

Article

## Cassava: The Drought, War and Famine Crop in a Changing World

Anna Burns <sup>1</sup>, Roslyn Gleadow <sup>1</sup>, Julie Cliff <sup>2</sup>, Anabela Zacarias <sup>3</sup> and Timothy Cavagnaro <sup>1,4,\*</sup>

<sup>1</sup> School of Biological Sciences, Faculty of Science, Monash University, Wellington Rd, Clayton, 3800 Victoria, Australia; E-Mails: anna.burns@monash.edu (A.B.); ros.gleadow@monash.edu (R.G.)

<sup>2</sup> Department of Community Health, Faculty of Medicine, Eduardo Mondlane University, C.P. 257, Maputo, Mozambique; E-Mail: julie.cliff@gmail.com

<sup>3</sup> Agricultural Research Institute of Mozambique (Instituto de Investiga ção Agr ária de Mo çambique), P.O. Box 3658 Mavalane, Maputo, Mozambique; E-Mail: anabela.zacarias@gmail.com

<sup>4</sup> Australian Centre for Biodiversity, Monash University, Wellington Rd, Clayton, 3800 Victoria, Australia

\* Author to whom correspondence should be addressed; E-Mail: timothy.cavagnaro@monash.edu; Tel.: +61-3-99055793; Fax: +61-3-99055613.

*Received: 18 October 2010; in revised form: 12 November 2010 / Accepted: 17 November 2010 / Published: 19 November 2010*

---

**Abstract:** Cassava is the sixth most important crop, in terms of global annual production. Cassava is grown primarily for its starchy tuberous roots, which are an important staple for more than 800 million people, mostly in sub-Saharan Africa, but also in other parts of Africa, Asia, the Pacific and South America. Cassava is important for both small-scale farmers and larger-scale plantations due to its low requirement for nutrients, ability to tolerate dry conditions and easy low-cost propagation. It is sometimes referred to as the “drought, war and famine crop of the developing world” and reliance upon this crop is expected to increase in the coming years as the global climate changes. As with all crops, cassava presents some challenges which need to be addressed, especially if its production is to continue to expand. We highlight here a number of key issues around the continued and increased reliance upon cassava as a staple food crop. Cassava contains cyanogenic glycosides that release hydrogen cyanide and many cultivars are toxic if not processed before consumption. The degree of toxicity is altered by plant breeding, agricultural practice, environmental conditions and methods of food preparation. We conclude that use

of cassava has the potential to help many countries achieve food security in a sustainable manner, in the face of significant environmental change, but that its introduction should be accompanied by appropriate education about its toxicity.

**Keywords:** cassava; cyanide; food security; drought; carbon dioxide; *Manihot esculenta*; sustainable agriculture; nitrogen use efficiency

---

## 1. Introduction

Producing enough food, in a sustainable manner, to meet the needs of an increasing global population is one of the greatest challenges we face. Recent estimates are that food production will need to double by 2050 [1]. The ability to achieve this goal is compounded by the decrease in arable land through environmental degradation and urban encroachment [1-3], increased cost and potential shortages of fertilisers [1,4], rising atmospheric carbon dioxide (CO<sub>2</sub>) and climate change [5-8]. In the context of food security, the climate impacts and adaptation debate has largely focused on yields [1,9]. However, by definition, food security is only achieved when “all people, at all times, have physical and economic access to sufficient safe and nutritious food to meet their dietary needs and food preferences for a healthy and active life” [10]. Thus, while yields need to be increased, this must not be achieved at the expense of the nutritive value of food [5,7,11] and must be in a way that is environmentally sustainable.

Most of the world’s population relies on a plant-based diet. Cassava (*Manihot esculenta* Crantz) is the third most important source of calories in the tropics [12] and the sixth most important food crop after sugar cane, maize, rice, wheat and potato, in terms of global annual production [13]. Due to its versatile nature, it is frequently referred to as the “drought, war and famine crop” to much of the developing world [14]. While it is already a major staple crop, it has the potential to be an important part of the solution to improving food security in a time of climate change [8,15,16]. It can grow on poor soils, is easily propagated, requires little cultivation and can tolerate periodic and extended periods of drought [17-20]. The main food product is the tuberous roots, which can be retrieved from the soil up to three years after maturity [21], allowing crops to be abandoned during periods of agricultural and social instability. This provides an important form of “insurance” against social disruption, prolonged droughts, or other periods of stress and unrest. Thus, cassava is an important means by which food production could be increased without the use of large amounts of agricultural inputs (e.g., fertilisers, water and pesticides). Cassava is also emerging as an important large-scale agricultural crop for use as a bio-fuel [22] and a source of industrial starch [21,23,24], although these are not considered here.

In this review, we consider the impacts of global change on this important staple crop in the context of sustainable development. The review is divided into four sections. Firstly, we provide background information on the origins of cassava, its cultivation and expansion across the globe. Secondly, we examine its cyanogenic nature and the impact on human health and pests. Thirdly, we consider the impact of environmental factors on cassava yield and toxicity, with an emphasis on climate change. Finally, we conclude that the potential for expanded use of cassava to help achieve food security is

considerable, but only if this is accompanied by transfer of cultural knowledge about effective methods for processing to reduce the risk of cyanide-related disorders; together with adequate access to a mixed diet. Equally important are the impacts of different agricultural strategies to increase cassava production in an environmentally sustainable manner. While the storage organs of cassava are in fact tuberous roots, and not derived from stem, they are commonly referred to as ‘tubers’, a convention that we follow here.

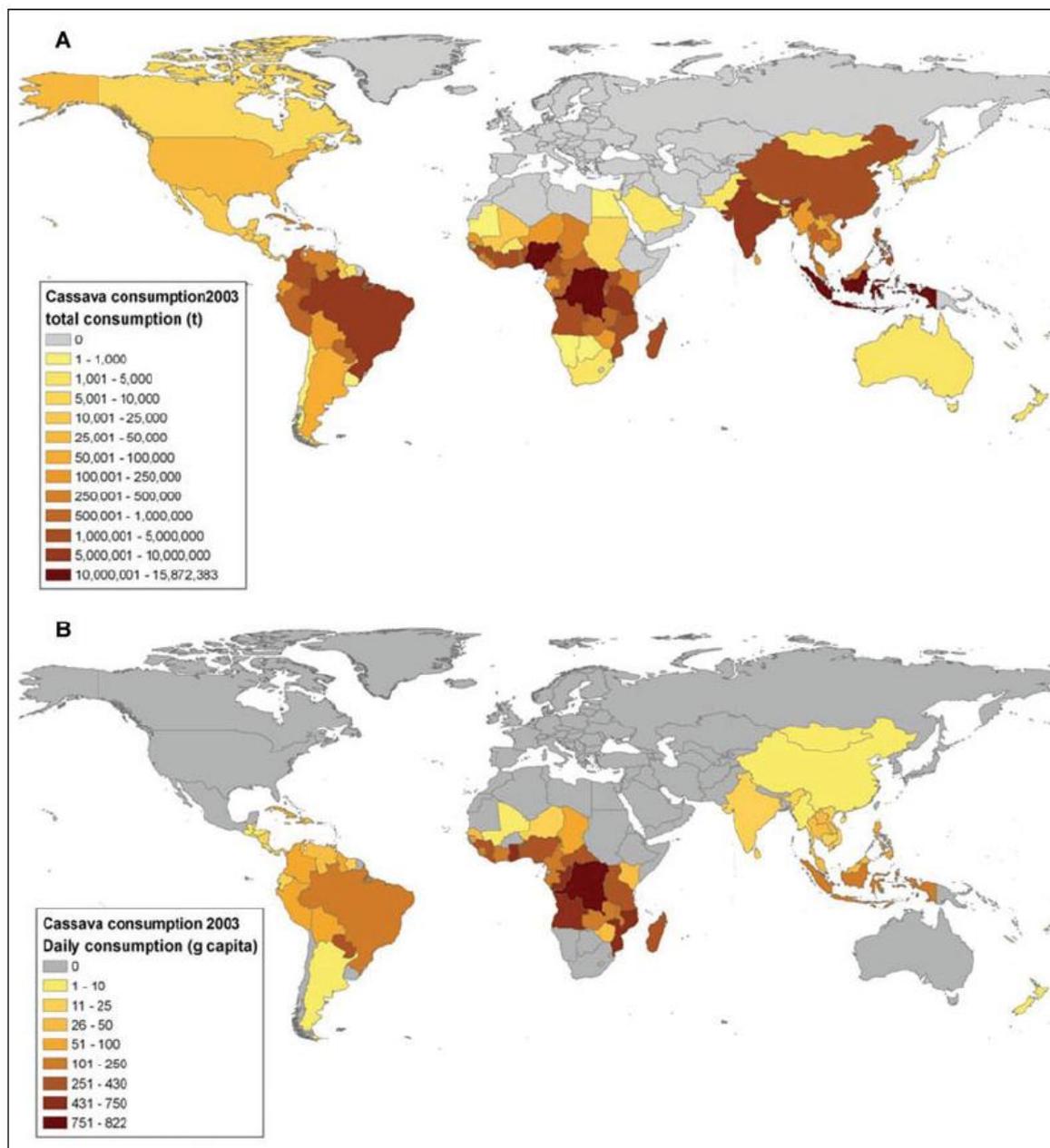
## 2. Cassava: Origins, Distribution and Food Products

Cassava (a tall shrub 1–4 m, Figure 1) is a domesticated plant derived from one or more species of the genus *Manihot*, in the Euphorbiaceae family [25,26]. *Manihot* belongs to the same sub-family as rubber (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg., Crotonoideae), and like rubber, contains both cyanogenic glycosides and latex [27-29]. Cultivated cassava is usually *Manihot esculenta* Crantz. The wild progenitors of cassava originated in areas marginal to the Amazon basin and domestication is thought to have occurred 5,000–7,000 years BC along the southern and/or northern rim of the Amazon basin [26,30-32]. Cassava was spread around the world between the 16th and 19th centuries by European explorers, who recognised its value as a food and cash crop [26]. Cassava is now widely grown for consumption in the tropics and subtropics, in a range of habitats including semi-arid savannas and montane regions (Figure 2). Its common names (e.g., manioc, mandioca and yuca) vary from country to country reflecting its long cultivation in different regions. Many thousands of cultivars have been developed that are adapted to local conditions and differ in their ability to tolerate pests and diseases, and in the nutritional and cooking qualities of food products [26,33].

**Figure 1.** Tuberous roots of cassava, harvested by a farmer in Mozambique (note the tall cassava plants in the background and re-planted stem in the foreground; photo: J. Cliff,).



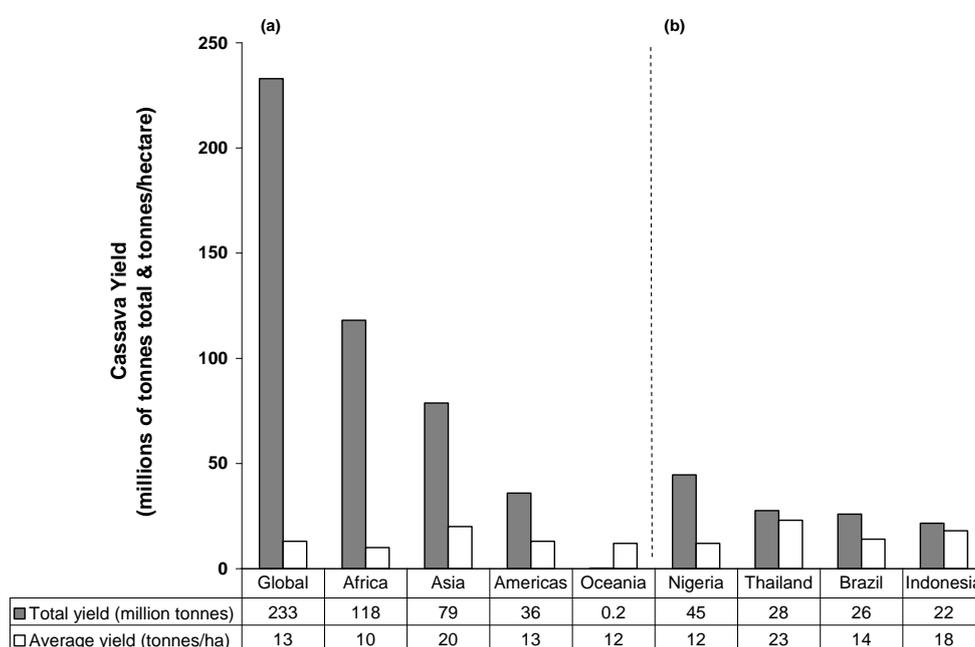
**Figure 2.** Total (A) and average per capita daily (B) consumption of cassava in 2003; from Montagnac *et al.* [33], produced by the International Institute for Tropical Agriculture Geospatial lab, based on FAOSTAT 2003 data. (Re-printed with permission from John Wiley & Sons, Copyright 2009).



Cassava is a major part of the diet for over 800 million people in approximately 80 countries [21,23,34]. Sixty to seventy percent of cassava produced globally is used for food [18,23]. The greatest per capita consumption of this crop is in sub-Saharan Africa (up to 800 g per person per day), where it is the main source of energy for over 40% of the population [23,35]. Consumption of cassava is also high in South America and parts of the South Pacific (Figure 2). Worldwide, production of this crop has doubled in the past 30 years from 118 million to 233 million tonnes (Figure 3) [13], with most of that increase on small-scale subsistence farms in Africa [35,36]. The primary food products come from the underground storage organs (Figure 1) that consist mostly of starch (up to 90% dry weight), but are

otherwise of low nutritional value [33]. They can be eaten as chips (fried or boiled) but more commonly they are processed into some kind of flour or granular product, such as tapioca, farinha or gari [24]. The type of processing depends on the cultivar (see Section 3.3), the food storage requirements and cultural traditions [21,23,33,34]. In many regions the leaves are also consumed, both fresh and cooked [21,37]. Although the leaves typically have higher concentrations of protein, minerals and vitamins than the tubers, the vitamins can be destroyed during cooking [33]. Leaves and tubers are also used as animal feed [24].

**Figure 3.** Total production (millions of tonnes, grey bars) and average yield (tonnes per hectare, white bars) of cassava in 2008 for: (a) cassava-growing regions and (b) the four highest producing countries. Data source: FAO STAT [13].



