Title: Elevated CO₂ concentrations alleviate the inhibitory effect of drought on physiology and growth of cassava plants

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ABSTRACT

Due to the rise in anthropogenic greenhouse gas concentrations, the earth's climate is expected to change, with precipitation being reduced in some areas resulting in growth-limiting droughts and, as a consequence, reduced plant productivity. We investigated the physiological and growth responses of cassava (*Manihot esculenta* Crantz) to approximate present-day ambient (390 $\mu\text{L L}^{-1}$) and elevated (750 $\mu\text{L L}^{-1}$) atmospheric CO₂ concentrations under well-watered and water deficit conditions, aiming at understanding how cassava would face those problems. Water deficits led to reductions in the Leaf Elongation Rate of plants grown at ambient as well as CO₂-enriched concentrations. However, plants grown at 750 $\mu\text{L L}^{-1}$ of CO₂ maintained leaf growth two days longer than plants grown at 390 $\mu\text{L L}^{-1}$. Three Days After Withholding Water (DAWW), photosynthesis and stomatal conductance were reduced in plants grown under ambient CO₂, while in plants under an elevated CO₂ concentration, these physiological functions remained similar to that of control plants grown under good water availability. Five DAWW plants grown with 750 $\mu\text{L L}^{-1}$ continued to have enhanced gas exchange compared with plants grown under 390 $\mu\text{L L}^{-1}$. Under drought stress, the instantaneous transpiration efficiency was always greatest for plants grown under elevated CO₂. The positive response of elevated CO₂ levels on total dry mass was 61% in the water-stressed plants and only 20% for the plants grown under good water availability. Stomatal limitation was an important factor reducing CO₂ assimilation in cassava growing under drought conditions.

Keywords: Water deficit, Gas exchange, Water-use efficiency, Transpiration, Biomass

1. INTRODUCTION

The atmospheric CO₂ concentration prevalent today is approximately 400 $\mu\text{L L}^{-1}$ and is expected to reach between 730 and 1020 $\mu\text{L L}^{-1}$ near the end of this century (Meehl et al., 2007). As a CO₂ level near 400 $\mu\text{L L}^{-1}$ limits the growth and photosynthesis of C₃ plants, its increase in the atmosphere is hypothesized to stimulate net photosynthetic CO₂ uptake and, consequently, promote increased biomass production and yield of C₃ crops (Kimball et al., 2002; Long et al., 2004; Rosenthal et al., 2012; Cruz et al., 2014).

In addition to its effects on photosynthesis and plant growth, the increase in atmospheric CO₂ concentration could potentially contribute to global warming due to its role as a greenhouse gas (Meehl et al., 2007; Forster et al., 2007). It is widely predicted that the amount and distribution of rainfall in various world regions are also responding to increasing atmospheric concentrations of CO₂ and other greenhouse gasses (Allen and Ingram, 2002). In fact, in many arid and semiarid regions, climate change has already contributed to a drop in rainfall intensity, with water deficit periods during the rainy season becoming longer, and evapotranspiration increasing due to increases in temperature (Batisani and Yarnal, 2010; Pingale et al., 2014). Thus, combined warmer temperatures and changes in precipitation amounts and dynamics are leading to increased risk of droughts for crops in many world regions.

