

## RESEARCH PAPER

# Growth and nutritive value of cassava (*Manihot esculenta* Cranz.) are reduced when grown in elevated CO<sub>2</sub>

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## Keywords

Acclimation; climate change; cyanogenesis; cyanogenic glycosides; food quality; konzo; leaf protein; manioc; photosynthesis; tapioca.

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## ABSTRACT

Global food security in a changing climate depends on both the nutritive value of staple crops as well as their yields. Here, we examined the direct effect of atmospheric CO<sub>2</sub> on cassava (*Manihot esculenta* Cranz., manioc), a staple for 750 million people worldwide. Cassava is poor in nutrients and contains high levels of cyanogenic glycosides that break down to release toxic hydrogen cyanide when damaged. We grew cassava at three concentrations of CO<sub>2</sub> (C<sub>a</sub>: 360, 550 and 710 ppm) supplied together with nutrient solution containing either 1 mM or 12 mM nitrogen. We found that total plant biomass and tuber yield (number and mass) decreased linearly with increasing C<sub>a</sub>. In the worst-case scenario, tuber mass was reduced by an order of magnitude in plants grown at 710 ppm compared with 360 ppm CO<sub>2</sub>. Photosynthetic parameters were consistent with the whole plant biomass data. It is proposed that since cassava stomata are highly sensitive to other environmental variables, the decrease in assimilation observed here might, in part, be a direct effect of CO<sub>2</sub> on stomata. Total N (used here as a proxy for protein content) and cyanogenic glycoside concentrations of the tubers were not significantly different in the plants grown at elevated CO<sub>2</sub>. By contrast, the concentration of cyanogenic glycosides in the edible leaves nearly doubled in the highest C<sub>a</sub>. If leaves continue to be used as a protein supplement, they will need to be more thoroughly processed in the future. With increasing population density, declining soil fertility, expansion into marginal farmland, together with the predicted increase in extreme climatic events, reliance on robust crops such as cassava will increase. The responses to CO<sub>2</sub> shown here point to the possibility that there could be severe food shortages in the coming decades unless CO<sub>2</sub> emissions are dramatically reduced, or alternative cultivars or crops are developed.

## INTRODUCTION

Two major problems facing the world are global climate change and food security. Globally, over 800 million people are undernourished and up to 2 billion people lack food security intermittently (FAO 2008). Projected changes in rainfall patterns, and an increase in the frequency and severity of drought, in many regions of the world will exacerbate this situation (Solomon *et al.* 2007; Funk *et al.* 2008; Liu *et al.* 2008). The hope is that the detrimental effects of climate change will be offset by higher plant growth rates as a result of rising atmospheric CO<sub>2</sub>, the

so-called 'CO<sub>2</sub> fertilisation effect' (Ziska & Bunce 2007; Jackson *et al.* 2008; Lobell & Field 2008). However, C3 plants acclimate to higher atmospheric CO<sub>2</sub> to some extent, resulting in less growth enhancement than originally predicted (Morison & Lawlor 1999). Moreover, responses vary between species (Kimball 1983). It is important, therefore, that the growth of major staple crops be tested under different emissions scenarios so that informed adaptive responses can be made in a timely manner (Ainsworth *et al.* 2008). To achieve this goal, a basic understanding of the responses of the world's key principal crops to all aspects of climate change is urgently needed.

Most research on the impact of climate change on crops has focused on yields. However, there is increasing recognition of the need to understand impacts on plant composition (e.g. Ziska *et al.* 2005; Raisanen *et al.* 2008). Tissue nitrogen (N) concentration of C3 and C4 plants grown at elevated CO<sub>2</sub> (eCO<sub>2</sub>) on average decreases 16% and 7%, respectively (Drake *et al.* 1997; Jackson *et al.* 2008). Given that most plant leaf N is allocated to protein, this affects the nutritional value. Grain protein content of wheat and rice, for example, is predicted to decrease 10–15% in the coming century and, indeed, may have already decreased as a result of atmospheric changes over the past century (Taub *et al.* 2008). Even less is known about how plant secondary metabolite production will change in response to rising atmospheric CO<sub>2</sub>. Several studies have found a significant increase in the production of C-based defence compounds (such as phenolics), in plants grown at elevated CO<sub>2</sub> (e.g. Lincoln *et al.* 1993; Coley *et al.* 2002) but there are few data on the effect of N-based secondary metabolites.

