbrium orientale), common lambsquarters (Chenopodium album) and fumitory (Fumaria agrarian).

During the growing season, dates for crop and weed assessment in both locations were 16-20th June, 17-22nd July, 18-23rd September, 4-7th November 2014 and 13 – 30th January 2015. On each sampling date in 2014, crop growth visual vigour rating, crop biomass, weed count and biomass, shoots, roots, rhizosphere and bulk soil from around the roots were sampled based on crop growth stages. In 2015 information on weed suppression, weed counts, bulk soil and stubble were collected postharvest from each plot. Crop and weed biomass cuts were performed using a 50cm x 50cm quadrat with 2 subplots per plot.

During the earlier pre-harvest sampling periods, crop canopy measurement were undertaken including normalized difference vegetation index (NDVI) using a Greenseeker device, photos and photosynthetically active radiation (PAR) and leaf area index (LAI) using light Ceptometer (AccuPAR LP-80 Ceptometer-Decagon Devices®). These parameters were assessed to gain valuable information regarding crop canopy architecture and photosynthetic efficiency as they related to crop growth and weed suppression.

Sampled shoots, stems and roots (taken from 4 replicates x 7 cultivars x 2 locations x 4 samples) were extracted in methanol using a Buchi high pressure extractor (Skoneczny et al., 2014; Weston et al., 2015) and stored at 40C awaiting further analysis using

the liquid (UPCL) column chromatography coupled with time of flight mass spectrometry (LC-MS QToF) to analyse, separate and identify targeted and nontargeted metabolites of interest based on relative abundance (Weston et al., 2015). Soil samples were stored at -800C until for future analysis for hydroxamic acids (Fomsgaard et al., 2006; Krogh et al., 2006). Hydroxamic acids are key secondary metabolites of importance in cereal crops; they are known to play important roles in plant defense against herbivory and in plant interactions including allelopathy and are active as soil siderophores as well (Wu et al., 2000; 2002: Belz, 2007; Macías et al., 2007).

RESULTS AND DISCUSSION

Figure 1 and 2 below reflect cultivar differences in crop vigour and average biomass based upon three biomass cuts in July, September and November. Cultivar differences were significant at each location for parameters assessed. This indicated the utility of performing experimentation with six replicates and two subplots per replicate in terms of ability to discern small differences in crop performance.

At Condobolin, Janz Cl and Mace produced greater biomass while Wedgetail produced the lowest followed by Condo, Whistler and Gregory. At Wagga Wagga, Espada, Condo and Livingston produced greater biomass while Whistler, Wedgetail, Suntop and Gregory produced the lowest. In general, cultivars which produced higher biomass also ranked highly for vigour in June and July 2014. Vigour ratings plotted in Figures 1 and 2 were collected in June and July 2014.



Mace at Wagga Wagga - June 2014.



Espada at Wagga Wagga – June 2014



Mace Wagga Wagga- Sept 2014



Espada Wagga Wagga- Sept 2014



Mace Wagga - 179 days after planting no herbicides



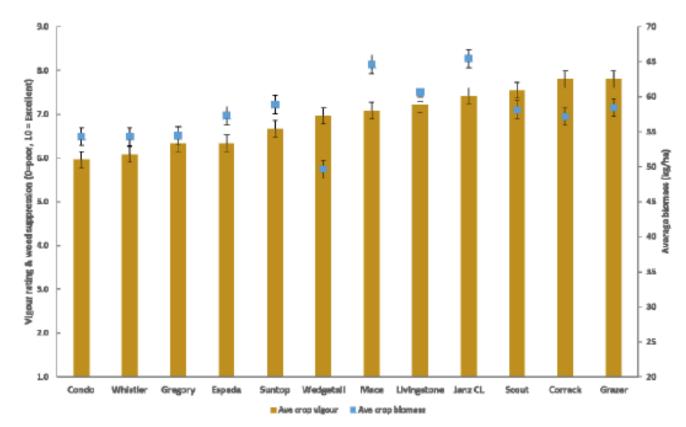
Mace at Wagga Wagga – Post-harvest 2015



Espada Wagga - 179 days after planting no herbicides



Espada at Wagga Wagga – Post- harvest 2015



WHEAT CULTIVAR VIGOUR RATING AND BIOMASS CONDOBOLIN 2014

Figure 1: Wheat cultivar visual vigour rating and biomass at Condobolin arranged in ascending order based upon average vigour rating (1 to 10) taken in June and July 2014.

