Effects of future atmospheric CO_2 concentration on the productivity and nitrogen fixation of pulses under Free Air CO_2 Enrichment

M. Bourgault[1], R. Armstrong[2], J. Brand[2], J. Nuttall[2], S. Tausz-Posch[1], S.K. Lam[3], M. Tausz[4], G. Fitzgerald[2]

[1] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, 4 Water St, Creswick, VIC 3363.
[2] Department of Economic Development, Jobs, Transport and Resources, Grains Innovation Park, 110 Natimuk Rd, Horsham, VIC 3401
[3] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, 500 Yarra Boulevard, Richmond, VIC 3121
[4] School of Ecosystem and Forest Sciences, The University of Melbourne, 4 Water St, Creswick, VIC 3363

Abstract
Future atmospheric CO_2 levels are expected to increase from current levels of approximately 400 µmol mol^{-1} to 550 µmol mol^{-1} by 2050. This is a very rapid increase in one of the fundamental resources for plant growth. To determine the effects that this will have on dryland agricultural systems, the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility was established in 2007 in Horsham, Victoria. AGFACE comprises 8 rings in a complete randomised block design treated with either ambient (a[CO_2] ~ 400 ppm) or elevated (e[CO_2] ~ 550 ppm) CO_2. From 2010 to 2012, five cultivars of field peas (Pisum sativum) were grown in AGFACE; in 2013 and 2014 lentils (Lens culinaris) were grown (6 cultivars). The research questions have moved from effect-type questions (does growth and yield increase under e[CO_2]?) to physiological questions trying to resolve whether genotypic differences exist in response to e[CO_2] with a particular emphasis on water and nitrogen (N) use efficiency related traits. Yields were increased by e[CO_2], from 16% to 39% for field peas, and from 34% to 147% in lentils. Small but significant reductions in leaf and grain N were found, contrary to expectations. In addition, N contribution from pulses appear to be increased by the higher biomass under e[CO_2], but not by a greater proportion of N derived from fixation.

Key words
Climate change adaptation, genotypic variability, pulse pre-breeding

Introduction
Atmospheric CO_2 concentrations ([CO_2]) have been increasing from about 280 ppm to 400 ppm from the pre-industrial era until now (March 2015; www.co2now.org). This increase in one of the fundamental resources of plant life, the substrate of photosynthesis, has direct implications for plant metabolism.

While it is well established that elevated [CO_2] (e[CO_2]) increases plant growth and yield, it is also thought to increase water use efficiency and nutrient use efficiency (Leakey et al 2009). However, these results have been observed in Free Air CO_2 enrichment (FACE) facilities in environments with higher natural rainfall and/or irrigated agricultural systems and there were concerns that they might not apply to relative low rainfall Australian dryland agriculture. The Australian Grains Free Air CO_2 Enrichment (AGFACE) facility was established in 2007. Over the last eight years, the research questions have evolved from effect-type questions (does growth and yield increase under e[CO_2]?) to physiological questions trying to resolve whether genotypic differences exist in response to e[CO_2] with a particular emphasis on water and nitrogen (N) use efficiency related traits.

Pulses are increasingly seen as an essential component of sustainable agricultural systems as a high value commodity in themselves as well as due to their ability to contribute N to the cropping system via symbiotic fixation. Elevated [CO_2] could help this symbiosis by providing extra assimilates to bacteria, and increase the N contribution of legumes to the subsequent crop. Under e[CO_2], cereals also tend to show decreased grain protein and nutrient concentrations (Högy and Fangmeier 2008), but legumes should not be susceptible to this dilution (Jablonski et al 2002).

Materials and Methods
The AGFACE facility is located near Horsham, Victoria on a cracking clay (Vertosol) soil. A detailed description of the site and the CO_2 exposure equipment is given in Mollah et al (2009). Briefly, the study site has approximately 35% clay content at the surface increasing to 60% at 1.4 m depth. Elevated CO_2 levels

(target 550 µmol mol^{-1} air) were maintained during daylight hours by injecting pure CO_2 into the air on the upwind side from horizontal stainless-steel tubes positioned about 150 mm above the canopy and following the growth of the crop (Figure 1). Concentrations were maintained within 90% target (495-605 µmol mol^{-1} air) for 93-98% of the time.

Pulses were grown in eight octagonal 'rings' in a randomised block design with four blocks. Within each block, there were one ambient (a[CO_2] ~ 390-400 µmol mol^{-1} air) and one elevated (e[CO_2] ~ 550 µmol mol^{-1} air) ring. From 2010 to 2012, peas were grown in rotation with wheat. Rings were 16 m in diameter, and split for a plus/minus supplemental irrigation treatment. Within each ring, cultivars of field peas were grown in sub-plots (4 by 1.4 m). In 2013, lentils were grown in 8-m rings, also split for a plus/minus supplemental irrigation treatment, in subplots of 2 rows (0.54 by 4 m). In 2014, lentils were grown in 4-m diameter rings and subplots of 4 rows (1 by 2 m) with no supplemental irrigation (Figure 1).

