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Elevated atmospheric [CO_2] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves

Elevated [CO_2] dramatically increases yields

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Primary Research Article

Abstract

Wheat production will be impacted by increasing concentration of atmospheric CO_2 [CO_2], which is expected to rise from about 400 μmol mol^{-1} in 2015 to 550 μmol mol^{-1} by 2050. Changes to plant physiology and crop responses from elevated [CO_2] (e[CO_2]) are well

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documented for some environments, but field-level responses in dryland Mediterranean environments with terminal drought and heat waves are scarce. The Australian Grains Free Air CO_2 Enrichment (AGFACE) facility was established to compare wheat (*Triticum aestivum*) growth and yield under ambient (~370 μmol^{-1} in 2007) and e[CO_2] (550 μmol^{-1}) in semi-arid environments. Experiments were undertaken at two dryland sites (Horsham and Walpeup) across three years with two cultivars, two sowing times and two irrigation treatments. Mean yield stimulation due to e[CO_2] was 24% at Horsham and 53% at Walpeup, with some treatment responses greater than 70%, depending on environment. Under supplemental irrigation, e[CO_2] stimulated yields at Horsham by 37% compared to 13% under rainfed conditions, showing that water limited growth and yield response to e[CO_2]. Heat wave effects were ameliorated under e[CO_2] as shown by reductions of 31 and 54% in screenings and 10 and 12% larger kernels (Horsham and Walpeup). Greatest yield stimulations occurred in the e[CO_2] late sowing and heat stressed treatments, when supplied with more water. There were no clear differences in cultivar response due to e[CO_2]. Multiple regression showed that yield response to e[CO_2] depended on temperatures and water availability before and after anthesis. Thus, timing of temperature and water and the crop's ability to translocate carbohydrates to the grain post-anthesis were all important in determining the e[CO_2] response. The large responses to e[CO_2] under dryland conditions have not been previously reported and underscore the need for field level research to provide mechanistic understanding for adapting crops to a changing climate.

Introduction

Global atmospheric CO_2 concentrations [CO_2] are expected to rise by 40% from near 400 μmol/mol in 2015 to about 550 μmol mol^{-1} in 2050 (RCP8.5 scenario; IPCC 2013) with a concomitant rise in mean global temperature of about 2^{o}C by 2050 (at 550 μmol/mol [CO_2])

and increased frequency and severity of droughts and heat waves in many cropping areas (IPCC 2014). These changes constitute significant challenges to meeting the demand of increasing global cereal production from 2.3 Gt in 2007 to about 4 Gt by 2050 (Tester and Langridge 2010) to feed a population expected to exceed 9 billion in 2050 (United Nations, 2013).

Effects of changing temperatures and rainfall aside, rising atmospheric [CO_2] alone will increase biomass and yield in C3 crops because photosynthesis of C3 plants is not currently CO_2-saturated and photosynthesis rates increase under elevated (e[CO_2]) (Kimball *et al.*, 2002; Leakey *et al.*, 2009). The two major plant responses to e[CO_2] are to (i) raise net photosynthesis with a consequent increase in crop growth and yield, and (ii) decrease stomatal conductance, increasing crop water use efficiency (Leakey *et al.*, 2009; Tausz-Posch *et al.*, 2012). These responses may become more important to agricultural production when water is limiting; and it has been suggested in some studies that the CO_2 response of plants is greater under drier conditions (Kimball *et al.*, 2002) because of the benefit of greater water use efficiency. However, assessment of impacts of interactions of drought with CO_2 are complicated by interactions with other crop factors such as nitrogen dynamics and phenology and there are studies that show more positive effects of greater soil moisture under e[CO_2] (Wu and Wang, 2000), though not under FACE conditions. Modelling studies have identified considerable uncertainty around the magnitude and even the direction of the response in water limited crop production environments (Ewert *et al.*, 2002). Mediterranean-type environments commonly have ample water supply during early growth phases, when crops often use stored soil water and experience a transition to drought towards later growth stages (Yang and Zhang 2006; Farooq *et al.*, 2014). Overly vigorous growth early in the season may be a disadvantage, because the resulting more rapid depletion of soil water may reduce grain yield. Under such conditions, carbohydrate supply is not maintained through grain filling during the late season terminal drought. This phenomenon, known as "haying off" (van Herwaarden *et al.*, 1998), has been the subject of a recent simulation study that concluded that e[CO_2] alone can stimulate early growth, but there was no evidence that e[CO_2] exacerbated haying off (Nuttall *et al.*, 2012). However, the combination of hotter and drier environments expected under future climate conditions may increase the risk of haying off, particularly in low rainfall areas.

Another environmental factor, heat stress, limits wheat yields globally (Cossani and Reynolds 2012) and is commonly a significant factor in water limited systems, which may further complicate the CO_2 fertilisation effect on crops. Heat stress from anthesis to grain maturity reduces yield through floret abortion, pollen sterility, increased photorespiration, and reduced time to capture resources due to accelerated growth (phenology) and senescence (Farooq *et al.*, 2011). The consequences of interactions of heat stress with [CO_2] on crop production are unclear. Because e[CO_2] induces stomatal closure (Bernacchi *et al.*, 2007) and therefore reduces canopy cooling, heat stress effects on the canopy could be exacerbated (Wall *et al.*, 2006). On the other hand, more efficient water use earlier in the season and reduced soil evaporation due to greater early season growth (Fischer 2011) may increase soil water availability later in the season (Kimball *et al.*, 1995; Ainsworth and Long, 2005; Leakey *et al.*, 2009). To add to the complexity, increased stem carbohydrate availability, a result of greater photosynthesis rates and biomass under e[CO_2], may buffer heat stress effects on wheat (Angus and van Herwaarden 2001; Farooq *et al.*, 2011). However, in previous FACE experiments on wheat, concentrations of soluble carbohydrates remained unchanged (Tausz-Posch *et al.*, 2015) or even decreased under e[CO_2] (Wall *et al.*, 2006).

Adapting crop responses to the effects of e[CO_2] will involve either changes in management or genetics. Selecting cultivars responsive to e[CO_2] and incorporating promising traits into breeding programs is one potential adaptive strategy (Ainsworth *et al.,* 2008; Ziska *et al.,* 2012; Tausz *et al.*, 2013). Wheat cultivars differ in their responses to e[CO_2] (Ziska, 2008), but there is little information on intraspecific responses to e[CO_2] under drought or high temperatures (e.g., in rice, Shimono *et al.*, 2009) and only limited understanding of the processes underlying this response**.** Identifying traits that are responsive to e[CO_2] under a range of environments may allow breeders to develop cultivars that can taking advantage of e[CO_2] and changing environmental conditions (Ainsworth *et al.*, 2008, Wang *et al.*, 2013).

When studying crop responses to e[CO_2], the Free Air CO_2 Enrichment (FACE) methodology is suggested as the most realistic option, because it lacks walls or enclosures and minimizes any changes to canopy or root microclimate (Okada *et al.*, 2001, Kimball *et al.*, 2002). Meta-analyses from FACE experiments studying wheat reported mean yield increases in the range of 15-17% (Ainsworth and Long 2005; Wang *et al.*, 2013) corrected for [CO_2] of about 550 μmol/mol. Most of these results were derived from FACE experiments in high yielding, high rainfall or irrigated wheat growing systems where mean yields are commonly greater than 5 t ha^{-1}. However, a significant proportion of global wheat production occurs in low yielding, water limited environments, often with pronounced terminal drought stress (Braun *et al.,* 1996; Farooq *et al.*, 2014). Results from a limited number of studies have shown yield increases by e[CO_2] of up to 33% (Ainsworth and Long, 2005) and 22% (Kimball, 2011) under water deficit. Thus, results from high yielding, higher rainfall systems might not be representative of low yielding conditions, highlighting the importance of undertaking FACE experiments in relevant agroecosystems (Ainsworth *et al.*, 2008).

Recently it has been noted that high frequency fluctuations in [CO_2] within FACE rings might cause lower responsiveness of crops to e[CO_2] (Bunce, 2012). In open-top chambers (OTC) comparing constant and fluctuating [CO_2] of 1-minute amplitude, Bunce (2012) found that photosynthetic rate and stomatal conductance were down-regulated in flag leaves of winter wheat in the fluctuating compared to constant [CO_2] chambers. Chambers with constant [CO_2] showed a yield increase of 19% while the fluctuating [CO_2] chambers were not different from the ambient chamber control treatment. In addition, flag leaf photosynthesis and stomatal conductance were lower after pulses of up to 1000 μmol mol^{-1} were applied to rice and wheat (Bunce, 2013). To date, only a study by Kimball et al. (1997) has compared results from FACE and OTC directly. They showed for continuously irrigated conditions that relative responses for wheat biomass were similar for FACE and OTC (increased 8-9%). There have not been other field tests comparing FACE and OTC systems directly so it is unclear whether high frequency variations in [CO_2] in FACE depressed the [CO_2] response or whether cultivar and environmental factors contribute more to variation in responses. Regardless, if FACE systems do underestimate [CO_2] response then increasing [CO_2] will cause greater changes to crops than currently estimated with FACE methodology.

