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Interactive effects of temperature and drought on cassava growth and toxicity: implications for food security?

Running head

Temperature × drought effects on cassava

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Key words

Manihot esculenta, cyanogenesis, climate change, nitrogen, nutrition, food security, plant defence, mycorrhizas

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Abstract

Cassava is an important dietary component for over 1 billion people, and its ability to yield under drought has led to it being promoted as an important crop for food security under climate change. Despite its known photosynthetic plasticity in response to temperature, little is known about how temperature affects plant toxicity or about interactions between temperature and drought, which is important because cassava tissues contain high levels of toxic cyanogenic glucosides, a major health and food safety concern. In a controlled glasshouse experiment, plants were grown at two daytime temperatures (23 °C and 34 °C), and either well-watered or subject to a one month drought prior to harvest at six months. The objective was to determine the separate and interactive effects of temperature and drought on growth and toxicity. Both temperature and drought affected cassava physiology and chemistry. While temperature alone drove differences in plant height and above-ground biomass, drought and temperature × drought interactions most affected tuber yield, as well as foliar and tuber chemistry, including C:N, nitrogen, and cyanide potential (CN_p; total cyanide released from cyanogenic glucosides). Conditions that most stimulated growth and yield (well-watered × high temperature) effected a reduction in tuber toxicity, whereas drought inhibited growth and yield, and was associated with increased foliar and tuber toxicity. The magnitude of drought effects on tuber yield and toxicity were greater at high temperature, thus increases in tuber CN_p were not merely a consequence of reduced tuber biomass. Findings confirm that cassava is adaptable to forecast temperature increases, particularly in areas of adequate or increasing rainfall; however, in regions forecast for increased incidence of drought, the effects of drought on both food quality (tuber toxicity) and yield are a greater threat to future food security and indicate an increasing necessity for processing of cassava to reduce toxicity.

Introduction

Cassava, *Manihot esculenta* Crantz, is eaten by approximately 1 billion people every day, mainly in the tropical and subtropical regions of Asia, Latin America, and Africa and is the major staple for 35-50% of people living in different areas of sub-Saharan Africa (FAO & IFAD, 2000). Cassava grows in a wide range of soil and climatic conditions, is easily propagated, resistant to drought and pests, and the tuberous roots have extended viability (up to 3 years) when left in soil (Nhassico *et al.*, 2008). These traits help explain the sizeable increase in the proportion of cultivated land taken up by cassava in Africa since the 1970s (Fermont *et al.*, 2008) and underpin its promotion as increasingly important for food security, particularly in the context of climate change (e.g. Jarvis *et al.*, 2012).

Although cassava yields well under poor conditions, the tuberous roots are low in nutritional quality - an important component of food security (Pinstrup-Andersen, 2009). Tubers are high in carbohydrate (80-90% dry matter), but low in protein (1-3% dry matter), low in micronutrients, and contain cyanogenic glucosides (Montagnac *et al.*, 2009). Cyanogenic glucosides, which are produced primarily as a defence against herbivores, are hydrolysed to release toxic hydrogen cyanide when the leaves and tubers are crushed or chewed (Conn, 1981). Consumption of cassava-based food stuffs that are inadequately processed to remove cyanogenic compounds can cause acute poisoning resulting in headaches and vomiting, and may lead to a type of permanent leg paralysis known as Konzo, or even death (Cliff, 1994). Increasing penetration of these products into communities without understanding the risks also poses a potential health hazard (Burns *et al.*, 2012a). Critically, cyanogenic glucoside concentrations in cassava vary with genotype and climatic factors (de Bruijn, 1973; Bokanga *et al.*, 1994), as evidenced by the correlation between drought periods in Africa and increased cassava flour toxicity. Further, these periods correspond with an increased incidence of cyanide poisoning and outbreaks of Konzo within communities reliant on cassava (Cliff, 1994; Cardoso *et al.*, 1999; Oluwole, 2015).

Climate change projections for cassava growing regions in Africa include mean surface air temperature increases of 3-4 °C, with seasonal increases of up to 7 °C by 2099 (Collins *et al.*, 2013; Niang *et al.*, 2014). In general, models forecast an increase in aridity over most of Africa (Dai, 2011), and decreases in soil moisture and increased risk of agricultural drought in

southern Africa (Collins *et al.*, 2013; Niang *et al.*, 2014). These forecasts highlight the need to investigate the effects of both drought and temperature on cassava.

A number of studies have investigated the impact of drought on cassava yield and productivity (e.g. Connor *et al.*, 1981; Keating *et al.*, 1982; El-Sharkawy & Cock, 1987; Baker *et al.*, 1989; El-Sharkawy *et al.*, 1992a; Bokanga *et al.*, 1994; El-Sharkawy & Cadavid, 2002; El-Sharkawy, 2006; Bakayoko *et al.*, 2009), but relatively few of these largely field-based studies have also measured cyanogenic capacity. Typically, the cyanide potential of tubers (CNp; the maximum amount of cyanide released from all endogenous cyanogenic glucosides) is higher when soil moisture is low (e.g. de Bruijn, 1973; Santisopasri *et al.*, 2001; Okogbenin *et al.*, 2003). Even fewer studies report CNp of leaves, even though leaves are an important animal feed or protein supplement for humans (Gomez *et al.*, 1985; Ngudi *et al.*, 2003). A controlled glasshouse study investigated the effects of drought on the growth and chemistry of cassava during the early stages of tuber development, and found significant increases in tuber and leaf CNp in droughted plants after 14-28 days of water deficit (Vandegeer *et al.*, 2013). This increase in toxicity was reported in plants grown at air temperatures at the lower end of the range at which cassava grows (18.8/16.9 °C mean day/night temperatures). Presumably higher temperatures would exacerbate the effects of drought.

