

# The impacts of climate change variables on vegetative and reproductive development of six genotypes of cacao

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## Abstract

Whilst climate change has the potential to effect cacao production, limited research has been performed examining the impact these changes are likely to have on cacao physiology and yield, and the potential to identify genetic variation in responses.

Data is presented from the culmination of a five-year, greenhouse-based research project on the effects of elevated CO<sub>2</sub> (ECO<sub>2</sub>) and water deficit stress in mature pod-bearing clones (CL 19/10, ICS 1, IMC 67, POUND 7/B, SCA 6, SPEC 54/1). Trees were grown for two years under CO<sub>2</sub> enrichment (700 ppm) and limited soil moisture.

Overall, water deficit caused a reduction in photosynthesis, stomatal conductance and a number of growth parameters, and an increase in water-use efficiency (WUE). Stem diameter increment, photosynthesis, quantum efficiency and WUE increased significantly in response to ECO<sub>2</sub>. The decline in quantum efficiency due to water stress was less severe under the elevated compared to the ambient CO<sub>2</sub> treatment. Similarly, the water deficit treatment resulted in a significant increase in WUE which was further enhanced under ECO<sub>2</sub> in particular genotypes.

In mature clones, the effects of ECO<sub>2</sub> on pod development were not apparent until the second year of exposure when pod size increased under ECO<sub>2</sub>. Water deficit caused a decrease in pod development in the first year of exposure but not the second, suggesting a potential adaptive response to water stress.

Genetic differences in fatty acid responses to ECO<sub>2</sub> were evident in SCA 6 and POUND 7/B. Genotypic variation in quantum efficiency, WUE and biomass partitioning between leaves and stems was also identified.

Overall, ECO<sub>2</sub> had a positive effect on a number of growth and photosynthetic parameters in cacao and it appears that ECO<sub>2</sub> could play an important role in mitigating against some of the negative impacts of water deficit stress through enhancement of WUE.

A new project is under way at the University of Reading (2017-2021) which builds upon this research. It aims to identify the physiological basis for tolerance to water deficit and high temperature stress. The interactive effects of elevated CO<sub>2</sub> and high temperature stress will be explored and screening

tools will be developed to speed up the identification of more resilient germplasm. Using data from past and ongoing research, models will be developed to predict physiological and yield responses to varying climate conditions.

## Introduction

Along with increases in CO<sub>2</sub> concentration, average global temperatures are expected to increase by approximately 2 to 5°C by the end of this century and variation in precipitation patterns is already affecting many areas (IPCC, 2013). Cacao is one of the most important tropical perennial crops but, considering its economic importance, relatively few studies have been conducted into how cacao is expected to respond to the progression of climate change. In C<sub>3</sub> plant species, such as cacao, elevated CO<sub>2</sub> (ECO<sub>2</sub>) generally increases growth and photosynthesis, reduces stomatal conductance (g<sub>s</sub>) and often improves intrinsic water use efficiency (WUE<sub>i</sub>). Previous studies in cacao seedlings have shown that ECO<sub>2</sub> resulted in an increase in vegetative growth and photosynthetic rate both in instantaneous exposure studies and more long-term growth studies (Baligar *et al.*, 2005; 2008; Lahive *et al.*, 2018). To date, growth under ECO<sub>2</sub> has not been studied in mature, pod-bearing cacao trees. Assimilate availability (altered either through changes in light levels or amount of vegetative growth) has been linked to changes in yield in cacao (Daymond *et al.*, 2002a; Daymond & Hadley, 2008). It is hypothesised that increased assimilation due to ECO<sub>2</sub> may impact on yield and bean quality characteristics.

In this study, mature pod bearing trees of different genotypes of cacao were grown under ECO<sub>2</sub> and water deficit (WD). Photosynthetic and growth responses were examined in response to these climate variables.