To address the uncertainty around wheat yield responses to CO_2 fertilization in water-limited, low-yielding wheat cropping systems, the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility commenced operation in 2007 (Mollah *et al.*, 2009). The AGFACE is located in the wheat growing region in South East Australia, representative of Mediterranean or semi-arid, water limited, low yielding wheat cropping systems worldwide, e.g. such as the Mega-Environment 4 as defined by the International Maize and Wheat Improvement Center (CIMMYT) (Braun *et al.*, 1996). This environment represents 15% of the area globally where wheat is grown (Fischer *et al.*, 2014).

This paper reports agronomic responses of two cultivars of wheat grown at two Mediterranean-type sites for three years at one site on heavy clay soils and for two years at a second site on less fertile, sandy soils. These locations represent some of the driest and lowest yielding agroecosystems tested in FACE experiments worldwide (Fig. 1). Additional environmental variation was achieved by adding supplementary irrigation treatments and employing an additional later time of sowing to shift the usual crop sowing time from early winter to mid-winter, forcing the crop to flower, set seed and mature during hotter conditions in the late spring; mimicking future climate conditions. Note that some of the data reported here were originally reported as mean yield responses (for the Yitpi cultivar) in O'Leary *et al.* (2015) as validation for crop simulation modelling for [CO_2] response and as site means in Nuttall *et al.* (2012). Here, the complete set of yield and yield component responses are reported with statistical analyses.

The set-up and environmental conditions of this experiment allowed testing of the following hypotheses:

1) Relative response of wheat biomass and yield to e[CO_2] will be greater in a semi-arid rainfed zone under drier and hotter conditions compared to responses from other agroecosystems for wheat;

2) Elevated [CO_2] will increase the incidence of haying-off;

3) Elevated [CO_2] will buffer adverse impacts on wheat yield components caused by high temperatures and dry conditions near anthesis;

4) Wheat cultivars will respond differently to e[CO_2].

Materials and Methods

Site description

An outdoor research facility was established in 2007 on the (at the time) Department of Primary Industries, Victoria research farm near Horsham (36°45′07″S latitude, 142°06′52E longitude, 128 m elevation) in Victoria, Australia on a Murtoa clay, a grey cracking Vertosol soil (Isbell, 1966). Mean clay content at the site was 52%, ranging from 37 to 66% to a depth of 1.8 m, and mean sand content was 21% across all depths. Mean lower limit for the root zone to 1.4 m depth was 0.313 g/cm^3 and upper limit (field capacity) was equal to 0.435 g/cm^3. This resulted in a maximum water holding capacity of 170 mm (O'Leary *et al*., 2015; Table S1). Long term (1981-2010) average annual rainfall is 435 mm, with 274 mm typically falling during the growing season (June – Nov). Typical unirrigated yields are 3-4 t ha^{-1} (range 1 to 6 t ha^{-1}). Mean growing season temperature is 16.5 °C (Jun – Nov).

A second site was operated in 2008 and 2009 at Walpeup (35°7′16″S latitude, 142°00′18E longitude, 94 m elevation), located approximately 200 km north of Horsham in a drier region, termed the 'Mallee'. The soil was a Calcarosol (Isbell, 1966) with a clay content of 8% and 91% sand (Vu *et al.*, 2009). Mean annual rainfall (30-yr average) is 320 mm (with about 188 mm in Jun-Nov) and growing season temperature is 18.3°C (Jun – Nov). Grain yields of wheat are typically 1-3 t ha^{-1} (range 0.4 to 4 t ha-1). The site was situated on the Mallee Research Station, run by the Department of Primary Industries, Victoria. The Walpeup site was chosen because its long term environment is generally drier and hotter compared to Horsham, especially near anthesis (Table 1), potentially mimicking future hotter environmental conditions.

General management

Agronomic management at both sites was according to local cultural practices, including spraying fungicides and herbicides, as needed. At Horsham, before the experiment, the field selected for the AGFACE experiment was irrigated and used for lucerne (alfalfa) production, then in the three years prior to the experiment used for annual grain crops. Soil tests taken before sowing in 2007 showed a 0-10 cm Colwell P of 43±12 mg kg^{-1}, and 0-10 cm soil organic carbon of 1.25±0.14%. Initial mean soil N values for the site in 2008 and 2009 were 233±114 kg and 164±98 kg NO_3-N ha^{-1}, respectively, for 0-60 cm depth. The site is considered N saturated due to a previous history of irrigation with communal effluent. The soil contained 0.14% total N in the top 0.20 m (Lam *et al.,* 2012). Increases in total N above 0.11% have no effect on grain yields in wheat in this region (Tuohey and Robson, 1980).

Superphosphate (9% P, 11% S) was drilled with the seed at sowing at 7-9 kg P ha^{-1} and 8-11 kg S ha^{-1} each year. Irrigation water (not commonly used in local practice) was applied at Horsham to create a range of environmental conditions within the experiment. It was not the intent to create the same water availability regime across the seasons but to replicate natural variability and provide a wide range of crop growth and yield responses. Supplemental irrigation was applied to the entire experiment on occasion during excessively dry periods (Table 1) to prevent crop loss. At Walpeup, crop rotation preceding the 2008 season was canola, wheat, pasture, wheat and field pea. In 2008, superphosphate was drilled at sowing at rates of 9 kg P ha^{-1} and 11 kg S ha^{-1}. Sulfate of ammonia (21% N, 24% S) was applied one year before the 2009 Walpeup experiment at rates of at 16 kg N ha^{-1} and 18 kg S ha^{-1}. The pre-sowing soil mineral nitrogen content was 76±26 kg NO_3-N ha^{-1} (0-50 cm depth) for 2009. Pre-season soil N was not measured in 2008.

At Horsham, standard meteorological data were collected either with an on-site weather station or from a nearby Bureau of Meteorology (BOM) station (Station #079023, Polkemmet), located about eight km from the Horsham site. All data were recorded at 0900 local time. The Polkemmet site data were used to fill in missing values from the AGFACE station. At Walpeup, meteorological data were collected from a BOM station located a few hundred meters from the experimental site (Station #076064, Mallee Research Station). Rainfall, irrigations, temperatures and sowing and sampling dates for each site-year are shown in Table 1 and Fig. 3. The total seasonal rainfall reported in Table 1 in some cases excludes significant rainfall amounts that fell within a few days of harvest and therefore would not have contributed to yield (Table 1).

Yitpi and Janz were chosen as test cultivars in this experiment because they are both widely grown in the region and are genetically distinct, coming from different regional breeding programs (Ogbonnaya *et al.,* 2007). They are both spring type wheats without significant vernalization requirements for phenological development, have similar phenology and were selected to avoid differences within the TOS and irrigated (Irr) treatments. Yitpi is a mid-maturity hard, white grained wheat with good early vigour and a semi-dwarf habit and is best adapted to low to medium rainfall areas (Seednet 2005). Janz is a widely adapted, prime hard quality, white grained wheat (Brennan *et al.,* 1991) with early to mid-season maturity. In the current experiments, the two cultivars flowered at similar times. Both cultivars are awned and have similar disease susceptibilities.

Experimental description

Horsham

The AGFACE facility at Horsham was arranged as a factorial split-split plot design with four blocks. In each replicate, there were two experimental main plots (‘rings’); one was e[CO_2] and the other was an ambient experimental plot (a[CO_2]). Plots were separated by 5.5 ring diameters (~60 m) to avoid wind-blown [CO_2] contamination to a[CO_2] rings. The areas immediately around plots were sown to wheat to act as a buffer (20 X 20 m areas total). Each year, the plots were relocated to adjacent areas, so wheat wasn’t grown consecutively on the same plot of land in order to minimize the possibility of soil borne root disease. In 2007, plots were split in half and each half randomly assigned for time of sowing (TOS) while in 2008 and 2009, each plot was randomly split for irrigation (Fig. 3) with a plastic barrier inserted along the north-south axis to 0.8 m depth to separate the Rainfed and Irrigated treatments. Cultivars were allocated to areas within each half of the plots. Fig. 3 shows all plots and cultivars for the experiment, but in this paper only Yitpi and Janz are discussed as the other treatments were part of separate studies. Yitpi was sown with 0 kg N or an addition 50 kg N (as urea) applied (N0 or N+ in Fig. 3), but the N+ treatment is part of a separate study and is not discussed here. In 2007 and 2008, the plots were 12-m in diameter, and in 2009, the plots were expanded to 16 m and additional cultivars added (not subject of this present study, Fig. 3).

Each plot had duplicate subplots of cvs. Yitpi and Janz, each 1.4 by 4.0 m (Fig. 3) and sown in a north-south direction as 8 rows spaced either 0.214 m (2007, 2008) or 0.195 m (2009) for growth (DC31 and DC65) and maturity (DC90) sampling (Zadoks, 1974). One of the duplicates of each treatment was used for destructive sampling at anthesis (DC65) and the other retained for harvest measurements (DC90) and in-season non-destructive measurements. Center rows were sampled, leaving the edge rows on each side as buffers. In 2007, destructive samples were taken randomly within each subplot as 1.0 m row segments at maturity. Beginning in 2008, all samples were collected from four rows by 1 m areas at maturity. Mean plant density as measured by plant counts about three weeks after emergence was 120 plants m^{-2} and ranged from 60-175 plants m^{-2}.