Rising atmospheric levels of CO₂ can have a profound impact on plant water relations, which will likely alter plant responses to especially warming and increased drought (Morgan et al., 2004; Ziska et al., 2012). The interaction between CO₂ concentration and plant-soil water is a particularly important issue since water deficits constitute one of the main factors limiting food production in various parts of the world (Wullschleger et al., 2002). Increases in ambient CO₂ levels have been demonstrated to reduce stomatal aperture, thereby curtailing consumptive plant water use (Battipaglia et al., 2013) and thus improving plant growth (Morgan et al., 2001; Wall et al., 2001). Normally, when plants are exposed to CO₂-enriched atmospheres the rate of net CO₂ assimilation per unit area is increased and/or transpiration is reduced, thereby increasing the leaf-water use efficiency (Ainsworth and Long, 2005; Barton et al., 2012; Morgan et al., 2004; 2011). However, the stomatal response to elevated CO₂ levels may or may not differ depending on the species and the interactive relationship between the CO₂ levels and soil-water content (Wall et al., 2001; Fernandez et al., 2002; Bernacchi et al., 2003). In addition, the increase in the leaf area that occurs as a result of elevated CO₂ levels (Oikawa et al., 2013) can also enhance whole canopy transpiration and nullify or reduce the positive effects of stomatal closure on WUE (Leakey et al., 2009). Higher CO₂ may also benefit plants through promoting osmotic adjustment (Danyagri and Dang, 2013) and increasing carbon allocation to root growth (Iversen, 2010), thus promoting

greater capacities for absorption of water stored in the deeper soil layers, which may enable and extend carbon assimilation during periods of water deficit. However, for some species and conditions, the potential benefit of water relations due to higher CO₂ may not be sufficient to reduce the negative impacts of climate change on plant growth (Vaz et al., 2012; Perry et al., 2013; da Silva et al., 2013).

Several species have been recommended to be strategically cultivated by farmers in regions where extensive periods of drought are common. Among these crops with drought tolerance traits, one of the most preferred choices by both researchers and producers is cassava. Even under adverse soil and climatic conditions, cassava can produce a satisfactory root yield, while other annual crops barely survive (El-Sharkawy and Cock, 1987). This ability to produce under adverse conditions has resulted in cassava being recognized as a strategic crop for mitigating famine in many regions across the globe, especially where drought is a chronic problem (El-Sharkawy and Cock, 1987; F.A.O., 2005; Burns et al., 2010). Furthermore, due to its relative drought tolerance, cassava also may be one of the better-suited tropical food crops for adapting to climatic changes because under stress conditions it can shift its optimum temperature upwards and reduce its water requirement for photosynthesis, growth and production (El-Sharkawy, 2014). However, little is known about how cassavas respond to predicted atmospheric global changes.

Due to the importance of cassava as a strategic crop for semi-arid regions and the predicted changes in climatic and atmospheric conditions, the present study was designed to help understand the interaction between elevated CO₂ levels and water deficit on growth, physiology and dry mass accumulation in cassava.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL CONDITIONS AND PLANT CULTURE

The experiment was conducted in a climate-controlled greenhouse, with two bays, at the USDA-ARS Crops Research Laboratory in Fort Collins, Colorado. *In vitro* plantlets, from 2- to 3-month-old shoot cultures, of the commercial cassava variety CM 507–37 were transferred to 200 mL plastic cups, containing a commercial potting mix (Fafard 52 mix Conrad Fafard, Inc, Agawam, MA). The purpose of using *in vitro* plantlets was to obtain more uniform and virus-free plants. The top of the cups was covered with a transparent plastic cup for two weeks to maintain high humidity around the canopy and the plants were acclimated in a greenhouse (25±1°C and relative humidity of 50±2%). After 20 days of acclimation, the plants were selected for uniformity