Cassava is highly plastic in its growth response to air temperature. Studies of the effects of temperature on cassava have tended to focus either on low temperature limitations to yield and effects on biomass allocation (Cock & Rosas, 1975; Irikura *et al.*, 1979; Manrique, 1992; Fermont *et al.*, 2009), or on its photosynthetic capacity and growth under higher temperatures (e.g. Cock *et al.*, 1979; Edwards *et al.*, 1990; El-Sharkawy & Cock, 1990; El-Sharkawy *et al.*, 1992b). These studies generally report reduced yields and growth at temperatures less than 17 °C (Cock & Rosas, 1975; El-Sharkawy *et al.*, 1992b), and broad photosynthetic temperature optima at leaf temperatures between 25–40 °C (Mahon *et al.*, 1977; El-Sharkawy *et al.*, 1984; El-Sharkawy & Cock, 1990; El-Sharkawy *et al.*, 1992b). In addition, greater stimulation of cassava yield by elevated atmospheric CO₂ concentrations (700 ppm) was found at higher growth temperatures in a glasshouse pot trial under well-watered conditions (Imai *et al.*, 1984), further highlighting the importance of interactions between temperature and other factors in the response of cassava to changing climates.

1 Despite the often substantial effects of temperature on growth, photosynthesis and biomass
2 partitioning of cassava, to our knowledge no studies to date have studied the effect of
3 temperature on the toxicity of cassava, nor investigated interactive effects of drought and
4 temperature on growth, yield and nutritional value. Given the importance of cyanogenic
5 capacity of this staple to human nutrition, the general importance of food quality to achieving
6 food security (Jarvis *et al.*, 2012; van Rijssen *et al.*, 2013), predictions of increased
7 temperatures and aridity in Africa, and the projected use of cassava (Scott *et al.*, 2000),
8 understanding environmental effects on cyanogenic glucoside content is crucial.

10 Here we present results of a controlled glasshouse experiment in which we examined the
11 effects of both temperature and drought on the toxicity (for the first time), growth and
12 biomass allocation of cassava. Specifically, we sought to address the following questions: (1)
13 Under drought conditions, does higher temperature exacerbate the effects of water deficit on
14 cyanogenic glucoside concentration? and (2) under well-watered conditions, is enhanced
15 growth under optimal (typically high) temperatures at the expense of investment in defence?
16 Results are discussed in the context of food security – both in terms of the amount and quality
17 of food – and climate change forecasts for cassava growing regions.

20 **Materials and Methods**

21 *Plant material and growing conditions*

22 Forty-three cassava plants (*Manihot esculenta* Crantz cv. MCol 1468) were propagated
23 clonally (as ~50 mm long cuttings) in sand, from a single parent plant. Thirty-eight cuttings
24 had sprouted 58 days after cultivation, and were transferred to individual 250 mm diameter,
25 plastic free-draining pots, containing 9 kg of a 1:4 (w/w) soil:sand mix. The soil:sand mix
26 comprised washed river sand and soil from 0-150 mm depth in Jock Marshall Reserve,
27 Monash University, Clayton, Victoria (37°54' S, 145°8' E), sieved to < 2 mm. This mixture,
28 referred to as 'soil' hereafter, had low endogenous levels of plant-available (Colwell)
29 phosphorus (3 mg kg⁻¹), mineral nitrogen (3 mg kg⁻¹ as the sum of NO₃⁻-N and NH₄⁺-N),
30 0.02% total nitrogen, 0.35% total carbon, a pH of 6.2, and high mycorrhizal fungal inoculum
31 potential (Miller, unpub data). A 10 mm layer of polystyrene beads was placed on the soil
32 surface to reduce evaporation.

For the first 85 days after planting (DAP), plants were watered as required. From 86 DAP plants were watered every second day to field capacity (FC) (Khan *et al.*, 2003) with a modified Hoagland's solution containing 5 mM nitrogen (supplied as NO_3^- and NH_4^+ in the ratio 5:1), which a preliminary experiment showed to be optimum for growth (data not shown). From 114 DAP plants were watered alternately with water and the nutrient solution; from 128 DAP plants were watered with nutrient solution every third watering; and after 144 DAP, at which point the drought treatment was applied, only water was applied to avoid differences in nutrient supply to droughted and well-watered plants.

For the first 80 DAP, plants were grown in a glasshouse with ambient temperature (mean day/night cycle of 22/18 °C) and natural light (22nd February to 12th May, 2011, Melbourne, Australia). At 81 DAP, plants were randomly allocated to one of two glasshouses and provided with supplemental lighting with a 16/8-hour day/night photoperiod (MK-1 Just-a-shade, Ablite, Melbourne, Australia), and a photosynthetically active radiation (PAR) of $400 \pm 100 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ (LI-1400 Light Meter, Li-Cor Environmental, Nebraska, USA). Temperatures were maintained at day/night mean temperatures of 25/20 °C, within cassava's optimal range (El-Sharkawy, 2004). These conditions were maintained until 132 DAP when temperature treatments were imposed.

Treatments

To quantify the combined effects of temperature and water supply on cassava, plants were randomly assigned to treatments in a 2×2 factorial design. From 133 DAP, two temperature treatments were imposed; in one glasshouse day/night mean temperatures were increased to 34/28 °C ($n = 19$ plants), referred to as 'high'; and in the other, 23/23 °C ($n = 19$), referred to as 'low'. The mean maximum day and minimum night temperatures in the high and low temperature glasshouses were 38 °C/25 °C and 26 °C/20 °C, respectively. The temperature in the high glasshouse was chosen based on IPCC (2013) projections of 7 °C warming in Africa by 2099 (Niang *et al.*, 2014), compared, for example, to present mean monthly temperatures of 29 °C in Mozambique (INAM., 2013). Temperatures in the low glasshouse were selected to provide a large difference in growth temperatures between treatments, without diverging too far from realistic growth conditions for cassava. Plants were swapped two times between adjacent glasshouses during the treatment period to reduce potential glasshouse effects. From 144 DAP, watering regimes were applied (Fig. 1). Plants allocated to the well-watered ($n = 9$) treatment were watered to field capacity for the duration of the experiment. The drought

treatment ($n = 10$) was imposed by withholding water until a soil moisture content of 25% field capacity was reached, following Vandegheer *et al.* (2013). Plants in the drought treatment were maintained at 25% field capacity for the remainder of the experiment. In summary, temperature treatments began four and a half months after striking cuttings and lasted six weeks, while the drought treatment began five months after striking and lasted four weeks.

Harvest and sampling

All plants were destructively harvested 176 DAP. As cassava was propagated clonally, the biomass of the original cutting was excluded from biomass measurements to account for variation in original cutting size (Vandegheer *et al.*, 2013). Leaf area was determined using a leaf area meter (LI-3000 Portable Area Meter and LI-3050A Belt Conveyor, Li-Cor inc., Nebraska, USA). Above-ground biomass (stems, leaves) was dried at 60 °C for 7 days, for dry weight determination and chemical analysis.