## Materials and Methods

Six different genotypes of *T. cacao* (CL 19/10, ICS 1, IMC 47, Pound 7/B, SCA 6 and SPEC 54/1) were used in this study. Clones of each genotype were propagated by patch budding onto seedlings of 'GU' clones (originating from French Guiana; Lachenaud *et al.*, 2007) at different dates between July 2009 and June 2012. In June and July 2013 all trees were transplanted into 50 L pots filled with a mixture of sand, gravel and vermiculite (1:2:2). The trees were watered to excess six times daily with a modified Long Ashton nutrient solution (End, 1990) until treatments were implemented. The experiments were conducted within four compartments in a 2\*2 square arrangement of a greenhouse suite specifically designed to study climate change variables on cocoa. Each compartment measured 10m x 6m. Two compartments were maintained at ECO<sub>2</sub> (700 ppm) and two at ambient CO<sub>2</sub> (ACO<sub>2</sub>), each treatment being represented on either side of the square. Four trees of each genotype were placed in each of four greenhouses (except for CL 19/10 and SPEC 54/1, *n* = 14 and 15, respectively). Well-watered

(WW) trees were irrigated with nutrient solution several times a day; the average soil moisture content for these trees was 36.3%. The WD treatment trees were irrigated to maintain the soil moisture within the pot to between 10 - 17% with re-watering events every 14 days to prevent solute build up within the pot. Temperature conditions within each compartment were set to mimic typical conditions in Ghana in January through a combination of heating and venting. The target temperature was set to cycle diurnally between a minimum of 19°C and a maximum of 32°C. Treatments were initiated on 02-09-13 and continued until 26-07-15 (23 months).

### Photosynthetic measurements

Photosynthetic light responses were measured on the youngest fully matured and hardened leaf on each plant, which had fully developed under experimental conditions (after approximately 10 weeks growth under experimental conditions), using an *LCpro+* portable infrared gas analyser fitted with a light attachment and an internal CO<sub>2</sub> source (*ADC BioScientific*, Great Amwell, Herts, UK). Net photosynthetic rate (A) was measured at eight irradiance intensities (696, 435, 261, 174, 87, 44, 26, 0 μmol m<sup>-2</sup> s<sup>-1</sup>) and at growth CO<sub>2</sub> concentration. Photosynthetic light response curves were then fitted by means of a non-rectangular hyperbola in the form:  $A = \{\Phi \cdot Q + A_{\max} - \sqrt{[(\Phi \cdot Q + A_{\max})^2 - 4 \cdot \Phi \cdot Q \cdot k \cdot A_{\max}] / 2k} - R$ , where  $\Phi$  is apparent quantum efficiency, Q is irradiance,  $A_{\max}$  is light saturated photosynthetic rate, k is convexity and R is respiration rate. *Photosyn Assistant* software (*Dundee Scientific*, Dundee, UK) was used to fit a non-rectangular hyperbola to the photosynthetic data. Stomatal conductance was recorded at saturating irradiance (696 μmol m<sup>-2</sup> s<sup>-1</sup>). WUE<sub>i</sub> was calculated as A/g<sub>s</sub>.

### Vegetative growth

Stem diameter measurements were made 10 cm from the base of each tree at the beginning of the experimental period and again after four months growth. The difference in diameter was calculated as stem diameter increment. After 23 months growth, each tree was harvested and dry above-ground biomass measured after drying at 70°C in a ventilated oven.

### Pod harvest

Manual pollinations were carried out using Amelonado flowers as pollen donors. There were two harvests of pods during the experimental period. Year 1 harvest took place between 01-09-14 and 07-11-14 and year 2 harvest between 27-05-15 and 18-07-15. Once mature, the pods were separated into beans, husk and placenta, and dried in a ventilated oven at 70°C for 4-7 days after which the dry weights were measured.

## Bean fat analysis

Beans from the second year harvest of SCA 6 and Pound 7/B were analysed for bean fat content. A sample of dried unfermented beans were digested in hydrochloric acid, filtered and dried and extracted with petroleum ether (Eurofins Scientific, Eurofins Food Testing, Wolverhampton, UK).