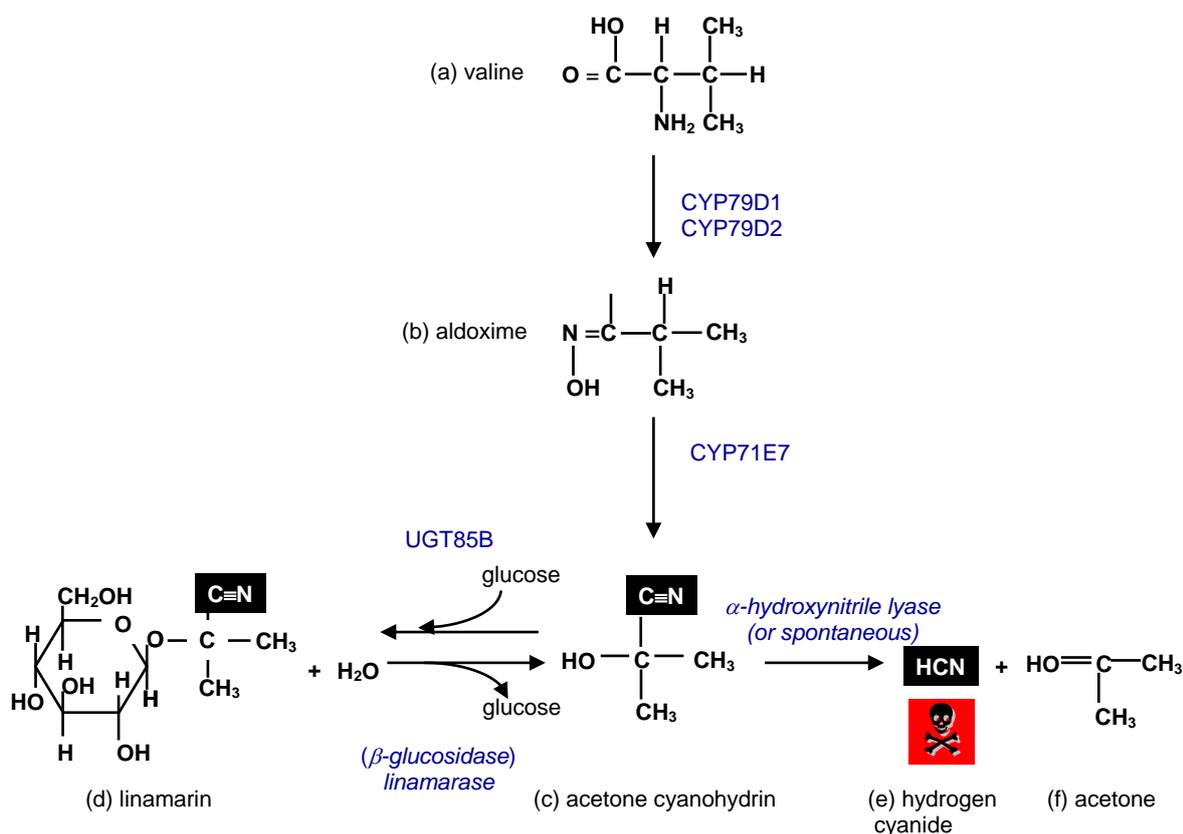
### 3. Cassava: Cyanide Production and Consequences for Consumers

#### 3.1. Cyanogenic Glycosides

While cassava has many positive attributes, which largely explain its widespread cultivation, it has a number of serious limitations. Firstly, there is the problem of rapid post-harvest deterioration of the tubers following removal from the soil, which limits its marketability [18,21,38]. Secondly, the tubers are low in protein [39-41] and some essential micro-nutrients [33], thus, an unbalanced diet can result in “hidden hunger” [42]. Thirdly, cassava contains a number of bioactive products that are harmful to human health [42-45]. The most important of these are the cyanogenic glycosides, which breakdown to release toxic hydrogen cyanide gas (HCN) in a process known as cyanogenesis (Figure 4) [46]. Cassava consumption can lead, therefore, to chronic health problems and death unless the food products are appropriately processed [41,47,48]. Indeed, it is the only staple food crop that can be lethally toxic unless it is correctly prepared [16,49,50]. In this section of this review, we consider the

role of these compounds in plant defence (against pests), as well as the impacts upon humans, in the context of achieving food security and environmental sustainability.

**Figure 4.** The biosynthetic pathway for the production of linamarin, the primary cyanogenic glycoside in cassava (a–d), and subsequent degradation to cyanide and acetone (d–f). The first committed synthetic step is catalysed by one of two P450 enzymes (CYP79D1 and D2) [27] to form an oxime (b). The oxime is then converted to a cyanohydrin (c), catalysed by another P450 (CYP71E7) [51] and then stabilised by a glucosidic bond, in a reaction catalysed by a glucosyltransferase (UGT85B). Cyanogenesis is the process whereby the cyanogenic glycoside (here, linamarin) comes into contact with a specific  $\beta$ -glucosidase (e.g., linamarase); glucose is released and the unstable  $\alpha$ -hydroxynitrile (c) disassociates (either spontaneously or in the presence of  $\alpha$ -hydroxynitrile lyase), releasing a ketone (acetone) and hydrogen cyanide, which is toxic [52]. Diagram is adapted from Møller [53] and Jørgensen *et al.* [27].

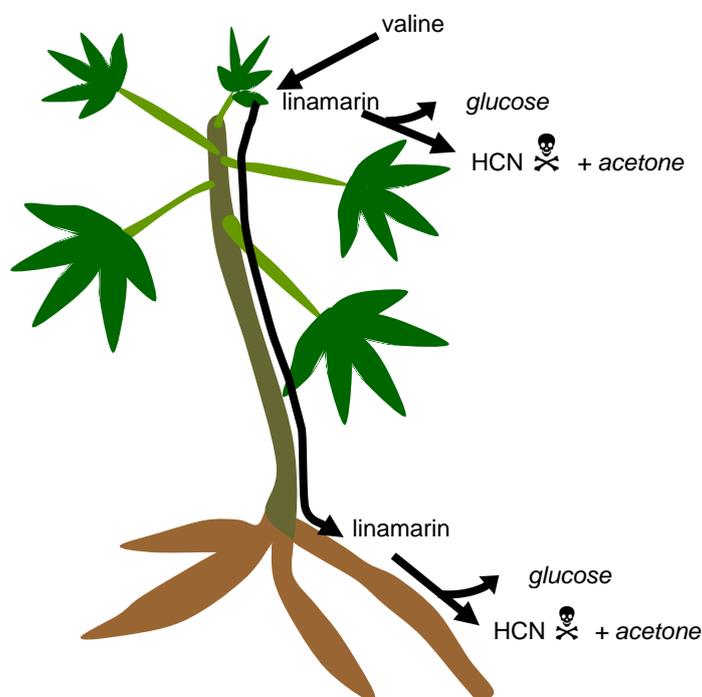


Cassava is one of over 2,000 plant species known to contain cyanogenic glycosides [53,54]. Cassava contains two different cyanogenic glycosides, linamarin and lotaustralin (ratio 93:7), synthesised from the amino acids valine and isoleucine, respectively [55,56]. Linamarin occurs throughout the tuber but the concentration is highest in the outer layers, immediately under the periderm [57,58]. The concentration of linamarin in the leaves can be more than ten times higher than in the tuber parenchyma (*i.e.*, flesh or pulp) [57]. Linamarin is synthesised in the leaves and then

transported to the roots, probably in the phloem, and some additional synthesis occurs in the periderm of the tubers [27] (represented diagrammatically in Figure 5).

The production of cyanogenic glycosides is thought to provide an immediate chemical defence for the plant against herbivore and pathogen attack through the release of toxic cyanide [53,59-64]. Cyanogenic glycosides also have a bitter taste that may function as a feeding deterrent [25,65]. The concentration of cyanogenic glycosides varies within individual plants, among cultivars and with environmental conditions [58,62,66,67]. The highest concentrations are in the young leaves, newly germinated seedlings and the outer layers of the tuber [27,57,68], consistent with a primary function in herbivore defence [59-61]. Cyanide released from the degradation of cyanogenic glycosides (Figure 4) is highly toxic to all aerobic organisms (including humans) because it binds to cytochrome oxidase, the last step in mitochondrial respiration, preventing oxygen uptake [52]. Degradation and auto-toxicity is prevented in the intact plant through spatial separation of the cyanogenic glycosides and the degradative enzymes at the tissue and organelle levels [69]. Cyanide is only released when the cyanogenic glycosides and specific enzymes are brought together. This typically occurs after tissue disruption caused by the chewing action of herbivores and is, therefore, only released when it is needed.

**Figure 5.** Linamarin and lotaustralin are synthesised in the leaves of cassava plants and transported to the tuberous roots [68]. Free cyanide is released when the cells storing linamarin (and lotaustralin) are disrupted and the contents mixed with linamarase from either the latex or the region outside of the cell known as the apoplast [27].



### 3.2. Cyanogens in Cassava: Defence against Pests and Diseases

All plants have to contend with complex assemblages of pests (both vertebrate and invertebrate) and disease-causing (pathogenic) organisms (e.g., bacteria, fungi and viruses). The production of

cyanogenic glycosides by cassava and many other crops provides an important defence against attack from both invertebrate and vertebrate pests [60,70], particularly generalist herbivores with chewing mouth parts [59]. Such natural defence chemicals are likely to be especially important in regions where farmers have limited or no access to synthetic pesticides. This reduced need for use of pesticides also has potentially important benefits in terms of environmental sustainability. Additionally, cassava also has to contend with a number of serious pests and pathogens that are specialists, able to either tolerate cyanide or circumvent its release from the cyanogenic glycoside. Major invertebrate pests of cassava include mites (particularly the cassava green mite), cassava mealybug, whiteflies, cassava hornworms and stemborers (*i.e.*, moth and beetle larvae), cassava burrowing bug and lacebugs [71]. Pests cause yield losses directly, by reducing biomass and consuming resources, and indirectly by spreading pathogens or increasing the plant's vulnerability to infections. Most of these pests feed on the leaves and stems of cassava, although the burrowing bug (*Cyrtomenus bergi*, Hemiptera) feeds on the tuberous roots. Cassava is one of only a few cyanogenic plants known to have cyanogenic glycosides in the roots (e.g., [72,73]), probably as an adaptation to protect these important storage organs. For example, early nymphal stages of cassava burrowing bugs, which bear short stylus (mouth parts), are deterred from feeding and have higher mortality than older nymphs [60]. Adults and older nymphs of these bugs are able to feed effectively on the roots by penetrating beyond the outer root layers that are particularly high in linamarin [60].

In Thailand, cassava yields have been severely curtailed by outbreaks of cassava mealybug (*Phenacoccus manihoti*) in recent years [74]. This and other related mealybug species are managed in South America and Africa through biological control with parasitoid wasps [71]. Pesticides can also be used to manage the pests of cassava, although access to pesticides may be limited in much of the world, and their excessive use can have serious negative environmental consequences. Many invertebrate pests of cassava, which are native to South America, have not yet been distributed to Asia, the Pacific islands or Africa [21]. However, the introduction of cassava pests to these areas could have rapid and potentially disastrous consequences.

The recent increase in incidence of diseases caused by viruses in cassava presents a serious threat to production [75]. The incidence of epidemics of cassava mosaic virus has increased over the last twenty years in East Africa, leading to losses of 47% of production and of US\$60 million per annum (in lost yield), and causing local famine [76,77]. Cassava brown streak virus has devastated cassava crops in East Africa and continues to spread [14,78]. This has resulted in significant investment in plant breeding programs to overcome this issue [14,76,79,80]. The incidence of plant diseases is affected by environmental conditions; for example, diseases caused by bacterial and fungal pathogens tend to be more severe when conditions are warmer and wetter, although some diseases are favoured by cool conditions [75,81]. Thus, in a changing climate the range of plant pathogens may change, as may the incidence and severity of plant diseases.

### 3.3. Cyanogens in Cassava: Variation within and Among Plants

Cassava is usually clonally propagated from stem cuttings, so there is minimal variation between individuals of the one cultivar when grown under the same environmental conditions (e.g., [82], but see below). There is, however, wide variation in the concentration of cyanogens among cultivars of

cassava, ranging from 1 to 2,000 ppm hydrogen cyanide equivalent [49]. This difference in cyanogenic concentration is thought to affect the taste, although other bioactive compounds may also affect taste [83,84]. Cassava cultivars are generally classified (on the basis of taste) as bitter or sweet (sometimes referred to as sour and cool [85]) depending on the concentrations of total cyanide [25,49]. Sweet cultivars are those with less than 100 ppm total cyanide fresh weight [25,86]. Cultivars can be further classified as innocuous (<50 ppm), moderately poisonous (50–100 ppm) and dangerously poisonous (>100 ppm) [87]. Tubers low in cyanide are peeled and boiled, then eaten as a vegetable or used for further cooking (e.g., as occurs in the Asia-Pacific) [24]. Lengthier processing techniques, including grating, fermentation, drying, and milling or pounding the tubers into granular products or flour, are common in regions where the tubers are higher in cyanide (e.g., in Africa and parts of Asia and Latin America) [24]. The toxicity of cassava leaves is reduced by pounding or fermenting, and then boiling the leaves, prior to consumption [88,89]. Women farmers with an intimate understanding of their crop can identify bitter and sweet cassava from the flavour and modify their treatment of the product accordingly ([90], see Section 3.4), although some studies have shown that taste is sometimes misleading [83]. Such cultural knowledge about the crop is essential and highlights the importance of appropriate education programs when cassava is introduced into new regions

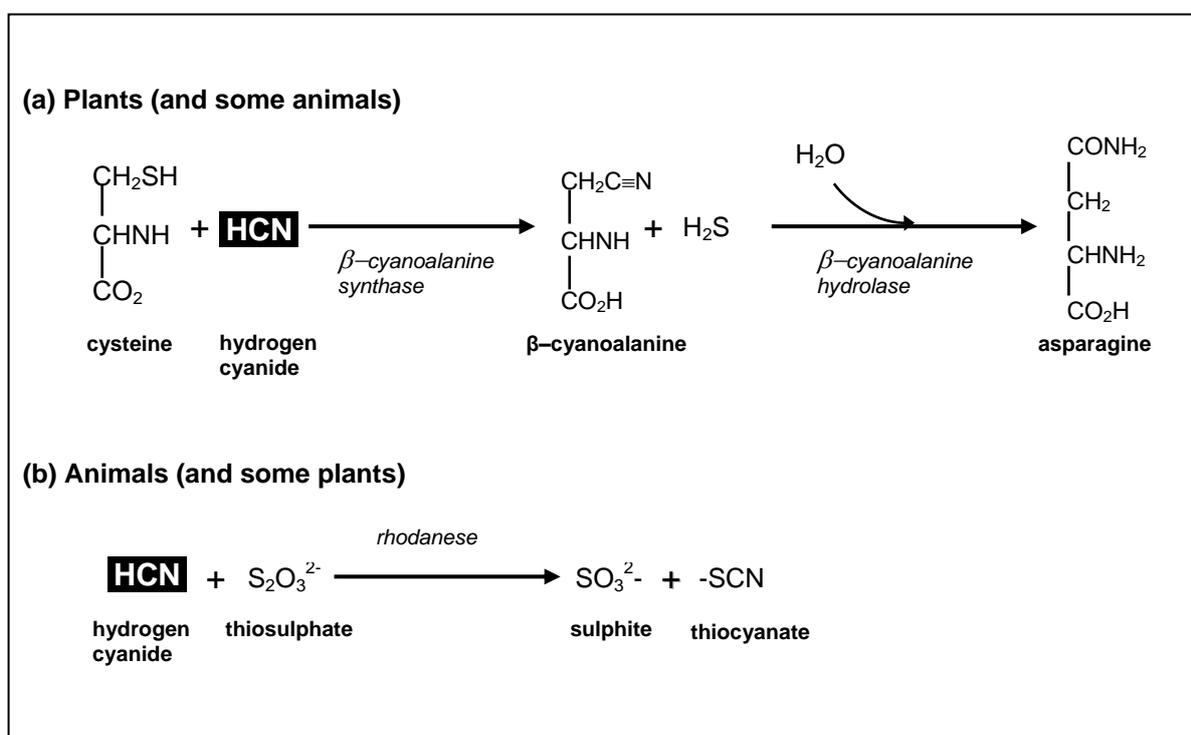
Bitter cultivars of cassava are often preferred by landowners, over sweet cultivars, due to better defence against pests and pathogens, theft (due to the time investment required to process stolen tubers), and a longer shelf-life after harvest [47,85,90,91]. Vertebrate pests of cassava, such as monkeys and wild pigs, are also deterred by bitter cultivars of cassava and this factor plays an important role in selection and planting configuration of cultivars by subsistence farmers ([41,47], J. Cliff personal observation). There is also a widespread belief among farmers in Africa and the Americas that bitter cultivars are higher yielding [25,47,91], but little experimental evidence to support this view [67,92]. From a physiological standpoint, this belief is surprising as there are significant energetic and nutrient costs (to the plant) in synthesising defence compounds such as cyanogenic glycosides [93-95]. In rubber, there is growing evidence that high linamarin production is related to higher latex production (*i.e.*, rubber yield), suggesting that there may be physiological advantages in being cyanogenic [29]. This view is supported by the fact that no acyanogenic individuals of rubber or cassava have been identified. Cyanogenic glycosides appear to play an important role in nitrogen storage in some species as well [28,53,96]. It remains to be seen whether correlations between higher yields and higher cyanide production are the result of improved nitrogen-use-efficiency, reduced herbivory or some other factor yet to be identified.