In addition, the SoilFACE array consists of eight 1-m deep bunkers dug in the soil (4 ambient and 4 elevated [CO_2]) with large intact soil cores (30 cm diameter x 100 cm deep) comprising three soil types: a Mallee Calcarosol, a Wimmera Vertosol and a High Rainfall Zone (HRZ) Chromosol (Figure 2) which permits the investigation of interactions between CO_2 level and soil type on crop growth.

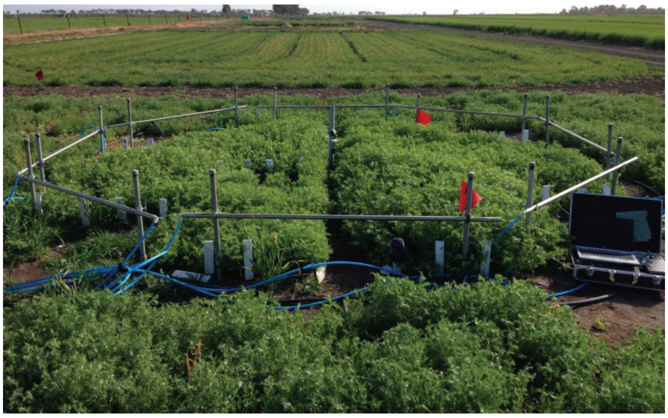

Figure 1: 4-m lentil elevated [CO_2] ring in 2014

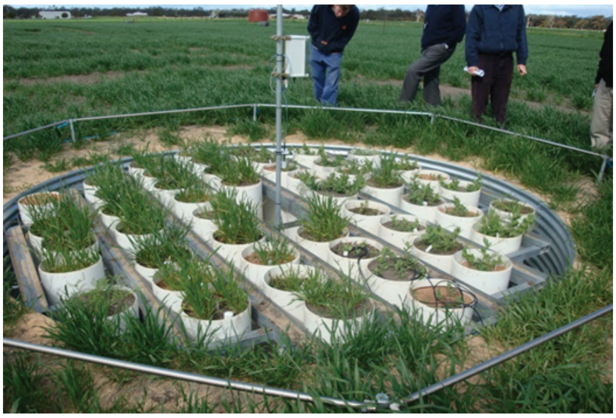

Figure 2: SoilFACE ring array

AGFACE 2010-2012: Field peas growth and grain yield

Five field pea cultivars were selected for their contrasting agronomic characteristics viz.leafiness, flowering time and duration, maturity, biomass accumulation, pod set and seed size. In particular, the cultivar PBA Hayman is a dual purpose, high biomass, small seeded cultivar (with low grain harvest index) representing an interesting contrast for evaluating potential sink limitations.

Averaged over the five cultivars, yields of field pea increased by 25% in 2010 (from 5.0 to 6.3 t ha^{-1}), 16% in 2011 (from 3.6 to 4.2 t ha^{-1}), and 39% in 2012 (from 2.6 to 3.6 t ha^{-1}) under e[CO_2]. There were no significant differences between cultivars in the yield response to e[CO_2] as illustrated in Figure 3 with cultivars aligning on or close to the line of average response. Similarly, e[CO_2] increased biomass at all growth stages observed but with no significant interaction between cultivar and CO_2 treatment..

However, there was genotypic variability in the grain N response to e[CO_2] in a three-way interaction with irrigation (Figure 4). Two cultivars (Bohatyr and Kaspa) consistently failed to maintain grain N concentrations under e[CO_2] regardless of water availability. By contrast, the cultivar Sturt maintained grain N concentration under rainfed conditions and PBA Twilight maintained grain N under supplemental irrigation. The dual-purpose cultivar PBA Hayman (small seeded and low harvest index) produced grains with higher N concentration and was better able to maintain grain N under e[CO_2] compared to other cultivars under both water regimes. Interestingly, the cultivars Sturt and PBA Twilight also differed in their yield response to irrigation with Sturt showing the greatest increase in yield and Twilight showing no response to the additional water. How some cultivars maintain grain N under e[CO_2] and contrasting irrigation treatments warrants further research.

SoilFACE 2009-2010: Field pea nitrogen fixation

Nitrogen fixation of field pea (line OZ0601) was assessed using the ^{15}N natural abundance technique with wheat as a reference species. Soil properties, especially soil nitrate supply, had a much greater effect on the amount of N fixed than e[CO_2] treatment, and significant differences were found in biomass, shoot N concentration, N uptake and N fixed in different soil types (Table 1).