Around 10% of all plants and 60% of crop species produce cyanogenic glycosides, constitutive N-based compounds that break down to release toxic cyanide (HCN) when plant tissue is crushed or chewed (Jones 1998; Gleadow *et al.* 2008). Cyanogenic glycosides are effective defence agents against generalist herbivores (Gleadow & Woodrow 2002a), including humans. While anti-nutritional factors are not normally a problem in human food, some are, and it is important to know what will happen under future climate scenarios. Very few studies have examined the effect elevated CO<sub>2</sub> on cyanogenic glycosides. Two studies (one on *Trifolium repens* L. and one on *Eucalyptus cladocalyx* F. Muell. seedlings) found that while the cyanogen concentration did not change in leaves of plants grown at elevated CO<sub>2</sub> on a per mass basis, the leaves overall became more toxic due to the decrease in leaf proteins required for detoxification (Gleadow *et al.* 1998, 2009). It appeared that the increase in nitrogen use efficiency of the plants grown at elevated CO<sub>2</sub> allowed N in excess of photosynthetic requirements to be reallocated to defence chemicals. If a similar response is found in staple food crops, there could be very serious consequences for food security.

Cassava (*Manihot esculenta* Cranz., manioc, tapioca) is the third most important food source in the tropics, after rice and maize, and is the staple food of ca. 750 million people in Africa, southern America, Asia and the Pacific Islands (FAO 2008). It contains two cyanogenic glycosides, linamarin and lotaustralin, in all plant tissues (McMahon *et al.* 1995). Tubers are the main agricultural product, although leaves are also eaten, primarily as a protein supplement (Gomez & Valdivieso 1985). Tubers and leaves are processed to remove the toxic cyanogens, but the process is not complete, often leading to chronic medical problems. One of these is konzo, an irreversible paralysis of the lower limbs caused by high concentrations of cyanide in the diet (Ernesto *et al.* 2002; Nhassico *et al.* 2008). Cyanide toxicity from cassava is becoming increasingly

widespread in developing countries as this crop is now grown more widely (Nhassico *et al.* 2008). The cyanogenic glycoside concentration of cassava is known to change with environmental conditions such as drought (El-Shakawy & Cadavid 2002). The World Health Organisation recommended limit for cyanide in food is 10 ppm (Cardoso *et al.* 2004). A study of flour collected from markets in northern Mozambique showed the cyanide levels in a typical year were approximately 20–40 ppm. A follow up study in a drought year found flour of 100–200 ppm cyanide (Cardoso *et al.* 2004). Since cassava can produce HCN at concentrations toxic to humans, it is essential to quantify effects of elevated CO<sub>2</sub> on its synthesis.

If cassava is to have a role in meeting global food demand in the future, it is important to know whether it will continue to be safe to consume and continue to be productive. The aim of this study was to determine: (i) whether the high yields observed in cassava at ambient CO<sub>2</sub> are sustained when plants are grown at elevated CO<sub>2</sub>; and (ii) whether the cyanogenic glycoside concentration of the tubers and leaves increases. Since acclimation to elevated CO<sub>2</sub> in C3 plants is thought to be mediated by accumulation of non-structural carbohydrates in leaves (Krapp *et al.* 1993), we hypothesised that the degree of acclimation in cassava would be moderated by the capacity of the tubers to accumulate excess carbohydrate.

## METHODS

### Plant material and growing conditions

Cassava (cv MCol 1468) was grown from cuttings in matched greenhouses (30/20 °C day/night) at three different concentrations of CO<sub>2</sub> (C<sub>a</sub>: 360, 550, 710 ppm, see below). Plants were supplied with Hewitt's nutrient solution containing either 1 mM or 12 mM nitrate three times per week (see Edwards *et al.* 2006). Mean day/night temperatures, measured at 5 min intervals, were (±1SE) 28.5 ± 0.2 °C/19.2 ± 0.1 °C in each greenhouse chamber. The CO<sub>2</sub> concentration was measured continuously with a Vaisala Carbocap IRGA GMT222 and fumigated as required between 06:00 and 18:00 h daily. The resulting mean concentration of CO<sub>2</sub> in each greenhouse (ppm ± 1SE) was 359 ± 2, 546 ± 1 and 709 ± 1. Cotton (*Gossypium hirsutum* L.) and soybean [*Glycine max* (L.) Merr.] plants grown in the greenhouse chambers showed increased biomass production progressively with increasing atmospheric CO<sub>2</sub> (not shown), in agreement with other published experiments (e.g. Ainsworth & Long 2005).