Walpeup

At Walpeup, the experiment was arranged as a randomised complete block with four replications and eight e[CO_2] plots (rings) and eight a[CO_2] plots. Each plot was 4.5 m diameter and was split for growth (DC31 and DC65) and maturity (DC90) sampling (Fig. 3). Plots were separated by 25 m within a field of wheat (cv Yitpi). Treatments were two TOS, and two [CO_2] levels, with the same two levels of [CO_2] by two TOS as at Horsham. Supplementary irrigation was applied as needed to the whole experiment to provide sufficient water to the crop to achieve a harvestable yield (Table 1), but there were no additional water or N treatments. The experiment was shifted to an adjacent area, which was sown to canola between seasons to avoid any disease carryover. The cultivar Yitpi was sown at a rate of 70 kg seed ha^{-1}. Row spacing was 0.25 m each year and the inner four (of eight) rows in each plot were sampled at the same growth stages as the experiment at Horsham.

Measurements

Biomass samples were collected at DC90 from sample areas (quadrats) described above. Plant material was initially air dried before threshing, and then dried at 70^{o}C, so that biomass and grain yield are expressed at 0% moisture content. Kernel number, plant number, spike number, biomass, spikes per plant, kernels per spike and kernel weight were derived from these quadrat samples for both sites and used to calculate the variables reported. Crop height was measured at anthesis (DC65) by using a meter stick in each plot to measure from soil to mean crop height at several points within each plot. Percent screenings is a standard measure of grain quality and is the amount of grain that passes through a 2 mm screen. Accumulated degree days (DD) from sowing to harvest were calculated on a daily temperature basis (T_d) using a 4^{o}C base (T_b): DD = $\Sigma(T_d - T_b)$; where $T_d = (T_{max} + T_{min})/2$ and T_{max} and T_{min} are daily maximum and minimum temperatures (^{o}C) (Loomis and Conner, 1992).

CO_2 injection and control

A detailed engineering description of the AGFACE control and monitoring system has previously been reported (Mollah *et al.*, 2009), but an overview is presented here. In the [CO_2] plots, each ring was composed of eight horizontal stainless steel tubes enclosing the plots. Pure CO_2 was injected into the prevailing wind from three or four of the windward segments, which quickly mixed with air and was blown across the rings. Computer control and feedback using the [CO_2], wind speed and direction at the center of each plot created the central target concentration of 550 μmol/mol. Each ring had its own sensor and control system, and [CO_2] was injected from sunrise to sunset starting from near germination. The CO_2 was measured and recorded every four seconds from sunrise to sunset (Table 1). As the crop grew, the fumigation tubes were raised periodically during the season so they were about 0.1 to 0.15 m above the crop canopy. The CO_2 was supplied from a large tank of pressurized CO_2, piped underground to the rings. Median 24 h [CO_2] in the ambient plots from 2007-2009 was 373 μmol/mol, and the daytime-only median concentration was 366 μmol/mol.

Statistics

Data were analyzed via ANOVA using the statistical software R (R Core Team, 2013). For Horsham, a four-way ANOVA model (CO_2 x TOS x Irrigation x Cultivar) was employed. For Walpeup, a two-way model (CO_2 x TOS) was used. Levene's test was used to check for homogeneity of variance across groups. If needed, data were then transformed (via Box-Cox power transformation) to meet the residual normalization criteria for ANOVA. One parameter, kernel weight, did not comply with the homogeneity rule. In this case, the analysis was performed separately for each TOS. There were four replications of each experimental treatment group at both sites. An analysis of variance for the full replicated design was performed with CO_2, TOS, Irrigation, and Cultivar as fixed factors in all years. The split-plot design for TOS or Irrigation was changed as appropriate (2007, plots split for TOS; 2008 and 2009, plots split for Irr) and cultivar was nested within each split in each year. Where a treatment effect was found to be significant in the ANOVA, a Welch t-test was performed to establish significant differences between treatment means and these are noted in the supplemental tables.

Multiple regression subset analysis (Afifi and Clark, 1990) was run to determine if there were common drivers for the yield response across all environments. Subset analysis allowed choosing variables that provided the "best" fit, based on highest adjusted R^2 to describe the yield response. Subset regression allows comparison of all possible variable combinations rather than just the one with the next highest correlation coefficient, as is done in stepwise regression, but this can exclude important parameter combinations with high R^2 values due to the nature of its sequential selection. The threshold for significance for inclusion of a factor was ($p < 0.05$). Input variables included all combinations of: temperatures (minimum, maximum and mean for the season plus pre- and post-anthesis only), water inputs (rain +

irrigation) for the season and pre- and post-anthesis only, number of days above 32°C and 36°C, degree days (season, pre- and post-anthesis) and number of cropping days in each season. The independent response variable (mean yield responsiveness) was included in the model at the treatment level (i.e., mean of four reps, Year-TOS-Irr-Cultivar).

Results

Growing conditions

Over the three-year period seasonal conditions varied in terms of rainfall amounts and timing, maximum temperatures (and timing), and accumulated thermal units (Table 1, Fig. 3). The Walpeup site was generally warmer than Horsham (Table 1). Mean daily maximum temperatures were greater at Walpeup, with 5-day pre-anthesis maximum temperatures 7-10°C greater than Horsham in 2008. Degree days pre-anthesis and seasonal values were greater at Walpeup than Horsham (Table 1), but in some cases degree day totals were greater at Horsham post-anthesis. The number of hottest days (≥ 36°C) was greater at Walpeup than Horsham each year.

In 2009 there was a prolonged heat wave during anthesis and grain filling at both locations (Fig. 3). At Walpeup 2009, for TOS2 maximum temperatures ranged from 31 to 40^{o}C during anthesis and from 34 to 42°C in the week before harvest (7 Nov to 15 Nov) (Fig. 3). Similarly, at Horsham in 2009 for TOS2, maximum temperatures near anthesis ranged from 29 to 40°C, with eight days over 35°C from 8 to 20 Nov. At Horsham in 2007, TOS2 maximum temperatures reached 35°C in the five days prior to anthesis (Fig. 3), with a mean of 32.5°C for this period (Table 1). At Horsham in 2009, TOS1 there were three days over 33.5°C just after anthesis. These temperatures are above reported critical thresholds (Fischer, 2011) for reducing grain set and filling and hence yield.

There were strong contrasts in growing season rainfall between all years with 2008 experiencing the driest conditions (109-208 mm water inputs) and 2009 the wettest (170-293 water inputs) and hottest; and crops experienced different conditions due to the two sowing times (Table 1). Timing of rainfall and irrigations also varied, creating a wide range of environments for crop response (Table 1, Fig. 3). Supplemental irrigation was applied to create a broad range of water environments rather than target specific input amounts, which resulted in contrasting conditions to assess crop response to e[CO_2] (Table 1).

Elevated [CO_2] main effects on yield, biomass, and yield components

Aboveground biomass and yield were greater ($p<0.001$ to 0.05, depending on season) under e[CO_2] compared to a[CO_2], except for yield in 2008 ($p=0.155$) at Horsham (Tables 2, S1a, S2a; Figs. 4a-f, 5a-b). The 3-year mean relative increases due to e[CO_2] in yield and biomass were 24% (2.29 to 2.85 t ha^{-1}, a[CO_2] to e[CO_2]) and 25% (6.85 to 8.57 t ha^{-1}), respectively, at Horsham, and 53% (1.26 to 1.93 t ha^{-1}) and 38% (4.55 to 6.30 t ha^{-1}) at Walpeup averaged across both years.

Since stimulation of biomass and yield by e[CO_2] were comparable, harvest index (HI) was not affected by e[CO_2] alone, except at Walpeup in 2009 ($p<0.05$; Tables 2, S2a) where HI was greater under e[CO_2] than a[CO_2], increasing by 18 and 38% respectively for TOS1 and TOS2 (Tables 2, S2a). Decreased HI could be indicative of greater hay-off under e[CO_2], but the effect was not different between a[CO_2] and e[CO_2].

On average, kernels m^{-2} increased by 22% at Horsham in 2009 ($p<0.05$), and 42% at Walpeup ($p<0.001$) due to e[CO_2] (Tables S1b, S2a). The mean increase in kernel weight under e[CO_2] was 10% at Horsham in 2009 ($p<0.05$) and 12% at Walpeup ($p<0.01$) (Table 2, Fig. 5c) and this was greater across all treatments at Horsham but one in 2009; but this was less consistent in other years (Figs. 4g-i). Kernel number and weight were not affected by e[CO_2] at Horsham in 2007 and 2008. At Walpeup kernel weight increased by 8% ($p<0.05$) in 2008 and 12% in 2009 ($p<0.01$) (Tables 2, S2a, S2b), while kernels m^{-2} increased by 12 and 61% in 2009 ($p<0.001$) for TOS1 and TOS2, respectively.. Increases were observed in kernels per spike in 2009 of 11% and 19% for TOS1 and TOS2 at Walpeup ($p<0.01$; Tables S2b, S2c).

Under e[CO_2], there were more plants m^{-2} at Horsham in 2007 and 2008 under TOS1 (mean 17% increase) but fewer for TOS2 (mean 12% reduction) as indicated by the TOS interaction at Horsham ($p<0.05$; Table S1b). There were no differences in plants m^{-2} at Walpeup. This did not follow the response of spikes m^{-2}, which was generally higher at Walpeup under e[CO_2] for both TOS in both years ($p<0.01$) (Tables S1b, S2b). This resulted in spikes/plant being 25% greater for TOS2 in 2007 and 2008 at Horsham and at Walpeup, 33% and 31% in 2008 and 0 and 26% in 2009 for TOS1 and TOS2, respectively (calculated from data in Table S1b).