prior to transplanting into 14 l pots (30 cm height x 24.5 cm diameter). Before transplanting, the pots were filled with a 2:2:1 mixture of perlite, peat moss and washed sand. After ten days in the 14 l pots, 50 % of the plants were transferred to a bay with an ambient CO₂ level (390 µL L⁻¹) and the others 50 % to an adjacent bay with an elevated CO₂ level (750 µL L⁻¹). Enrichment with CO₂ was performed between 06:00 h and 18:00 h and the CO₂ supply was continuously computer-controlled (Argus Control Systems Ltd., White Rock, BC). During the experimental period, the CO₂ level inside the bays ranged from 390± 10 µL L⁻¹ (ambient CO₂ treatment) and from 740±12 µL L⁻¹ (elevated CO₂ treatment). Plants were irrigated daily to reach field capacity and fertilized three times a week with modified Hoagland's nutrient solution (Hoagland and Arnon, 1950), as suggested by Cruz et al. (2003). The pH of the nutrient solutions was adjusted to maintain values between 6.3 and 6.5. Every 15 days, all the pots were thoroughly flushed with tap water to avoid substrate salinization and freshly-constituted nutrient solutions were then applied to all. To avoid possible spatial bays differences, the pots were re-positioned within each bay once a week, and every 15 days the CO₂ treatments and the corresponding pots were switched between the two bays (Perry et al., 2013). Air temperature inside both bays was maintained between 28.5±1.5 °C during the day and between 24±1°C during the night, using computer controlled air conditioners and heaters (York International, York, PA), while the relative humidity was maintained at 35±4%. Light inside the greenhouse was supplemented (12:12 h, day to night photoperiod) with 600 W lights (PL Light Systems, Beamsville, Ontario) which maintained a light intensity between 1150±50 µmol m⁻² s⁻¹ at bench level.

Thirty days after transplanting, water deficit cycles were initiated. Irrigation was suspended for seven days, followed by daily irrigation, to reach field capacity, for seven days. Following this procedure, five water deficit cycles of seven days each were induced. So, the treatments were: (i) Elevated CO₂ well-watered (750_WW), (ii) Elevated CO₂ with water deficit (750_WS), (iii) Ambient CO₂ well-watered (390_WW), and (iv) Ambient CO₂ with water deficit (390_WS). According to Tyree and Alexander (1993), small drying cycles followed by well-irrigated periods is the preferred approach for understanding how plants response to elevated CO₂ under drought than the unique drying cycle.

2.2. MEASUREMENTS

At the beginning of the fourth deficit cycle, 72 days post transplanting, the pots were sealed with a plastic film to prevent water evaporation, which left water loss to occur only from the plants. For an estimation of transpirational water loss, all pots were weighed daily, at approximately 07:00 h, using an electronic balance. After weighing, the well-watered pots were irrigated to reach field capacity. Evaluations of leaf growth were also initiated at this time. On each

plant, one folded leaf at a similar nodal position was tagged (Alves and Setter, 2000) and their growth measured over time. The central lobe length of leaf blades were measured daily with a ruler. Leaf-lobe Elongation Rate (LER) was calculated from the 1-d incremental increase in leaf length, according Alves and Setter (2000), and expressed in $\text{cm}^2 \text{d}^{-1}$. Leaf water potential and relative water content were not measured because in cassava these parameters remain relatively unchanged during water deficit episodes. In fact, Alves and Setter (2000), citing several authors, report that studies have indicated that, in cassava, stomatal closure is an early event in the response to water-limited conditions, usually well in advance of a detectable decrease, if any, in leaf water potential. These results imply that gas exchange, is a sensitive and excellent parameter for detecting physiological response of cassava to drought. For gas exchange, measurements were taken at one, three and five days after withholding water and one day after re-watering. The net photosynthesis (P_n), stomatal conductance (g_s) and transpiration rate (E) were determined on the central lobe of the youngest fully-expanded leaf. Evaluations were done using the CIRAS-1 steady state portable gas analysis system with a PLC (U) leaf chamber (PP systems, United Kingdom). The CIRAS-1 system provided the temperature and vapor pressure control, which were adjusted on the day of measurement to approximate growth conditions. The CIRAS-1 was set to record the measurements of the gas exchange parameters using the concentrations of 390 and 750 $\mu\text{L L}^{-1}$ of CO_2 for plants grown under both CO_2 treatments. The leaf chamber was connected to an artificial light source to provide a photosynthetic photon flux density of 1100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on the leaf surface. Measurements were taken between 09:00 h and 12:00 h. Gas exchange measurements were recorded after a stable CO_2 exchange rate was obtained, which occurred 4-5 min after the leaf was placed in the cuvette. The leaf area within the CIRAS-1 cuvette was measured and photosynthesis was expressed on a leaf area basis. The instantaneous water-use efficiency (ITE) was calculated as the ratio of photosynthesis and transpiration. All the photosynthetic parameters were calculated according to Farquhar et al. (1980). Pots were allowed to dry through transpirational water loss during seven days, and daily values of transpirable soil water (FTSW) for each pot was calculated by dividing the daily pot weight minus the final pot weight (Ray and Sinclair, 1998). According to these authors the use of FTWS in studies with drought, in greenhouse, can dispense measurement of soil water potential. The experiment was terminated 100 days after transplanting the acclimated tissue culture shoots into the 14 l pots. At this time, plants were harvested, oven-dried at 75°C for 96 h, and subsequently weighed.