### Harvesting and gas exchange

Photosynthetic parameters were measured on the first fully expanded leaf of 3-month-old plants in February 2009 using a Li-Cor 6400 portable photosynthesis system (Lambda Instruments, Blacksburg, VA, USA) at saturating PPFD (1500 µmol quanta m<sup>-2</sup>s<sup>-1</sup>), leaf temperature typical of field condition (30 °C) and growth

CO<sub>2</sub>. Net assimilation ( $A_{net}$ ), stomatal conductance ( $g_s$ ), and the concentration of CO<sub>2</sub> inside the leaf ( $C_i$ ) were calculated using standard equations (Von Caemmerer & Farquhar 1981). A leaf was then sampled from five plants per treatment ( $N = 5$ ). Leaf discs (5 mm diameter) were excised for analysis of cyanogenic glycoside concentration (see below). Leaf area and dry weight (oven-dried at 70 °C for 48 h) were measured on leaves from which the discs had been removed to facilitate conversion of area to mass-based units. Dried leaves were finely ground and analysed for total leaf N (see below).

Whole plants were destructively harvested after 6 months and separated into tubers, roots and shoots, dried at 70 °C for 2 weeks, and weighed ( $N = 6$ ). Tubers were defined as roots >1 cm diameter. Before drying, cores (5 mm diameter) were taken midway between tip and base of tubers and 8–10 mm below the peel for total N and cyanogenic glycoside analysis ( $N = 3$ ).

### Chemical analysis

Total N was determined on finely ground dried leaves and tuber segments (5–10 mg) using using an elemental analyser (EA 1110 CHNO; Carlo-Erba Instruments, Milan, Italy). For cyanogenic glycoside analysis, two leaf discs (diameter = 5 mm) or two tuber segments (5 mm diameter, 2-mm thick) were placed in sealed vials containing 0.5 ml 0.1 M citrate buffer (pH 5.5) containing  $\beta$ -glucosidase (1.12 units ml<sup>-1</sup>) from almond (EC 3.2.1.21, Sigma-Aldrich, St. Louis, MA, USA), and a separate well containing 200  $\mu$ l 1 M NaOH. Cells were lysed using two freeze–thaw cycles (K. Jørgenson & B. Møller, pers. comm.). The intact vials were then incubated at 37 °C for 15 h, and evolved HCN trapped in the NaOH wells was assayed colorimetrically using NaCN as standard (Gleadow & Woodrow 2002b). Cyanide concentration was expressed on a dry mass basis using the leaf mass area (leaf area per dry mass) of the leaf from which the discs were taken.

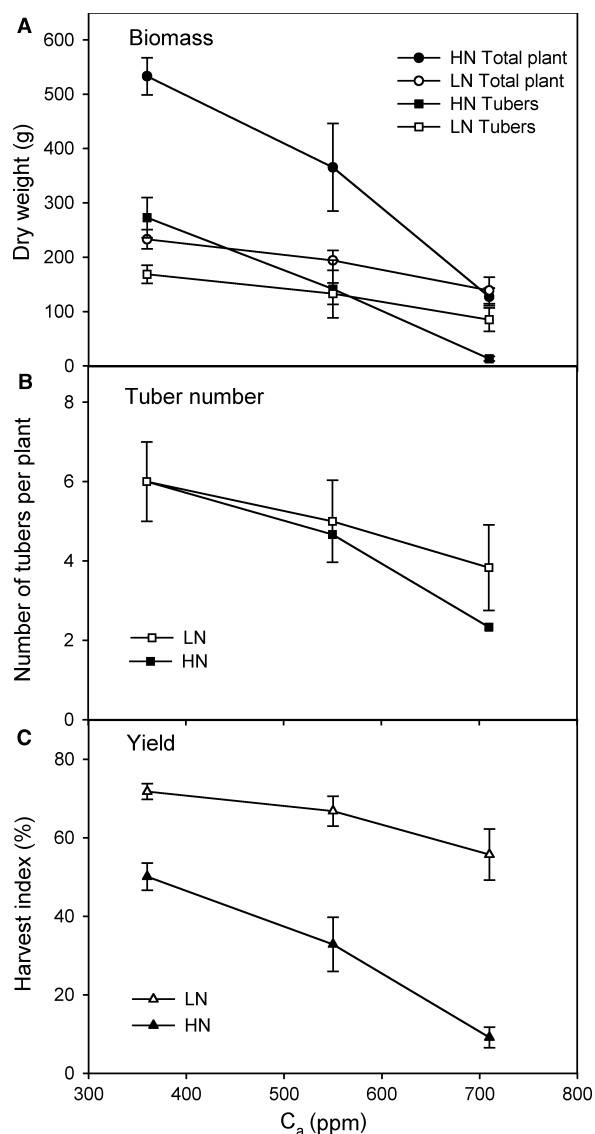
### Calculations and statistical analysis