At Horsham in 2009 TOS1, absolute screening values (percent of grain < 2 mm) were higher than in other years (Table S1c, Figs. 4j-l) and e[CO_2] produced a 31% reduction in mean screening values ($p<0.05$; Tables S1c, 2). Screening values at Walpeup were very high during the heat wave in 2009 TOS2 and e[CO_2] values were 54% of a[CO_2] values ($p<0.01$; Tables 2, S2b).

Crop height at anthesis (DC65) was greater (Tables S1c, S2c) under e[CO_2] in all site years (on average by 9% at Horsham and 18% at Walpeup). Plants were almost always shorter in 2008 compared to 2009. Crop phenology was not affected by e[CO_2] although differences of less than two days would not have been detected. This is consistent with Kimball *et al.*, (1995) who reported one day difference in maturity between a[CO_2] and e[CO_2] treatments for their 'dry' treatment.

Time of Sowing (TOS)

As expected, the late sowing (TOS2) significantly shortened the duration of growth periods (Table 1) and caused anthesis and grain filling to occur under hotter and drier conditions (Fig. 3). Thus, TOS affected virtually all growth and yield response parameters measured (Tables 2, S1, S2, Figs. 4,5). For example, mean 3-year e[CO_2] wheat yields grown under TOS2 conditions were 43% and 14% less than those of TOS1 at Horsham and Walpeup respectively. At Horsham for 2007 and 2009 (when yields were different), mean yield increases due to e[CO_2] for TOS1 and TOS2 were 19% and 33%, respectively, while at Walpeup these were 46% and 67%, respectively (Table 2). In 2008, the biomass increase under e[CO_2] was less at TOS2 (16%) ($p<0.001$) compared to TOS1 (36%; Table 2), whereas this was not the case for the other years when TOS2 response to e[CO_2] was greater.

At Walpeup, the only interactions for [CO_2] X TOS occurred in 2009 for kernels m^{-2} ($p<0.01$). At Horsham, [CO_2] X TOS interactions occurred for HI in 2007, where TOS1 showed a mean decrease of 3% while for TOS2 HI increased by 1%. The other [CO_2] X TOS interaction was for plants m^{-2} in 2007 and 2008 ($p<0.05$), where plant number increased by 14% and 19%, respectively for TOS1 in 2007 and 2008 and decreased by 18% and 5% for TOS2 in 2007 and 2008.

Supplemental irrigation (Irr)

Supplemental irrigation (only applied at Horsham) increased biomass in all years and yield in 2007, as well as yield components such as number of kernels m^{-2}, which was expected in a water limited cropping system. Yield response to e[CO_2] was greater with supplemental irrigation (20% versus 57% for rainfed vs supplemental) (Tables 2, S1a).

The 3-year mean biomass responses were 19% and 30% greater due to e[CO_2] for rainfed and supplemental treatments (Table 2). In 2007, the e[CO_2] response to rainfed and supplemental treatments were 5% and 27%.

Cultivars (Cult)

Although there were significant cultivar differences (Tables 2, S1a-d), there were no noteworthy interactions between [CO_2] and cultivar (data not shown).

Yield response to water inputs and temperature

Multiple regression for yield responsiveness to e[CO_2] (ratio of e[CO_2]/a[CO_2]) identified three significant input variables: mean minimum temperature pre-anthesis ($p<0.001$), water input pre-anthesis ($p<0.001$) and number of days equal to or greater than 32°C ($p=0.004$) with an adjusted R^2 of 0.62, which is a measure of the success of the regression in predicting y from x adjusted to account for the number of predictors in the model. The constant was not different from zero ($p=0.25$). The resulting regression was:

$$\mathrm{Yresp} = \mathrm{Tmin\_pre} * (0.278) + \mathrm{W\_pre} * (6.11*10^{-3}) - \mathrm{Days32} * (5.26*10^{-2}) - 0.267$$

Yresp is a fraction based on yield calculated as: e[CO_2]/a[CO_2].

Tmin_pre is the mean minimum temperature from sowing to anthesis.

W_pre is amount of water (mm) applied (rain + irrigation) from sowing until anthesis.

Days32 is number of days equal to and greater than 32°C.

Discussion

Hypothesis 1: Biomass and yield response to e[CO_2] is greater in semi-arid agroecosystems

The large biomass and yield responses (up to 79%) measured in this study at both sites support this hypothesis and have not been observed previously under field conditions, lying well above the highest responses measured to date, greater even than that of the 34% response of hybrid rice (Liu *et al.,* 2008). Elevated [CO_2] lowers stomatal conductance under drought conditions, reducing transpiration (Kimball *et al.*, 1995; Wall *et al.,* 2006) and potentially increasing soil water later in the season (Leakey *et al.,* 2009). Kirkegaard *et al.,* (2007) reported that post-anthesis soil water contribution to yield represented a water use efficiency of 60 kg ha^{-1} mm^{-1}, three times that expected for seasonal water use under similar environments. Thus, in dryland conditions, yield would be expected to respond strongly to even small amounts of additional available soil water and could contribute to enhanced translocation of carbon to the grain.

The TOS treatment was a surrogate for future climate conditions, with higher temperatures at anthesis together with reduced rainfall, allowing testing of effects of temperature changes and changes of water availability under e[CO_2]. The greater stimulation of e[CO_2] on grain yield and yield components under TOS2 compared to TOS1 from e[CO_2] also supports hypothesis 1, with the hotter and drier conditions for TOS2 at and after anthesis leading to greater yield response to e[CO_2] across both sites. The stimulation of growth due to additional carbon supply early in the season increased kernel numbers m^{-2}, spike numbers and grain size depending on season and treatment, which resulted in yield increases under e[CO_2].

Determining the mechanisms for e[CO_2] response is critical to modelling responses across varieties and environments and for pre-breeding trait selection. Previous studies on wheat under e[CO_2] reported increased grain yield with increased number of spikes (Wang *et al.,* 2013), whereas results on kernel weight were inconsistent (Kimball *et al.*, 1995; Högy *et al.,* 2009; Tausz-Posch *et al.,* 2015). Results from chamber experiments (Wang *et al.*, 2013), but not FACE (Högy *et al.,* 2010) found increased kernel numbers per spike. Our results appear consistent with the observations that tillering capacity, and hence the formation of additional spikes, is important in determining [CO_2] response (Ziska 2008; Tausz-Posch *et al.*, 2012; Tausz *et al.*, 2013), especially under hotter, drier conditions, such as observed for spikes m^{-2} at Walpeup and TOS2 produced more spikes/plant under e[CO_2] at both sites. This was also supported by increases in grain weight across sites for the hotter conditions (Walpeup and Horsham in 2009). High tillering is, however, not necessarily a desired trait in dryland varieties as it may lead to wasteful early biomass production and poor tiller economy (Mitchell *et al.*, 2012). In environments where terminal stress is severe, the trade-offs between the traits that are used in breeding to increase yield potential, such as kernel number, spikes m^{-2}, kernel weight and kernels per spike may have to be reconsidered under e[CO_2] (Tausz-Posch *et al.*, 2015).

While hypothesis 1 is supported in comparisons between sites (other FACE studies, including the comparison between Horsham with Walpeup in this study), comparisons *within* our set of experiments at Horsham across seasons and water treatments appear to contradict this. As stated previously, a larger response would be expected under e[CO_2] in drier conditions, that is, significant interactions for [CO_2] X Irr, where irrigation lowers the relative response to [eCO_2], whereas yield response increased in this experiment with greater irrigation (Horsham). In previous FACE studies, water inputs may have been less limiting to growth even in 'dry' treatments (e.g., 350 mm water inputs in Maricopa FACE, Kimball *et al.*, 1995) and hence the hypothesis of responsiveness to drought and, in particular, terminal drought has not been adequately tested. In our system, the wheat was clearly water limited, as demonstrated by the strong yield response when extra water was applied under supplemental irrigation with e[CO_2] providing the opportunity for additional growth and grain development and pointing out the importance of not only amount but timing of water inputs to take advantage of the e[CO_2] response.

In order to understand the mechanism of response to e[CO_2], a multiple regression analysis was performed using key environmental variables, including temperature and water. Results indicated a complex relationship among effects of water, temperature and, importantly, timing of the factors. The positive response to pre-anthesis minimum temperatures and pre-anthesis amount of water input and negative dependence on post-anthesis high temperatures provides a possible mechanism for the apparent contradiction in yield responsiveness across the two sites. As minimum temperatures increase and if more water is available in these semi-arid environments, there is stimulation of early season growth, which allows plants to take advantage of the extra C available for photosynthesis**,** in turn stimulating tiller and spike formation, providing more assimilate for translocation to grain. This response, however, is decreased by high temperature after anthesis and during grain filling (Farooq *et al.,* 2011).

Current crop models have performed well at estimating mean responses of wheat to e[CO_2] (Nuttall *et al.*, 2012; O'Leary *et al.*, 2015) and may therefore be suitable for estimating future mean crop responses. However, estimating crop responses for more specific environments, such as the dryland areas described in this current study, still elude crop models, which may not adequately consider the impacts of high temperature, heat stress, water and [CO_2] simultaneously (McGrath and Lobell, 2013). This situation may in part arise because interactions with [CO_2] have not been well documented through experimentation (Asseng *et al.*, 2004; Parry *et al.*, 2004; Asseng *et al.*, 2015). This type of data, from FACE systems under realistic field conditions, unfettered by enclosures is critical to improving our understanding of environment-specific crop responses to climate change factors, providing impetus to improving crop models for estimating future yields and to better direct selection strategies for crop breeding programs.