Roots were washed free from the soil with water and separated into tubers (roots >5 mm diameter) and fine roots. A sub-sample of fine roots was stored in 70% ethanol for determination of mycorrhizal colonization of roots (Supp. Info.). Sub-samples of the inner tuber flesh (parenchyma) were taken from the middle, longitudinally. Two samples (ca. 2 g fresh weight) were taken from the centre of these slices, avoiding the tuber peel (cortex). One sample was used for determination of cyanogenic glucosides and the other for nitrogen and carbon analyses. Sections of tuber peel were also sampled from the middle of each tuber for the same analyses. All fine and coarse root material was dried at 60 °C for 7 days for determination of dry weight and percent dry matter of all tubers. Harvest index was calculated by dividing the total tuberous root dry weight by the total plant dry weight.

Analytical methods

Cyanogenic glucoside concentration

At 176 DAP, prior to destructive harvesting two leaf discs of 5 mm diameter were sampled from the middle of the centre lobe (avoiding the midrib) of the third fully expanded leaf of each plant, for analysis of cyanogenic glucosides. Cyanogenic glucoside concentrations were also determined for the two largest tubers from each plant. To avoid potential confounding effects of intra-tuber variation (Bradbury *et al.*, 1991), cyanide content of the middle section, longitudinally, of each tuber was used for analysis. Cyanogenic glucosides were measured as cyanide potential (CNp), that is the total amount of cyanide (CN) evolved from fresh leaf or

tuber tissue, according to Vandegeer *et al.* (2013). Cyanide captured in a well of 1M NaOH was quantified using a colorimetric assay. Absorbance was measured at 595 nm with NaCN as the standard. Leaf disks and tuber samples were rinsed and dried in a 60 °C oven for 48 h to enable determination of mass based cyanide concentrations.

In order to ensure there was no potential epigenetic effect of tissue age CNp (Jørgensen *et al.*, 2005) foliar and tuber flesh CNp were compared in plants derived from cuttings taken from different parts of the parent plant. CNp was not dependent on the position from which the cutting was obtained (data not shown). Further, both within and across all treatments, no significant size effect on tuber CNp was found, thus differences in tuber CNp were not a consequence of any differences in tuber developmental stage (data not shown).

Elemental analyses

Dried leaf, tuber flesh and peel sub-samples were ground to a fine powder in a cooled IKA Labortechnik A10 Analytical Mill (Janke & Kunkel, Stanfen, Germany). For each tissue the concentration of nitrogen (N%) was determined for 4-7 mg dwt of tissue by dry combustion using an elemental analyser (Elementar Vario Micro Cube CHNS analyser, DKSH Australia). In order to estimate the degree of water stress (Farquhar *et al.*, 1989), 3 mg dwt of tuber flesh was analysed for carbon isotopes ($\delta^{13}\text{C}\text{‰}$) using an ANCA GSL2 elemental analyser coupled to a Hydra 20-22 isotope ratio mass-spectrometer (Sercon Ltd., UK) with a precision of 0.1‰. Since cassava drops leaves in response to drought (e.g. Vandegeer *et al.*, 2013), we measured tuber $\delta^{13}\text{C}$ rather than foliar $\delta^{13}\text{C}$ to provide integrated measure of plant WUE over the entire time of tuber development (Farquhar *et al.*, 1989; Jefferies & MacKerron, 1997)

Foliar chlorophyll fluorescence, foliar chlorophyll concentration and root arbuscular mycorrhizal colonization were also measured. Method information for these analyses is in Supporting Information.

Statistical analysis

Data were analysed using two-factor General Linear Models (GLMs) in JMP v.9 (SAS Institute Inc., Cary, NC., 2010) and IBM SPSS Statistics for Windows 21.0 (IBM Corp, Armonk, NY., 2012) statistical software. Where necessary, data were transformed to satisfy the assumptions of GLMs. Tukey's HSD tests were used post-hoc to compare means at $P < 0.05$ where no significant interaction between water regime (W) and temperature (T) was

detected. Where a significant $T \times W$ interaction was detected, simple main effects tests were conducted within temperature treatments to compare drought and well-watered treatments.

Results

Plant Growth and Physiology

At the final harvest, both temperature and drought treatments had influenced the growth of plants, as indicated by a significant interaction for total plant biomass ($F_{1,34} = 6.330$, $P = 0.017$; Table 1); the reduction in biomass with drought was greater at high temperature than low temperature. Total biomass was greatest in the high temperature well-watered treatment (42 ± 3.5 g dwt; mean $\pm$ 1SE), two-fold greater than plants from both drought treatments and 1.5 times greater than plants from the low temperature well-watered treatment. Irrespective of water treatment, plants grown at high temperature had a 1.5-fold increase in height ($F_{1,34} = 50.82$, $P < 0.001$; Fig. 2) and above-ground biomass ($F_{1,34} = 17.916$, $P < 0.001$; Fig. 3), compared with plants grown at low temperatures. There was no difference in the height of plants assigned to different temperature treatments prior to the application of temperature treatments; however, within one week of changing glasshouse temperatures the growth rate of plants in the high temperature glasshouse (20 mm day^{-1}) was twice that of plants in the low temperature glasshouse (10 mm day^{-1}), a difference which persisted until harvest (Fig. 2).

Plants produced significantly more leaf biomass ($F_{1,34} = 7.720$, $P = 0.009$) and increased leaf area ($F_{1,34} = 4.480$, $P = 0.042$; Table 1) in the high temperature treatment, and well-watered treatment ($F_{1,34} = 10.977$, $P = 0.002$; and $F_{1,34} = 5.577$, $P = 0.024$, respectively) with no interactive effects. Specific leaf area of leaves retained on the plant at harvest ranged from $250 - 290 \text{ cm}^2 \text{ g}^{-1}$ dwt, with no difference between treatments. Plants produced 15% more leaves (Table 1) in the high temperature glasshouse ($F_{1,34} = 4.777$, $P = 0.036$); however, within two weeks of applying the drought treatment, drought-treated plants shed 40% more leaves ($F_{1,34} = 6.935$, $P = 0.013$; Table 1).