### 3.4. Cyanogens in Cassava: Impacts on Human Health

Consumption of cassava products with high concentrations of cyanogens can lead to illness or even death [97,98]. While many food crops are cyanogenic, including apples, wheat, almonds, sorghum and clover [70], unlike in cassava, the toxic element is generally either in parts of the plant that are not consumed (e.g., apple seeds and wheat leaves) or are eaten in small amounts (e.g., almonds) [70]. The risk of cyanide toxicity from cassava is increased because not only is the part that is consumed highly cyanogenic but it also frequently forms a large proportion of the overall diet. Sulphur-containing amino acids (methionine and cysteine) are required to detoxify cyanide in humans [70,99,100]

(see Figure 6). Cassava tubers are a poor source of protein, and the leaves, which are eaten as a protein supplement, are particularly low in these amino acids [101]. Not only is the overall proportion of ingested protein low in a high cassava diet, but the need for S-amino acids for detoxification restricts the proportion of that protein that can be assigned to growth, which may result in stunting of children [47,102,103]. Thus, where cassava is the main source of food in human diets, there is a need to ensure that there is adequate sulphur nutrition.

**Figure 6.** Detoxification of cyanide in both plants and animals uses sulphur. (a) In plants and some insects, detoxification of cyanide primarily takes place via the beta-cyanoalanine pathway [104]. (b) In animals, cyanide reacts with thiosulphate (derived from the amino acids cysteine or methionine) in a reaction catalysed by the enzyme rhodanese, resulting in the less toxic thiocyanate, which is excreted [105]. Some plants, including cassava, also contain some rhodanese [54,104].



Cases of cyanide poisoning are most common among people who subsist on a monotonous diet of cassava [47,103,106]. Acute intoxication symptoms, which occur within hours of consumption of insufficiently processed cassava, include dizziness, fatigue, headache, nausea and diarrhoea [97]. Too much cyanide over a relatively short time interval (*i.e.*, weeks), and insufficient protein, can cause irreversible spastic paraparesis (*i.e.*, weakness of the legs, [107]) due to upper motor neuron damage, resulting in konzo [47,106]. Interestingly, in South and Central America, where cassava has been cultivated for thousands of years, cassava toxicity is rare and konzo is unknown [25,108,109]. By contrast, konzo occurs over wide areas in southern, central and eastern Africa, especially in times of drought, war and social upheaval [47,97,110,111]. War and displacement in the Democratic Republic of Congo continue to cause large numbers of konzo cases [109,112]. Konzo is more frequent in children (greater than 2 years of age) and women (less than 45 years of age) [47,97,113], due to the

high nutritional demands of pregnant and lactating women, and children, who have been weaned but have only limited access to alternative foods. Adult males, by contrast, have better access to other foods. The chronic neurological disease, tropical ataxic neuropathy (TAN), is also associated with eating a staple diet of cassava [35,114], but there is some debate about a causal link with cyanide exposure [115,116]. Symptoms of TAN include deafness, reduced visual perception, and an ataxic gait [114,117]. TAN is common among older age groups in West Africa who have depended on a long-term cassava-based diet with moderate amounts of cyanogenic glycosides [49,114]. High cyanide exposure also exacerbates conditions associated with iodine deficiency such as goitre and cretinism [35].

The acute lethal dose of cyanide for humans is 0.5–3.5 mg kg<sup>-1</sup> body weight [70,118]. To control the level of cyanogenic glycosides in cassava products, the World Health Organisation has set the safe level of total cyanogens in cassava flour at 10 mg kg<sup>-1</sup> dry weight (*i.e.*, 10 ppm)[35,49]. In Indonesia, the acceptable limit of total cyanide in cassava flour is 40 ppm [49]. Therefore, since the toxic effect of cyanide depends on body weight, a child of approximately 20 kg could safely eat up to 1 kg of flour with a concentration of 10 ppm total cyanide, in a meal; and an adult of 60 kg, up to 3 kg of flour with 10 ppm cyanide (Table 1). Considering that the daily per capita consumption of cassava products is up to 700–800 g in parts of Africa [35], a child and an average adult (60–80 kg) would start to reach their minimum lethal dose of cyanide if the cassava products contained 40 ppm or more of total cyanide (Table 1); and thus, probably experience symptoms of illness (see above) at this concentration. Field kits have been developed in Australia by Dr J.H. Bradbury to allow producers and consumers in developing countries to easily measure the cyanogenic content of cassava products, free of charge [119–121]. These kits provide an invaluable resource and their use should be considered as part of the sustainable expansion of this crop, especially because cassava production is likely to continue to increase (see above) and potentially become more toxic under certain climate change scenarios (see below).

**Table 1.** Minimum lethal dose ranges of hydrogen cyanide (HCN) in humans per body weight [70,118] and the amount of cassava product, containing 10 ppm and 40 ppm HCN, required to reach these lethal doses.

Body weight (kg)	Lethal dose range of HCN (mg)	Lethal amount of cassava product (kg)	
		10ppm HCN	40ppm HCN
10	5–35	0.5–3.5	0.13–0.88
20	10–70	1–7	0.25–1.75
40	20–140	2–14	0.50–3.50
60	30–210	3–21	0.75–5.25
80	40–280	4–28	1.00–7.00
100	50–350	5–35	1.25–8.75

The final amount of total cyanide in cassava products depends on the initial concentrations of cyanogens in the cassava plants and the type of processing used [49]. All methods include some way to disrupt the cells followed by an incubation period to allow the cyanide to dissipate [122]. Simply cooking tubers and leaves of high-cyanogenic cassava does not decrease the risk of intoxication [25]

because high temperatures ( $>70\text{ }^{\circ}\text{C}$ ) denature the enzymes in the plant parts, preventing further decomposition of the cyanogenic glycosides [123]. Cyanide is released later, during digestion, due to hydrolysis of the cyanogenic glycosides by bacterial enzymes in the gut [70]. Processing methods vary among cultures and countries, and even among communities within particular regions [21,25,49]. Women and children most often process the cassava and, therefore, are vulnerable to the cyanide released during the detoxification process [25,90]. Processing can involve a combination of methods and take up to several weeks before the final product is ready for consumption [24,88]. Typically, the peel is removed from the tubers, which are then chopped or grated, and sun-dried, soaked or fermented. The dried cassava is pounded into flour, or the fermented root pulp is roasted to produce a coarser textured product (called “gari” or “rale” in Africa, depending on the country). Additionally, starch extracted from the tuber can be fermented, dried and used to make bread and biscuits [24]. A problem with most of these methods is that beneficial nutrients, such as B vitamins (thiamine, riboflavin and niacin),  $\beta$ -carotene (a source of vitamin A) and vitamin C, can be leached out or destroyed, which reduces the already low nutritional value of cassava [33]. There is also evidence that waste water from cassava processing (e.g., for starch production) can be toxic to the environment, with significant toxicity in fish reported [124,125].

While time-consuming, the processing methods are relatively effective at reducing the concentration of cyanogens in cassava products. Heap fermentation removes 45–95% of the cyanogens in the tubers [35,88], whereas sun-drying is less effective. In contrast, grating combined with fermentation and drying results in a product virtually free of cyanogens [88]. Grating is particularly effective as it mixes the cyanogens with the enzymes in the tubers, which releases the cyanide, but it is very time consuming and can expose the people grating to cyanide poisoning by inhalation. The South American cassava sleeve press, or “tipiti”, employed by Amazonian Amerindians appears to be highly effective in detoxifying cassava, typically removing 97–99% of cyanogens [126]. Such effectiveness explains, in part, why chronic toxicity and neuropathic diseases are rarely observed there [126–128]. While effective, the tipiti requires a plentiful supply of water, which is often limited in cassava-growing areas of Africa [25], especially in times of drought. There is limited processing of tubers in the Pacific Islands, where sweet cultivars predominate, and then only to produce preferred food types rather than for detoxification [21]. However, more bitter cultivars do occur in the Pacific Islands [129] and cases of acute cyanide toxicity have been reported in Papua New Guinea (J. Cliff, pers. comm.). If the more highly toxic cultivars currently used in parts of Southeast Asia make their way further east and south, it will be important that their dissemination be accompanied by suitable cultural knowledge for detoxification [21,25].

The newly developed “wetting method” by Bradbury and co-workers, reduces the total cyanide concentration of cassava flour by over 80% [122,130,131]. The method consists of spreading a thin layer of wet flour in a tray for 2 hours in the sun or 5 hours in the shade before cooking [122,131]. This allows time for the degradative enzymes to react with the cyanogenic glycosides in an aqueous environment and then for the cyanide to volatilise in the warm environment. This method is particularly useful to ensure the safety of flour of unknown origin purchased in markets, or produced in times of drought when the flour contains more cyanogens [111]. Considerable benefit could be gained from outreach programs seeking to educate consumers of cassava in the correct use of this method.

Sustainable expansion of this crop must be accompanied by education programs about appropriate processing of cassava, and, ideally, routine testing of the toxicity of cassava products (e.g., with the relatively inexpensive cyanide testing kits specifically developed for cassava). In addition, further agricultural development with a variety of crops is required, particularly in poor rural communities, to expand the dietary options of such communities and thus reduce their dependence on cassava.

### *3.5. Use of Genetic Engineering to Reduce Cyanogens in Cassava*

Given the impacts of cyanide on humans and animals, there is a need to reduce the amount of total cyanogens ingested by consumers of cyanogenic crops. Breeding of cassava cultivars with reduced cyanogen levels, while retaining other positive traits, is a desirable goal but the consequences of changes in cyanogen production must be fully considered in terms of impacts upon other aspects of plant physiology and performance in the field (e.g., pest resistance or internal N cycling). Genetic technologies have been used to create cultivars with reduced capacity to synthesise cyanogenic glycosides. Reduced expression of the genes governing the first step of the biosynthetic pathway of linamarin (*CYP79D1/D2*) has been achieved using RNAi technology, resulting in very low levels of linamarin in the leaves and roots of transgenic cassava plants [27,132]. However, the growth and development of the transgenic plants were also somewhat altered [27,132]. If a reduction in chemical defence properties of RNAi cassava results in reduced resistance to pests then it is possible that a greater reliance on pesticides will be required. An alternative strategy has focussed on reducing the concentration of cyanogenic glycosides in processed cassava by enhancing the rate of expression of  $\alpha$ -hydroxynitrile lyase, the enzyme that catalyses the conversion of acetone cyanide to hydrogen cyanide [133]. This is advantageous because it accelerates volatilisation of hydrogen cyanide in processed tubers, while the modified plants retain cyanogenic glycosides for use in defence or nitrogen metabolism. These examples highlight the need for careful consideration of the impacts of research and development activities on all aspects of ecosystems and cropping and food systems. Further research, including field trials, is required before such transgenic plants can be released for agricultural use. This is likely to require significant investment by government and philanthropic agencies because the clonal nature of this crop makes it an unattractive investment option for agribusiness companies. Regulatory and intellectual property issues associated with genetically modified crops will also need to be considered.

## **4. Cassava: Sustainable Production in a Time of Environmental Change**

Cassava is already one of the world's most important crops and its production is expected to expand further in the coming years. It is essential that we begin to consider how this crop will respond to changes in its environment, particularly if it is introduced into new areas where growers are unaware of its potential toxicity, and in the context of global climate change. In this section, we review current knowledge relating to the impacts of environmental factors on the growth, nutrition and production of cyanogens in cassava.

#### 4.1. Sustainable Production

Cassava yields vary considerably among regions of the world (Figure 3). African subsistence farmers achieve yields of 8–10 tonnes of fresh roots per hectare over a 12–18 month crop cycle [36]. In contrast, average yields in Indonesia and Thailand are twice that, with recorded yields of 18–23 tonnes per hectare in 2008 (Figure 3). Yields of 90 tonnes per hectare have been recorded under ideal growing conditions in Colombia [18,134]. This suggests that the yield potential of cassava is not being fully realized in some regions, including those where a modest increase in yield could go a long way to help achieve food security. The difference in yields around the world is likely to be the result of agronomic practices, as well as environmental conditions. Cassava requires little active management after the first 4–6 months following planting (*i.e.*, after canopy establishment and closure [86]) and is compatible with many types of intercrops [21,36,135]. In Africa, for example, cassava is often grown as an intercrop on subsistence farms on marginal land [20,36,136,137]. Access to agricultural inputs, such as fertilisers and pesticides are often limited or non-existent in such situations [138]. Therefore, intercropping needs to be carefully managed to minimise depletion of soil nutrients [135]. However, intercropping has the benefit of increasing the diversity of food consumed, which has implications for human capacity to detoxify cyanide in the diet (see Section 3.4). Cassava is also grown as an imitation fallow, *i.e.*, a cassava crop is planted instead of leaving the land to rest and the discarded plant material is mulched into the soil [137,139,140]. Using cassava as an imitation fallow allows for agricultural intensification without rapid degradation of soil quality [139]. However, the potential environmental impacts from use of cyanogenic species as a green manure, such as leaching of cyanogenic glycosides into groundwater [141,142] and allelopathic effects [143], should be considered.

Competition between cassava and weeds, for space, water and nutrients, can also significantly reduce cassava yields [135,144]. While weeds can be controlled with herbicides where available, they typically need to be removed by hand in subsistence farming systems, which represents a significant expenditure of time and energy [135]. If cassava production were to shift away from subsistence-based to profit-based farming systems, there would almost certainly be a need to use herbicides to control weeds. This must, however, be accompanied by knowledge of their safe use, both in terms of human and environmental safety.

The world is currently undergoing significant environmental change associated with increasing emissions of greenhouse gases such as CO<sub>2</sub>, methane and nitrous oxide, primarily from anthropogenic activities [145]. This will have a significant impact on agricultural productivity in many regions of the world [146]. Africa is expected to be particularly vulnerable to the impacts of climate change due to its geography and dependence on small-scale agriculture [3]. Farmers readily adapt to the vagaries and changes in climate from year to year, within a certain framework [147]. However, the key to empower farmers to adapt to the long-term changes that lie ahead is to provide them with reliable and relevant information [3]. Cassava currently plays a major role in global food supply and has a fundamentally important role to play in meeting the world's increasing demand for food. However, we contend that if the full potential of cassava is to be realized, these issues need to be fully addressed so as to maximise crop yields but not at the expense of environmental sustainability.