Hypothesis 2: Elevated [CO_2] will increase the incidence of hay-off

It has been hypothesized that elevated levels of [CO_2] could lead to haying-off (van Herwaarden *et al.*, 1998; Nuttall *et al.*, 2012) and yield reductions because the larger biomass cannot be sufficiently supplied with water to support the larger yields. The negative yield responses under TOS2, lower harvest indices and high anthesis biomass response (data not shown) compared to harvest yield response in 2008 were consistent with the haying-off effect. The TOS2 treatment in Horsham 2008 had very little seasonal water (109 mm rain+irrigation) and this likely resulted in the high screenings expressed as poor grain fill (Angus and van Herwaarden 2001). However, because there were no differences in grain yield response for e[CO_2] , conclusions about the effect of [eCO_2] on the incidence of haying-off were inconclusive.

Hypothesis 3: Elevated [CO_2] will buffer the negative impacts of heat shocks on yield

Our third hypothesis posited that e[CO_2] modifies crop yield response to heat shocks that occur near anthesis. In 2009, there was a significant heat wave that affected both sites. This occurred during grain fill (TOS1) and flowering/grain set (TOS2) and there was a less severe period of temperatures above 32°C near anthesis for 2007 TOS2 during flowering. Heat during sensitive grain filling phases can reduce kernel size (Farooq *et al.*, 2011) and kernel weight, thereby increasing screenings.

It is noteworthy that 2009 was the only year in which e[CO_2] decreased screenings and screening values were very high due to the heat wave. In addition, kernel weights and kernels m^{-2} under e[CO_2] were greater in 2009 and kernels/spike and HI at Walpeup. Further, some of the highest yield responses occurred at these times. Crops that have sufficient water but are heat-stressed can maintain grain-filling rate, duration and size (Dupont *et al.*, 2006). The e[CO_2] treatment created plants that were larger (greater biomass and height) and, given that there was more water applied in 2009, they may have had more ability to translocate carbohydrate reserves compared to the a[CO_2] treated plants, allowing them to increase grain size and reduce screenings and therefore buffer heat stress during grain filling (Angus and van Herwaarden 2001). These results, obtained during opportunistic observations in naturally occurring heat waves during the experiment require confirmation through experimentally imposed treatments.

Hypothesis 4: Cultivars will respond differently when grown under e[CO_2]

It has been proposed that selection for yield response to e[CO_2] will be important to capitalize on the CO_2 fertilization effect (Ziska *et al.*, 2012). Cultivar differences in the yield response to e[CO_2], an important pre-requisite for selection strategies, have been shown for different crop species (Tausz *et al.*, 2013). Targeted selection would appear to be a productive avenue for selection if there are traits expressed that can support the positive effects of e[CO_2]. For example transpiration use efficiency, tillering and stem carbohydrate storage capacity (Mitchell *et al.*, 2012; Dreccer *et al.*, 2013) have been proposed as useful traits for incorporation into future wheat breeding lines and given the changes to tillering, biomass accumulation and water use efficiency that occur under e[CO_2], these traits are relevant to future atmospheric conditions (Tausz-Posch *et al.*, 2012, Tausz-Posch *et al.*, 2015). However, in the present study, there was no evidence that the two cultivars differed in their response to e[CO_2]. It appears that these cultivars did not differ in particular functional traits, even though they were genetically quite distinct, coming from breeding programs using different germplasm and selected under different conditions. In contrast, when cultivars were specifically selected for contrasting expression of a transpiration use efficiency trait in an otherwise similar genetic background, interactions between [CO_2] and cultivar were found (Tausz-Posch *et al.*, 2012).

Statistical discussion

It is not uncommon for many field based experiments to lack statistical power, where the number of feasible replicates is limited and variability relatively high. In this experiment, a statistical analysis that combined years and locations would increase the statistical power for the CO_2 main effect and some interactions. We decided against this approach for three reasons: (1) the designs were different between years and locations, (2) we did find a number of significant effects of [CO_2] (and lack of power is only of concern for the interpretation of negative results), but most importantly, (3) it may be misleading to treat the three years and two locations as replications, because the conditions were highly variable (and typical for the region). The experiment was designed to essentially generate a series of environments that would differ in accumulated heat units and water supply (among other) under which the responses to e[CO_2] could be assessed. Thus, even though treatments were nominally replicated, their biological importance may be very different in different years. For example, in 2009, the crop experienced significant heat stress at sensitive development stages, which imposed an additional very specific stress factor on the crop not experienced in other years. Thus, despite the potential difficulties to detect interactions, it is more instructive to report results separately for different years.

It should also be noted that it has become usual practice to conduct further synthesis and analyses on published data, and for that purpose it is most important to report detailed data and even in complex conditions “simply describing what was done and why, and discussing the possible interpretations of each result, should enable the reader to reach a reasonable conclusion” (Perneger 1998).

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Supporting information captions

Table S1a. Horsham site. Mean values for key factors for four replications. Standard deviations (n=4) are in parentheses. P value significance: ***<0.001, **<0.01, *<0.05, +<0.1, ns= not significant. (TOS = Time of Sowing, Irr = irrigation, Cult = cultivar). e/a = fractional response of e[CO_2] expressed as: e[CO_2]/a[CO_2].

Table S1b. Horsham site. Mean values for key factors for four replications. Standard deviations (n=4) are in parentheses. P value significance: ***<0.001, **<0.01, *<0.05, +<0.1, ns= not significant.

(TOS = Time of Sowing, Irr = irrigation, Cult = cultivar). e/a = fractional response of e[CO_2] expressed as: e[CO_2]/a[CO_2].

Table S1c. Horsham site. Mean values for key factors for four replications. Standard deviations (n=4) are in parentheses. P value significance: ***<0.001, **<0.01, *<0.05, +<0.1, ns= not significant. (TOS = Time of Sowing, Irr = irrigation, Cult = cultivar). e/a = fractional response of e[CO_2] expressed as: e[CO_2]/a[CO_2].

Table S1d. Horsham site. Mean values for key factors for four replications. Standard deviations (n=4) are in parentheses. P value significance: ***<0.001, **<0.01, *<0.05, +<0.1, ns= not significant. (TOS = Time of Sowing, Irr = irrigation, Cult = cultivar). e/a = fractional response of e[CO_2] expressed as: e[CO_2]/a[CO_2].

Table S2a. Walpeup site. Mean values for key factors for four replications. Standard deviations (n=4) are in parentheses. P value significance: ***<0.001, **<0.01, *<0.05, +<0.1, ns= not significant. (TOS = Time of Sowing, Irr = irrigation, Cult = cultivar). e/a = fractional response of e[CO_2] expressed as: e[CO_2]/a[CO_2].

Table S2b. Walpeup site. Mean values for key factors for four replications. Standard deviations (n=4) are in parentheses. P value significance: ***<0.001, **<0.01, *<0.05, +<0.1, ns= not significant. (TOS = Time of Sowing, Irr = irrigation, Cult = cultivar). e/a = fractional response of e[CO_2] expressed as: e[CO_2]/a[CO_2].

Table S2c. Walpeup site. Mean values for key factors for four replications. Standard deviations (n=4) are in parentheses. P value significance: ***<0.001, **<0.01, *<0.05, +<0.1, ns= not significant. (TOS = Time of Sowing, Irr = irrigation, Cult = cultivar). e/a = fractional response of e[CO_2] expressed as: e[CO_2]/a[CO_2].

Table 1. Agronomic events and dates at each site, year and Time of Sowing (TOS). DC refers to crop decimal code (Zadoks, 1974), Rain and Sup refer to rainfed and supplemental irrigation treatments. ‘Seasonal’ refers to period from sowing to harvest for each environment.

