Influence of elevated [CO_2] on competition between annual ryegrass and wheat under water deficit: biomass production and total antioxidant capacity defence response

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Abstract
Annual ryegrass (*Lolium rigidum* Gaud.), a troublesome weed in winter cropping systems is likely to increase competitiveness with major crops under climate change. To test this hypothesis, we grew wheat (*Triticum aestivum* L. cv. Yitpi) and annual ryegrass to measure the level of competition and competition-mediated plant defence responses. The experimental design was factorial with two [CO_2] (a[CO_2] *vs* e[CO_2]), two watering levels (80% field capacity and drought during DC 60; heading stage) and two competition treatments (wheat only; W only, wheat × ryegrass; W × R) with eight replicates. In the W × R treatment, wheat (by 39%) and ryegrass (by 105%) produced higher biomass under e[CO_2] compared to a[CO_2]. Total biomass of wheat grown in W × R, increased by 31% at DC 90 compared to DC 60 under a[CO_2] and wheat biomass increased only by 5% at DC 90 compared to DC 60 under e[CO_2]. Ryegrass biomass increased at DC 90 compared to DC 60 under both a[CO_2] (by 62%) and under e[CO_2] (by 157%). Total antioxidant capacity (TAC) of wheat grains at maturity was higher in all the experimental conditions under e[CO_2] than at a[CO_2] except wheat grown in W × R under drought conditions. These results confirm that wheat can compete better with ryegrass during the vegetative growth under e[CO_2]. However, during reproductive development, ryegrass showed greater competition with wheat under e[CO_2] than at a[CO_2]. Further, these findings suggest that additional management strategies to control ryegrass under future climate change will be required.

Keywords
Intra-specific competition, photosynthesis, drought.

Introduction
Annual or rigid ryegrass (*Lolium rigidum* Gaud) is an annual, diploid, C_3 grass, native to the Mediterranean and has become the most economically damaging, troublesome winter weed in Australia (Owen et al. 2014). It is a competitive weed, which appears to have a greater competitive advantage in later sown crops. Moreover, annual ryegrass has evolved resistance to numerous classes of herbicides with distinct modes of action and can also exhibit cross-resistance to multiple herbicide classes (Owen et al. 2014).

Atmospheric CO_2 concentration [CO_2] is predicted to increase up to 550 μmol mol^{-1} by 2050 according to the Intergovernmental Panel on Climate Change (IPCC) emission scenario A1B. This increase is likely to affect the global and regional climates and weather patterns. In light of these challenges on future climates, agronomic and genetic adjustments will be required to consider both the indirect climate effects on global food production, as well as the direct effects of increasing [CO_2] and other extreme climate events on crop-weed competition. The competition between weed and crops has been altered under elevated atmospheric [CO_2] (e[CO_2]) (Ziska 2003).

Weeds indirectly determine the crop yield potential because most weed species are superior in adapting to environmental stresses such as water deficit and high temperature and thus compete for resources with the main crop species (Patterson and Flint 1990). Many adaptive strategies like avoidance and/or acclimation to cope with heat and drought stresses have been identified. Some of these strategies include accumulation of antioxidants, osmoprotectants, heat shock proteins, signalling cascades, and transcriptional control which involved the offsetting of stress-induced biochemical and physiological alterations. It has been demonstrated that the competition between weed species and crops is also dependent on [CO_2] level of the atmosphere

(Patterson and Flint 1990). Therefore, as important as it is to capture the CO_2 fertilisation effect, a thorough understanding of how crop-weed competition determines wheat grain yield potential under predicted changing climate scenario is important. The objectives of the current study were to identify the competition between annual ryegrass and wheat in terms of (i) grain yield (ii) total antioxidant capacity (TAC) in vegetative and reproductive organs under elevated atmospheric [CO_2] and drought conditions.