The total below-ground biomass of plants was influenced by the interactive effects of temperature and water treatments ($F_{1,34} = 9.461$, $P = 0.004$; Table 1, Fig. 3), with a greater magnitude reduction in root biomass at high temperature than low temperature. Specifically, and similar to above-ground biomass, total below-ground biomass was greatest in the high

temperature well-watered treatment (27.4 ± 2.7 g dwt; mean $\pm$ 1SE), four times higher than plants in the high temperature drought treatment. The same pattern was observed for tuber biomass ($F_{1,34} = 9.717$, $P = 0.004$; Table 1). The difference in tuber mass was not a consequence of differences in the number of tubers, which was similar across all treatments (overall mean $\pm$ 1SE of 2.3 ± 0.15 tubers per plant). Significant water $\times$ temperature effects were detected for root:shoot ($F_{1,34} = 27.01$, $P < 0.001$) and harvest index ($F_{1,34} = 25.57$, $P < 0.001$; Table 1). Specifically, harvest index (23 ± 1.7 , mean $\pm$ 1SE) and root:shoot (0.6 ± 0.05 , mean $\pm$ 1SE) of plants from the high temperature drought treatment were less than half all other treatments. Plants produced more fine roots (Table 1) in the high temperature ($F_{1,34} = 11.64$, $P = 0.002$) and well-watered treatments ($F_{1,34} = 10.76$, $P = 0.002$), with no interactive effects. Under well-watered conditions, increases in above- and below-ground biomass with high temperature were proportional, temperature therefore had no detectable effect on biomass partitioning (harvest index and root:shoot; Table 1). Across all treatments, mycorrhizal fungi colonized over 95% of fine root length, with no difference between treatments detected (data not shown).

Changes in mean percent dry matter of tubers reflected a significant interaction between drought and temperature treatments ($F_{1,34} = 21.537$, $P < 0.001$; Table 1). At low temperature, tuber dry matter (%) was similar between droughted and well-watered plants, whereas at high temperature drought effected a significant reduction in tuber dry matter (%) from a maximum of $30.5 \pm 0.5\%$ (mean $\pm$ 1SE) under well-watered conditions to a minimum of $20.6 \pm 0.9\%$ (mean $\pm$ 1SE) under drought (Table 1).

The photosynthetic efficiency (F_v/F_m) of all plants one week before harvest was 0.76 ± 0.1 (mean $\pm$ 1SE) with no difference between drought or temperature treatments (data not shown). Total chlorophyll concentration in the third fully expanded leaf of each plant was 1.3-fold higher in droughted plants and did not differ with temperature ($F_{1,34} = 14.15$, $P < 0.001$; Supp. Info.).

Plant chemical composition

$\delta^{13}\text{C}$ was determined on tubers that were likely initiated under equivalent conditions (approx. 3 months after planting; Alves, 2002) but developed for 6 weeks under treatment conditions. Plants had tuber $\delta^{13}\text{C}$ values ranging from -25.9% to -22% , with highest values in the low temperature ($F_{1,34} = 436.3$, $P < 0.001$) and droughted treatments ($F_{1,34} = 131.4$, $P < 0.001$;

Table 2), with no interactive effects. There was no significant correlation between tuber $\delta^{13}\text{C}$ and tuber cyanide concentration within or across all treatments (data not shown).

The nitrogen (N) concentration of leaves (28.35 - 37.17 mg g⁻¹ dwt; Fig. 4a) was higher than that of tuber peel (3.14 – 8.26 mg g⁻¹ dwt; Table 2) and tuber flesh (1.52 – 3.51 mg g⁻¹ dwt; Fig. 4c), across all treatments. There was a significant interactive effect of temperature and water treatments on N concentration in leaves ($F_{1,33} = 8.286$, $P = 0.006$), tuber flesh ($F_{1,34} = 7.538$, $P = 0.010$) and tuber peel ($F_{1,21} = 22.03$, $P < 0.001$). Nitrogen concentrations in all tissues were highest in the high temperature drought treatment and lowest in the high temperature well-watered treatment, with a trend towards higher N in tissues of droughted plants. The magnitude of the drought effect differed between temperature treatments such that at high temperature tuber flesh and peel N increased 2.3-fold and 2.6-fold with drought, respectively, but at low temperature no differences with drought were detected (Table 2, Fig. 4c). Changes in tissue carbon to nitrogen ratios (C:N) reflected the changes in N concentrations and not in C, with a significant reduction in C:N under drought in all tissues that was of greater magnitude under high temperature than low temperature (Table 2).

A significant effect of temperature alone on the concentration of cyanogenic glucosides (CNp) was only observed in well-watered plants. Across all treatments, CNp was highest in tuber peel (0.77 - 3.19 mg g⁻¹ dwt; Table 2) and leaves (1.78 – 3.21 mg g⁻¹ dwt; Fig. 4b), and lowest in the tuber flesh (0.05 – 0.35 mg g⁻¹ dwt; Fig. 4d). For all tissues, a significant main effect of watering regime was detected, with significant increases in CNp under drought (Table 2, Fig. 4). Drought effected an increase in leaf cyanide concentration, irrespective of temperature ($F_{1,34} = 8.778$, $P = 0.0061$), with 1.8-fold and 1.5-fold increases in leaf CNp in the low and high temperature treatments, respectively (Fig. 4b). No significant main effect of temperature on leaf CNp was detected. By contrast, significant differences in CNp with temperature were detected in tuber tissues. In tuber flesh, the magnitude of the drought effect differed significantly between temperature treatments, with 6.7-fold greater CNp in tubers from droughted plants at high temperature, whereas at low temperature, the trend towards increased CNp with drought was not significant (Fig. 4d). Significant main effects of temperature ($F_{1,34} = 8.963$, $P = 0.005$) and watering treatment ($F_{1,34} = 7.136$, $P = 0.012$) were found on tuber peel CNp, which was greater under drought and at low temperature (Table 2). Pooling watering treatments, tuber peel CNp of low temperature-grown plants was double that of high temperature grown plants.

The proportion of foliar N allocated to cyanogenic glucosides (CN-N/N%) was similar across all treatments (mean 4.1%), despite significant differences in CNp with drought (Table 2). By contrast, significant effects of temperature and drought on CN-N/N% were detected in tuber tissues. Specifically, at high temperature, a significantly greater proportion of N was allocated to CN under drought, whereas at low temperature CN-N/N% was similar between watering treatments. There was a significant main effect of temperature on tuber peel CN-N/N%, with on average higher CN-N/N% at low temperature (mean 30.9%) than high temperature (mean 12.2%; Table 2).