#### 4.2. Cassava and Sustainable Water Management

Much of the focus for predicting crop yields during the 21<sup>st</sup> century has focussed on water availability and increasing temperature. Average global precipitation, evaporation and water vapour are expected to increase in the future, but changes will be region-specific. Annual precipitation and run-off are expected to increase in the moist tropical areas and high latitudes, but to decrease in the semi-arid tropics, sub-tropics, and mid-latitude regions [145,148]. Furthermore, the intensity of precipitation events is predicted to increase during this century, with more high rainfall events in tropical and high latitude areas, and extended periods of low rainfall and droughts in the sub-tropical and mid-latitude areas [148]. Thus, cassava-growing areas in southern Africa, north-east Brazil and Latin America are likely to experience less annual rainfall and runoff, whereas central Africa, Asia and parts of South America may receive greater rainfall and runoff [145,148]. Already the growing-season rainfall in eastern and southern Africa has declined by approximately 15% in the last 30 years, and droughts have seemed to be more severe [6]. These phenomena will directly affect both crop production and the nutritional value of food products.

Cassava is well-known for its capacity to withstand significant periods of water stress but with an associated reduction in growth and yields [86,134,149]. The response of cassava plants to water stress is a function of both the duration and severity of water deficit and the cultivar. Under experimental conditions, root and shoot biomass can be 70% less in water-stressed plants [86,134]. Not only is the severity of the water deficit important but also its timing, with effects most pronounced if water deficit occurs in the first 1–5 months after planting [86,150,151]. This observation is not surprising since this is the period of expansion of the plant canopy and the initiation and development of the tuberous roots. Full recovery is most likely if more than one wet season occurs in a year, in which case tuber yields of stressed plants can be similar to those of non-stressed plants at final harvest, depending on the cultivar, due to greater nutrient- and water-use-efficiency of the stressed plants [86,134]. Many of these issues can be addressed if crop irrigation is a viable option; however, this requires considerable planning around the complex issues associated with sustainable water management.

Cassava has a number of physiological adaptations that allow it to tolerate extended periods of water stress [86,134]. Mechanisms of drought tolerance include a high degree of control over stomatal aperture, leaf movement and stimulation of root growth [86,134]. The leaves are oriented to maximise light interception at the beginning and end of the day when temperatures are low, but fold and droop at midday when it is warmer [134]. Together, these mechanisms maximise photosynthesis while reducing water loss by transpiration [18]. Furthermore, cassava's fine root system can penetrate below two metres, allowing plants to exploit deep water sources, slowly [134].

Cassava can still produce a reasonable yield under drought conditions [134], but there is evidence that the concentration of cyanogens increases in water-stressed plants. Monitoring cassava toxicity amongst cassava-dependent communities in Mozambique revealed that total cyanide concentration in flour made from cassava in drought years tripled compared to flour produced in normal years [35,49,111]. Ernesto *et al.* [111] found that flour produced from cassava grown in years with average rainfall in northern Mozambique contained, on average, 40 ppm total cyanide, compared with about 120 ppm in a drought year. Both of these values are well above the World Health Organisation safe level of 10 ppm, but the three fold increase in dry years is alarming [49,111].

Coupled with the fact that protein intake is lower in times of drought, these observations are consistent with the increased incidence of acute cyanide poisoning and outbreaks of konzo during droughts in cassava-dependent communities [48,110,111]. Not only is this a problem for human nutrition, but for animal production as well, because cassava is used for animal feed in many countries [21,152-155].

Although the effect of drought on the yield and productivity of cassava has been studied extensively (e.g., [18,86,156]), only a handful of studies have included measurements of the cyanogenic glycosides (e.g., [67,134,149,157]). This is a significant knowledge gap. The increase in cyanogens in cassava products during periods of drought is thought to be the result of high endogenous levels in the source plants, but much of the evidence to support this is anecdotal [48]. Field studies generally find an increase in the concentration of cyanogens in tubers in response to water stress but the degree of increase appears to be cultivar-dependent [67,134,149,157]. For example, El-Sharkawy [134] reported that prolonged water-stress imposed from three to six months after planting resulted in an average increase of 40% in the amount of cyanide per unit plant dry weight across four cultivars. Furthermore, Bokanga *et al.* [67], in a study of ten cassava cultivars grown in different locations with varying natural rainfall, found that the cyanogenic potential of tubers of all cultivars was higher at the driest locations, and was five times higher in one cultivar at the driest sites compared to the highest rainfall sites. The increases in cyanogen concentrations seen in these studies could be a simple “concentration effect” from reduced yields (*i.e.*, increased cyanide per mass), but an irrigation experiment found that the amount of cyanogens produced per hectare of cultivated land was greater in non-irrigated plots, suggesting a direct increase in cyanogenic glycoside production [67]. In assessing the impact of drought, it is important to distinguish between experiments in which plants are subject to water deficits over a long period or to instantaneous drought, as can occur at the time of harvest. Santisopasri *et al.* [157], for example, found that the cyanogen concentration of stressed plants harvested in a high rainfall period, was lower than those from non-stressed plants harvested during a low rainfall period.

Given the projected increase in the severity and frequency of droughts under current climate change scenarios, there is a clear need to undertake more detailed systematic investigations on the response of cassava to different kinds of water stress. Further research is required to determine whether the genes encoding the cyanogenic glycosides are up-regulated in response to water-stress and/or if water-stress triggers translocation of cyanogenic glycosides from senescing leaves to the roots [158]. Investigating the link between the production of cyanogens and drought in cassava is a high research priority, especially since much of the world is projected to experience a drier climate under future climate change scenarios. The results of such research could be extended to modelling of drought responses, with emphasis on cassava regions where drought and cyanide-related diseases are currently a problem, and regions where cassava cropping is expected to be introduced in the future.

#### 4.3. Cassava and Sustainable Nutrient Management

The increase in area under cassava cultivation in Africa has primarily been on low quality soils, especially in East Africa [144]. As with most crops, application of fertilisers leads to improved growth and nutrition of cassava, with increased plant biomass, tuber number and overall yield [18,144,159]. Interestingly, the biomass of cassava crops appears to be linked to the nutritional status of the plants from which the cuttings (or “stakes”) were taken [160]. Thus, correcting a minor nutrient imbalance

could dramatically increase yields for both current and subsequent crops. Although variation in local soil conditions is important, increases in cassava yield can be readily achieved with a strong response to application of potassium (K) and moderate responses to nitrogen (N) and phosphorus (P) [161]. In the developing world this can be especially important, as access to synthetic fertilisers is often limited or non-existent. Furthermore, excess application of nutrients can have negative environmental impacts via leaching of mobile nutrients into waterways. The nutritional optima for most crops, including cassava, have been determined [162]. This information is invaluable in determining the type and amount of nutrients required to maximise yields, and the best time to apply them. Such tables, however, are of little use to farmers who are unable to access services to determine the nutrient status of their plants or to buy fertilisers. Improved access to reliable plant nutrient analyses represents an important development opportunity. On a larger scale, however, such information could be used to relate soils data to the crop nutrient data with a view to improve access to critical nutrients on a regional scale (*i.e.*, prioritisation of development efforts). We consider this an important priority area to increase cassava yields.

Since N is a key component of cyanide, it may follow that changes in plant N nutrition could have an impact upon cyanogen production in cassava, as found for other cyanogenic species [72,163]. However, since nutrient acquisition also affects plant growth and biomass accumulation, correlations between nutrient availability and concentrations of cyanogens are not always clear-cut. For example, in one instance, use of combined N, P and K fertiliser resulted in a decrease in the concentration of total cyanogens in cassava roots, along with an increase in root biomass [159]. In contrast, use of N fertiliser by itself has been reported to result in higher concentrations of cyanogens in cassava roots [67,158]. In most cyanogenic species, there is a correlation between N supply and the concentration of cyanogenic glycosides in the leaves [67,72,163-166], although this is not universally true [167-169]. In our work on cassava [169], we found that leaves from plants grown under a high N treatment only had higher concentrations of cyanogenic glycosides when plants were grown at higher concentrations of atmospheric CO<sub>2</sub>. Given the contradictions in the literature, there is a need for more studies investigating the putative link between N and cyanogens, especially under field conditions where cassava is grown.

Surprisingly little is known about the effect of other important nutrients such as P and K on the production and storage of cyanogenic glycosides. We are aware of few published papers regarding the effect of the availability of K alone on cyanogens in cassava [67,158,164,170], and none that report on the effect of P alone. The reduction of total cyanogens in cassava roots following application of K fertiliser (by c.25%) appears to be related to increases in starch content and dry matter of the tuberous roots rather than an decrease in synthesis of cyanogens [67,158,164,170].

Over 80% of all terrestrial plant species, including cassava, form beneficial associations with a specialised group of soil fungi, the arbuscular mycorrhizal fungi (AMF). The formation of arbuscular mycorrhizas (AM) can result in increased plant acquisition of nutrients (including P, N, Zn and other nutrients), with potential benefits to both the plants, and those who consume them [171]. For example, using another crop species, Cavagnaro *et al.* [172] demonstrated that the Zn content in the edible portions was increased up to 50% when plants formed AM. Since 30% of the world's soils are Zn deficient and an estimated 40% of the world's population suffer from inadequate dietary Zn intake [4,173], the role of AM in improving the nutritive value of crops may be very important, especially in

the developing world, where farmers have limited access to (expensive) synthetic fertilisers [138]. It is also important to note that these beneficial fungi are found in most soils, and through careful land management, their potential benefits to plants can be achieved.

The role of AM in cassava growth and nutrition has received some attention, with improvements in plant growth, developmental rates and yield reported [174-178]. In most cases, plant responses to the formation of mycorrhizas were dependent on the cassava cultivar and the species of mycorrhizal fungi [174,175,179]. The effect of mycorrhizal association on the yield of tuberous roots of cassava cultivars is also dependent on the availability of soil nutrients [179]. That is, when soil nutrients are readily available, the effect of mycorrhizal association on yield is lower, as is the case with other crops [172,180]. Similarly, the diversity of AMF in soils where cassava is grown tends to be lower where the availability of soil nutrients is high [181]. Taken together, these examples suggest that AM may have a role to play in improving cassava yields and nutrient contents, especially in soils of relatively low nutrient status. Thus, management of cassava should take AM into consideration. It remains to be seen how AM formed by cassava are impacted by climate change, and whether or not the formation of AM has an impact upon cyanogenesis in cassava.

In summary, cassava production in many parts of the world, especially Africa, may be limited by an inadequate supply of nutrients to plants. Further work is required to identify which nutrients are limiting production on a regional basis, and the potential to correct these deficiencies, such as via fertiliser inputs, crop breeding (not considered here in detail), changes in crop rotations (e.g., including legumes to build soil nitrogen), or by capitalizing on beneficial associations with naturally occurring symbiotic (arbuscular mycorrhizal) soil fungi. In addition to improving cassava yields, this may also help to increase the nutrient content of tubers and (more likely) leaves, and thereby help to satisfy nutritional aspects of food security. It is important that we explore fully the link between the concentration of cyanogens and fertiliser application, as any improvements in agricultural practice introduced to increase cassava yield would almost certainly include increased use of fertilisers. Also needed is investigation of soil fertility in regions where people want to grow cassava, so that potential nutrient-related problems can be identified early on through assessment of regional soil properties.

#### *4.4. Cassava Production and Temperature*

Increasing concentrations of greenhouse gases in the atmosphere and changes in land-use are expected to lead to an increase in global average air temperature of up to 4 °C in the next century, along with associated increases in evapo-transpiration [148]. Although cassava can tolerate a wide range of environmental conditions, it grows best in conditions where annual rainfall is greater than 600 mm and where there is high solar radiation and humidity [18]. Optimal growth of cassava occurs under annual mean air temperatures of 25–29 °C but it can tolerate temperatures of 16–38 °C [18,86]. Low air temperatures can delay sprouting, reduce the rate of leaf production and reduce total plant dry weight [86]. High air temperatures can lead to greater crop growth and photosynthetic rates, with the maximum photosynthetic rate recorded at 30–40 °C for three cultivars from different habitats [182]. However, temperatures greater than 30 °C reduce leaf longevity [86]. Such physiological impacts are likely to have an impact upon plant yields. Although at the moment it appears that higher global

temperatures will favour cassava production [8], this remains to be widely assessed and represents an increasingly important development issue for the coming decades.

Cassava cultivars respond in different ways to changes in growing temperatures. In a study by Irikura *et al.* [183], for example, three cultivars had their greatest tuber yield at 24 °C or 28 °C but one cultivar yielded more at 20 °C. This variation suggests that as the climate changes, or when cassava is introduced into new areas with different climates, there is an opportunity to maximise yields through the careful matching of cultivars to the environment into which they are introduced. Other features of cultivars must also be taken into consideration, but this would provide a good starting point, for example in regional suitability field trials.

We are not aware of any studies investigating the effects of growing condition temperatures on cyanogenesis in cassava. Given the importance of this crop and the certainty of increases in global temperatures, this is a major knowledge gap—as are the interactions between temperature, atmospheric CO<sub>2</sub> concentrations and drought on cyanogenesis and yields of cassava. Little insight can be gained from studies of other species, as results are contradictory. For example, two studies on clover were completely opposite in their results. Stockmal and Oleszek [184] found higher concentrations of cyanogens in plants that had recently experienced low temperatures, whereas Collinge and Hughes [185] found that cyanogen concentrations were highest in moderate temperature conditions (16–22 °C). The higher cyanogen levels may simply reflect temperature-limited growing conditions. Clearly, this is an area where additional knowledge is urgently needed. Thus, investigation of the impact of variation in temperature on cyanogenesis and growth of different cassava cultivars will provide valuable information in the context of achieving food security under future climate change. The outcomes of such research could include matching new areas of cassava production to cultivars with suitable temperature optima for the region.

#### 4.5. Cassava Production in a High CO<sub>2</sub> World

The final environmental variable considered here is the concentration of atmospheric CO<sub>2</sub>. Over the past 250 years the global atmospheric concentration of CO<sub>2</sub> has increased exponentially from 280 to 380 ppm [145] and is expected to double in the next 50 years, given current emission scenarios [148]. The increase in atmospheric CO<sub>2</sub> concentrations globally, has the potential to boost plant growth and crop yields (since CO<sub>2</sub> is an essential ingredient in photosynthesis), depending on the availability of soil nutrients and water [5,186–189]. Estimates of 28–35% increases in yield of four major crops (rice, wheat, sorghum and cotton) were originally reported. These projected increases were based on results from CO<sub>2</sub> chamber studies, but have since been down-graded to an average of 17% on the basis of field studies conducted in open-air conditions (Free Air CO<sub>2</sub> Enrichment, FACE) [146]. The degree of enhancement in yield is species-dependent and moderated by access to fertilisers and water supply [146,188]. This further serves to highlight the link between CO<sub>2</sub> and plant nutrition and is especially important to the developing world where farmers have limited access, if any, to fertilisers.

If cassava is to help meet global food demands in the future then we need to determine how increasing concentrations of atmospheric CO<sub>2</sub> will affect the growth of cassava and the flow-on effects on its nutritional quality and yield. Cassava is a woody C3 plant, with a normal CO<sub>2</sub>-sensitive photosynthetic system [190], although El-Shakawy [186] considers it to have some C4 characteristics.