WHEAT CULTIVAR VIGOUR RATING AND BIOMASS WAGGA WAGGA 2014

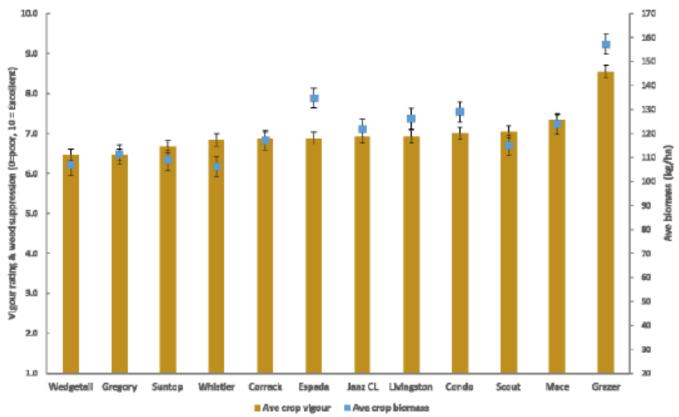


Figure 2: Wheat cultivar visual vigour rating and biomass at Wagga Wagga arranged in ascending order based upon average vigour rating (1 to 10) taken in June and July 2014.

Figures 3 and 4 show weed biomass, count and yield differences among the cultivars at both locations. Average yields were 3.2 and 1.7 t/ha, respectively, in Wagga Wagga and Condobolin. Yield differences among locations reflect typical trends observed for overall yields in each region based on rainfall received and plant density at each location, with Wagga high density plantings producing up to 2 times greater yields than Condobolin. The cultivars have been arranged in

ascending order based upon weed biomass (yellow bars). Espada, Janz CI and Whistler produced consistently lower weed counts in both locations. Whistler, Wedgetail and Janz CI produced lower grain yield while Espada produced highest yields in both locations. Condo also produced reasonable yields and limited weed biomass in both locations. Gregory and Mace produced high levels of weed biomass and weed numbers at both locations.

WHEAT CULTIVAR WEED BIOMASS, COUNTS AND YIELD CONDOBOLIN 2014

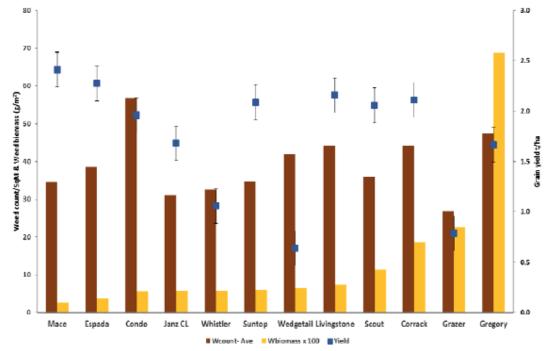
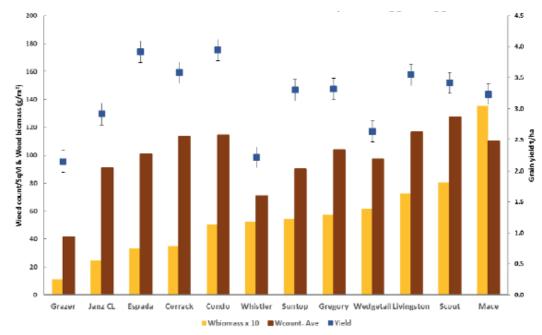


Figure 3: Wheat cultivar weed biomass, counts and yield at Condobolin 2014.