2.3. STATISTICAL DESIGN AND ANALYSIS

Plants were distributed over a randomized block design involving a 2X2 factorial combination (two CO₂ concentrations x two water deficit regimes) with six replicates. Each experimental plot consisted of a single plant. Analysis of variance was performed with the statistical analysis software SISVAR (Ferreira, 2008) and where significant effects were observed the means were contrasted and separated by Tukey's test.

3.0 RESULTS

3.1 FRACTION OF TRANSPIRABLE SOIL WATER (FTSW)

After seven days of water deficit, FTSW were of 0.02 and 0.07, respectively, for plants in the ambient and elevated CO₂ (Fig. 1). The total amount of transpired water was not significantly different among these CO₂ treatments (data not presented). There was, however, difference in the rate which daily FTSW decreased in each treatment, with pots in ambient CO₂ drying more rapidly than pots in elevated CO₂ (Fig. 1). For example, four days after withholding water, FTSW was 0.14 and 0.32, respectively, for 390_WS and 750_WS plants. Hence, ambient CO₂ plants experienced water-deficit stress before plants cultivated under elevated CO₂.

3.2. LEAF GROWTH AND DRY MASS PRODUCTION

The central Leaf-lobe Elongation Rate (LER) of well-watered plants (390_WW and 750_WW) stabilized between seven and eight days after onset of measurements (Fig. 2A). No differences were observed between CO₂ treatments in the well-watered plants, indicating that the CO₂ had little influence on leaf growth of plants growing under these conditions.

For up to four Days After Withholding Water (DAWW), no differences were observed in LER among the CO₂ treatments (390_WS x 750_WS; Fig. 2B). However, on the fifth DAWW, LER of plants cultivated under the 390_WS treatment was nil, while the central leaf-lobe of the 750_WS plants continued to grow (Fig. 2B). Stalling of leaf elongation of the plants grown under the 750_WS occurred on the seventh day of measurements, two days after leaf growth of 390_WS plants had ceased. One day after rewatering, the central leaf-lobe resumed growth, with the 390_WS plants presenting a significantly higher LER value than the 750_WS plants.

Total dry mass of plants grown under well-watered and water deficit conditions was significantly higher in 750 $\mu\text{L L}^{-1}$ than in 390 $\mu\text{L L}^{-1}$ of CO₂, showing that growth at higher CO₂ concentrations reduced the effects of water deficit.

3.3. GAS EXCHANGE

One DAWW, the stomatal conductance (g_s , Fig. 4A) and transpiration (E , Fig. 4B) of 390_WW and 390_WS plants were greater than of the plants grown under the 750_WW and 750_WS. The g_s and E of the 390_WS plants were, respectively, 77% and 34% greater than that of the 750_WS plants. However, the photosynthetic rate (P_n), around $20 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, did not differ between treatments (Fig. 4C). The Instantaneous Transpiration Efficiency (ITE) was higher for plants grown under elevated CO_2 levels (Fig. 4D).