## Statistical analysis

Unbalanced ANOVA was performed to test the effects of CO<sub>2</sub> treatment, water treatment and genotype and interactions on each of the parameters. Data transformation was carried out as necessary to normalise residuals before analysis. Means and standard errors were calculated from untransformed data and are presented here. All analyses were carried out using Genstat 13th edition.

## Results

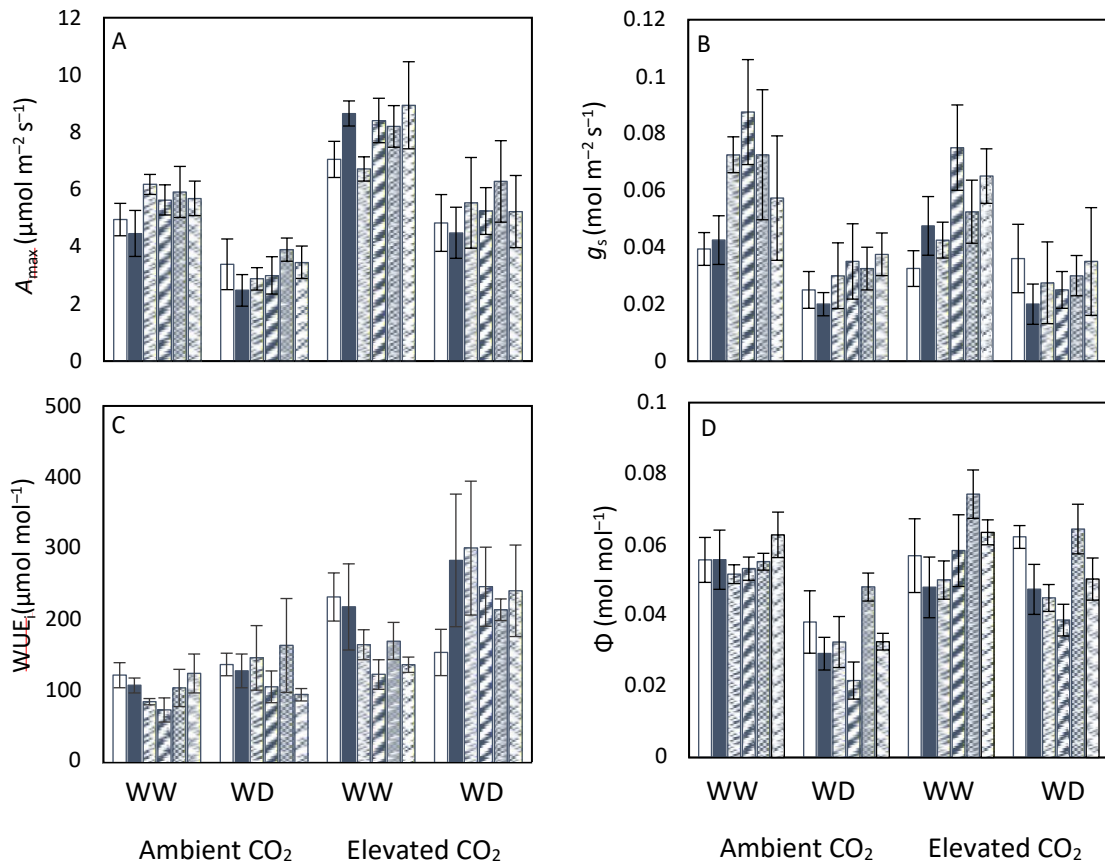
### Photosynthetic parameters

Gross light-saturated photosynthetic rate ( $A_{\max}$ ) increased by an average of 54 %, from  $4.31 (\pm 0.25) \mu\text{mol m}^{-2} \text{s}^{-1}$  in ACO<sub>2</sub>-grown trees to  $6.62 (\pm 0.35) \mu\text{mol m}^{-2} \text{s}^{-1}$  in trees grown at ECO<sub>2</sub> ( $P < 0.001$ ) (Fig. 1A). The water deficit treatment resulted in a significant reduction (- 60 %) in  $A_{\max}$  ( $P < 0.001$ ). There were no genotypic differences in  $A_{\max}$  and no significant interactions between treatments.