	2007 – Horsham		2008 - Horsham		2009 - Horsham		2008 - Walpeup		2009 - Walpeup	
Agronomic event	TOS1	TOS2	TOS1	TOS2	TOS1	TOS2	TOS1	TOS2	TOS1	TOS2
Sowing	18 June	23 Aug	4 Jun	5 Aug	23 Jun	19 Aug	19 May	30 Jun	14 May	20 Jul
Emergence (50%)	3 July[1]	31 Aug[1]	18 Jun	24 Aug[1]	5 July[1]	31 Aug[1]	26 May	13 Jul	23 May	31 Jul
Tillering (DC31)	6 Sep	25 Oct	20 Aug	10 Oct	2 Sep	13 Oct	30 Jul	10 Sep	8 Jul	15 Sep
Anthesis (DC65)	29 Oct	20 Nov	20 Oct	6 Nov	27 Oct	13 Nov	30 Sep	21 Oct	15 Sep	2 Nov
Harvest (DC90)	12 Dec	20 Dec, 24 Dec, 2 Jan	8 Dec	15 Dec	8 Dec	15 Dec	10 Nov	25 Nov	19 Nov	19 Nov
Irrigation dates & amounts (mm)	Rainfed: 17 Sep (10) 8 Oct (10) 16 Oct (28) Sup: 17 Sep (10) 24 Sep (10) 2 Oct (10) 8 Oct (10) 16 Oct (28) 14 Nov (28)	Rainfed: 17 Sep (10) 8 Oct (10) 17 Oct (28) Sup: 17 Sep (10) 2 Oct (10) 8 Oct (10) 16 Oct (28) 14 Nov (28) 4 Dec (10)	Rainfed: 3 Oct (10) Sup: 8 Sep (20) 25 Sep (20)	Rainfed: 16 Oct (15) 26 Oct (10) Sup: 8 Sep (10) 24 Sep (20) 16 Oct (30) 25 Oct (20)	Rainfed: None Sup: 6 Oct (10) 22 Oct (30) 3 Nov (30)	Rainfed: None Sup: 22 Oct (30) 3 Nov (30)	8 Oct (12)	24 Sep (12) 8 Oct (12) 23 Oct (20)	10 Oct (12)	10 Oct (12)
CO_2 on	19 Jul	19 Jul	24 Jun	9 Sep	6 Jul	3 Sep	25 Jun	14 Jul	22 May	27 Jul
CO_2 off	21 Dec 2007 to 2 Jan 2008	21 Dec 2007 to 2 Jan 2008	27 Nov	15 Dec	30 Nov	14 Dec	30 Oct	24 Nov	19 Nov	19 Nov
Seasonal rainfall (mm, sowing to harvest)	171	111	168	84	223	170	109	124	247	129
Rainfall excluded from seasonal rainfall and dates[2]		20-23 Dec, 24.6 mm		12-14 Dec, 50 mm	8 Dec, 17.4 mm		7-8 Dec, 23.6 mm			
Seasonal water inputs: rain + irrigations (mm, Rain/Sup)	219/267	159/207	178/208	109/164	223/293	170/230	121	168	259	141
Degree days (sowing to anthesis/anthesis to harvest)[3]	835/626	841/567	741/596	649/470	801/645	791/459	979/569	911/538	988/956	1069/373
Mean daily minimum seasonal temperature (°C)	5.2	6.9	4.5	5.0	6.3	7.2	6.6	7.2	8.1	8.7
Mean daily maximum seasonal temperature (°C)	19.3	23.1	17.7	20.0	19.5	22.2	19.0	20.2	20.3	22.8
Mean max temperature, 5 day period pre-anthesis (°C)	23.7	32.5	24.5	21.6	21.2	35.7	26.0	28.8	25.3	35.4
Mean max temperature, 10 day period post-anthesis (°C)	20.4	28.0	23.6	26.7	27.1	33.6	21.6	28.6	21.9	32.7
No. days max ≥ 32°C	12	16	7	7	15	15	6	9	19	19
No. days max ≥ 36°C	1	1	1	1	7	7	2	4	13	13

[1] Date not recorded so estimated via crop simulation model.
[2] Some rainfall data does not include very late season rainfall that would not have contributed to yield and is included here (see text for details).
[3] Base temperature 4°C (Loomis and Conner, 1992).

Table 2. Fraction change due to eCO_2 ($e[CO_2]/a[CO_2]$) in yield, biomass, kernel weight, screenings and harvest index at Horsham and Walpeup (summarized from data in Tables S1 and S2).

			Yitpi				Janz			
			TOS1		TOS2		TOS1		TOS2	
Factor	Location	Year	Rain	Sup	Rain	Sup	Rain	Sup	Rain	Sup
Yield ($g\ m^{-2}$)	Horsham	2007	1.05	1.13	1.08	1.28	1.05	1.25	1.02	1.44
		2008	1.23	1.38	0.88	1.04	1.16	1.78	1.23	0.83
		2009	1.07	1.46	1.40	1.70	1.17	1.40	1.17	1.72
Biomass ($g\ m^{-2}$)	Horsham	2007	1.11	1.14	1.12	1.20	1.11	1.22	1.07	1.39
		2008	1.31	1.37	1.04	1.17	1.27	1.50	1.32	1.11
		2009	1.16	1.37	1.30	1.42	1.27	1.17	1.16	1.54
Kernel weight (mg)	Horsham	2007	0.98	1.05	1.04	1.07	1.02	0.99	1.02	1.14
		2008	1.02	1.10	0.98	1.00	1.03	1.08	1.00	1.03
		2009	0.95	1.16	1.12	1.15	1.04	1.13	1.11	1.12
Screenings (%<2mm)	Horsham	2007	1.31	0.96	1.10	0.71	0.97	0.96	1.04	0.55
		2008	0.82	0.75	1.13	1.39	0.38	0.54	1.11	1.18
		2009	1.01	0.60	0.64	0.42	0.95	0.77	0.45	0.64
Harvest Index	Horsham	2007	0.95	0.98	1.00	1.05	0.95	1.00	0.93	1.08
		2008	0.94	1.00	0.82	0.90	0.89	1.25	0.72	0.73
		2009	0.93	1.04	1.04	1.11	0.94	1.14	1.03	1.09
Yield ($g\ m^{-2}$)	Walpeup	2008	1.64		1.54					
		2009	1.28		1.79					
Biomass ($g\ m^{-2}$)	Walpeup	2008	1.74		1.57					
		2009	1.09		1.34					
Kernel weight (mg)	Walpeup	2008	1.10		1.05					
		2009	1.12		1.12					
Screenings (%<2mm)	Walpeup	2008	1.08		0.70					
		2009	0.20		0.54					
Harvest Index	Walpeup	2008	0.95		1.00					
		2009	1.18		1.38					

Figure captions

Figure 1. Wheat (*Triticum aestivum*) yields under ambient CO_2 and average annual rainfall for each location plus irrigation (where applicable) at sites of major agricultural Free Air CO_2 Enrichment trials. In Maricopa, crops were continuously irrigated, but with different amounts and in Changping they received some supplemental irrigation. Yield ranges are between minimum and maximum averages from different years or treatments (high or low nutrients) reported. Horsham yields do not include irrigated plots. Data in this graph are from the following references: 1) this paper, 2) this paper; Tausz-Posch *et al.*, 2012, 3) Lam *et al.*, 2012, 4) Kimball *et al.*, 1995; Kimball *et al.*, 2002, 5) Weigel and Manderscheid 2012, 6) Högy *et al.*, 2009, 7) Ma *et al.*, 2007.

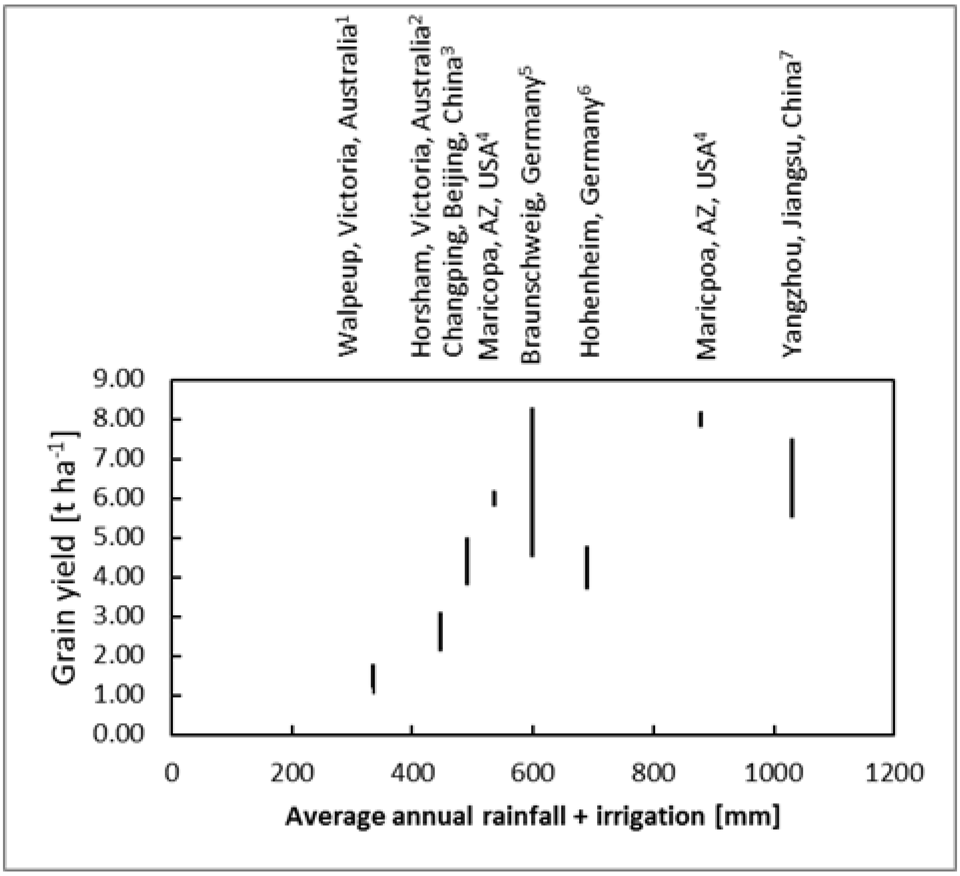
Figure 2. Rainfall, irrigation amounts (mm) and temperatures (°C) at Horsham and Walpeup, 2007-2009. The horizontal line at 32°C is the threshold above which heat can cause damage to wheat kernels (see text). Tic marks along TOS1 and TOS2 lines show dates of stem elongation (DC31) and flowering (DC65), respectively from left to right. Lines begin at sowing and end at harvest (DC90) and dates are shown in Table 1.