Discussion

We report here, for the first time, the effects of temperature, and temperature combined with drought on growth, biomass partitioning, and nitrogen allocation to cyanogenic glucosides in cassava leaves and tubers under controlled conditions. Increases in tuber toxicity with drought and at lower growth temperatures point to potential trade-offs between growth and secondary metabolism in resource allocation. Greater complexity arises in the different responses of cyanogenesis in above- and below-ground tissues to drought and temperature. Findings are consistent with models that predict cassava to be adaptable and resilient to forecast temperature increases (Jarvis *et al.*, 2012), but show that irrespective of growth temperature, drought effects on tuber yield and toxicity are the greater threat to future food security.

Temperature effects on growth of well-watered plants

The high (34 °C) and low (23 °C) temperature regimes used here, represent the low and high ends of the temperature range where cassava is cultivated (El-Sharkawy & Cock, 1990; El-Sharkawy *et al.*, 1992b). In accord with El-Sharkawy *et al.* (1992b), who found the optimum temperature range for cassava photosynthesis is 30 – 40 °C, and Mahon *et al.* (1977) who found higher photosynthetic and growth rates at 29/24 °C compared to 24/19 °C, the high temperature treatment had significant and rapid effects on plant growth under well-watered conditions, clearly indicating that the low temperature treatment was suboptimal for growth of this cultivar. Leaf growth in cassava is known to decrease at lower temperatures (Irikura *et al.*, 1979). We found no effect of temperature on biomass partitioning under well-watered conditions (Table 1); no consistent effect of temperature on biomass partitioning is evident (e.g. Mahon *et al.*, 1976; Keating *et al.*, 1982).

Impact of temperature on drought responses - yield, biomass and physiology

Above-ground, the physical responses to drought observed here are similar to previous studies with smaller, fewer leaves, at both growth temperatures. While drought alone drove changes in leaf loss and retention, both temperature and drought affected leaf area and biomass (Table 1). Leaf formation and growth in cassava are known to be highly sensitive to even small decreases in soil moisture (Connor & Cock, 1981; Connor *et al.*, 1981; Baker *et al.*, 1989; De Tafur *et al.*, 1997; Okogbenin *et al.*, 2003; Alves & Setter, 2004; Vandegeer *et al.*, 2013). Rapid closure of stomata, combined with leaf abscission in response to more prolonged water deficit, enable cassava to retain photosynthetically active, turgid leaves (El-Sharkawy & Cock, 1984; Palta, 1984; Alves, 2002; Turyagyenda *et al.*, 2013; Vandegeer *et al.*, 2013). Consistent with this, there was no reduction in photosynthetic efficiency (F_v/F_m) of leaves retained under drought (Calatayud *et al.*, 2002; see also Vandegeer *et al.*, 2013; but see Zhao *et al.*, 2015). By contrast, changes in tuber $\delta^{13}\text{C}$ across all treatments reflect the physiological effects of both temperature and drought treatments (Table 1). Consistent with high temperature stimulation of photosynthesis and growth (discussed above), lower tuber $\delta^{13}\text{C}$ under well-watered and high temperature conditions is indicative of higher intercellular CO_2 concentration (C_i) and stomatal conductance (g_s) (Farquhar *et al.*, 1989). In contrast, the less negative $\delta^{13}\text{C}$ of tubers of plants in the drought treatment indicates that the plants experienced some stress.

Tuber yield declines in response to drought are generally considered a consequence of reduction in canopy area and assimilate production (Connor & Cock, 1981; Baker *et al.*, 1989; De Tafur *et al.*, 1997; Setter & Fregene, 2007). Here, the relative yield decline with drought was greater at high temperature (80%) than at low temperature (35%, ns), but this was not explained by differences in leaf area, leaf biomass, and leaf loss, which were largely similar at high and low temperature. Only 24% of total biomass was in the tubers in the high temperature-droughted plants - approximately half that in all other treatments which ranged from 50-58%. While some studies similarly report a greater relative decrease in tuber yield (93%) compared to shoot biomass (59%) with water stress (e.g. Aina *et al.*, 2007), others report a greater relative decrease in shoot biomass (e.g. El-Sharkawy, 2007; 18% and 57% in tubers and shoots, respectively in one cultivar). Our data highlight the importance of growth temperature in affecting drought impacts on biomass allocation within a cultivar.

1
2 The reduction in tuber yield of 80% at high temperature here is within the range of yield
3 reductions reported elsewhere with drought under both natural and controlled field conditions.
4 For example, mean percentage declines in tuber biomass of 82-96% were found for nine
5 cultivars of cassava when a water deficit (25% FC) was imposed early (28 DAP) and
6 sustained until harvest between 3-6 months later (Aina *et al.*, 2007). Importantly, our data
7 demonstrate that growth temperature, as well as timing and duration of water deficit, is
8 important in determining the effect of drought on tuber yield (e.g. see Alves, 2002). While
9 there have been no prior studies of temperature $\times$ drought effects on cassava, there is some
10 indication that yield reductions in response to drought are more substantial under warmer
11 temperatures. The yield reductions of 82-95% reported by Aina *et al.* (2007), for example,
12 were at a mean maximum temperature of 32 °C, whereas at a mean growth temperature of 23
13 °C tuber yield only declined 0-25%, albeit using different cultivars (El-Sharkawy *et al.*,
14 1992a). In the latter study, similar to findings here, the absence of substantial yield declines
15 (0-25%) with drought at 23 °C occurred despite droughted plants showing other effects of
16 water deficit including lower photosynthetic rates and reduced leaf area and shoot biomass at
17 that temperature.