The WD treatment caused a significant reduction in  $g_s$  ( $P < 0.001$ ) (Fig. 1B). Average  $g_s$  decreased from  $0.058 (\pm 0.004) \text{mol m}^{-2} \text{s}^{-1}$  in WW trees to  $0.029 (\pm 0.003) \text{mol m}^{-2} \text{s}^{-1}$  in the WD trees. Stomatal conductance also varied among genotypes ( $P < 0.05$ ). CO<sub>2</sub> concentration did not significantly affect  $g_s$  ( $P > 0.05$ ).

Average WUE<sub>i</sub> in ACO<sub>2</sub>-grown trees was  $116.77 (\pm 8.3) \mu\text{mol mol}^{-1}$ . This increased to  $208.5 (\pm 15.57) \mu\text{mol mol}^{-1}$  in those grown at ECO<sub>2</sub> (+ 79 %) ( $P < 0.001$ ) (Fig. 1C). The WD treatment increased WUE<sub>i</sub> compared to WW trees ( $P < 0.01$ ).

There was a significant interaction between CO<sub>2</sub> and water treatment in relation to  $\Phi$  ( $P = < 0.01$ ) (Fig. 1D). Under WW conditions  $\Phi$  increased by about 5% in response to ECO<sub>2</sub> ( $0.056 (\pm 0.002)$  to  $0.058 (\pm 0.003) \text{mol mol}^{-1}$ ), however, in WD conditions  $\Phi$  increased by 52% in response to ECO<sub>2</sub> ( $0.034 (\pm 0.003)$  to  $0.051 (\pm 0.003) \text{mol mol}^{-1}$ ). Quantum efficiency also varied between genotypes ( $P < 0.001$ ), with values ranging from  $0.043 (\pm 0.005)$  to  $0.061 (\pm 0.004) \text{mol mol}^{-1}$  for the genotypes Pound 7/B and SCA 6, respectively.

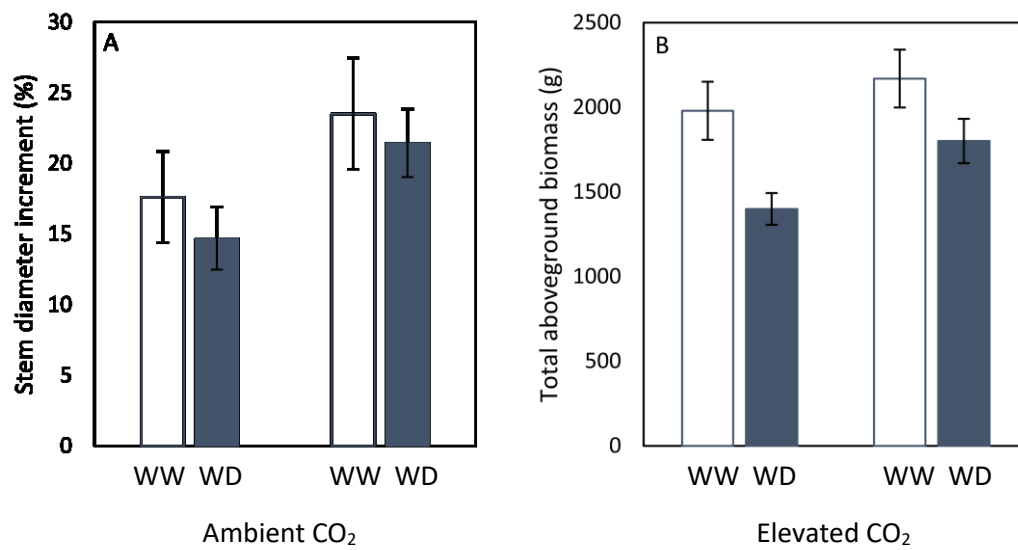


**Figure 1.** Photosynthetic parameters measured in six genotypes of cacao grown under elevated CO<sub>2</sub> and water deficit treatment. A: Light-saturated photosynthetic rate; B: stomatal conductance; C: intrinsic water use efficiency; D: quantum efficiency. WW: well-watered; WD: water deficit. □ CL19/10 ■ ICS1 ▨ IMC47 ▩ POUND7/B ▒ SCA6 ▤ SPEC54/1