Harvest index was calculated by dividing the total mass of tubers by whole plant biomass (both on a dry mass basis). Data were analysed using ANOVA (General Linear Model) in Minitab15.1 (Minitab Inc). Data were log-transformed where necessary to satisfy requirements for normality. Pair-wise comparisons were made using Tukey's test where significant effects were observed.

## RESULTS

### Biomass and harvest index

Total plant biomass decreased linearly with increasing CO<sub>2</sub> ( $P < 0.01$ , Fig. 1A). Mass decreased approximately 1.2 g for every 100 ppm increase in CO<sub>2</sub> for plants grown with 12 mM N ( $r^2 = 0.66$ ,  $P < 0.001$ ), and about 0.28 g per 100 ppm for the 1 mM N-grown plants ( $r^2 = 0.40$ ,



**Fig. 1.** Biomass of cassava grown at three concentrations of CO<sub>2</sub> (C<sub>a</sub>) and supplied with nutrient solution containing either 1 mM N (open) or 12 mM N (closed): A: Total dry mass of whole plants (circles) and tubers (squares); B: Number of tubers per plant; and C: Harvest index (yield; tuber dry mass as a proportion of total biomass (roots, shoots and tubers)). Each point is the mean of six replicates  $\pm$  1SE.

$P < 0.001$ ). Plants grown with 12 mM N were twice as big as those supplied with 1 mM N at ambient CO<sub>2</sub> ( $P < 0.001$ ), but this difference decreased with increasing CO<sub>2</sub> until there was no significant effect of N supply on plant biomass at 710 ppm CO<sub>2</sub> (Fig. 1A).

There were half as many tubers in plants grown in 710 ppm CO<sub>2</sub> compared to those grown in 360 ppm (Fig. 1B). This difference alone was not enough to account for the order of magnitude decrease in total mass of these storage organs in the high-N treatment. Tuber mass decreased almost linearly with increasing CO<sub>2</sub>



**Fig. 2.** Tubers of cassava grown at ambient CO<sub>2</sub> (360 ppm) and approximately twice-ambient CO<sub>2</sub> (710 ppm) and supplied with 12 mM N. (Concentrations noted on tags were from preliminary data.)