18 19 ***Temperature and drought effects on plant chemistry and nutritional value***

20 Global temperatures are rising but there are relatively few papers on the likely impact on
21 cassava yield (e.g. Lobell *et al.*, 2008; Knox *et al.*, 2012), and none to our knowledge on the
22 direct effects of temperature on cyanogenic glucosides. Knowledge of temperature effects on
23 cyanogenic glucosides in general is limited to a few studies on clover that have low optimum
24 growth temperatures (e.g. Stochmal & Oleszek, 1997; Hayden & Parker, 2002). In the present
25 study, enhanced growth of cassava at higher temperature under well-watered conditions was
26 associated with a significant reduction in tuber CNp and in the proportion of N allocated to
27 CN, but there was no change in foliar chemistry or N allocation (Fig. 4; Table 2), confirming
28 field studies showing that foliar chemistry is not a suitable proxy for estimating tuber toxicity
29 (Bokanga *et al.*, 1994; Jørgensen *et al.*, 2005; Burns *et al.*, 2012b). This result also supports
30 the assertion that environmentally driven changes in foliar defence metabolites cannot be
31 assumed to be representative of the whole plant, an important consideration for root food
32 crops (Parker *et al.*, 2012; Miller *et al.*, 2014). The lower tuber CNp at high temperature may
33 indicate a reallocation of N away from defence under conditions that stimulate growth (Hermis
34 & Mattson, 1992; Neilson *et al.*, 2013). A reallocation of resources is plausible, given that

1 differences in tuber CNp with temperature under well-watered conditions could not be
2 explained either by changes in biomass (i.e. tissue dilution) or nitrogen concentration.
3
4 Trade-offs between growth and defence are more likely to be evident under resource
5 limitation (Coley *et al.*, 1985), as was the case here, where plants were N deficient (< 4% leaf
6 N) at the time of harvest (Reuter & Robinson, 1997; Howeler, 2002). Tissue N is positively
7 correlated with foliar cyanogenic glucoside concentrations in some species (e.g. *Eucalyptus*
8 *cladocalyx*, Gleadow & Woodrow, 2000; *Sorghum bicolor*, Busk & Møller, 2002), but not in
9 others (e.g. *Prunus turneriana*, Miller *et al.*, 2004; *Beilschmiedia collina*, Miller & Tuck,
10 2013). Surprisingly few studies have addressed the relationship between N and CN in
11 cassava, or have tested the tubers. Some studies indicate a relationship between foliar N and
12 CNp in the shoot apex and leaves of cassava (Jørgensen *et al.*, 2005) whereas other field and
13 glasshouse studies report no correlation between N and CNp in either tubers or foliage
14 (Gleadow *et al.*, 2009; Burns *et al.*, 2012b). Here, unlike for CNp, we could largely account
15 for the decrease in tuber N by the increase in tuber size. Thus, it appears that tuber CNp is
16 regulated independently of tuber (or foliar) N concentrations. This is not surprising given that
17 while some synthesis of cyanogenic glucosides occurs in the roots (Du *et al.*, 1995; McMahon
18 *et al.*, 1995), cyanogenic glucosides in cassava are predominantly synthesised in the shoots
19 and transported to the roots (Jørgensen *et al.*, 2005).

20
21 For a plant such as cassava, with a high optimum growth temperature, a major consequence of
22 climate change will arise from the interaction between rising temperatures and drought on
23 yield and, importantly, the concomitant changes in toxicity and nutritional value. Reduction in
24 tuber quality, as measured by percent dry matter, and starch yield under water stress have
25 previously been reported (e.g. Santisopasri *et al.*, 2001; El-Sharkawy, 2007; Bakayoko *et al.*,
26 2009), consistent with the significant reduction in tuber quality reported here under high
27 temperature/drought conditions, from 31.5% to 20.5% dry matter (Table 1).

28
29 Of almost 30 papers on cassava describing controlled environmental and field-based studies
30 in which drought responses were reported, only seven measured the effect on tuber
31 cyanogenic glucosides (El-Sharkawy & Mwanza, 1993; Bokanga *et al.*, 1994; Santisopasri *et al.*
32 *et al.*, 2001; Okogbenin *et al.*, 2003; El-Sharkawy, 2006; Hular-Bograd *et al.*, 2011; Vandegeer
33 *et al.*, 2013), and none report the interactive effects of temperature and drought. Four studies
34 report mean relative increases in tuber toxicity ranging from 54 to 82% across 27 cultivars, in

1 12 month old plants subject to drought varying in timing and duration (El-Sharkawy &
2 Mwanza, 1993; Bokanga *et al.*, 1994; Okogbenin *et al.*, 2003; El-Sharkawy, 2006). Here,
3 within a population of clones from a single cultivar, we demonstrate the importance of the
4 interactive effects of temperature and drought, with greatest relative increase in tuber toxicity
5 with drought (600%) found at high temperature, in part as a consequence of lower tuber CNp
6 under well-watered conditions. As was the case with differences between temperature
7 treatments under well-watered conditions, tissue dilution of cyanogenic glucoside content did
8 not account for differences between treatments (see also Bokanga *et al.*, 1994). Changes in
9 tuber mass with drought did not account for changes in tuber N either, as tuber N
10 concentrations increased, but tuber N content (per tuber) halved.

11
12 Increases in foliar cyanogenic glucoside concentrations with drought are known from other
13 species (e.g. Gleadow & Woodrow, 2002), but very little is known about how temperature
14 may affect that response. Whereas temperature $\times$ drought effects on tuber flesh (and peel)
15 chemistry were more complex, drought alone drove changes in foliar CNp, with similar
16 increases (mean 62%) at both temperatures (Fig. 4; Table 2). Further, changes in foliar N and
17 foliar CNp were largely proportional across all treatments (Fig. 4), thus increased foliar N,
18 CNp and chlorophyll in droughted plants could be consistent with reclamation of constituents
19 from abscising leaves (Aerts, 1996; Munné-Bosch & Alegre, 2004), a process which has also
20 been hypothesised to contribute to increased tuber CNp under drought (Vandegeer *et al.*,
21 2013).

22
23 It is likely that the changes in above- and below-ground cassava tissue chemistry in response
24 to temperature $\times$ drought treatments here reflect a combination of factors, including changes
25 in biomass, reclamation and reallocation of nutrients, trade-offs in N allocation, as well as
26 more direct environmental effects on biosynthesis and transport of cyanogenic glucosides.
27 Many of these processes and factors are not yet fully understood. For example, we require a
28 greater understanding of factors affecting transport, remobilisation, and biosynthesis of
29 cyanogenic glucosides (Møller, 2010; Neilson *et al.*, 2013) which may be independently
30 regulated in roots and shoots (Blomstedt *et al.*, 2012; Miller *et al.*, 2014). Moreover,
31 interpreting distribution and allocation of N to cyanogenic glucosides with respect to their
32 defensive function may be further limited by the increasingly recognised non-defensive roles
33 of these metabolites in storage and moderating stress (Neilson *et al.*, 2013; Selmar &
34 Kleinwächter, 2013; Gleadow & Møller, 2014).