### Vegetative growth

Trees grown at ECO<sub>2</sub> showed a significantly greater increase in stem diameter over the first four months of exposure compared with those grown under ACO<sub>2</sub> ( $P = 0.012$ ) (Fig. 2A). The magnitude of the response to ECO<sub>2</sub> varied between genotypes but the interaction was not significant.

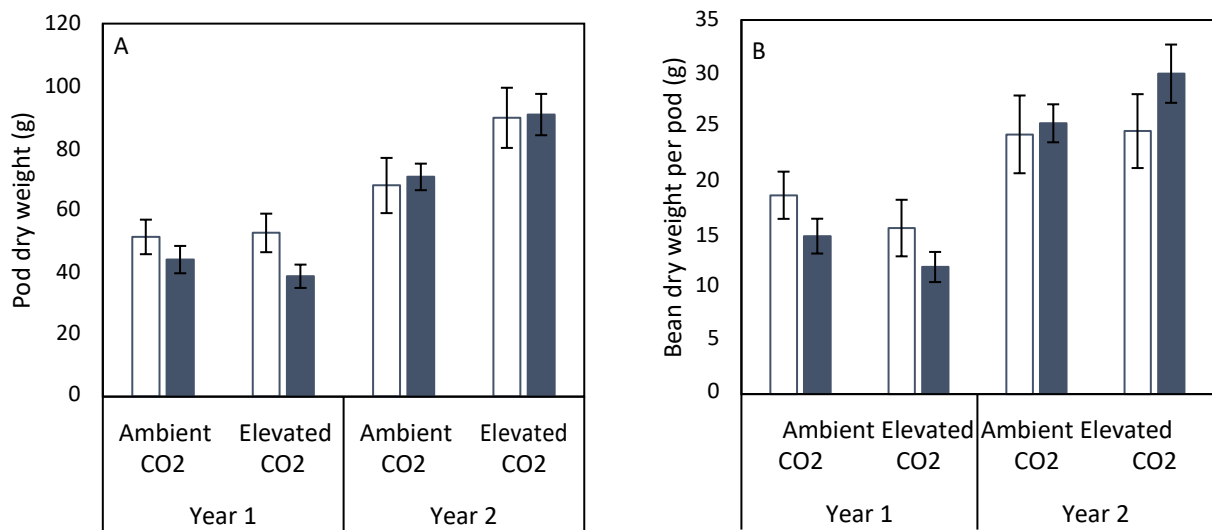
After 23 months trees grown under ECO<sub>2</sub> accumulated 17.5 % more aboveground biomass than those grown under ACO<sub>2</sub> ( $P < 0.01$ ) (Fig. 2B). The WD treatment resulted in a 30.6 % reduction in aboveground biomass compared to the WW treatment ( $P < 0.001$ ) and there were also significant differences in biomass between genotypes ( $P < 0.001$ ), ranging from an average of 1 ( $\pm 0.11$ ) Kg (SCA 6) to 2.42 ( $\pm 0.29$ ) Kg (CL 19/10).



**Figure 2.** Stem diameter increment during four months of growth under elevated CO<sub>2</sub> and water deficit stress (A) and total aboveground biomass production after 23 months growth under experimental conditions (B). Error bars show standard error.