Methods

Growing conditions and plant materials

This study was conducted between January and August 2015 using two identical environmental control growth chambers (Steriudium e2400; 3.1 m long × 2.4 m wide × 2.6 m high) located at the Federation University, Australia. The [CO_2] in one chamber was maintained at 400 µmol mol^{-1} (a[CO_2]), whereas the other chamber was maintained at 700 µmol mol^{-1} (e[CO_2]) during day time, throughout the experiment. Wheat cultivar (*Triticum aestivum* L. cv. Yitpi) and annual ryegrass (*Lolium rigidum* Gaud.) were grown in 5 L opaque polyethylene cylindrical pots (40 cm height × 18 cm diameter). The experimental design was factorial with two [CO_2] treatments (a[CO_2] *vs* e[CO_2]), and two levels of soil water supply (80% soil field capacity and drought) and two levels of weed competition (wheat only; 2 wheat plants per pot (W only hereafter), wheat × ryegrass (W × R hereafter); 2 wheat and 2 ryegrass plants per pot) with eight replicates. Four seeds of wheat cv. Yitpi were sown 2 cm below the soil surface of each pot on 02 February 2015. Ryegrass seedlings were raised in an environmentally controlled glasshouse at Federation University, Australia. Two weeks after the wheat was sown, ryegrass seedlings were transplanted into the wheat pots and wheat seedlings were thinned as follows. In the pots of wheat grown without weed competition, wheat seedlings were thinned to two plants per pot based on wheat plant density under field conditions. In the pots of wheat grown with ryegrass competition, wheat seedlings were thinned to two plants and two ryegrass seedlings were transplanted per pot.

Water treatment

All the pots were watered with reverse osmosis water to constant weight (80% field capacity) by weighing each pot with the plants every second day. Drought experiment was started at DC 60 growth stage of wheat, and plants were grown without watering until the wheat plants showed negative assimilation rates. After the drought treatment, all the plants grown under well-watered and drought conditions were watered every second day until grain maturity.

Plant harvesting

At DC 60, four replicates from each treatment were harvested and separated into leaves, stems, and spikes. At grain maturity, plants were harvested and were separated as above. Ryegrass was separated into spikes and vegetative biomass. Wheat spikes were dried at room temperature and dry weights were measured. Grains were separated from spikes and aspirated (Vacuum separator, Kimseed, Australia) to remove the remaining husk and dust, and stored at 4 ºC in plastic containers until further analysis.

Antioxidant measurements

The total antioxidant capacity (TAC) was determined using an adaptation of the CUPRAC method as used by Apak et al. (2013). Absorbance was subsequently read on a UV-visible spectrophotometer at 450nm and standardised with Trolox®. Total antioxidant capacity was expressed as mg Trolox/100 g dry weight (DW).

Statistical analyses

Data were analysed with MINITAB 17 statistical package using a General Linear Model analysis of variance. Homogeneity of variances was checked using Levene's test and loge-transformed where necessary to equalise variances between treatments. A three way ANOVA was conducted testing the overall effects and their interactions of [CO_2] (e[CO_2] *vs*. a[CO_2]), competition (W only *vs*. W × R- wheat *vs*. W× R - ryegrass), water supply (well-watered *vs*. drought). The least significant difference (LSD) at $p = 0.05$ was used to compare the means between treatments unless otherwise stated.

Results

Plant biomass at DC 90

Competition × [CO_2] and competition × drought interactions were significant for the total biomass production at DC 90. Among all the treatments, highest biomass was produced by wheat grown without ryegrass competition under e[CO_2]. Wheat biomass produced in the wheat only treatment at DC 90 was 38%

higher at e[CO_2] than at a[CO_2] (Figure 1). In W × R, wheat (by 39%) and ryegrass (by 105%) produced higher biomass under e[CO_2] than at a[CO_2] (Figure 1). When considering competition × drought interaction, wheat produced a lower biomass under drought conditions in both W only (by 22%) and W × R (by 38%) compared to well-watered conditions. In contrast, ryegrass produced almost similar biomass under both well-watered and drought conditions (Figure 1). However, in W × R, stem and leaves dry matter of wheat decreased by 32% under drought compared to well-watered treatment (Figure 1). Competition × drought interaction was significant for spike dry weight at DC 90. Wheat spike weight decreased under drought in W only (by 28%) and W × R (by 41%) than in the well-watered conditions. However, ryegrass produced similar spike biomass under both well-watered and drought conditions.