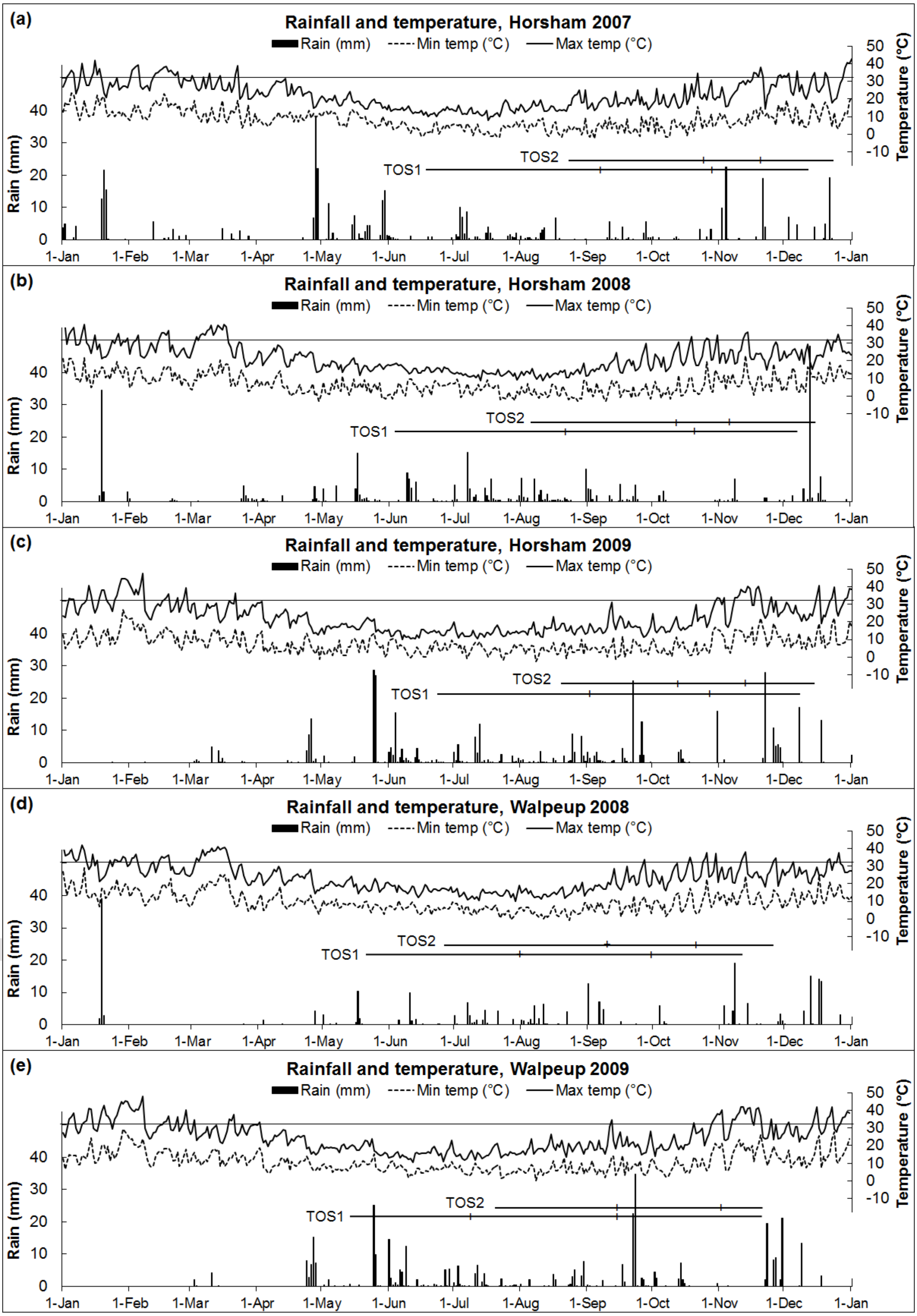
Figure 3. Example rings for each year at Horsham and Walpeup. (a) Horsham, 2007 and 2008, 12-m diameter; (b) Horsham, 2009, 16m diameter (c) Walpeup 2008-09, 4.5 m diameter. At Horsham, each ring was split (dotted line) for Time of Sowing (2007) or Irrigation (2008 and 2009) treatments. Sub-plots were randomized within each half ring. The above represent one ring within each experiment. At Horsham there were nitrogen input and growth and maturity destructive sampling treatments (see text) and in 2009 multiple cultivars were sown but only treatments Yitpi N0 and Janz N0 are reported here. At Walpeup, each ring had two sub-plots: growth (DC31 and DC65) and maturity (DC90) destructive samplings.

Figure 4. Horsham grain yield, above ground biomass, kernel weight and screenings (kernels < 2mm) of wheat (*Triticum aestivum* cv. Yitpi and cv. Janz), 2007-2009, e[CO_2] and a[CO_2]. Summarized

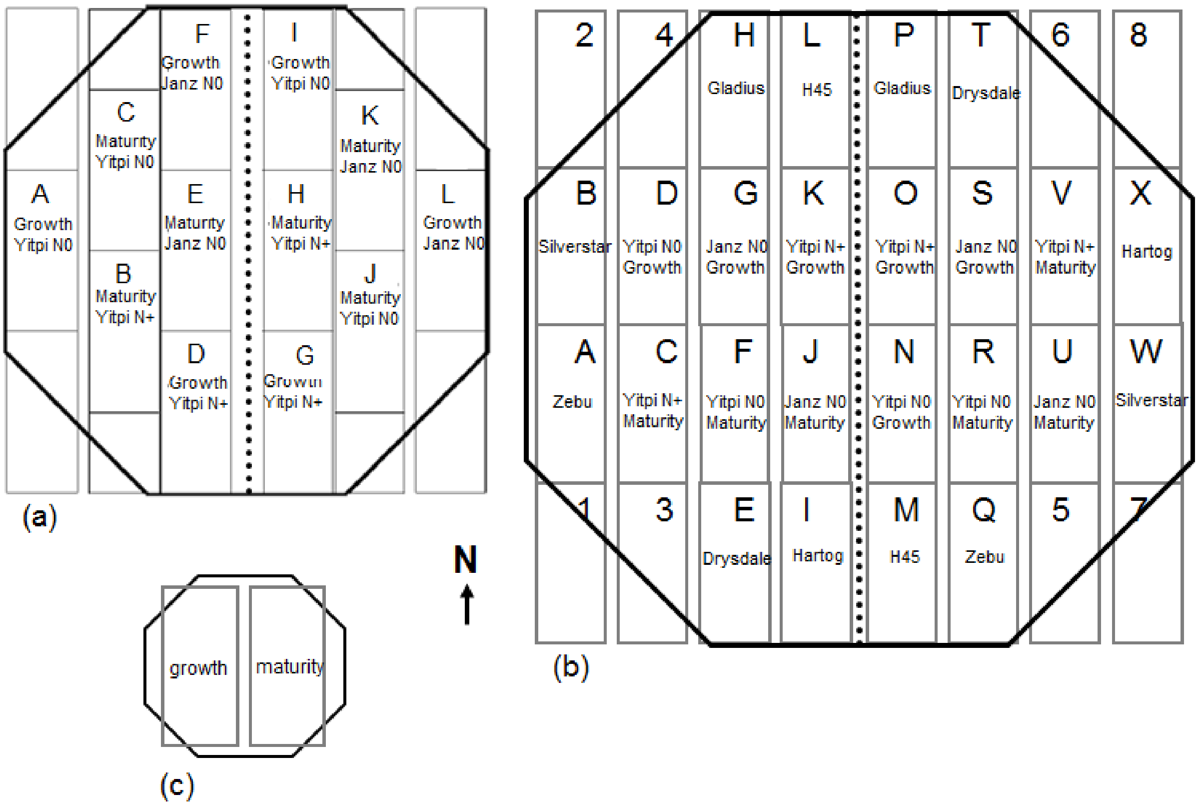
from Table S1. Error bars are standard deviations of the mean of n=4 replicates for each column. T1 = TOS1, T2 = TOS2, R = Rainfed, S = Supplemental irrigation, J = Janz cultivar and Y = Yitpi cultivar. Significance of ANOVA effects for elevated and ambient [CO_2] (CO_2), supplemental irrigation vs. rainfed only (Irr), normal versus late time of sowing (TOS), and cultivars Yitpi and Janz (Cult) are indicated as follows: *** $p<0.001$, **$p<0.01$, *$p<0.05$, +$p<0.10$. Only significant interactions with [CO_2] are presented. Effects and interactions not listed were not significant ($p>0.10$) for the year in question.