Implications for food security in a changing climate

Achieving food security is not only about increasing yields, but also ensuring that food is safe and of sufficient nutritive value. Cassava yields are expected to be positively impacted by a warming climate, within the range currently projected (Lobell *et al.*, 2008; Jarvis *et al.*, 2012). We found that tuber yields were increased and CNp was decreased when plants were grown under higher temperatures with adequate water supply, however, the combined effects of higher temperature with drought stress had a negative effect on tuber yields and nutritive value. This plasticity highlights the importance of considering climate change as a multifactor phenomenon, and has important implications for those that currently rely on cassava as their main source of food and income (see Nhassico *et al.*, 2008; Muoki & Maziya-Dixon, 2010). Little is known about how drought and temperature might interact with the effects of increasing atmospheric [CO₂] on cassava growth and chemistry. Greater elevated [CO₂] (700 ppm) stimulation of yield was found at a higher growth temperature (33 °C/31 °C day/night) than lower growth temperature (28 °C/21 °C) under well-watered conditions in a glasshouse pot study, pointing towards potentially additive or synergistic effects of increasing temperature and [CO₂] on cassava growth and yield (CNp was not measured; Imai *et al.*, 1984). However, inconsistent findings with respect to elevated [CO₂] effects on cassava growth and chemistry have been reported in glasshouse (pot) and field based studies (e.g. Gleadow *et al.*, 2009; Rosenthal *et al.*, 2012). The only CO₂ enrichment study under field conditions was in fertile soils in the absence of drought and found a substantial (104%) increase in tuber biomass, as well as enhanced water use efficiency (WUE) and lower foliar N concentrations under elevated [CO₂] (585 ppm; Rosenthal *et al.*, 2012). How the physiological responses of cassava to increasing [CO₂], temperature and drought combine to affect both yield and nutritional value (toxicity) requires attention.

In addition to the effects of increasing [CO₂], the combined effects of high temperature and drought investigated here also have implications for efforts seeking to expand production of cassava as a security crop in the face of a changing climate (e.g. Rufino *et al.*, 2013). The importance of this is emphasised by projections suggesting that we will see an almost doubling of cassava utilization in sub-Saharan Africa from the 1990s to 2020 (Scott *et al.*, 2000). Over the latter part of the 20th Century an increase in temperature (1 – 2 °C/50 years) and decrease in precipitation (up to 1 mm/day/50 years) has been observed in much of southern Africa, and over this century climate models forecast an increase in aridity across

Africa, and faster increases in temperature in Africa than the global average (Dai, 2011; Niang *et al.*, 2014). These projections, coupled with our findings, suggest that careful attention needs to be paid to the yield and nutritive responses of cassava in major cassava growing regions. This will be especially important where subsistence farmers have sole reliance on cassava in times of environmental and social stress (Cliff, 1994; Burns *et al.*, 2010).

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Supporting Information caption

Supporting information file includes foliar chlorophyll concentrations at final harvest, as well as methods information for the determination of mycorrhizal colonization and foliar chlorophyll concentrations, and for chlorophyll fluorescence measurements.

Figure captions

Figure 1. Soil moisture content of cassava grown in two temperature $\times$ watering treatments. Plants were grown in high (mean 34 °C) or low temperature (mean 23 °C) glasshouses for six weeks from 133 DAP (solid arrow), and under either well-watered (100% field capacity) or droughted (25% field capacity) conditions for four weeks from 144 DAP (dashed arrow). Treatments were low temperature well-watered (○), low temperature drought (●), high temperature well-watered (▽) and high temperature drought (▼). Data are means $\pm$ SE of $n = 9 - 10$.

Figure 2. Height (cm) of cassava plants subject to two temperature $\times$ watering treatments measured at intervals throughout the experiment until harvest 176 DAP. Plants were grown in high temperature (mean 34 °C) or low temperature (mean 23 °C) glasshouses for six weeks from 133 DAP (solid arrow), and under either well-watered (100% field capacity) or droughted (25% field capacity) conditions for four weeks from 144 DAP (dashed arrow). Treatments were low temperature well-watered (○), low temperature drought (●), high temperature well-watered (▽) and high temperature drought (▼). Data are means $\pm$ SE of $n = 9 - 10$. *indicates significant differences between temperature treatments at $P < 0.05$.

Figure 3: Above- and below-ground biomass of cassava plants grown in two temperature $\times$ watering treatments. Treatments were high (HT, mean 34 °C; white bars) or low temperature (LT, mean 23 °C; light grey bars) imposed for six weeks from 133 DAP, and well-watered (W, 100% field capacity; open bars) or droughted (D, 25% field capacity; hatched bars) imposed for four weeks from 144 DAP. Fine root biomass (dark grey) and tuber biomass are shown. Data are means $\pm$ SE of $n = 9 - 10$. Results of two-way GLMs are shown for total

shoot and total root biomass, letters indicate significant differences at $P < 0.05$. Where the $T \times W$ interaction was significant, letters indicate significant differences between drought and well-watered treatments at each temperature.

Figure 4. Nitrogen concentration (a, c) and cyanide potential (CNp) (b, d) of cassava leaves and tubers from plants grown in two temperature $\times$ watering treatments. Plants were grown in high (mean 34 °C; white bars) or low temperature (mean 23 °C; light grey bars) glasshouses for six weeks from 133 DAP, and in well-watered (100% field capacity; open bar) or droughted (25% field capacity; hatched) conditions for four weeks from 144 DAP. Data are means $\pm$ SE of $n = 9 - 10$. Results of two-way GLMs are shown; different letters indicate significant differences at $P < 0.05$. Where the $T \times W$ interaction was significant, letters indicate significant differences between drought and well-watered treatments at each temperature.

1 **Table 1.** Mean ($\pm$ SE) plant growth characteristics for cassava grown in low temperature (mean 23 °C/23 °C, day/night) or high temperature
2 (mean 34 °C/28 °C) glasshouses, and either well-watered (100% field capacity, $n = 9$) or droughted (25% field capacity, $n = 10$) treatments.
3 Results (P values) of two-way GLMs of temperature (T) and water regime (W) are shown. Significant differences between means are indicated
4 by superscript letters (Tukey's HSD; $P < 0.05$) across all treatments, or within temperature treatments when the $T \times W$ interaction was
5 significant.