Three DAWW g_s and E of the 390_WS plants were reduced compared to the first DAWW (Figs 5A and 5B). On the same day, P_n of 750_WS plants was similar to that of 390_WW and 750_WW plants (Fig 5C). In contrast, P_n of 390_WS plants was only $12.1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, a value 34% lesser than of that 750_WS plants. When the leaf cuvette CO_2 concentration was increased to $750 \mu\text{L L}^{-1}$, g_s (Fig 5A) and P_n (Fig 5C) of 390_WS plants were similar to that of 750_WW and 390_WW plants (Fig. 6).

Five DAWW, g_s of 390_WS and 750_WS plants were, respectively, 12.75 and $13.4 \text{ mmol m}^{-2}\text{s}^{-1}$ (Tab. 1), values well below those observed for the first DAWW. Also, E was significantly reduced. Photosynthesis of 750_WS plants was $6.7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, a value 165 % greater than 390_WS plants (Table 1). As a result, ITE was again greater for plants grown under $750 \mu\text{L L}^{-1}$ of CO_2 . Compared to the first and third days of evaluation these results for g_s , E and P_n , taken together, show that there was a strong negative effect of the water deficit, especially on plants grown under $390 \mu\text{L L}^{-1}$ of CO_2 .

One day after rewatering, the 390_WS and 750_WS plants had P_n (Fig 6A), g_s (Fig 6B), E (Fig 6C), and ITE (Fig 6D) values similar to their respective well irrigated treatments.

4.0 - DISCUSSION

4.1 - LEAF GROWTH AND DRY MASS PRODUCTION

Water deficits led to reductions in LER of plants grown under ambient as well as CO₂-enriched conditions. Leaf growth inhibition in cassava grown under water deficit has been associated with reductions in the numbers of cells produced by the leaf meristems and less expansion of existing cells (Alves and Setter, 2004). However, plants grown at 750 $\mu\text{L L}^{-1}$ CO₂ continued leaf elongation two days after growth had completely ceased in plants grown at 390 $\mu\text{L L}^{-1}$. Other studies have also found a reduction or delay in the leaf or stem growth rate by CO₂ enrichment when plants are subjected to water deficit (Clifford et al., 1993; Manderscheid and Weigel, 2007). The partial stomatal closure and, consequently, reduced transpiration, allowed the 750_WS plants to maintain favorable moisture balances for longer periods of time, thereby delaying the cessation of leaf growth when compared to 390_WS plants. After rewatering, however, both the 750_WS and 390_WS recovered their leaf growth capacities. Several researchers have previously noted a strong capacity of stressed cassava plants to initiate leaf growth and recover leaf area after cessation of stress (Alves and Setter, 2004; Connor et al., 1981; El Sharkawy and Cook, 1987). Alves and Setter (2004) suggested that this rapid restoration of elongation is due to continuous cell wall loosening during the periods of water deficit followed by rapid water absorption, which result in an increase in turgor pressure and, consequently, a rapid recovery of cassava leaf growth after rewatering (Ike and Thurtell, 1981; Alves and Setter, 2000; Alves and Setter, 2004). Such adjustments may be greater in plants that are under greater stress, helping to explain why the 390_WS plants showed greater growth leaf rate at one day after rewatering.

For most C₃ plants, dry mass accumulation increases with increased ambient CO₂ concentration, although the response rate will vary depending on the species and genotype (Ainsworth and Long, 2005; Reddy et al., 2010). In our study, the increase from 390 to 750 $\mu\text{L L}^{-1}$ of CO₂ induced a 20% increase in the DMP in the well-irrigated plants (750_WW x 390_WW). Similar increases in cassava biomass were reported by Cruz et al. (2014). The positive effect of elevated CO₂ levels on the DMP was 61% higher for the stressed plants (750_WS x 390_WS), indicating that the stimulating effect was more pronounced under water deficit conditions. Likewise, the negative effect of water deficit was greater for dry mass accumulation of plants grown under ambient CO₂ (390_WW x 390_WS) than for the plants grown under high concentrations of CO₂ (750_WW x 750_WS). The CO₂-enriched plants sustained greater *Pn* during the water stress period. We suggest that this ability to maintain *Pn* may reduce the effects of drought in cassava as the levels of CO₂ in the atmosphere increase, at least in the concentration used in this experiment. In an extensive review, Poorter and Perez-Soba (2001) also observed that most herbaceous species had greater relative growth rates under elevated CO₂ when exposed to water deficits than under well-watered conditions. One of the main explanations is that, similar to our observations, elevated CO₂ levels induce stomatal closure and thus improve the internal water