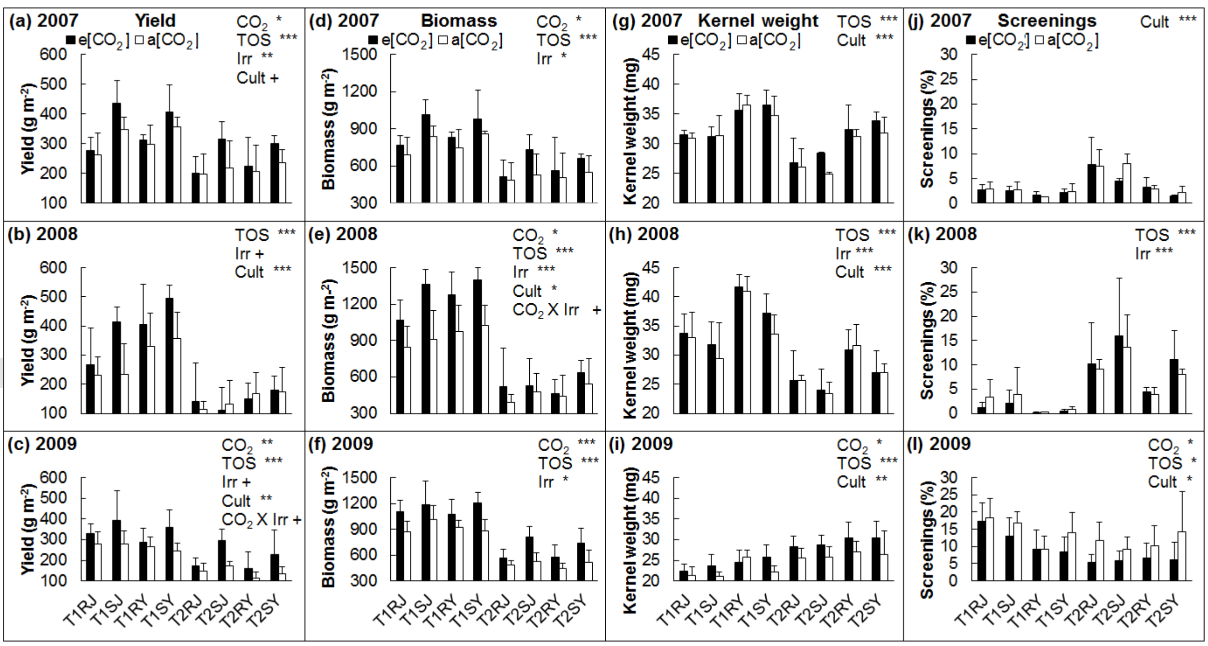
Figure 5. Walpeup grain yield, above ground biomass, kernel weight and screenings (kernels < 2mm) of wheat (*Triticum aestivum* cv. Yitpi), 2008-2009, e[CO_2] and a[CO_2]. Summarized from Table S2. Error bars are standard deviations of the mean of n=4 replicates for each column. T1 and T2 = Time of sowing, R = Rainfed, S = Supplemental irrigation. Significance of ANOVA effects for elevated and ambient [CO_2] (CO_2) and normal versus late time of sowing (TOS) are indicated as follows: *** $p<0.001$, **$p<0.01$, *$p<0.05$, +$p<0.10$. Only significant interactions with CO_2 are presented. Effects and interactions not listed were not significant ($p>0.10$) for the year in question.

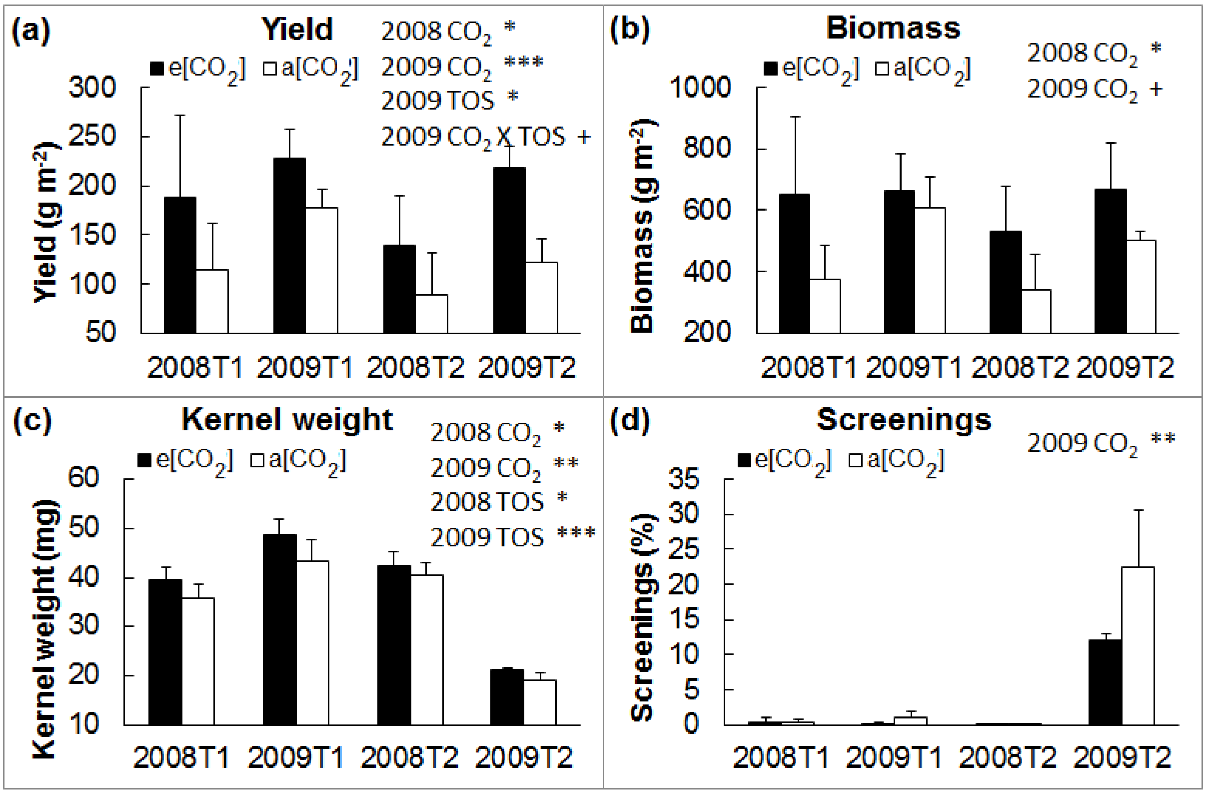

Walpeup, Victoria, Australia[1]
Horsham, Victoria, Australia[2]
Changping, Beijing, China[3]
Maricopa, AZ, USA[4]
Braunschweig, Germany[5]
Hohenheim, Germany[6]
Maricpoa, AZ, USA[4]
Yangzhou, Jiangsu, China[7]
Grain yield [t ha^{-1}]
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
200
400
600
800
1000
1200
Average annual rainfall + irrigation [mm]



(a)
Rainfall and temperature, Horsham 2007
(b)
Rainfall and temperature, Horsham 2008
(c)
Rainfall and temperature, Horsham 2009
(d)
Rainfall and temperature, Walpeup 2008
(e)
Rainfall and temperature, Walpeup 2009
Rain (mm)
Min temp (°C)
Max temp (°C)
Temperature (°C)
TOS1
TOS2
1-Jan
1-Feb
1-Mar
1-Apr
1-May
1-Jun
1-Jul
1-Aug
1-Sep
1-Oct
1-Nov
1-Dec


F
Growth
Janz N0
I
Growth
Yitpi N0
C
Maturity
Yitpi N0
K
Maturity
Janz N0
A
Growth
Yitpi N0
E
Maturity
Janz N0
H
Maturity
Yitpi N+
L
Growth
Janz N0
B
Maturity
Yitpi N+
J
Maturity
Yitpi N0
D
Growth
Yitpi N+
G
Yitpi N+
(a)
N
growth
maturity
(c)
2 4 H L P T 6 8
Gladius
H45
Gladius
Drysdale
B D G K O S V X
Silverstar
Yitpi N0 Growth
Janz N0 Growth
Yitpi N+ Growth
Yitpi N+ Growth
Janz N0 Growth
Yitpi N+ Maturity
Hartog
A C F J N R U W
Zebu
Yitpi N+ Maturity
Yitpi N0 Maturity
Janz N0 Maturity
Yitpi N0 Growth
Yitpi N0 Maturity
Janz N0 Maturity
Silverstar
1 3 E I M Q 5 7
Drysdale
Hartog
H45
Zebu
(b)


(a) 2007 Yield
e[CO2] a[CO2]
CO2 * TOS *** Irr ** Cult +
Yield (g m-2)
(b) 2008
TOS *** Irr + Cult ***
(c) 2009
CO2 ** TOS *** Irr + Cult ** CO2 X Irr +
(d) 2007 Biomass
CO2 * TOS *** Irr *
Biomass (g m-2)
(e) 2008
CO2 * TOS *** Irr *** Cult * CO2 X Irr +
(f) 2009
CO2 *** TOS *** Irr *
(g) 2007 Kernel weight
TOS *** Cult ***
Kernel weight (mg)
(h) 2008
TOS *** Irr *** Cult ***
(i) 2009
CO2 * TOS *** Cult **
(j) 2007 Screenings
Cult ***
Screenings (%)
(k) 2008
TOS *** Irr ***
(l) 2009
CO2 * TOS * Cult *
T1RJ T1SJ T1RY T1SY T2RJ T2SJ T2RY T2SY



(a)
Yield
■e[CO2] □a[CO2]
2008 CO2 *
2009 CO2 ***
2009 TOS *
2009 CO2 X TOS +
Yield (g m-2)
300
250
200
150
100
50
2008T1 2009T1 2008T2 2009T2
(b)
Biomass
■e[CO2] □a[CO2]
2008 CO2 *
2009 CO2 +
Biomass (g m-2)
1000
800
600
400
200
2008T1 2009T1 2008T2 2009T2
(c)
Kernel weight
■e[CO2] □a[CO2]
2008 CO2 *
2009 CO2 **
2008 TOS *
2009 TOS ***
Kernel weight (mg)
60
50
40
30
20
10
2008T1 2009T1 2008T2 2009T2
(d)
Screenings
■e[CO2] □a[CO2]
2009 CO2 **
Screenings (%)
35
30
25
20
15
10
5
0
2008T1 2009T1 2008T2 2009T2