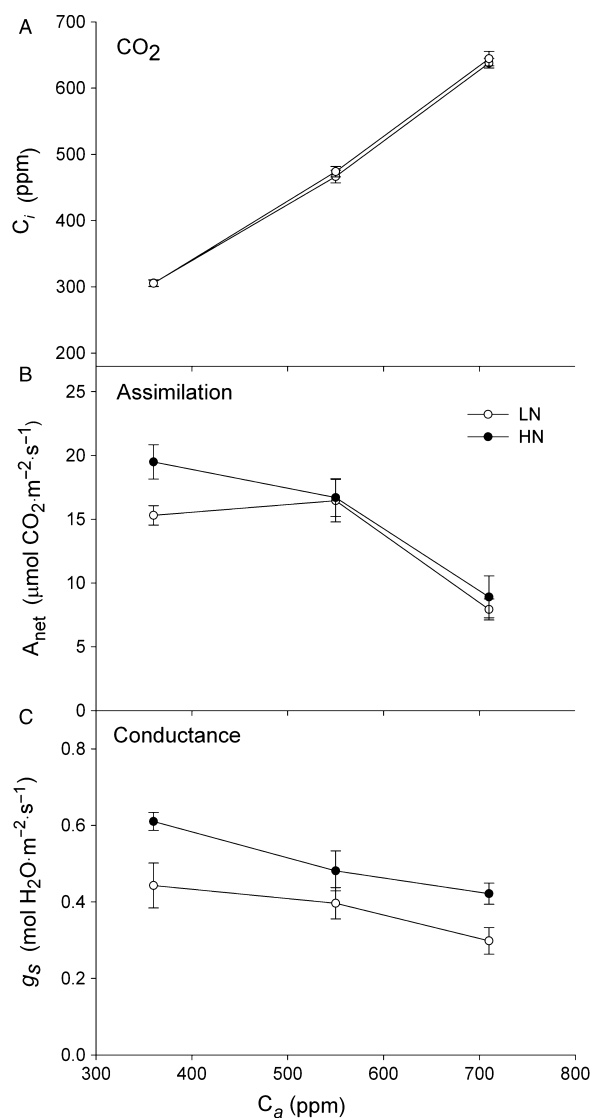
(Figs 1A and 2;  $P < 0.001$ ). Plants supplied with 12 mM N were much more severely impacted by the increase in CO<sub>2</sub>, decreasing by about 7 g for every 100 ppm increase in CO<sub>2</sub> ( $r^2 = 0.84$ ;  $P < 0.001$ ), with a reduction in harvest index of 80% (Fig. 1C).

#### Photosynthetic parameters

In all treatments,  $C_i$  varied in direct proportion to  $C_a$  ( $P < 0.001$ ; Fig. 3A). Despite the higher  $C_i$ , assimilation rates were lower in plants grown at 710 ppm than either the 550 ppm or 360 ppm CO<sub>2</sub> treatments (Fig. 3A;  $P < 0.05$ ). Photosynthetic capacity of the leaves was, therefore, lowest for plants grown in the highest CO<sub>2</sub> scenario (Fig. 3B). Assimilation rates were slightly higher in plants grown with 12 mM N compared with those grown with 1 mM N, but the difference was not statistically significant. Stomatal conductance ( $g_s$ ) was significantly lower in plants grown at 710 ppm CO<sub>2</sub> compared with those grown at 360 ppm, with intermediate values for plants grown at 550 ppm (Fig. 3B;  $P < 0.05$ ). Plants from the 1 mM N treatments had consistently lower  $g_s$  than those from the 12 mM N treatment.

#### Leaf and tuber chemistry

Cassava leaves (which are edible) became more cyanogenic when grown at 710 ppm CO<sub>2</sub> (Fig. 4A;  $P < 0.01$ ). The cyanogenic glycoside concentration of the first fully expanded leaf in the high N plants was more than twice as high ( $P < 0.001$ ) as when grown at approximately twice-ambient CO<sub>2</sub> ( $1544 \mu\text{g CN g}^{-1}$ ), compared to the ambient ( $586 \mu\text{g CN g}^{-1}$ ) and intermediate ( $680 \mu\text{g CN g}^{-1}$ ) CO<sub>2</sub> scenarios (Fig. 4A). N supply correlated with an increase in leaf cyanogenic glycoside concentration at the highest  $C_a$ . The cyanogenicity of the tubers, by contrast, was similar in plants from all N and CO<sub>2</sub> treatments, with an overall mean of  $37 \mu\text{g CN g}^{-1}$ , an order of magnitude less than the concentrations in leaves.



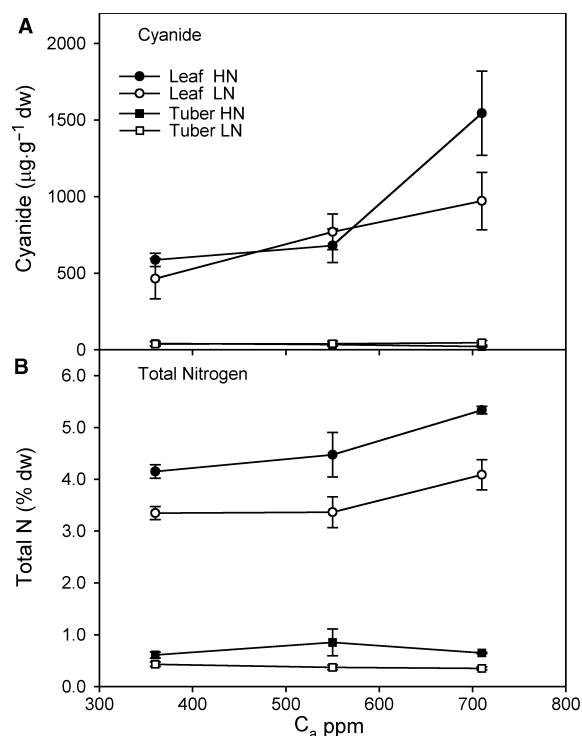
**Fig. 3.** Photosynthetic parameters for cassava grown at three concentrations of CO<sub>2</sub> ( $C_a$ ) and supplied with nutrient solution containing either 1 mM N (open) or 12 mM N (closed): A: Internal concentration of CO<sub>2</sub>,  $C_i$ ; B: Net assimilation rate,  $A_{\text{net}}$ ; C: Stomatal conductance,  $g_s$ . Each point is the mean of six replicates  $\pm$  1SE.