As such, cassava would be expected to show a significant increase in growth rates at elevated CO<sub>2</sub>. We have only identified three published experimental studies directly addressing this issue [169,191,192], which give contrasting results (see Table 2). Two of these studies were conducted in glasshouses with variable CO<sub>2</sub> concentrations, and one in the field (using small open-top CO<sub>2</sub> chambers), over durations of 3 to 8 months (which is shorter than the average cassava crop cycle of 12 months [86]). One of the main points from these studies is that the ratio of root to shoot (*i.e.*, leaves and stems) biomass, which is a measure of potential yield, increased, decreased or remained unchanged under elevated CO<sub>2</sub> compared to control conditions (Table 2). The differences in results between the studies may have arisen from variation in experimental duration, soil nutrient availability and soil environments, the degree of CO<sub>2</sub> enhancement or other growing conditions. Of these studies, only the one of shortest duration [191] showed an increase in mass of tuberous roots. In contrast, in our recent work [169], we found that tuberous roots were smaller and fewer in number in plants grown under elevated CO<sub>2</sub> conditions (710 ppm). Interestingly, the growth rates were even more depressed in plants supplied with higher N (12 mM nitrate solution). Variable results have also been reported from elevated CO<sub>2</sub> experiments with other root crops such as potato [189,193-195]. These studies highlight the complex interactions between changes in atmospheric composition, soil nutrition and plant biology. Clearly, there is a need for further experimentation, particularly under field conditions, such as FACE, in the types of environments in which cassava is cultivated.

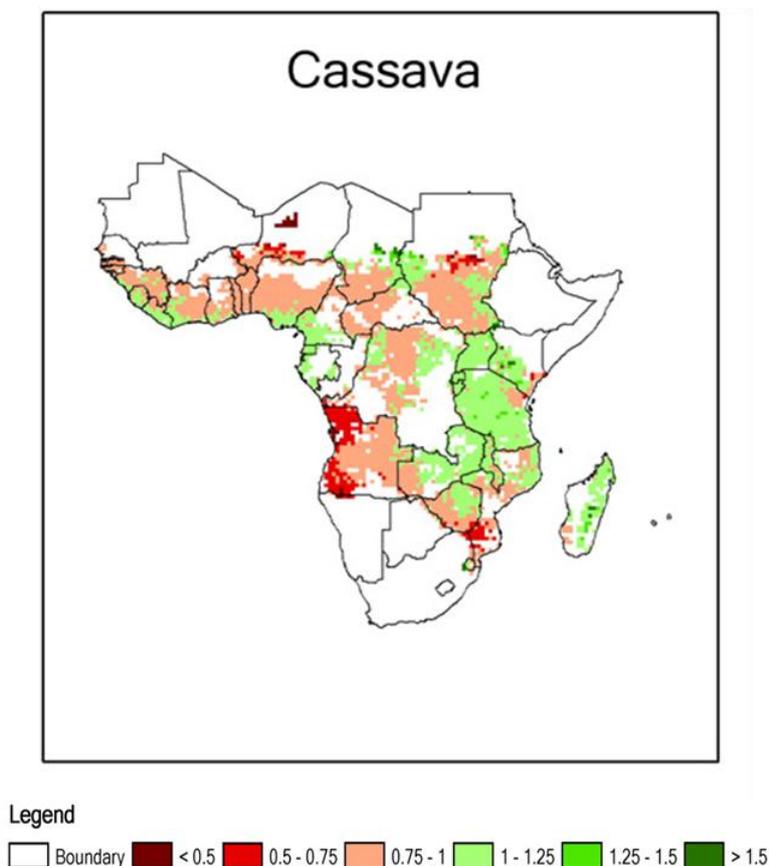
**Table 2.** Direction of change in net carbon dioxide (CO<sub>2</sub>) assimilation rate and root:shoot ratio of cassava plants grown in experimental conditions, with different concentrations of atmospheric CO<sub>2</sub> (ambient and elevated) and soil N supply, from three published studies.

CO <sub>2</sub> concentrations	Duration & Experiment type	Net CO <sub>2</sub> assimilation rate	Root: shoot ratio	N supply	Reference
350 ppm 700 ppm	3 months, Glasshouse	Lower at elevated CO <sub>2</sub> (measured after 2 months) <sup>a</sup>	Increased	Supplied as compound fertiliser twice during experiment	Imai <i>et al.</i> 1984 [191]
360 ppm 550 ppm 710 ppm	9 months, Glasshouse	Lower at 710 ppm than 550 and 360 ppm *, N.S. between 550 and 360 ppm (measured after 3 months) <sup>b</sup>	Decreased	1 mM & 12 mM N in Hewitt's solution, 3 times per week	Gleadow <i>et al.</i> 2009 [169]
480 ppm 680 ppm	8 months, Field	Higher at elevated CO <sub>2</sub> but N.S (measured after 2.5 and 7 months) <sup>c</sup>	No change	N:P:K:Mg (18:18:18:3) 4.1 kg/ha, once at beginning of experiment	Fernandez <i>et al.</i> 2002 [192]

\*  $P < 0.05$ ; N.S.: not significantly different; a. See Figure 1 of [191]; b. see Figure 3 of [169]; c. see Figure 1 and Table 2 of [192].

The results of the experiments outlined above can be compared with models simulating changes in crop production under various atmospheric CO<sub>2</sub>, temperature and precipitation scenarios. Models of cassava production by both Liu *et al.* [8] and Lobell *et al.* [196] predict that there will be very little overall impact on cassava yield by 2030 globally, due to cassava's ability to tolerate drought and variable growing conditions. However, changes are likely to be country-specific; thus some parts of sub-Saharan Africa are likely to experience a reduction in cassava yield (Figure 7). These models assumed agriculture under a “business as usual framework” and further assumed that elevated CO<sub>2</sub> would be beneficial to plant growth through the so-called “CO<sub>2</sub>-fertilisation effect” [197]. The study by Liu *et al.* [8], an extensive GIS-based analysis of major crops, economic development and population growth in sub-Saharan Africa, allowed them to predict that certain regions of Tanzania, Mozambique and the Democratic Republic of Congo could face more serious malnutrition in the future. Furthermore, parts of Ethiopia, Uganda, Rwanda and Burundi, south-western Niger, and Madagascar are likely to remain hotspots of food insecurity unless strong adaptive measures are taken.

**Figure 7.** Expected changes in cassava crop yield in Africa in response to climate change (modelled with the A2 climate scenario, IPCC 2007, by Liu *et al.* [8]). The impact ratio (IR, see range displayed in the legend), is the ratio of yield expected in 2030 compared to observed yield in 1990. An IR value less than one means crop yield will decrease due to climate change; IR = 1: no change expected; IR > 1: increased yield expected. (Re-printed from [8] with permission from Elsevier, Copyright 2008).



An often neglected consequence of changes in atmospheric CO<sub>2</sub> on plants is the impact on the chemical composition of plants. When plants are grown under elevated CO<sub>2</sub>, changes in their nutrient acquisition and use affect the amount of N-containing compounds in the plant, such as protein and cyanogens, thus altering a plant's nutritional value [165,169,188,198]. Many crop plants (such as rice, wheat and potato) grown at elevated CO<sub>2</sub> contain less nitrogen and protein (typically 7–15% less N on average) than plants grown at ambient CO<sub>2</sub> [192,198-200]. This means that herbivores, including humans, need to consume more biomass to take in the same amount of protein when consuming plants grown under elevated CO<sub>2</sub> conditions. The reduction in plant N can be explained by increased rates of photosynthesis (thus C gain) per unit of plant N, potentially increased plant size, and changes in soil N cycling [201-203]. These changes in the balance of C and N in the plant may in turn have implications for the synthesis of N-containing compounds, including protein and cyanogenic glycosides. The impacts of changes in CO<sub>2</sub> concentrations on micronutrients, especially those implicated in “hidden hunger” [42], in food plants also need to be considered. Trends are emerging that decreases in micronutrients such as iron and zinc may occur in the grains of rice and wheat in response to elevated CO<sub>2</sub>, although this is not always the case [204-207]. Again, these issues have received relatively little attention (compared to yields) in the context of the nutritional aspects of achieving food security.

To our knowledge, there are only four published papers that describe the effect of CO<sub>2</sub> on cyanogenic glycosides, and only one of these treats cassava. Leaves of clover (*Trifolium repens*) [165,208] and sugar gum (*Eucalyptus cladocalyx*) [209] seedlings grown at elevated CO<sub>2</sub> (520 ppm and 800 ppm, respectively) showed no change in the cyanogen concentration on a per mass basis. However, the nutritional value of such plants decreased due to lower concentrations of protein [165,209]. In contrast, Gleadow *et al.* [169] found that the concentration of total cyanide in cassava leaves more than doubled (from 600 to 1,600 ppm) in response to doubling of the CO<sub>2</sub> concentration, in the presence of sufficient N fertiliser, and almost doubled when N was limited. The concentration of cyanogens in the tubers, however, was not significantly affected by the CO<sub>2</sub> treatment. It would be premature, however, to draw any firm conclusion with respect to the impacts of elevated CO<sub>2</sub> on cyanogenesis in cassava given the limited number of studies. Given that plants grown at elevated CO<sub>2</sub> generally have improved water use efficiency [187,210,211], it is possible that any increase in cyanogens in plants in response to future increases in CO<sub>2</sub> will be countered by improved plant water-status in the field. Again, this is an area we consider significant and further studies should be of high priority. Taken together, integrated studies of the impacts of elevated CO<sub>2</sub> on changes in plant growth, yield and nutrient acquisition, and the flow-on effects on concentrations of nutrients and cyanogens in cassava, would make valuable contributions to understanding the potential role of cassava production in achieving food security in the future. Ideally, such studies should be conducted under field conditions, such as free-air carbon dioxide enrichment experiments, in conditions under which cassava is typically grown.

## 5. Conclusions

Cassava is a remarkable plant—it has the capacity to withstand tough conditions with relatively little need for active agricultural management. This is largely why it has spread rapidly around the globe to become one of the world's most important food crops. The expansion of cassava cropping is

likely to continue in the future and is likely to improve food security for the world's increasing human population. In this review, we have sought to highlight a number of issues before this can occur, with a view to informing prioritisation of research and development activities.

We have presented clear evidence that environmental conditions can have a significant impact upon cyanogenesis in, and yield of, cassava. There is, however, a high degree of uncertainty around many of these impacts, and there are clearly important questions that remain unanswered. These questions can be readily addressed through a systematic assessment of cassava's responses to environmental change. We consider studies of drought, temperature, nutrient supply and atmospheric CO<sub>2</sub> concentrations to be of particular importance. While each of these parameters alone can significantly influence the growth, nutrition and cyanogenesis of cassava, it will be the interactions between these factors, which are likely to be most important. Such information needs to be matched with projected changes in environmental conditions under various climate change scenarios. The outcomes of such work could involve matching cultivars with suitable growth characteristics to new and existing areas of cassava production. Environmental conditions also modulate the interactions between cassava and its pests and disease-causing pathogens. Thus, agricultural development activities should consider changes in pest and pathogen ranges as regional climates change. Furthermore, identification of both agricultural and social factors that lead to increased risks of cyanide poisoning (e.g., drought, war and limited access to agricultural inputs), will be important in maintenance of food safety and health of cassava consumers, and to inform the distribution of appropriate new cultivars.

As cassava production expands into new regions, there is a need for a concomitant expansion of knowledge relating to the effective detoxification of cassava. Together with use of simple cyanide testing kits, specifically developed for cassava, these measures could significantly reduce the risk of cyanide poisoning in both new and existing cassava-growing regions. Breeding and genetic modification of cassava cultivars with reduced cyanogen levels could also contribute to development of safer cassava food products. However, we caution that the consequences of changes in cyanogen production must be fully considered, along with regulatory implications and end-user perceptions of genetically modified crops. Furthermore, integrated agricultural development is required, particularly in poor rural communities, to expand the dietary options of such communities and thus reduce their dependence on cassava.

In conclusion, this review has sought to highlight a number of key issues around the continued and increased reliance upon cassava as a staple food crop. It is our view that by addressing some of the issues raised here, there is the capacity to enhance the role of cassava production and utilisation in achieving food security in a time of significant environmental change. Furthermore, such efforts must focus on an integrated approach that applies the best available knowledge to implement real changes on the ground. This will be of most value where it is undertaken in the regions where cassava is grown, and by collaborating with, and building capacity of, people already involved in the safe production and consumption of cassava.

## **Acknowledgements**

We wish to thank Howard Bradbury AM for valuable discussions and technical and intellectual advice. We gratefully acknowledge The Australian Government's aid agency, AusAID, for providing

the financial support that made this review possible. We also thank the Finkel Foundation, Monash University School of Biological Sciences and Monash University (Strategic Initiative Grant) for supporting our other work on cassava.

## References

1. Baulcombe, D.; Crute, I.; Davies, B.; Dunwell, J.; Gale, M.; Jones, J.; Pretty, J.; Sutherland, W.; Toulmin, C.; Green, N.; *et al.* *Reaping the Benefits: Science and the Sustainable Intensification of Global Agriculture*; The Royal Society: London, UK, 2009.
2. Gregory, P.J.; Ingram, J.S.I.; Brklacich, M. Climate Change and Food Security. *Phil. Trans. R. Soc. B* **2005**, *306*, 2139-2148.
3. Challinor, A.; Wheeler, T.; Garforth, C.; Craufurd, P.; Kassam, A. Assessing the vulnerability of food crop systems in Africa to climate change. *Climatic Change* **2007**, *83*, 831-399.
4. Cordell, D.; Drangert, J.O.; White, S. The story of Phosphorus: Global food security and food for thought. *Global Environ. Change* **2009**, *19*, 292-305.
5. Ainsworth, E.A.; Beier, C.; Calfapietra, C.; Ceulemans, R.; Durand-Tardif, M.; Farquhar, G.D.; Godbold, D.L.; Hendrey, G.R.; Hickler, T.; Kaduk, J.; *et al.* Next generation of elevated [CO<sub>2</sub>] experiments with crops: A critical investment for feeding the future world. *Plant Cell Environ.* **2008**, *31*, 1317-1324.
6. Funk, C.; Dettinger, M.D.; Michaelsen, J.C.; Verdin, J.P.; Brown, M.; Barlow, M.; Hoell, A. Warming of the Indian Ocean threatens eastern and southern African food security but could be mitigated by agricultural development. *Proc. Nat. Acad. Sci. USA* **2008**, *105*, 11081-11086.
7. Edgerton, M.D. Increasing crop productivity to meet global needs for feed, food, and fuel. *Plant Physiol.* **2009**, *149*, 7-13.
8. Liu, J.G.; Fritz, S.; van Wesenbeeck, C.F.A.; Fuchs, M.; You, L.Z.; Obersteiner, M.; Yang, H. A spatially explicit assessment of current and future hotspots of hunger in Sub-Saharan Africa in the context of global change. *Global Planet. Change* **2008**, *64*, 222-235.
9. Gleadow, R.M. Food Security in a Warming World. *Australasian Science*, Jan/Feb 2010, pp. 31-33.
10. Declaration on world food security. In *Declaration on World Food Security*; Food and Agriculture Organisation of the United Nations: Rome, Italy, 1996.
11. Ziska, L.H.; Bunce, J.A. Predicting the impact of changing CO<sub>2</sub> on crop yields: Some thoughts on food. *New Phytol.* **2007**, *175*, 607-618.
12. FAO. *Why Cassava?* Available online: [http://www.fao.org/ag/AGP/agpc/gcgs/index\\_en.html](http://www.fao.org/ag/AGP/agpc/gcgs/index_en.html) (accessed on 9 July 2010).
13. FAOSTAT. *Food and Agricultural Commodities Production*; Available online: <http://faostat.fao.org/> (accessed on 1 April 2010).
14. Pearce, F. Cassava comeback. *New Scientist*, 21 April 2007, pp. 38-39.
15. Nassar, N.M.A.; Ortiz, R. Cassava improvement: Challenges and impacts. *J. Agric. Sci.* **2007**, *145*, 163-171.
16. Gallo, M.; Sayre, R. Removing allergens and reducing toxins from food crops. *Curr. Opin. Biotechnol.* **2009**, *20*, 191-196.