### Pod & bean dry weight

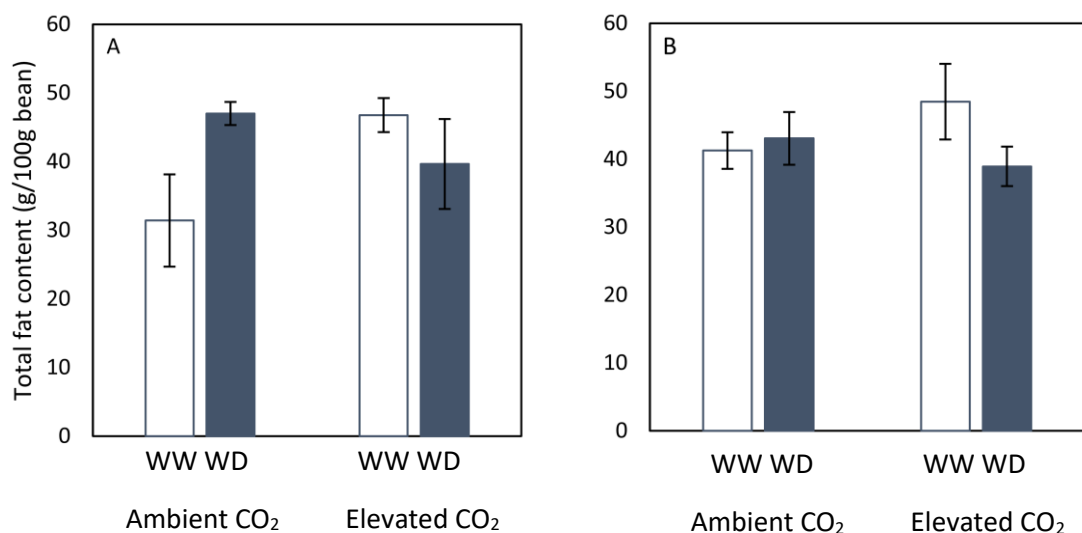
In general, pod and bean dry weights were significantly greater in the second harvest. In the first year's harvest there was no effect of CO<sub>2</sub> on pod dry weight ( $P > 0.05$ ) and the WD treatment caused a slight, but not significant, reduction in pod dry weight ( $P = 0.053$ ) (Fig. 3A). However, in the second year, pods which developed under ECO<sub>2</sub> conditions had significantly greater dry weight than those grown under ACO<sub>2</sub> ( $P < 0.005$ ), and there was no effect of water treatment ( $P > 0.05$ ). Both the WD and ECO<sub>2</sub> treatments caused a decline in bean dry weight in the first year but this was not significant, while there was no effect of treatments on bean dry weight in the second year (Fig. 3B).



**Figure 3.** Pod dry weight (A) and bean dry weight per pod (B) from cacao trees growing under elevated CO<sub>2</sub> and water deficit stress. Error bars show standard error. White bars: WW; black bars: WD

#### Bean fat content

There was a significant interaction between the water and CO<sub>2</sub> treatments in Pound 7/B but not SCA 6. Under ACO<sub>2</sub>, the WD treatment resulted in a significant increase in bean fat content from 31.4 ( $\pm$  6.7) g/100g bean to 47 ( $\pm$  6.6) g/100g bean. However, under ECO<sub>2</sub> no such increase in fat content was observed due to WD (Fig. 4A). In SCA 6 no main effects or interactive effects were observed (Fig. 4B).



**Figure 4.** Total fat content of beans, from the second year harvest, formed under elevated CO<sub>2</sub> and water deficit conditions in cacao genotypes Pound 7/B (A) and SCA 6 (B). Error bars show standard error.

## Discussion

In this study there was a significant enhancement of photosynthetic rate in trees grown at  $ECO_2$ . The average increase of 54 % is similar to previously reported values for juvenile trees and seedlings of cacao (Baligar *et al.*, 2008; Lahive *et al.*, 2018).  $WUE_i$  was also improved in trees grown at  $ECO_2$  and this could prove to be particularly beneficial in the future, enabling increased carbon uptake per unit water loss, in this way, the reduction in productivity due to WD could be reduced under  $ECO_2$  compared to the same WD conditions in  $ACO_2$  conditions. The WD treatment also increased  $WUE_i$  in these trees through limitation of stomatal aperture which limits water loss to a greater extent than carbon uptake.  $ECO_2$  may have the potential to reduce some of the negative effects of soil moisture deficit through enhancement of quantum efficiency ( $\Phi$ ) (a measure of photosynthetic performance under sub-saturating light levels), which in this study was most evident in WD trees. Growing cacao under shade is a management technique which may be necessary to protect against high temperature stress. Improved carbon uptake at low light levels will be beneficial under such conditions. The genetic variation observed in  $\Phi$  could also be useful in terms of identifying germplasm which respond most favourably to these conditions.