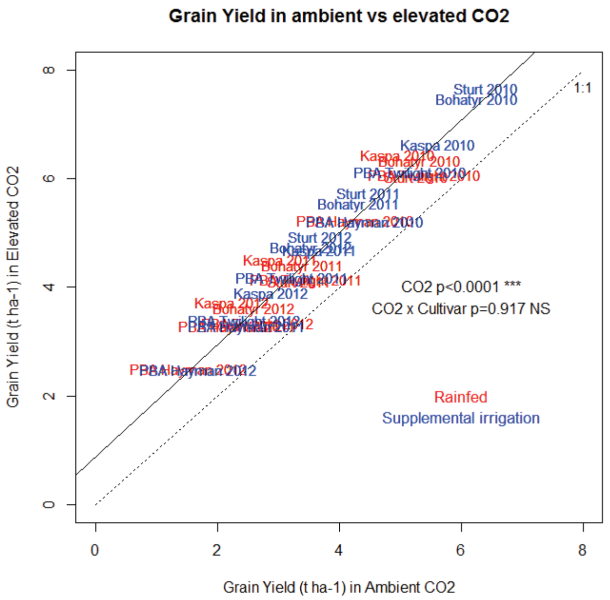

Figure 3: Lack of genotypic differences in the responsiveness of grain yield to e[CO_2] in field peas

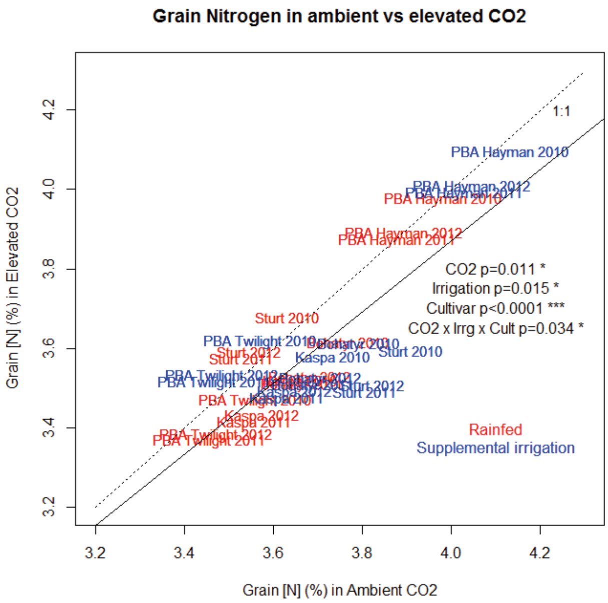

Figure 4: Genotypic differences in the response of grain nitrogen to e[CO_2] in field peas

The shoot biomass recorded in a Mallee Calcarosol in both 2009 and 2010 was significantly lower than that in the HRZ Chromosol but the proportion of legume N derived from the atmosphere (%Ndfa) was the opposite (e.g. 54.4% in the Calcarosol vs 9.9% in the Chromosol, when averaged across ambient and e[CO_2] in 2010). These differences in %Ndfa reflected the much higher soil nitrate levels recorded in the Chromosol at sowing, which can significantly inhibit N_2 fixation, compared to the Calcarosol.

Whereas there was no direct effect of e[CO_2] on the amount of N fixed in 2009 (which is the product of shoot N uptake and %Ndfa), in 2010 the overall amount of N fixed was more than doubled in the e[CO_2] treatment, but only when grown in the Vertosol; the Calcarosol and the Chromosol showed no stimulation with e[CO_2]. These results suggest that the effect of e[CO_2] on nitrogen fixation by field pea is not clear cut and that background environmental properties such as soil fertility will interact with e[CO_2] (see Armstrong et al 2015).

Table 1: Analysis of Variance for the SoilFACE experiment

	Dry matter (g/core)	Shoot N (%)	N uptake (g/core)	%Ndfa	N fixed (g/core)
	2009				
[CO_2]	NS	NS	NS	NS	NS
Soil type	**	*	*	NS	**
[CO_2] x Soil type	NS	NS	NS	NS	NS
	2010				
[CO_2]	0.06		NS	NS	NS
Soil type	***		***	***	***
[CO_2] x Soil type	*		0.06	NS	*

Significance levels: p>0.05 NS, 0.05>p>0.01 *, 0.01>p>0.001 **, p<0.001 ***

AGFACE 2013-2014: Lentils growth and grain yield

Six lentil cultivars were chosen for their genetic diversity and contrasting quality and agronomic characteristics differentiating in seed type, flowering time, maturity and vigour. The lentil line 05H010L-07HS3010 is a high biomass line that has a low harvest index.