status, especially during water deficit (Poorter and Perez-Soba, 2001; Wall et al., 2011; Morgan et al., 2011). However, there are situations where elevated CO₂ had no positive effect on the physiological parameters and growth of water stressed plants (Vaz et al., 2012; Perry et al., 2013). The net effects of CO₂ on growth and dry mass production under water stress depend on the balance between direct effects of CO₂ on both photosynthesis and stomatal aperture (Manderscheid and Weigel, 2007). In some cases, the effect of increased CO₂ concentration on this balance is not sufficient to allow some species to improve their growth under water deficit (Perry et al., 2013), partially explaining the different responses between species.

4.2 - GAS EXCHANGE

In the first evaluation of gas exchange P_n was not significantly different between CO₂ treatments. This result, along with the fact that in the second evaluation 390_WW plants, measured with 750 μmol of CO₂, had higher photosynthesis than 750_WW, confirms previous results that growth of cassava plants in CO₂-enriched atmospheres can result in photosynthetic acclimation, thereby reducing the short-term potential benefit of higher CO₂ concentration (Cruz et al., 2014). Under field conditions such acclimation may occur during the early growth stage, disappearing when storage roots become a strong sink (Fernandez et al., 2002 and Rosenthal et al., 2012). Under elevated CO₂ a key factor, perhaps the most important, that characteristically influences the acclimation, its occurrence and extent, is the plant source–sink balance (Irigoyen et al., 2014).

By three DAWW, stomatal conductance and transpiration of 390_WS plants had dropped significantly, confirming that reductions in g_s and E is an early response of cassava when subjected to even moderate water deficit (Itani et al., 1999; Calatayud et al., 2000; El-Sharkawy et al., 1984). Water deficit also reduced P_n of 390_WS plants, while plants growing under elevated CO₂ had offset the effect of moderate water deficit, resulting in P_n rates in 750_WS plants being similar to those of well-watered plants (390_WW and 750_WW). Elevated CO₂ concentrations have often been found to reduce stomatal conductance and increase transpiration efficiency, especially under water deficit conditions, conserving water and thus maintaining photosynthetic capacity (Wand et al., 1999; Morgan et al., 2011). Photosynthesis was similar to that of unstressed plants when the stressed plant leaves were treated with elevated CO₂. This demonstrates that a rise in the external CO₂ concentration can completely compensate the physical leaf resistances imposed by a short-term non-lethal water deficit, thus enabling the 390_WS/750 plants to exhibit the same P_n level as plants grown with good water availability. That result of P_n suggests that water stress affected the CO₂ diffusion from the atmosphere to the carboxylation site, while the carboxylation capacity of the mesophyll remained unaffected, confirming that physical resistance of gas transfer explains

almost exclusively the reduction in P_n at the initial stage of water deficit (Chaves et al., 2009; Flexas et al., 2014).