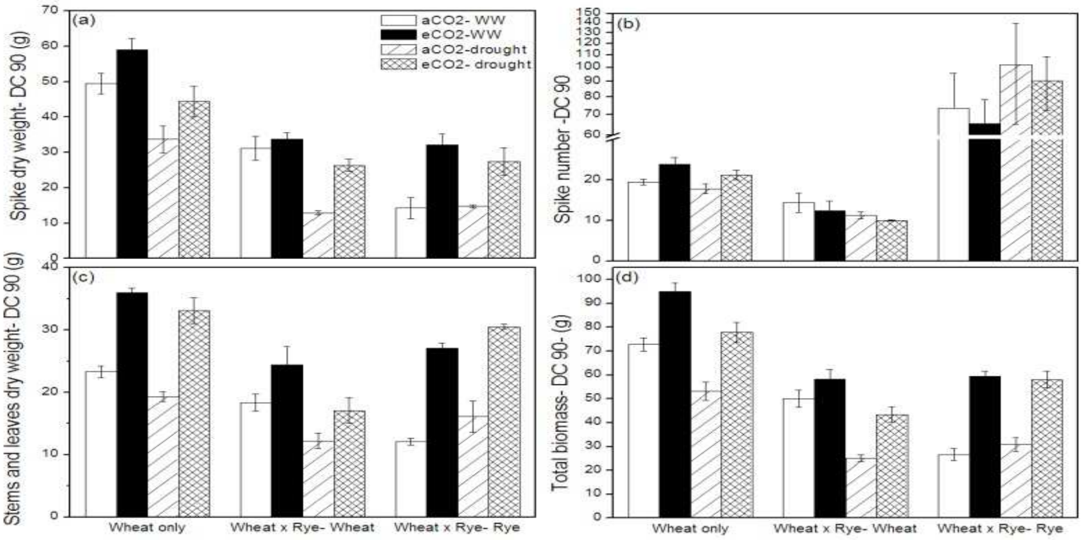

Figure 1. Wheat and ryegrass (a) spike dry weight, (b) spike number, (c) stem and leaves dry weight and (d) total biomass when wheat grown individually (wheat only) and in a competition with annual ryegrass (wheat rye –wheat; wheat grown with ryegrass competition, wheat rye- rye; ryegrass grown with wheat) under ambient [CO_2] and elevated [CO_2] at well-watered and drought conditions. Data presented are the mean ±standard errors of n=4 replicates.

Total biomass of wheat grown in W × R, increased by 31% at DC 90 compared to DC 60 under a[CO_2] and wheat biomass increased only by 5% at DC 90 compared to DC 60 under e[CO_2]. Ryegrass biomass increased by 62% at DC 90 compared to DC 60 under a[CO_2] while ryegrass biomass increased by 157% at DC 90 compared to DC 60 under e[CO_2] (DC 60 data not shown).

Total antioxidant analysis

The three way interaction of [CO_2] × competition × drought was significant for the TAC of leaves at DC 60. Highest TAC was observed in wheat leaves in the W × R treatment grown at e[CO_2] under well-watered conditions and this was 22% higher than TAC of wheat leaves grown in W only under well-watered conditions. Under a[CO_2], TAC of wheat leaves grown in W only was 34% higher under well-watered conditions than under drought conditions (data not shown), while TAC of wheat leaves grown in W × R was similar under well-watered and drought conditions. However, under a[CO_2] ryegrass leaves produced higher total antioxidant capacity under well-watered condition, compared to drought conditions. In contrast, under e[CO_2], TAC of wheat leaves grown in W × R was higher in well-watered conditions (by 19%) than under drought conditions (data not shown). Three way interaction of [CO_2] × competition × drought was significant for the total anti-oxidant capacity of wheat grains at maturity. TAC of wheat grown in W only and in W × R was higher by 29% and 194% respectively under drought than under well-watered conditions with a[CO_2] (data not shown). In contrast, under e[CO_2], TAC of wheat grown in W only was not different under drought and well-watered conditions, while TAC of wheat grains in W × R was 14% higher under drought rather than the well-watered conditions (data not shown).