17. Hillocks, R.J.; Thresh, J.M.; Bellotti, A.C. *Cassava: Biology, Production and Utilization*; CABI Publishing: Oxon, UK, 2002.
18. El-Sharkawy, M.A. Cassava biology and physiology. *Plant Mol. Biol.* **2003**, *53*, 621-641.
19. De Tafur, S.M.; El-Sharkawy, M.A.; Cadavid, L.F. Response of cassava (*Manihot esculenta* Crantz) to water stress and fertilization. *Photosynthetica* **1997**, *34*, 233-239.
20. Dahniya, M.T. An overview of cassava in Africa. *Afr. Crop Sci. J.* **1994**, *2*, 337-343.
21. Lebot, V. *Tropical Root and Tuber Crops: Cassava, Sweet Potato, Yams and Aroids*; CABI: Wallingford, UK, 2009.
22. de Vries, S.C.; van de Ven, G.W.J.; van Ittersum, M.K.; Giller, K.E. Resource use efficiency and environmental performance of nine major biofuel crops, processed by first-generation conversion techniques. *Biomass Bioenerg.* **2010**, *34*, 588-601.
23. *The World Cassava Economy: Facts, Trends and Outlooks*; Food and Agriculture Organization of the United Nations and International Fund for Agricultural Development: Rome, Italy, 2000; pp. 1-59.
24. Balagopalan, C. Cassava Utilization in Food, Feed and Industry. In *Cassava: Biology, Production and Utilization*; Hillocks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: Oxon, UK, 2002; pp 301-318.
25. McKey, D.; Cavagnaro, T.R.; Cliff, J.; Gleadow, R.M. Chemical ecology in coupled human and natural systems: People, manioc, multitrophic interactions and global change. *Chemoecology* **2010**, *20*, 109-133.
26. Allem, A.C. The Origins and Taxonomy of Cassava. In *Cassava: Biology, Production and Utilization*; Hillocks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: Oxon, UK, 2002; pp. 1-16.
27. Jørgensen, K.; Bak, S.; Busk, P.K.; Sørensen, C.; Olsen, C.E.; Puonti-Kaerlas, J.; Møller, B.L. Cassava plants with a depleted cyanogenic glucoside content in leaves and tubers. Distribution of cyanogenic glucosides, their site of synthesis and transport, and blockage of the biosynthesis by RNA interference technology. *Plant Physiol.* **2005**, *139*, 363-374.
28. Selmar, D.; Lieberei, R.; Biehl, B. Mobilization and utilization of cyanogenic glycosides: The linustatin pathway. *Plant Physiol.* **1988**, *86*, 711-716.
29. Kongsawadworakul, P.; Viboonjun, U.; Romruensukharom, P.; Chantuma, P.; Ruderman, S.; Chrestin, H. The leaf, inner bark and latex cyanide potential of *Hevea brasiliensis*: Evidence for involvement of cyanogenic glucosides in rubber yield. *Phytochemistry* **2009**, *70*, 730-739.
30. Rival, L.; McKey, D. Domestication and diversity in manioc (*Manihot esculenta* Crantz ssp *esculenta*, Euphorbiaceae). *Curr. Anthropol.* **2008**, *49*, 1116-1125.
31. Leotard, G.; Duputie, A.; Kjellberg, F.; Douzery, E.J.P.; Debain, C.; de Granville, J.J.; McKey, D. Phylogeography and the origin of cassava: New insights from the northern rim of the Amazonian basin. *Mol. Phylogenet. Evol.* **2009**, *53*, 329-334.
32. Duputie, A.; Massol, F.; David, P.; Haxaire, C.; McKey, D. Traditional Amerindian cultivators combine directional and ideotypic selection for sustainable management of cassava genetic diversity. *J. Evol. Biol.* **2009**, *22*, 1317-1325.
33. Montagnac, J.A.; Davis, C.R.; Tanumihardjo, S.A. Nutritional value of cassava for use as a staple food and recent advances for improvement. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 181-194.

34. Balagopalan, C.; Padmaja, G.; Nanda, S.K.; Moorthy, S.N. *Cassava in Food, Feed and Industry*; CRC Press: Boca Raton, FL, USA, 1988.
35. Nhassico, D.; Muquingue, H.; Cliff, J.; Cumbana, A.; Bradbury, J.H. Rising African cassava production, diseases due to high cyanide intake and control measures. *J. Sci. Food Agric.* **2008**, *88*, 2043-2049.
36. Hillocks, R.J. Cassava in Africa. In *Cassava: Biology, Production and Utilization*; Hillocks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: Oxon, UK, 2002; pp. 41-54.
37. Achidi, A.U.; Ajayi, O.A.; Bokanga, M.; Maziya-Dixon, B. The use of cassava leaves as food in Africa. *Ecol. Food Nutr.* **2005**, *44*, 423-435.
38. van Oirschot, Q.E.A.; O'Brien, G.M.; Dufour, D.; El-Sharkawy, M.A.; Mesa, E. The effect of pre-harvest pruning of cassava upon root deterioration and quality characteristics. *J. Sci. Food Agric.* **2000**, *80*, 1866-1873.
39. Kolapo, A.L.; Sanni, M.O. A comparative evaluation of the macronutrient and micronutrient profiles of soybean-fortified gari and tapioca. *Food Nutr. Bull.* **2009**, *30*, 90-94.
40. Shewry, P.R. Tuber storage proteins. *Ann. Bot.* **2003**, *91*, 755-769.
41. Cock, J.H. Cassava: A basic energy source in the tropics. *Science* **1982**, *218*, 755-762.
42. Bouis, H.E.; Welch, R.M. Biofortification—A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Sci.* **2010**, *50*, S20-S32.
43. Bayoumi, S.A.L.; Rowan, M.G.; Beeching, J.R.; Blagbrough, I.S. Constituents and secondary metabolite natural products in fresh and deteriorated cassava roots. *Phytochemistry* **2010**, *71*, 598-604.
44. Cliff, J.; Martensson, J.; Lundqvist, P.; Rosling, H.; Sorbo, B. Association of high cyanide and low sulfur intake in cassava-induced spastic paraparesis. *Lancet* **1985**, *2*, 1211-1213.
45. Montagnac, J.A.; Davis, C.R.; Tanumihardjo, S.A. Nutritional value of cassava for use as a staple food and recent advances for improvement. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 181-194.
46. Poulton, J.E. Localization and Catabolism of Cyanogenic Glycosides. In *Cyanide Compounds in Biology*; Evered, D., Harnett, S., Eds.; John Wiley & Sons: Chichester, UK, 1988; pp. 67-91.
47. Cliff, J.; Nicala, D. Long term follow-up of konzo patients. *Trans. Roy. Soc. Trop. Med. Hyg.* **1997**, *91*, 447-449.
48. Mlingi, N.; Poulter, N.H.; Rosling, H. An outbreak of acute intoxications from consumption of insufficiently processed cassava in Tanzania. *Nutr. Res.* **1992**, *12*, 677-687.
49. Cardoso, A.P.; Mirione, E.; Ernesto, M.; Massaza, F.; Cliff, J.; Haque, M.R.; Bradbury, J.H. Processing of cassava roots to remove cyanogens. *J. Food Compos. Anal.* **2005**, *18*, 451-460.
50. McKey, D.; Beckerman, S. Chemical Ecology, Plant Evolution and Traditional Manioc Cultivation Systems. In *Tropical Forests, People and Food: Biocultural Interactions and Applications to Development*; Hladick, A., Linares, O.F., Hladik, C.M., Pagezy, H., Semple, A., Hadley, M., Eds.; UNESCO: Paris, France, 1993; pp. 83-112.
51. Jørgensen, K.; Moranta, A.V.; Kannangara, R.; Morant, M.; Jensen, N.B.; Olsen, C.E.; Motawia, M.S.; Møller, B.L.; Bak, S. CYP71E7: The oxime-metabolizing P450 in *Manihot esculenta* Crantz, cassava. *Plant Physiol.* 2010, in press.

52. Solomonson, L.P. Cyanide as a Metabolic Inhibitor. In *Cyanide in Biology*; Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J., Wissing, F., Eds.; Academic Press: London, UK, 1981; pp. 11-28.
53. Møller, B.L. Functional diversifications of cyanogenic glucosides. *Curr. Opin. Plant Biol.* **2010**, *13*, 338-347.
54. Conn, E.E. Biosynthesis of Cyanogenic Glycosides. In *Cyanide in Biology*; Vennesland, V., Conn, E.E., Knowles, C.J., Westly, J., Wissing, F., Eds.; Academic Press: New York, NY, USA, 1981; pp. 1-10.
55. Bissett, F.H.; Clapp, R.C.; Coburn, R.A.; Ettliger, M.G.; Long, L. Cyanogenesis in manioc: Concerning lotaustralin. *Phytochemistry* **1969**, *8*, 2235-2247.
56. Nartey, F. Studies on cassava, *Manihot utilissima* Pohl—I. Cyanogenesis: The biosynthesis of linamarin and lotaustralin in etiolated seedlings. *Phytochemistry* **1968**, *7*, 1307-1312.
57. Nambisan, B.; Sundaresan, S. Distribution of linamarin and its metabolizing enzymes in cassava tissues. *J. Sci. Food Agric.* **1994**, *66*, 503-507.
58. de Bruijn, G.H. The cyanogenic character of cassava (*Manihot esculenta*). In *Chronic Cassava Toxicity, Proceedings of an Interdisciplinary Workshop*, London, UK, 29–30 January 1973; Nestel, B., MacIntyre, R., Eds.; International Development Research Centre (Ottawa): Ottawa, Canada, 1973; pp. 43-48.
59. Gleadow, R.M.; Woodrow, I.E. Constraints on effectiveness of cyanogenic glycosides in herbivore defense. *J. Chem. Ecol.* **2002**, *28*, 1301-1313.
60. Bellotti, A.C.; Riis, L. Cassava cyanogenic potential and resistance to pests and diseases. *Acta Hort.* **1994**, *375*, 140-150.
61. Kakes, P. The function of cyanogenesis in cassava. *Acta Hort.* **1994**, *375*, 79-85.
62. Jones, D.A. Cyanogenesis in Animal-Plant Interactions. In *Cyanide Compounds in Biology*; Evered, D., Harnett, S., Eds.; John Wiley & Sons: Chichester, UK, 1988; pp. 151-170.
63. Morant, A.V.; Jorgensen, K.; Jorgensen, B.; Dam, W.; Olsen, C.E.; Moller, B.L.; Bak, S. Lessons learned from metabolic engineering of cyanogenic glucosides. *Metabolomics* **2007**, *3*, 383-398.
64. Tattersall, D.B.; Bak, S.; Jones, P.R.; Olsen, C.E.; Nielsen, J.K.; Hansen, M.L.; Høj, P.B.; Møller, B.L. Resistance to an herbivore through engineered cyanogenic glucoside synthesis. *Science* **2001**, *293*, 1826-1828.
65. Poulton, J.E. Cyanogenesis in plants. *Plant Physiol.* **1990**, *94*, 401-405.
66. Vetter, J. Plant cyanogenic glycosides. *Toxicon* **2000**, *38*, 11-36.
67. Bokanga, M.; Ekanayake, I.J.; Dixon, A.G.O.; Porto, M.C.M. Genotype-environment interactions for cyanogenic potential in cassava. *Acta Hort.* **1994**, *375*, 131-139.
68. Koch, B.; Nielsen, V.S.; Halkier, B.A.; Olsen, C.E.; Moller, B.L. The biosynthesis of cyanogenic glucosides in seedlings of cassava (*Manihot esculenta* Crantz). *Arch. Biochem. Biophys.* **1992**, *292*, 141-150.
69. McMahon, J.M.; White, W.L.B.; Sayre, R.T. Cyanogenesis in cassava (*Manihot esculenta* Crantz). *J. Exp. Bot.* **1995**, *46*, 731-741.
70. Jones, D.A. Why are so many food plants cyanogenic? *Phytochemistry* **1998**, *47*, 155-162.

71. Bellotti, A.C. Arthropod Pests. In *Cassava: Biology, Production and Utilization*; Hillocks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: Oxon, UK, 2002; pp. 209-236.
72. Gleadow, R.M.; Woodrow, I.E. Polymorphism in cyanogenic glycoside content and cyanogenic beta-glucosidase activity in natural populations of *Eucalyptus cladocalyx*. *Aust. J. Plant Physiol.* **2000**, *27*, 693-699.
73. Hughes, M.A. The cyanogenic polymorphism in *Trifolium repens* L (white clover). *Heredity* **1991**, *66*, 105-115.
74. Mydans, S. Wasps to Fight Thai Cassava Plague. *The New York Times*, 18 July 2010; Available online: [http://www.nytimes.com/2010/07/19/world/asia/19thai.html?\\_r=2](http://www.nytimes.com/2010/07/19/world/asia/19thai.html?_r=2) (accessed on 21 July 2010).
75. Calvert, L.A.; Thresh, J.M. Viruses and Virus Diseases of Cassava. In *Cassava: Biology, Production and Utilization*; Hillocks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: Oxon, UK, 2002; pp. 237-260.
76. FAONewsroom. *Combating Cassava Mosaic*; Available online: <http://www.fao.org/newsroom/en/field/2007/1000693/index.html> (accessed on 22 July 2010)
77. Legg, J.P.; Owor, B.; Sseruwagi, P.; Ndunguru, J. Cassava Mosaic Virus Disease in East and Central Africa: Epidemiology and Management of a Regional Pandemic. In *Plant Virus Epidemiology*; Elsevier Academic Press: San Diego, CA, USA, 2006; Volume 67, pp. 355-418.
78. Alicai, T.; Omongo, C.A.; Maruthi, M.N.; Hillocks, R.J.; Baguma, Y.; Kawuki, R.; Bua, A.; Otim-Nape, G.W.; Colvin, J. Re-emergence of cassava brown streak disease in Uganda. *Plant Dis.* **2007**, *91*, 24-29.
79. Mtunda, K.J. *Breeding, Evaluation and Selection of Cassava for High Starch Content and Yield in Tanzania*; University of KwaZulu-Natal: Pietermaritzburg, South Africa, 2009.
80. Dixon, A.G.O.; Ssemakula, G. Prospects for cassava breeding in Sub-Saharan Africa in the next decade. *J. Food Agric. Environ.* **2008**, *6*, 256-262.
81. Hillocks, R.J.; Wydra, K. Bacterial, Fungal and Nematode Diseases. In *Cassava: Biology, Production and Utilization*; Hillocks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: Oxon, UK, 2002; pp. 261-280.
82. Okolie, P.N.; Obasi, B.N. Diurnal variation of cyanogenic glucosides, thiocyanate and rhodanese in cassava. *Phytochemistry* **1993**, *33*, 775-778.
83. Oluwole, O.S.A. Cyanogenicity of cassava varieties and risk of exposure to cyanide from cassava food in Nigerian communities. *J. Sci. Food Agric.* **2008**, *88*, 962-969.
84. King, N.L.R.; Bradbury, J.H. Bitterness of cassava: Identification of a new apiosyl glucoside and other compounds that affect its bitter taste. *J. Sci. Food Agric.* **1995**, *68*, 223-230.
85. Mkumbira, J.; Chiwona-Karlton, L.; Lagercrantz, U.; Mahungu, N.M.; Saka, J.; Mhone, A.; Bokanga, M.; Brimer, L.; Gullberg, U.; Rosling, H. Classification of cassava into 'bitter' and 'cool' in Malawi: From farmers' perception to characterisation by molecular markers. *Euphytica* **2003**, *132*, 7-22.
86. Alves, A.A.C. Cassava Botany and Physiology. In *Cassava: Biology, Production and Utilization*; Hillocks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: Oxon, UK, 2002; pp. 67-89.