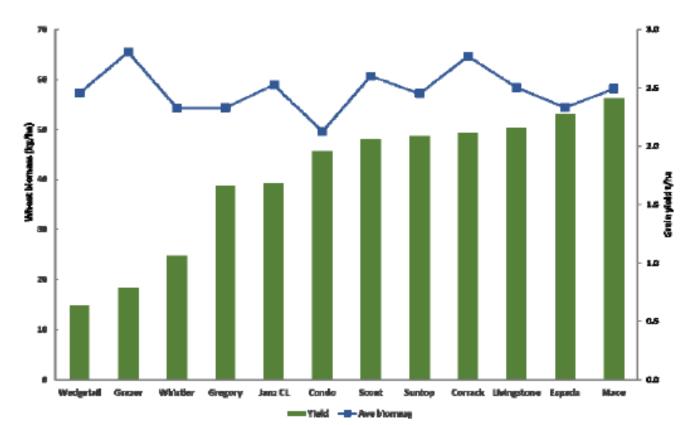


WHEAT CULTIVAR WEED BIOMASS, COUNTS AND YIELD WAGGA WAGGA 2014



Figures 5 and 6 show wheat cultivar biomass and grain yield at both locations. The cultivars have been arranged in ascending order according to yield. Espada and Condo produced the highest yields at Wagga Wagga with Livingston and Corrack yielding

second best. At Condobolin Mace and Espada yielded the most grain with Livingston, Corrack, Suntop and Scout yielding the second highest. At Wagga Wagga, crop biomass was positively related to yield.



WHEAT CULTIVAR BIOMASS AND YIELD CONDOBOLIN 2014

Figure 5: Wheat cultivar biomass and yield at Condobolin 2014

WHEAT CULTIVAR BIOMASS AND YIELD WAGGA WAGGA 2014

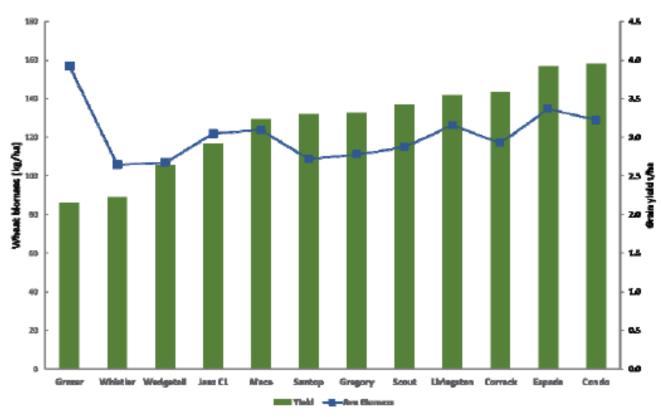


Figure 5: Wheat cultivar biomass and yield at Wagga Wagga 2014

Figures 7 and 8 show the differences in wheat cultivar biomass, weed count and biomass at both locations. The cultivars have been arranged in ascending order according to weed biomass grams persquare meter g/m2). Janz Cl, Espada and Condo had lower weed

biomass in both locations and the poor performers were Gregory and Mace at Condobolin and Wagga Wagga respectively. At Wagga Wagga, there was a strong negative relationship between crop biomass and weed biomass.