Tissue/Parameter	High temperature		Low temperature		GLM (P)		
	Well	Drought	Well	Drought	T	W	T $\times$ W
<u>Whole plant</u>							
Total biomass (g dwt)	41.9 $\pm$ 3.5 ^a	19.4 $\pm$ 1.9 ^b	28.4 $\pm$ 4.6 ^A	21.5 $\pm$ 2.0 ^A	ns	<0.001	0.017
Root:Shoot	1.9 $\pm$ 0.1 ^a	0.6 $\pm$ 0.1 ^b	1.9 $\pm$ 0.2 ^A	1.6 $\pm$ 0.1 ^A	<0.001	<0.001	<0.001
Harvest Index (%)	54.7 $\pm$ 1.9 ^a	23.1 $\pm$ 1.7 ^b	53.5 $\pm$ 3.7 ^A	48.7 $\pm$ 2.98 ^A	<0.001	<0.001	<0.001
<u>Shoots</u>							
Total number leaves*	27.0 $\pm$ 1.4 ^a	27.6 $\pm$ 1.4 ^a	23.9 $\pm$ 1.7 ^b	24.0 $\pm$ 1.6 ^b	0.036	ns	ns
Number leaves dropped	10.2 $\pm$ 0.5 ^{ab}	14.3 $\pm$ 1.4 ^a	8.7 $\pm$ 1.0 ^b	11.5 $\pm$ 1.8 ^{ab}	ns	0.013	ns
Number leaves retained	16.8 $\pm$ 1.2 ^a	13.3 $\pm$ 0.4 ^b	15.2 $\pm$ 0.9 ^{ab}	12.5 $\pm$ 0.6 ^b	ns	<0.001	ns
Leaf mass (g dwt)**	5.2 $\pm$ 0.3 ^a	3.8 $\pm$ 0.2 ^b	3.9 $\pm$ 0.5 ^{ab}	3.2 $\pm$ 0.3 ^b	0.009	0.002	ns
Leaf area (cm ²)**	1292 $\pm$ 77.3 ^a	1005 $\pm$ 69.8 ^{ab}	1026 $\pm$ 119.7 ^{ab}	911 $\pm$ 69.0 ^b	0.042	0.024	ns
<u>Roots</u>							
Tuber % dry matter	30.5 $\pm$ 0.5 ^a	20.6 $\pm$ 0.9 ^{ba}	28.1 $\pm$ 1.6 ^A	27.6 $\pm$ 0.6 ^A	0.029	<0.001	<0.001
Tuber biomass (g dwt)	23.3 $\pm$ 2.6 ^a	4.6 $\pm$ 0.7 ^b	16.4 $\pm$ 3.2 ^A	10.7 $\pm$ 1.4 ^A	ns	<0.001	0.004
Fine root mass (g dwt)	4.1 $\pm$ 0.3 ^a	2.8 $\pm$ 0.2 ^b	2.8 $\pm$ 0.4 ^b	2.4 $\pm$ 0.2 ^b	0.002	0.002	ns

1 *Total number of leaves over course of experiment; **Leaf mass and leaf area measured for leaves retained at harvest. **Table 2.** Mean ($\pm$ SE)
2 chemical composition of tissues from cassava grown in low temperature (mean 23 °C/23 °C, day/night) or high temperature (mean 34 °C/28 °C)
3 glasshouses, and either well-watered (100% field capacity, $n = 9$) or droughted (25% field capacity, $n = 10$) treatments. Results (P values) of
4 two-way GLMs of temperature (T) and water regime (W) are shown. Significant differences between means are indicated by superscript letters
5 (Tukey's HSD; $P < 0.05$) across all treatments, or within temperature treatments when the $T \times W$ interaction was significant.

Tissue type/parameter	High temperature		Low temperature		GLM (P)		
	Well	Drought	Well	Drought	T	W	T $\times$ W
<u>Leaves</u>							
CN-N/N (%)*	3.9 $\pm$ 0.5	4.3 $\pm$ 0.5	3.2 $\pm$ 0.4	4.9 $\pm$ 0.9	ns	ns	ns
C:N	17.6 $\pm$ 0.2 ^a	13.3 $\pm$ 0.2 ^b	16.8 $\pm$ 0.5 ^A	14.7 $\pm$ 0.4 ^B	ns	<0.001	0.002
<u>Tuber</u>							
CN-N/N (%)*	1.5 $\pm$ 0.2 ^a	5.5 $\pm$ 1.0 ^b	7.1 $\pm$ 1.8 ^A	6.9 $\pm$ 1.3 ^A	0.002	0.020	0.007
C:N	275.7 $\pm$ 16.2 ^a	125.2 $\pm$ 10.4 ^b	197.4 $\pm$ 24.2 ^A	148.3 $\pm$ 7.6 ^A	ns	<0.001	0.002
$\delta^{13}\text{C}$ (‰)	-25.9 $\pm$ 0.14 ^a	-24.6 $\pm$ 0.11 ^b	-23.5 $\pm$ 0.11 ^c	-22.0 $\pm$ 0.09 ^d	<0.001	<0.001	ns
<u>Tuber Peel</u>							
CNp (mg g ⁻¹ dwt)	0.77 $\pm$ 0.20 ^a	1.63 $\pm$ 0.25 ^{ab}	1.77 $\pm$ 0.60 ^{ab}	3.19 $\pm$ 0.52 ^b	0.005	0.012	ns
Nitrogen (mg g ⁻¹ dwt)	3.14 $\pm$ 0.22 ^a	8.26 $\pm$ 0.74 ^b	5.94 $\pm$ 0.64 ^A	6.40 $\pm$ 0.43 ^A	ns	<0.001	<0.001
CN-N/N (%)*	13.1 $\pm$ 5.3 ^a	11.3 $\pm$ 2.5 ^a	25.9 $\pm$ 8.3 ^b	35.8 $\pm$ 3.7 ^b	0.001	ns	ns
C:N	135.7 $\pm$ 7.0 ^a	54.8 $\pm$ 4.5 ^b	75.9 $\pm$ 7.2 ^A	66.3 $\pm$ 4.2 ^A	0.0003	<0.001	<0.001

6 *CN-N/N (%) is the proportion of total N that is present as CN in each tissue type.

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