Five DAWW water deficit induced severe stress. Compared with the first DAWW, the g_s reduction was greater than 94% for 390_WS plants and greater than 84% for 750_WS plants. Stomatal closure limits transpiration rate, but at the same time, leads to a higher leaf temperature and VPD, which tends to increase transpiration. While the net effect of CO₂-induced stomatal closure is generally lowered transpiration, this response in an increasingly warmer world may change. Our results conducted with greenhouse-grown plants indicate that leaves and plants grew more with elevated CO₂ levels when stressed. At the field level, Porto (1983) suggests that drought stressed cassava plants can avoid excessive heating caused by stomatal closure by simply changing leaf orientation in relation to the sun and increasing reflectance. This would suggest that the trade-offs of CO₂-induced stomatal closure on cassava gas exchange, in the present study, may not be as severe as in other crops (Manderscheid and Weigel, 2007), although further field studies will be needed to strengthen this argument. At that stress level, the elevated [CO₂] continued to moderate the effect of the water deficit; P_n of the 750_WS plant was 165% greater than that of the 390_WS plants. However, the compensation was only partial because P_n of 750_WS plants was still significantly much lower than P_n observed for the 750_WW and 390_WW plants in previous days. In this more severe phase of water stress, the concentration of 750 $\mu\text{L L}^{-1}$ of CO₂ likely was not sufficient to compensate the diffusional resistances that eventually inhibited the CO₂ transport from the ambient air to carboxylation sites. Further, under such stress, biochemical limitations in addition to diffusional resistances likely existed (Chaves et al., 2009). Similar to the results presented here, Fleisher et al. (2008) also observed that under severe water deficit conditions, the ability of increased CO₂ levels to offset the negative effect of water deficit on photosynthesis was greatly reduced. Despite this, at five DAWW the positive effect of elevated CO₂ on photosynthesis was still observed. This result suggests that in cassava the relative stimulation of photosynthesis by CO₂ elevation occurs even under severe water stress, a result contrary to previously reported findings (Thiagarajan and Lada, 2007; Warren et al., 2011; Bauweraerts et al., 2013). The great tolerance of the photosynthetic apparatus of cassava to water deficit (El-Sharkawy, 2012; 2014) may explain these different results between species.

4.3 INSTANTANEOUS TRANSPIRATION EFFICIENCY (ITE)

Under all conditions, the ITE was consistently higher in plants grown under elevated CO₂ (750_WW and 750_WS). An increase in ITE is one of the most consistently documented responses of plants to elevated atmospheric CO₂ levels (de Kauwe et al., 2013; Ainsworth and Long, 2005). From the first to fifth DAWW, ITE of 390_WS plants remained stable (approximately 7.3), while

ITE of 750_WS plants increased approximately 83% (from 10.1 to 18.5). Higher ITE under elevated CO₂ level was potentially important in ameliorating negative impacts of water deficit on cassava. Higher ITE of water stressed plants cultivated under elevated CO₂ was due to both increased P_n and reduced g_s . Higher P_n resulted from higher ambient CO₂, while reduced g_s could have resulted from both high CO₂ as well as a response to drought stress (Morgan et al., 2011; Leakey et al., 2009). In our study, when the 750_WS plants were grown under good water availability (first DAWW) the higher ITE value was due solely to the lower E , while under moderate (three DAWW) and severe water deficit (five DAWW) a higher P_n could explain the higher ITE. Instantaneous transpiration efficiency derived from a greater P_n is a positive aspect for plants growing under water deficit because it can contribute more meaningfully to increase the dry matter accumulation.

4.4 - RECOVERY

One day after rewatering, the 390_WS and 750_WS plants fully recovered their P_n to levels equal to the well-watered controls. Galmés et al. (2007) studied the extent of photosynthesis recovery in several plants adapted to drought environment and observed that one day after rewatering the recoveries were species-dependent, with herbs showing the greatest recovery, but of only 49 to 64% of that in well irrigated plants. Our results reveal the great capacity of cassava to rapidly recover from water deficit conditions even when growing under a severe water deficit, as was used in this study. The rapid ability to restore stomatal function and reactions of carboxylation at satisfactory levels (Connor et al., 1981; El-Sharkawy, 2012; 2014) explains the recovery of cassava plants just one day after rewatering. Additionally, the pool of carbohydrates stored within the plant, particularly the stem, may be rapidly metabolized and used as a carbon source for the rapid recovery of important physiological functions immediately after re-watering (Duque and Setter, 2013). Rapid recovery after a severe drought makes the cassava crop a good option for environments with water deficit (Okogbenin et al., 2013; El-Sharkawy, 2014).