Yields of lentil cultivars were increased by 34% in 2013 (from 2.7 t ha^{-1} in ambient to 3.6 t ha^{-1} under e[CO_2]) and by 147% in 2014 (from 0.38 t ha^{-1} in ambient to 0.94 t ha^{-1} under e[CO_2]), which suggests an ameliorating effect of e[CO_2] under severe drought terminal stress in the 2014 season. Cultivars differences were not significant (p=0.13 in 2013 and p=0.10 in 2014), but PBA Ace and PBA Jumbo tended to be higher yielding while 05H010L-07HS3010 tended to be consistently lower (Figure 5). Once again, we could not detect any significant differences in yield responsiveness to e[CO_2] between cultivars.

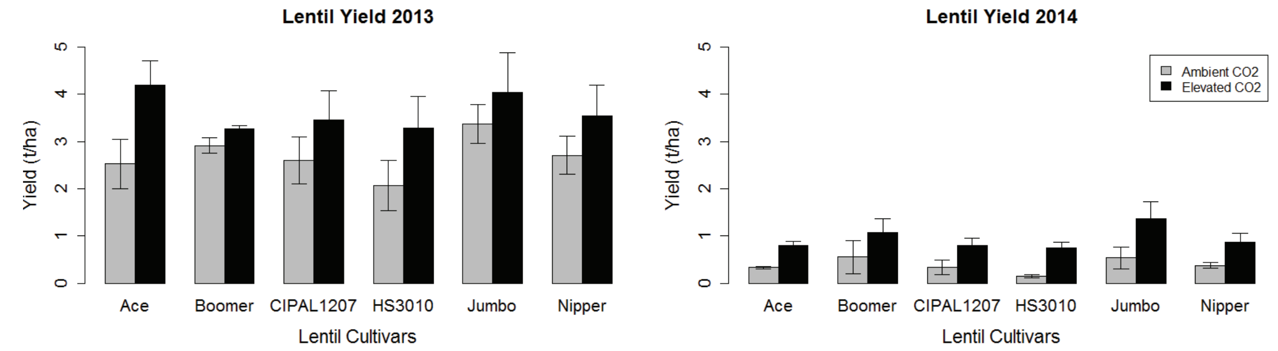

Figure 5: Grain yield in six lentil cultivars grown in AGFACE (2013-2014)

Current experimentation

In 2014, the cultivar PBA Ace and the lentil line 05H010L-07HS3010 were subjected to a 3-day heat shock under closed-top chambers with temperature raised to 40ºC during the day. These same plots were also equipped with mini-rhizotrons to scan images of the root system throughout the season. Preliminary results suggest that the heat shock has increased yields slightly from 0.51 to 0.65 t ha^{-1}. We also found cultivar differences in rooting depth, with 05H010L-07HS3010 reaching deeper layers faster while we could not find roots below 50 cm in PBA Ace. In addition, e[CO_2] increased root growth at all layers and particularly so in the 37.5 to 50 cm layer. These measurements will be collected again in the 2015 season.

Conclusion

Clearly, eCO_2 should increase yields of pulse crops in the absence of temperature increases. In very dry years, eCO_2 may make the difference between a commercial crop failure and a crop worth harvesting. However, we have also noted potential decreases in leaf and grain N and there genotypic variability for such traits provides the potential for better adapted varieties via breeding. While the amount of N fixed is increased through greater biomass accumulation under eCO_2, the proportion of N derived from the atmosphere remains similar, with soil type and soil N content appearing to have more effect on fixation.

Acknowledgements

Research at the AGFACE facility is jointly run by the Victorian Government and the University of Melbourne and receives substantial additional funding from the Grains Research & Development Corporation and the Australian Department of Agriculture. We wish to acknowledge the crucial contributions of Samuel Henty, Russel Argall, Peter Howie, Mahabubur Mollah, Justine Ellis, Jennifer Briggs in running the AGFACE facility.

References

Armstrong RD, Bourgault M, Lam SK (2015). Soil type influences N_2 fixation in fieldpeas more than elevated CO_2. In Proceedings of 17th ASA Conference, 20-24th September 2015, Hobart Australia. www.agronomy2015.com.au

Högy P, A Fangmeier, 2008. Effects of atmospheric CO_2 on grain quality of wheat. Journal of Cereal Science 48, 580-591.

Jablonski LM, X Wang, P Curtis, 2002. Plant reproduction under elevated CO_2 conditions: a meta-analysis of reports on 79 crops and wild species. New Phytologist 156: 9-26.

Leakey ADB, EA Ainsworth, CJ Bernacchi, A Rogers, SP Long, DR Ort, 2009. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany 60(10): 2859-2876.