WHEAT CULTIVAR AND WEED BIOMASS CONDOBOLIN 2014

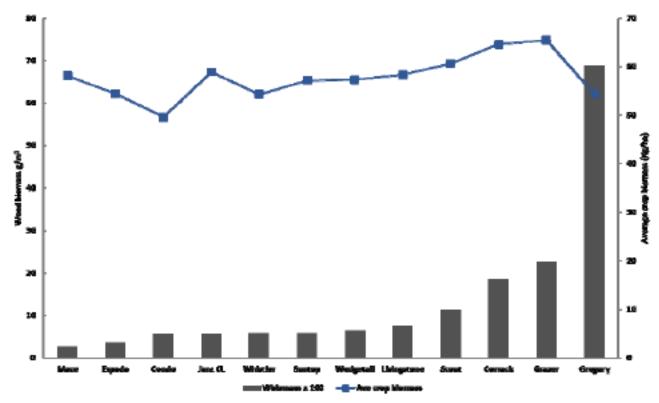


Figure 7: Wheat cultivar and weed biomass at Condobolin

WHEAT CULTIVAR AND WEED BIOMASS WAGGA WAGGA 2014

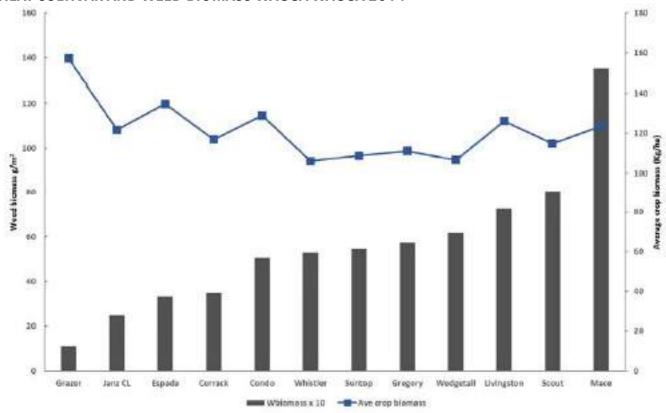
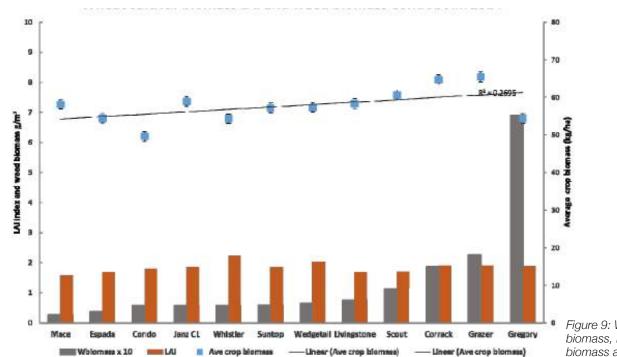


Figure 7: Wheat cultivar and weed biomass at Wagga Wagga.

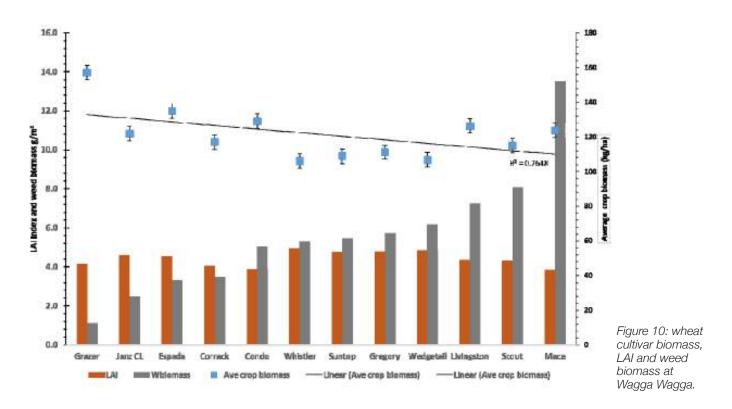
Figures 9 and 10 show the differences in cultivar biomass, LAI and weed biomass at both locations. The cultivars are show in ascending order based upon weed biomass. Wedgetail and Whistler had the highest LAI in both locations while Mace had the lowest. Interestingly, in Condo, those factors which resulted in improved crop biomass resulted in higher weed biomass in the same cultivars. In this more extreme climatic location, the cultivars that generated more biomass also provided conditions and environment which favoured weed growth; this could potentially be due to canopy architecture and impact of shading on temperature and moisture availability beneath the canopy. However, in Wagga Wagga the converse was true; in less moisture limiting situations and under cooler temperatures, the cultivars which produced the highest biomass also suppressed weeds most significantly.



WHEAT CULTIVAR BIOMASS LAI AND WEED BIOMASS CONDOBOLIN 2014

Figure 9: Wheat cultivar biomass, LAI and weed biomass at Condobolin

WHEAT CULTIVAR BIOMASS LAI AND WEED BIOMASS WAGGA WAGGA 2014



CONCLUSION

In year 1 of this experiment, we demonstrated that genetically diverse wheat cultivars performed differently in two locations with varying rainfall patterns. Significant differences in crop biomass, LAI, weed count and biomass between cultivars was also location dependent. These results show that although weed suppression in wheat is influenced by genotype, the genotypic response in wheat is clearly influenced by environmental factors as well, when it comes to growth, yield and weed suppression. However, certain cultivars were excellent performers in both locations in terms of weed suppression and crop yields and these included Espada and Condo and to a lesser extent Janz. Additional results will be obtained from metabolomic analyses to evaluate both primary and secondary biosynthetic pathways operational in crop genotypes and to determine if these pathways influence weed suppression in either location over time. The role of hydroxamic acids in weed suppression will be further examined in detail through targeted metabolic profiling in both soils and plant tissue. Additional field experiments will be repeated over the next 3 years to determine impacts of year and location upon wheat cultivar performance and weed suppression.

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