87. Bourdoux, P.; Seghers, P.; Mafuta, M.; Vanderpas, J.; Vanderpas-Rivera, M.; Delange, F.; Ermans, A.M. Cassava products: HCN content and detoxification processes. In *Nutritional Factors Involved in the Goitrogenic Action of Cassava*; Delange, F., Iteke, F.B., Ermans, A.M., Eds.; International Development Research Centre (IDRC): Ottawa, Canada, 1982; pp. 51-58.
88. Montagnac, J.A.; Davis, C.R.; Tanumihardjo, S.A. Processing techniques to reduce toxicity and antinutrients of cassava for use as a staple food. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 17-27.
89. Kobawila, S.C.; Louembe, D.; Keleke, S.; Hounhouigan, J.; Gamba, C. Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. *Afr. J. Biotechnol.* **2005**, *4*, 689-696.
90. Chiwona-Karltun, L.; Tylleskar, T.; Mkumbira, J.; Gebre-Medhin, M.; Rosling, H. Low dietary cyanogen exposure from frequent consumption of potentially toxic cassava in Malawi. *Int. J. Food Sci. Nutr.* **2000**, *51*, 33-43.
91. Wilson, W.M.; Dufour, D.L. Why “bitter” cassava? Productivity of “bitter” and “sweet” cassava in a Tukanoan Indian settlement in the northwest Amazon. *Econ. Bot.* **2002**, *56*, 49-57.
92. Kayode, G.O. Effects of various planting and harvesting times on the yield, HCN, dry-matter accumulation and starch content of four cassava varieties in a tropical rainforest region. *J. Agric. Sci.* **1983**, *101*, 633-636.
93. Gershenzon, J. The Cost of Plant Chemical Defense Against Herbivory: A Biochemical Perspective. In *Insect-plant Interactions*; Bernays, E.A., Ed.; CRC Press: New York, NY, USA, 1989; Volume 5, pp. 105-173.
94. Goodger, J.Q.D.; Choo, T.Y.S.; Woodrow, I.E. Ontogenetic and temporal trajectories of chemical defence in a cyanogenic eucalypt. *Oecologia* **2007**, *153*, 799-808.
95. Kakes, P. An analysis of the costs and benefits of the cyanogenic system in *Trifolium repens* L. *Theor. Appl. Genet.* **1989**, *77*, 111-118.
96. Jenrich, R.; Trompetter, I.; Bak, S.; Olsen, C.E.; Moller, B.L.; Piotrowski, M. Evolution of heteromeric nitrilase complexes in Poaceae with new functions in nitrile metabolism. *Proc. Nat. Acad. Sci. USA* **2007**, *104*, 18848-18853.
97. Tylleskar, T.; Banea, M.; Bikangi, N.; Fresco, L.; Persson, L.A.; Rosling, H. Epidemiologic evidence from Zaire for a dietary etiology of konzo, an upper motor neuron disease. *Bull. World Health Organ.* **1991**, *69*, 581-589.
98. Cliff, J.; Coutinho, J. Acute intoxication from newly-introduced cassava during drought in Mozambique. *Trop. Doct.* **1995**, *25*, 193.
99. Cardoso, A.P.; Ernesto, M.; Nicala, D.; Mirione, E.; Chavane, L.; N'Zwalo, H.; Chikumba, S.; Cliff, J.; Mabota, A.P.; Haque, M.R.; *et al.* Combination of cassava flour cyanide and urinary thiocyanate measurements of school children in Mozambique. *Int. J. Food Sci. Nutr.* **2004**, *55*, 183-190.
100. Cliff, J.; Nicala, D.; Saute, F.; Givragy, R.; Azambuja, G.; Taela, A.; Chavane, L.; Gani, A. Ankle clonus and thiocyanate, linamarin, and inorganic sulphate excretion in school children in communities with Konzo, Mozambique. *J. Trop. Pediatr.* **1999**, *45*, 139-142.
101. Yeoh, H.H.; Chew, M.Y. Protein content and amino-acid composition of cassava leaf. *Phytochemistry* **1976**, *15*, 1597-1599.

102. Stephenson, K.; Amthor, R.; Mallowa, S.; Nungo, R.; Maziya-Dixon, B.; Gichuki, S.; Mbanaso, A.; Manary, M. Consuming cassava as a staple food places children 2–5 years old at risk for inadequate protein intake, an observational study in Kenya and Nigeria. *Nutr. J.* **2010**, *9*, doi:10.1186/1475-2891-9-9.
103. Banea-Mayambu, J.P.; Tylleskar, T.; Tylleskar, K.; Gebre-Medhin, M.; Rosling, H. Dietary cyanide from insufficiently processed cassava and growth retardation in children in the Democratic Republic of Congo (formerly Zaire). *Ann. Trop. Paediatr.* **2000**, *20*, 34-40.
104. Zagrobelny, M.; Bak, S.; Møller, B.L. Cyanogenesis in plants and arthropods. *Phytochemistry* **2008**, *69*, 1457-1468.
105. Westley, J. Mammalian Cyanide Detoxification with Sulphane Sulphur. In *Cyanide Compounds in Biology*; Evered, D., Harnett, S., Eds.; John Wiley & Sons: Chichester, UK, 1988; pp. 212-218.
106. Tylleskar, T.; Banea, M.; Bikangi, N.; Cooke, R.D.; Poulter, N.H.; Rosling, H. Cassava cyanogens and konzo, an upper motoneuron disease found in Africa. *Lancet* **1992**, *339*, 208-211.
107. Howlett, W.P.; Brubaker, G.R.; Mlingi, N.; Rosling, H. Konzo, an epidemic upper motor neurone disease studied in Tanzania. *Brain* **1990**, *113*, 223-235.
108. Dufour, D.L. Biocultural approaches in human biology. *Amer. J. Hum. Biol.* **2006**, *18*, 1-9.
109. Cliff, J. *The Burden of Cassava Cyanide-induced Disease: Estimates for the World Health Organization*; CCDNN News: Canberra, Australia, 2010; p. 4.
110. Cliff, J. Cassava safety in times of war and drought in Mozambique. *Acta Hort.* **1994**, *375*, 372-378.
111. Ernesto, M.; Cardoso, A.P.; Nicala, D.; Mirione, E.; Massaza, F.; Cliff, J.; Haque, M.R.; Bradbury, J.H. Persistent konzo and cyanogen toxicity from cassava in northern Mozambique. *Acta Trop.* **2002**, *82*, 357-362.
112. Banea-Mayambu, J.P.; Gitebo, N.D.; Nkuadi, M.A. Bitter cassava consumption and konzo in Kahemba territory, Bandudu Province, DRC. In *Proceedings of the Workshop on Toxic-nutritional Neurodegenerations Konzo and Lathyrism*, Ghent, Belgium, 21–22 September 2009; Bradbury, J.H., Ed.; CCDNN News: Canberra, Australia, 2009; Volume 14, pp. 5-6.
113. Howlett, W.P. Konzo: A new human disease entity. *Acta Hort.* **1994**, *375*, 323-329.
114. Osuntokun, B.O. Chronic cyanide intoxication of dietary origin and a degenerative neuropathy in Nigerians. *Acta Hort.* **1994**, *375*, 311-321.
115. Oluwole, O.S.A.; Onabolu, A.O.; Cotgreave, I.A.; Rosling, H.; Persson, A.; Link, H. Incidence of endemic ataxic polyneuropathy and its relation to exposure to cyanide in a Nigerian community. *J. Neurol. Neurosurg. Psychiat.* **2003**, *74*, 1417-1422.
116. Oluwole, O.S.A.; Onabolu, A.O.; Cotgreave, I.A.; Rosling, H.; Persson, A.; Link, H. Low prevalence of ataxic polyneuropathy in a community with high exposure to cyanide from cassava foods. *J. Neurol.* **2002**, *249*, 1034-1040.
117. Onabolu, A.O.; Oluwole, O.S.A.; Bokanga, M.; Rosling, H. Ecological variation of intake of cassava food and dietary cyanide load in Nigerian communities. *Public Health Nutr.* **2001**, *4*, 871-876.
118. Halstrøm, F.; Møller, K.D. The content of cyanide in human organs from cases of poisoning with cyanide taken by mouth. With a contribution to the toxicology of cyanides. *Acta Pharmacol. Toxicol.* **1945**, *1*, 18-28.

119. Bradbury, J.H. *Monitoring of Cyanide Content in Cassava Roots and Flour Using Picrate Kits*; CCDNN News: Canberra, Australia, 2010; Available online: <http://online.anu.edu.au/BoZo/CCDN/three.html> (accessed on 7 October 2010).
120. Bradbury, M.G.; Egan, S.V.; Bradbury, J.H. Picrate paper kits for determination of total cyanogens in cassava roots and all forms of cyanogens in cassava products. *J. Sci. Food Agric.* **1999**, *79*, 593-601.
121. Egan, S.V.; Yeoh, H.H.; Bradbury, J.H. Simple picrate paper kit for determination of the cyanogenic potential of cassava flour. *J. Sci. Food Agric.* **1998**, *76*, 39-48.
122. Bradbury, J.H.; Denton, I.C. Rapid wetting method to reduce cyanogen content of cassava flour. *Food Chem.* **2010**, *121*, 591-594.
123. Nambisan, B.; Sundaresan, S. Effect of processing on the cyanoglucoside content of cassava. *J. Sci. Food Agric.* **1985**, *36*, 1197-1203.
124. Adewoye, S.O.; Fawole, O.O.; Owolabi, O.D.; Omotosho, J.S. Toxicity of cassava wastewater effluents to African catfish: *Clarias gariepinus* (Burchell, 1822). *Ethiopian J. Sci.* **2005**, *28*, 189-194.
125. Ehiagbonare, J.E.; Adjarhore, R.Y.; Enabulele, S.A. Effect of cassava effluent on Okada natural water. *Afr. J. Biotechnol.* **2009**, *8*, 2816-2818.
126. Dufour, D.L. Effectiveness of cassava detoxification techniques used by indigenous peoples in northwest Amazonia. *Interciencia* **1989**, *14*, 86-91.
127. Dorea, J.G. Fish are central in the diet of Amazonian riparians: Should we worry about their mercury concentrations? *Environ. Res.* **2003**, *92*, 232-244.
128. Dorea, J.G. Cassava cyanogens and fish mercury are high but safely consumed in the diet of native Amazonians. *Ecotoxicol. Environ. Safty* **2004**, *57*, 248-256.
129. Aalbersberg, W.G.L.; Limalevu, L. Cyanide content in fresh and processed Fijian cassava (*Manihot esculenta*) cultivars. *Trop. Sci.* **1991**, *31*, 249-256.
130. Bradbury, J.H. Simple wetting method to reduce cyanogen content of cassava flour. *J. Food Compos. Anal.* **2006**, *19*, 388-393.
131. Cumbana, A.; Mirione, E.; Cliff, J.; Bradbury, J.H. Reduction of cyanide content of cassava flour in Mozambique by the wetting method. *Food Chem.* **2007**, *101*, 894-897.
132. Siritunga, D.; Sayre, R. Engineering cyanogen synthesis and turnover in cassava (*Manihot esculenta*). *Plant Mol. Biol.* **2004**, *56*, 661-669.
133. Siritunga, D.; Arias-Garzon, D.; White, W.; Sayre, R.T. Over-expression of hydroxynitrile lyase in transgenic cassava roots accelerates cyanogenesis and food detoxification. *Plant Biotechnol. J.* **2004**, *2*, 37-43.
134. El-Sharkawy, M.A. Drought-tolerant cassava for Africa, Asia and Latin-America. *Bioscience* **1993**, *43*, 441-451.
135. Leihner, D.E. Agronomy and Cropping Systems. In *Cassava: Biology, Production and Utilization*; Hillocks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: Oxon, UK, 2002; pp. 91-113.
136. Olasantan, F.O.; Ezumah, H.C.; Lucas, E.O. Effects of intercropping with maize on the micro-environment, growth and yield of cassava. *Agr. Ecosyst. Environ.* **1996**, *57*, 149-158.

137. Saidou, A.; Kuyper, T.W.; Kossou, D.K.; Tossou, R.; Richards, P. Sustainable soil fertility management in Benin: Learning from farmers. *NJAS-Wagen. J. Life Sci.* **2004**, *52*, 349-369.
138. Cardoso, I.M.; Kuyper, T.W. Mycorrhizas and tropical soil fertility. *Agric. Ecosyst. Environ.* **2006**, *116*, 72-84.
139. Fermont, A.M.; van Asten, P.J.A.; Giller, K.E. Increasing land pressure in East Africa: The changing role of cassava and consequences for sustainability of farming systems. *Agric. Ecosyst. Environ.* **2008**, *128*, 239-250.
140. Onwueme, I.C. Cassava in Asia and the Pacific. In *Cassava: Biology, Production and Utilization*; Hillocks, R.J., Thresh, J.M., Bellotti, A.C., Eds.; CABI Publishing: Oxon, UK, 2002; pp. 55-65.
141. Refsgaard, K.; Bjarnholt, N.; Møller, B.L.; Saddik, M.M.; Hansen, H.C.B. Dissipation of cyanogenic glucosides and cyanide in soil amended with white clover (*Trifolium repens* L.). *Soil Biol. Biochem.* **2010**, *42*, 1108-1113.
142. Bjarnholt, N.; Laegdsmand, M.; Hansen, H.C.B.; Jacobsen, O.H.; Møller, B.L. Leaching of cyanogenic glucosides and cyanide from white clover green manure. *Chemosphere* **2008**, *72*, 897-904.
143. Huang, J.H.; Fu, R.; Liang, C.X.; Dong, D.F.; Luo, X.L. Allelopathic effects of cassava (*Manihot esculenta* Crantz) on radish (*Raphanus sativus* L.) and ryegrass (*Lolium perenne* L.). *Allelopathy J.* **2010**, *25*, 155-162.
144. Fermont, A.M.; Tittonell, P.A.; Baguma, Y.; Ntawuruhunga, P.; Giller, K.E. Towards understanding factors that govern fertilizer response in cassava: Lessons from East Africa. *Nutr. Cycl. Agroecosyst.* **2010**, *86*, 133-151.
145. Forster, P.; Ramaswamy, V.; Artaxo, P.; Berntsen, T.; Betts, R.; Fahey, D.W.; Haywood, J.; Lean, J.; Lowe, D.C.; Myhre, G.; *et al.* Changes in Atmospheric Constituents and in Radiative Forcing. In *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*; Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L., Eds.; Cambridge University Press: Cambridge, UK/New York, NY, USA, 2007.
146. Ainsworth, E.A.; Long, S.P. What have we learned from 15 years of free-air CO<sub>2</sub> enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO<sub>2</sub>. *New Phytol.* **2005**, *165*, 351-371.
147. Agricultural Productivity and Climate Change. In *Proceedings of the Theo Murphy High Flyers Think Tank*, Melbourne, Australia, 22–23 October 2009; Australian Academy of Science: Canberra, Australia, 2010.
148. Meehl, G.A.; Stocker, T.F.; Collins, W.D.; Friedlingstein, P.; Gaye, A.T.; Gregory, J.M.; Kitoh, A.; Knutti, R.; Murphy, J.M.; Noda, A.; *et al.* Global Climate Projections. In *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*; Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L., Eds.; Cambridge University Press: Cambridge, UK/ New York, NY, USA, 2007.