Total N concentration in the tubers was also similar in plants from all treatments, with an overall mean of  $0.54 \pm 0.07\%$  dry weight. Total leaf N, measured on the same leaves as the cyanogen concentrations, was marginally higher in plants grown at high N ( $P < 0.01$ ; Fig. 4B), and also in those grown at the highest  $C_a$  ( $P < 0.01$ ).

#### DISCUSSION

Plant growth and development is affected by increasing concentrations of CO<sub>2</sub> in the atmosphere, both indirectly through changes in climate, and directly *via* its affect on photosynthesis. A recent analysis of productivity of food





**Fig. 4.** Chemical analysis of cassava leaves (circles) and tubers (squares) grown at three concentrations of CO<sub>2</sub> (C<sub>a</sub>) and supplied with nutrient solution containing either 1 mM N (open) or 12 mM N (closed). A: Cyanogenic glycoside concentration measured as evolved cyanide, and B: total leaf nitrogen. Both were measured on a dry weight basis (dw). Each point is the mean ± 1SE.

crops predicts that crops such as wheat and rice will decrease in yield in the coming century due to climate change, but that cassava would be relatively unaffected (Liu *et al.* 2008), based on cassava's ability to tolerate episodic drought. Here, we present the finding that yield of edible cassava roots may be drastically reduced as a direct response to rising atmospheric CO<sub>2</sub> (Fig. 1). This is in contrast to the accepted view that increased CO<sub>2</sub> leads to an increase in root mass (Shimon & Bunce 2009).

A reduction in the production of edible tubers in a crop known as the 'drought, war and famine crop of the developing world' (Pearce 2007) is of grave concern. Over 750 million people, including 45% of sub-Saharan Africans, rely upon cassava as their primary food source (Nhassico *et al.* 2008). The UN has estimated that total food output must rise by 60% in the next 40 years to meet worldwide demand (FAO 2008). Predicted changes in climate alone are expected to lead to a *per capita* decrease in calorie intake in much of Africa (Liu *et al.* 2008). Global cassava production more than doubled between 1995–2005 (FAO 2008; Nhassico *et al.* 2008) because of its rapid and reliable growth on poor soils, drought tolerance, ability to be harvested at virtually any time, and little need for tending. This increasing reliance on cassava is expected to continue, with it being heavily promoted as an option in the face of

climate change (FAO 2008). The data presented here suggest that this may not be a viable option. An order of magnitude decrease in yields, such as found here could lead to significant food shortages. There is, therefore, a need to identify the mechanisms underlying this unexpected response to elevated CO<sub>2</sub>.

Plant growth is typically enhanced at high levels of C<sub>a</sub> (Morison & Lawlor 1999). While unexpected, our observations may not be unique. Several studies found that potato tubers were smaller in elevated CO<sub>2</sub>-grown plants (*e.g.* Miglietta *et al.* 1998; Lawson *et al.* 2001). This was balanced to some extent by an increase in the overall number of tubers, something we did not observe in cassava (Fig. 1). Lawson *et al.* (2001) suggested that the large amounts of stored carbohydrates might have resulted in a more pronounced down-regulation of photosynthesis. We found that assimilation rates decreased as C<sub>a</sub> increased, despite increasing C<sub>i</sub>, indicating that the photosynthetic capacity of the leaves was, indeed, lower in plants grown under elevated CO<sub>2</sub>. This is the opposite of our prediction – that the large carbohydrate sink provided by the tubers would reduce the likelihood of acclimation. The rationale behind the prediction was that the transport of carbohydrates away from the leaves would moderate the down-regulation of *rubS*, a key step in Rubisco synthesis (Krapp *et al.* 1993). We did not find any evidence that cassava behaved like a C<sub>4</sub>-intermediate, as suggested by (El-Shakawy 2004).