Despite the large increase in photosynthetic rate, the increase in above-ground biomass production was not as great. It is likely that changes in respiration rates may have an impact on the net carbon uptake by these trees. Although increases in photosynthetic rate were evident within the first few months of growth at  $ECO_2$  there was a lag before these effects were seen in relation to pod growth. In terms of reproductive development, the effects of  $ECO_2$  were not evident in the first year's harvest. However, in the second year the  $ECO_2$  treatment significantly increased pod dry weight, but bean dry weight per pod was unaffected, suggesting an alteration in biomass allocation patterns under  $ECO_2$ . It is possible that the lag in the observed growth response could be due to the increased assimilate being allocated toward woody biomass accumulation as after four months stem diameter increase was greater in the  $ECO_2$  treatment compared to ambient, and after 23 months growth vegetative biomass was significantly greater in the  $ECO_2$  grown trees relative to those grown at ambient  $CO_2$ . In sour orange trees grown under  $ECO_2$ , strong increases in trunk and branch volume were observed initially. However, later the increase in vegetative growth began to decline and a greater of biomass was allocated to fruits (Kimball *et al.*, 2007).

There was a trend towards decreased pod and bean weight in the first year in response to the WD treatment. The reduction in assimilation rate seen in response to water stress in the first year is most



likely to be responsible for the reduction in pod and bean development. However, the impact of the WD appears to have been stronger on vegetative growth compared to reproductive growth.

The fat content of the beans is the most valuable part of the bean. A range of factors such as climate, geographic region and genetics, are known to impact on bean fat content (Wood 1985). In beans of Pound 7/B there was an interaction between the water and CO<sub>2</sub> treatments such that the ECO<sub>2</sub> increased fat content but only under WW conditions. Unexpectedly, the WD treatment resulted in higher fat content in ambient CO<sub>2</sub> conditions. This result is at odds with observations of field grown cacao in Nigeria where beans which developed during drier conditions had lower bean weight and fat content compared to those which developed in wetter conditions (Wessel & Toxopeus 1967). As assimilation rates increased in trees growing under ECO<sub>2</sub>, this could account for greater bean fat content in Pound 7/B. There was also a significant increase in vegetative biomass production under ECO<sub>2</sub>, suggesting vegetative sinks may have been stronger than reproductive sinks in SCA 6 as there was no effect of CO<sub>2</sub> in this genotype. Identification of germplasm with strong reproductive sinks which favour carbon allocation towards the bean rather than toward vegetative growth, could ensure improved bean yields under ECO<sub>2</sub> conditions. Daymond *et al.* (2002b) have shown genetic variation exists in cacao in relation to yield partitioning, and this current study suggests that there is also variation in partitioning in response to CO<sub>2</sub>.

This study has shown that, across a number of genotypes, ECO<sub>2</sub> stimulates photosynthesis in mature cacao trees to a similar extent as it does in cacao seedlings. ECO<sub>2</sub> appears to ameliorate some of the negative effects of water deficit through improvements in WUE<sub>i</sub> and  $\Phi$  which may be beneficial under future climate change. Enhancements in vegetative growth are also seen as a result of photosynthetic enhancement under ECO<sub>2</sub>, however, pod and bean production do not appear to be as strongly influenced by ECO<sub>2</sub>. Further research at University of Reading is building on this research to assess the impacts of high temperature stress and CO<sub>2</sub> on growth and yield and to develop screening tools to speed the identification of more resilient germplasm.

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