5.0 - CONCLUSIONS

Our results showed that elevated CO₂, at least in the concentration used in the present experiment, delayed the onset of water deficit in cassava by reducing the stomatal conductance and decreasing the transpiration rate, as evidenced by pots drying slower, and thus maintaining a higher FTSW level. In addition, elevated CO₂ levels also increase the P_n and ITE and, as a result, biomass

production is greater for plants grown under elevated CO₂ levels during water deficit. In other words, an increase in the atmospheric CO₂ level will increase water use efficiency of cassava and hence the crop performance under water deficit conditions. These results, however, should be used as a baseline, and field studies must be developed to determine the relationship between elevated CO₂ and drought in longer term experiments and in conditions under which stress develops more gradually.

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Fig. 1 Effect of CO₂ level (390 and 750 $\mu\text{L L}^{-1}$) on fraction of transpirable soil water (FTSW) in cassava plants grown under water deficit treatments. Each point is the mean of six replicates $\pm$ s.e.m. (standard error of the *mean*).

Fig. 2 Effect of CO₂ level (390 and 750 $\mu\text{L L}^{-1}$) on the Leaf-lobe Elongation Rate (LER) of cassava plants grown under well-watered (WW, Fig. 1A) and water deficit (WS, Fig. 1B) treatments. Each point is the mean of six replicates $\pm$ s.e.m. (standard error of the *mean*). * significant statistical differences between treatments within the same day ($p < 0.05$); N.S. implies no statistical differences. LER was calculated based on the increment of lobe length at each 1-d interval and the values are plotted at the last day the data were collected.

Fig. 3 Effect of CO₂ (390 and 750 $\mu\text{L L}^{-1}$) on the Total Dry Mass Production of cassava plants grown under well-watered (WW) and water deficit (WS) treatments. Each point is the mean of six replicates $\pm$ s.e.m. Different letters accompanying each bar indicate a significant difference ($p \leq 0.05$).

Fig. 4 Effect of CO₂ level (390 and 750 μL L⁻¹) on stomatal conductance (A), transpiration (B), net photosynthesis (C), and instantaneous transpiration efficiency (D) of cassava plants grown under well-watered (WW) and water deficit (WS) treatments one DAWW. Each point is the mean of six replicates ± s.e.m. Different letters accompanying each bar indicate a significant difference ($p \leq 0.05$). N.S. is not significant

Fig. 5 Effect of CO₂ level (390 and 750 $\mu\text{L L}^{-1}$) on stomatal conductance (A), transpiration (B), and net photosynthesis of cassava plants grown under well-watered (WW) and water deficit (WS) treatments after three DAWW. Each point is the mean of six replicates $\pm$ s.e.m. Different letters accompanying each bar indicate a significant difference ($p \leq 0.05$).

Table 1. Effect of the CO₂ level (390 and 750 $\mu\text{L L}^{-1}$) on stomatal conductance, transpiration, net photosynthesis, and instantaneous transpiration efficiency of the cassava plants grown under drought (WS) treatments five DAWW. Each point is the mean of six replicates. Means followed by different letters are significantly different at $p \leq 0.05$.

Treatments	Parameters			
	Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)	Transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	Net photosynthesis ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	Instantaneous transpiration efficiency ($\mu\text{mol CO}_2 \text{mmol H}_2\text{O}^{-1}$)
390_WS	12.75 a	0.33 a	2.53 b	7.30 b
750_WS	13.40 a	0.37 a	6.70 a	18.5 a

Fig. 6 Effect of CO₂ level (390 and 750 $\mu\text{L L}^{-1}$) on net photosynthesis (A), stomatal conductance (B), transpiration (C), and instantaneous transpiration efficiency (D) of cassava plants grown under well-watered (WW) and water deficit (WS) treatments one day after rewatering. Each point is the mean of six replicates $\pm$ s.e.m. Different letters accompanying each bar indicate a significant difference ($p \leq 0.05$).