149. Okogbenin, E.; Ekanayake, I.J.; Porto, M.C.M. Genotypic variability in adaptation responses of selected clones of cassava to drought stress in the Sudan savanna zone of Nigeria. *J. Agron. Crop Sci.* **2003**, *189*, 376-389.
150. Bakayoko, S.; Tschannen, A.; Nindjin, C.; Dao, D.; Girardin, O.; Assa, A. Impact of water stress on fresh tuber yield and dry matter content of cassava (*Manihot esculenta* Crantz) in Cote d'Ivoire. *Afr. J. Agric. Res.* **2009**, *4*, 21-27.
151. Baker, G.R.; Fukai, S.; Wilson, G.L. The response of cassava to water deficits at various stages of growth in the subtropics. *Aust. J. Agric. Res.* **1989**, *40*, 517-528.
152. Hahn, S.K.; Reynolds, L.; Egbunike, G.N. Cassava as Livestock Feed in Africa. In *Proceedings of the IITA/ILCA/University of Ibadan Workshop on the Potential Utilization of Cassava as Livestock Feed in Africa*, Ibadan, Nigeria, 14–18 November 1988; Hahn, S.K., Reynolds, L., Egbunike, G.N., Eds.; International Institute of Tropical Agriculture: Ibadan, Nigeria, 1992; pp. 7-15.
153. Chauynarong, N.; Elangovan, A.V.; Iji, P.A. The potential of cassava products in diets for poultry. *World Poultry Sci. J.* **2009**, *65*, 23-35.
154. Oso, A.O.; Oso, O.; Bamgbose, A.M.; Eruvbetine, D. Utilization of unpeeled cassava (*Manihot esculenta*) root meal in diets of weaner rabbits. *Livest. Sci.* **2010**, *127*, 192-196.
155. Thang, C.M.; Ledin, I.; Bertilsson, J. Effect of using cassava products to vary the level of energy and protein in the diet on growth and digestibility in cattle. *Livest. Sci.* **2010**, *128*, 166-172.
156. Alves, A.A.C.; Setter, T.L. Abscisic acid accumulation and osmotic adjustment in cassava under water deficit. *Environ. Exp. Bot.* **2004**, *51*, 259-271.
157. Santisopasri, V.; Kurotjanawong, K.; Chotineeranat, S.; Piyachomkwan, K.; Sriroth, K.; Oates, C.G. Impact of water stress on yield and quality of cassava starch. *Ind. Crop. Prod.* **2001**, *13*, 115-129.
158. Nambisan, B. *Impact of Environmental Factors on Cyanide Content in Cassava*; CCDNN News: Canberra, Australia, 2003; pp. 2-3.
159. Cadavid, L.F.; El-Sharkawy, M.A.; Acosta, A.; Sanchez, T. Long-term effects of mulch, fertilization and tillage on cassava grown in sandy soils in northern Colombia. *Field Crop. Res.* **1998**, *57*, 45-56.
160. Molina, J.L.; El-Sharkawy, M.A. Increasing crop productivity in cassava by fertilizing production of planting material. *Field Crop. Res.* **1995**, *44*, 151-157.
161. Howeler, R.H. Long-term effect of cassava cultivation on soil productivity. *Field Crop. Res.* **1991**, *26*, 1-18.
162. Reuter, D.J.; Robinson, J.B.; Dutkiewicz, C. *Plant Analysis: An Interpretation Manual*, 2nd ed.; CSIRO Publishing: Melbourne, Australia, 1997.
163. Busk, P.K.; Moller, B.L. Dhurrin synthesis in sorghum is regulated at the transcriptional level and induced by nitrogen fertilization in older plants. *Plant Physiol.* **2002**, *129*, 1222-1231.
164. Endris, S. *Modification of Nutritional Quality of Cassava Through Plant Nutrition*; CCDNN News: Canberra, Australia, 2007; pp. 2-3.
165. Gleadow, R.M.; Edwards, E.J.; Evans, J.R. Changes in nutritional value of cyanogenic *Trifolium repens* grown at elevated atmospheric CO<sub>2</sub>. *J. Chem. Ecol.* **2009**, *35*, 476-478.

166. Simon, J.; Gleadow, R.M.; Woodrow, I.E. Allocation of nitrogen to chemical defence and plant functional traits is constrained by soil N. *Tree Physiol.* **2010**, *30*, 1111-1117.
167. Webber, B.L.; Woodrow, I.E. Intra-plant variation in cyanogenesis and the continuum of foliar plant defense traits in the rainforest tree *Ryparosa kurrangii* (Achariaceae). *Tree Physiol.* **2008**, *28*, 977-984.
168. Miller, R.E.; Gleadow, R.M.; Woodrow, I.E. Cyanogenesis in tropical *Prunus turneriana*: Characterisation, variation and response to low light. *Funct. Plant Biol.* **2004**, *31*, 491-503.
169. Gleadow, R.M.; Evans, J.R.; McCaffery, S.; Cavagnaro, T.R. Growth and nutritive value of cassava (*Manihot esculenta* Cranz.) are reduced when grown in elevated CO<sub>2</sub>. *Plant Biol.* **2009**, *11*, 76-82.
170. Susan John, K.; Ravindran, C.S.; George, J. *Long Term Fertilizer Experiments: Three Decades Experience in Cassava*; John, S.K., Ravindran, C.S., George, J., Eds.; Central Tuber Crops Research Institute: Kerala, India, 2005; p. 83.
171. Cavagnaro, T.R. The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: A review. *Plant Soil* **2008**, *304*, 315-325.
172. Cavagnaro, T.R.; Jackson, L.E.; Six, J.; Ferris, H.; Goyal, S.; Asami, D.; Scow, K.M. Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. *Plant Soil* **2006**, *282*, 209-225.
173. Brown, K.H.; Wuehler, S.E. *Zinc and Human Health: Results of Recent Trials and Implications for Program Interventions and Research*; International Development Research Centre: Ottawa, Canada, 2000.
174. Azcón-Aguilar, C.; Cantos, M.; Troncoso, A.; Barea, J.M. Beneficial effect of arbuscular mycorrhizas on acclimatization of micropropagated cassava plantlets. *Sci. Hortic.* **1997**, *72*, 63-71.
175. Khasa, P.; Furlan, V.; Fortin, J.A. Response of some tropical plant-species to endomycorrhizal fungi under field conditions. *Trop. Agric.* **1992**, *69*, 279-283.
176. Sieverding, E. Should VAM inocula contain single or several fungal species? *Agric. Ecosyst. Environ.* **1990**, *29*, 391-396.
177. Carretero, C.L.; Cantos, M.; Garcia, J.L.; Azcon, R.; Troncoso, A. Arbuscular-mycorrhizal contributes to alleviation of salt damage in cassava clones. *J. Plant Nutr.* **2008**, *31*, 959-971.
178. Liasu, M.O.; Atayese, M.O.; Osonubi, O. Effect of mycorrhiza and pruning regimes on seasonality of hedgerow tree mulch contribution to alley-cropped cassava in Ibadan, Nigeria. *Afr. J. Biotechnol.* **2006**, *5*, 1341-1349.
179. Sieverding, E.; Howeler, R.H. Influence of species of VA mycorrhizal fungi on cassava yield response to phosphorus fertilization. *Plant Soil* **1985**, *88*, 213-221.
180. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: Cambridge, UK, 2008.
181. Straker, C.J.; Hilditch, A.J.; Rey, M.E.C. Arbuscular mycorrhizal fungi associated with cassava (*Manihot esculenta* Crantz) in South Africa. *S. Afr. J. Bot.* **2010**, *76*, 102-111.
182. El-Sharkawy, M.A.; Tafur, S.M.D.; Cadavid, L.F. Potential photosynthesis of cassava as affected by growth conditions. *Crop Sci.* **1992**, *32*, 1336-1342.
183. Irikura, Y.; Cock, J.H.; Kawano, K. The physiological basis of genotype—Temperature interactions in cassava. *Field Crop. Res.* **1979**, *2*, 227-239.

184. Stockmal, A.; Oleszek, W. Changes of cyanogenic glucosides in white clover (*Trifolium repens* L.) during the growing season. *J. Agric. Food Chem.* **1997**, *45*, 4333-4336.
185. Collinge, D.B.; Hughes, M.A. Developmental and physiological studies on the cyanogenic glucosides of white clover, *Trifolium repens* L. *J. Exp. Bot.* **1982**, *33*, 154-161.
186. El-Sharkawy, M.A. International research on cassava photosynthesis, productivity, eco-physiology, and responses to environmental stresses in the tropics. *Photosynthetica* **2006**, *44*, 481-512.
187. Drake, B.G.; Gonzalez-Meler, M.A.; Long, S.P. More efficient plants: A consequence of rising atmospheric CO<sub>2</sub>? **1997**, *48*, 609-639.
188. Jackson, L.E.; Burger, M.; Cavagnaro, T.R. Roots, nitrogen transformations, and ecosystem services. *Annu. Rev. Plant Biol.* **2008**, *59*, 341-363
189. Craigon, J.; Fangmeier, A.; Jones, M.; Donnelly, A.; Bindi, M.; De Temmerman, L.; Persson, K.; Ojanpera, K. Growth and marketable-yield responses of potato to increased CO<sub>2</sub> and ozone. *Eur. J. Agron.* **2002**, *17*, 273-289.
190. Edwards, G.E.; Sheta, E.; Moore, B.D.; Dai, Z.; Franceschi, V.R.; Cheng, S.H.; Lin, C.H.; Ku, M.S.B. Photosynthetic characteristics of cassava (*Manihot esculenta* Crantz), a C<sub>3</sub> species with chlorenchymatous bundle sheath cells. *Plant Cell Physiol.* **1990**, *31*, 1199-1206.
191. Imai, K.; Coleman, D.F.; Yanagisawa, T. Elevated atmospheric partial pressure of carbon dioxide and dry matter production of cassava (*Manihot esculenta* Crantz). *Jpn. J. Crop Sci.* **1984**, *53*, 479-485.
192. Fernandez, M.D.; Tezara, W.; Rengifo, E.; Herrera, A. Lack of downregulation of photosynthesis in a tropical root crop, cassava, grown under an elevated CO<sub>2</sub> concentration. *Funct. Plant Biol.* **2002**, *29*, 805-814.
193. Heagle, A.S.; Miller, J.E.; Pursley, W.A. Growth and yield responses of potato to mixtures of carbon dioxide and ozone. *J. Environ. Qual.* **2003**, *32*, 1603-1610.
194. Lawson, T.; Craigon, J.; Black, C.R.; Colls, J.J.; Tulloch, A.M.; Landon, G. Effects of elevated carbon dioxide and ozone on the growth and yield of potatoes (*Solanum tuberosum*) grown in open-top chambers. *Environ. Poll.* **2001**, *111*, 479-491.
195. Miglietta, F.; Magliulo, V.; Bindi, M.; Cerio, L.; Vaccari, F.P.; Loduca, V.; Peressotti, A. Free air CO<sub>2</sub> enrichment of potato (*Solanum tuberosum* L.): Development, growth and yield. *Global Change Biol.* **1998**, *4*, 163-172.
196. Lobell, D.B.; Burke, M.B.; Tebaldi, C.; Mastrandrea, M.D.; Falcon, W.P.; Naylor, R.L. Prioritizing climate change adaptation needs for food security in 2030. *Science* **2008**, *319*, 607-610.
197. Idso, S.B. The aerial fertilization effect of CO<sub>2</sub> and its implications for global carbon cycling and maximum greenhouse warming. *Bull. Amer. Meteorol. Soc.* **1991**, *72*, 962-965.
198. Hogy, P.; Fangmeier, A. Atmospheric CO<sub>2</sub> enrichment affects potatoes: 2. Tuber quality traits. *Eur. J. Agron.* **2009**, *30*, 85-94.
199. Taub, D.R.; Miller, B.; Allen, H. Effects of elevated CO<sub>2</sub> on the protein concentration of food crops: a meta-analysis. *Global Change Biol.* **2008**, *14*, 565-575.
200. Cotrufo, M.F.; Ineson, P.; Scott, A. Elevated CO<sub>2</sub> reduces the nitrogen concentration of plant tissues. *Global Change Biol.* **1998**, *4*, 43-54.

201. de Graaff, M.A.; van Groenigen, K.J.; Six, J.; Hungate, B.; van Kessel, C. Interactions between plant growth and soil nutrient cycling under elevated CO<sub>2</sub>: A meta-analysis. *Global Change Biol.* **2006**, *12*, 2077-2091.
202. Hu, S.; Chapin, F.S.; Firestone, M.K.; Field, C.B.; Chiariello, N.R. Nitrogen limitation of microbial decomposition in a grassland under elevated CO<sub>2</sub>. *Nature* **2001**, *409*, 188-191.
203. Reich, P.B.; Hungate, B.A.; Luo, Y.Q. Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. *Annu. Rev. Ecol. Evol. Syst.* **2006**, *37*, 611-636.
204. Lieffering, M.; Kim, H.Y.; Kobayashic, K.; Okadad, M. The impact of elevated CO<sub>2</sub> on the elemental concentrations of field-grown rice grains *Field Crop. Res.* **2004**, *88*, 279-286.
205. Seneweera, S.P.; Conroy, J.P. Growth, grain yield and quality of rice (*Oryza sativa* L.) in response to elevated CO<sub>2</sub> and phosphorous nutrition. *Soil Sci. Plant Nutr.* **1997**, *43*, 1131-1136.
206. Manderscheid, R.; Bender, J.; Jaiger, H.J.; Weigel, H.J. Effects of season long CO<sub>2</sub> enrichment on cereals. II. Nutrient concentrations and grain quality. *Agric. Ecosyst. Environ.* **1995**, *54*, 175-185.
207. Fangmeier, A.; DeTemmerman, L.; Mortensen, L.; Kemp, K.; Burke, J.; Mitchell, R.; van Oijen, M.; Weigel, H.J. Effects on nutrients and on grain quality in spring wheat crops grown under elevated CO<sub>2</sub> concentrations and stress conditions in the European, multiple-site experiment 'ESPACE-wheat'. *Eur. J. Agron.* **1999**, *10*, 215-229.
208. Frehner, M.; Luscher, A.; Hebeisen, T.; Zanetti, S.; Schubiger, F.; Scalet, M. Effects of elevated partial pressure of carbon dioxide and season of the year on forage quality and cyanide concentration of *Trifolium repens* L. from a FACE experiment. *Acta Oecol.* **1997**, *18*, 297-304.
209. Gleadow, R.M.; Foley, W.J.; Woodrow, I.E. Enhanced CO<sub>2</sub> alters the relationship between photosynthesis and defence in cyanogenic *Eucalyptus cladocalyx* F. Muell. *Plant Cell Environ.* **1998**, *21*, 12-22.
210. Brouder, S.M.; Volenec, J.J. Impact of climate change on crop nutrient and water use efficiencies. *Physiol. Plant.* **2008**, *133*, 705-724.
211. Wall, G.W.; Garcia, R.L.; Kimball, B.A.; Hunsaker, D.J.; Pinter, P.J.; Long, S.P.; Osborne, C.P.; Hendrix, D.L.; Wechsung, F.; Wechsung, G.; *et al.* Interactive effects of elevated carbon dioxide and drought on wheat. *Agron. J.* **2006**, *98*, 354-381.