Discussion

Under the predicted climate change conditions such as droughts and changing rainfall pattern, weed species are likely to out-compete crop species for available resources (Patterson and Flint 1990). In our experiment, we have clearly demonstrated that wheat is more competitive with ryegrass, one of the most troublesome and costly weeds in annual cropping systems in Australia, in the early growth stages under e[CO_2] than at a[CO_2]. However, in the later stages of plant growth, ryegrass became more competitive under e[CO_2] than at a[CO_2]. Percentage reduction of total wheat biomass due to ryegrass competition at DC 60 was lower under e[CO_2] than at a[CO_2] (data not shown), while percentage reduction of total wheat biomass due to ryegrass competition at DC 90 was higher under e[CO_2] than at a[CO2] (Figure 1). This indicates that wheat can compete with ryegrass better in the early growth stages under e[CO_2] than at a[CO_2]. Moreover, at DC 60, in W × R competition, ryegrass aboveground biomass production in response to wheat growth under e[CO_2] was much lower. In contrast, ryegrass aboveground biomass production in response to wheat growth under e[CO_2] at DC90 was almost 2.5 times higher. Slow growth of ryegrass compared to wheat in the early growth stages under e[CO_2] may be due to lower light interception and reduced temperatures under the canopy for photosynthesis. Therefore, growth conditions may not be ideal for ryegrass particularly in the early stage of plant development. Models that quantify the effect of crop- weed competition based on ecophysiological principles have shown that light interception and leaf area expansion are the main traits that determine crop competitiveness. However, the differences in optimum temperature for different species for photosynthesis cannot be excluded. During the later growth stage, the competitive ability of wheat was substantially decreased under e[CO_2]. It has been reported that the competitiveness of wheat with weeds can be shifted after the vegetative stage. Ryegrass response to e [CO_2] in terms of total biomass production was much higher than wheat at the later growth stage. This clearly demonstrates that ryegrass' competitiveness increased during the later growth stages of wheat under e[CO_2].

TAC of wheat leaves grown in W only treatment at DC 60 growth stage, under well-watered conditions, was 13% lower at e[CO_2] compared to a[CO_2]. These results are in general agreement with a number of other studies where decreases in antioxidative defence capacity were reported in wheat leaves grown under e[CO_2] (Aranjuelo et al. 2008). Aranjuelo et al. (2008) concluded in their study that decreased TAC was the result of lower levels of photooxidative stress in plants grown under e[CO_2], hence a lack of oxidative stimuli for antioxidant production. It has been suggested that most physiological stresses lead to disturbance in plant metabolism and cause oxidative injuries by increasing the production of reactive oxygen species (ROS). Elevated [CO_2] may alleviate the formation of ROS, because for a given electron transport rate, relatively more electrons can be consumed in carbon fixation, and fewer are channelled into ROS producing pathways, such as photorespiration or the Mehler reaction (Asada 1999).

In a summary, we conclude that wheat is more competitive with ryegrass during the early stages of plant development; however, at later stages, ryegrass responded well to high [CO_2] and water stress in contrast to ryegrass at a[CO_2]. Furthermore, we have identified a close relationship between TAC and the competitive ability of wheat when subjected to ryegrass infestation at both growing stages. These findings will be useful for implementation of appropriate ryegrass management strategies under increased atmospheric [CO_2] and drought stress.

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