All plants close their stomata to some extent as a direct response to increasing CO<sub>2</sub> (Morison & Gifford 1983; Lake & Woodward 2008). In cassava, stomata are known to be very sensitive to changes in vapour pressure deficit and ABA, even if there is adequate soil moisture content (Alves & Setter 2004; El-Shakawy 2004). It could be that the stomata are also exceptionally sensitive to CO<sub>2</sub>, resulting in a concomitant decrease in net assimilation, such as observed here.

El-Shakawy (2004) attributes environmentally induced changes in photosynthesis in cassava to non-stomatal factors, such as biochemical resource allocation. We found that the cyanogenic glycoside concentration in leaves increased with increasing CO<sub>2</sub>, accounting for 1.5% of total leaf N in plants grown at 710 ppm, up from *c.* 0.7% in plants grown at ambient CO<sub>2</sub>. This is well below the 10–15% of leaf N allocated to cyanogenic glycosides in some cyanogenic plants (*e.g.* Gleadow & Woodrow 2000), but it is likely to account for some of the N that would otherwise be allocated to Rubisco. Reallocation of N resources to defence alkaloids may also explain the low growth rates observed in potatoes (*e.g.* Miglietta *et al.* 1998), although this has not been measured to date.

For those who rely on cassava as a staple, it is an 'empty food'; that is, it is high in calories but low in micronutrients and protein. Consequently, leaves are eaten to complement the low protein concentrations in the tubers (Lancaster & Brooks 1983). The increase in leaf cyanide we report here clearly has implications for such practices. While it is possible to detoxify cassava leaves (and tubers), leaves are generally only processed before eating in Africa, whereas in parts of southern Asia and

South America they are eaten as a salad. Furthermore, even where people have long experience of growing cassava and are aware of detoxification procedures, cyanide toxicity still occurs (Nhassico *et al.* 2008). The increases we have shown here are likely to be exacerbated by the expected increase in cyanogens that are a common consequence of water stress (El-Shakawy & Cadavid 2002; Gleadow & Woodrow 2002b). On the other hand, cassava tubers do not appear to be more cyanogenic when plants are grown at elevated CO<sub>2</sub>. However, supplementary crops that are eaten to provide protein are likely to be adversely impacted by the predicted climatic changes over the coming century (Liu *et al.* 2008; Taub *et al.* 2008).

Previous studies on cyanogenic plants have found only a marginal increase in leaf cyanide and a significant decrease in leaf N in plants grown at twice-ambient CO<sub>2</sub> (Gleadow *et al.* 1998, 2009). In cassava, the cyanogenic glycosides are primarily synthesized in the leaves and transported to the roots (McMahon *et al.* 1995; Jørgensen *et al.* 2005). If cyanogenic glycosides are an integral part of the N turnover in cyanogenic organisms, as proposed by Zagrobelny *et al.* (2008), then the reallocation of N away from the photosynthetic apparatus observed in plants grown at elevated CO<sub>2</sub> could unsettle the transport and distribution of metabolites within the leaves, and also to the roots. Acyanogenic cassava has been produced using RNAi technologies (Siritunga & Sayre 2003; Jørgensen *et al.* 2005) but it is unclear whether they will be as productive under field conditions, particularly in the light of the newly discovered role for cyanogenic compounds in N metabolism and transport (Jenrich *et al.* 2007). The preference for high cyanide varieties by the predominantly female subsistence farmers in parts of Africa also raises doubts about the acceptance of completely non-cyanide producing cultivars (Chiwona-Karlton *et al.* 2002).

With increasing population density, declining soil fertility, expansion into marginal farmland, together with the predicted increase in extreme climatic events, reliance on robust crops such as cassava will increase. The responses to CO<sub>2</sub> shown here point to the possibility that there could be severe food shortages in the coming decades unless CO<sub>2</sub> emissions are dramatically reduced, or alternative cultivars or crops are developed.

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