

## Limited effects of shade on physiological performances of cocoa (*Theobroma cacao* L.) under elevated temperature

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### ABSTRACT

Shade is one of the recommended management solutions to mitigate the effects of heat stress, which is a major challenge for cocoa production globally. Nevertheless, there are limited studies to verify this hypothesis. Here, we evaluate the effects of heat and shade on cocoa physiology using experimental plots with six-month old potted seedlings in a randomized complete block design. Infrared heaters were applied for one month to increase leaf temperatures by an average of 5–7 °C (heat treatment) compared with no heat (unheated treatments), and shaded plants were placed under a shade net removing 60% of the light compared with no shade (sun treatments). Plants under heat treatments in sun and in shade showed severe reduction in photosynthesis. Measurements of chlorophyll fluorescence and photosynthetic light response curves indicated that heat caused damages at photosystem II and additionally resulted in lower rates of maximal photosynthesis. Temperature optima for photosynthesis were at 31–33 °C with only small differences between treatments, and as light saturation was reached at low PAR levels of 325–380  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in shade and 427–521  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in sun, ambient rates of photosynthesis were comparable between sun and shade treatments. Heat treatments resulted in decreased concentrations of chlorophyll and changed pigment composition, reduced specific leaf areas, and plant biomass. While shade may benefit cocoa seedlings, our results indicate that the positive effects may not be sufficient to counteract the negative effects of increased temperatures on cocoa physiology.

### 1. Introduction

Emissions of greenhouse gases have caused a rise in global mean surface temperatures of about 0.95–1.2 °C above averages from pre-industrial levels (Masson-Delmotte et al., 2018; IPCC, 2021). Temperatures are changing faster in Africa than the global average (Hutchins et al., 2015) and are expected to increase between 0.3 and 4.8 °C by the end of 21st century depending on the representative concentration pathways (RCPs) proposed by IPCC (Stocker et al., 2013). The global temperature rise is likely to affect cocoa (*Theobroma cacao* L.) and the vulnerable farmers whose main economic and social interventions depend on proceeds from cocoa production. For example, Ghana has

experienced a progressive rise of annual mean temperature of 26.8 °C in 1970–28.0 °C in 2017 while the Northern parts of the cocoa belts in Cote d'Ivoire, Togo and Nigeria have been projected to reach maximum daytime temperatures above 36.0 °C, a temperature earlier confined to savannah areas (Schroth et al., 2016; Ameyaw et al., 2018).

Given the pace and volume by which greenhouse gases are getting into the atmosphere, global warming is likely to challenge global food security, including decreasing yields in cocoa production (Sultan et al., 2019; Stocker et al., 2013). Reports from other crops (Lamaoui et al., 2018; Allakhverdiev et al., 2008; Erge et al., 2008) have indicated that increased temperatures may lead to increased evaporative demands and need of water. It is possible that this will also affect cocoa production

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especially when climate forecasts indicate rising temperatures but decreasing rainfalls in the cocoa production zones (Schroth et al., 2016). Where soil moisture is inadequate to meet daily requirements by the plant, quality and quantity of produce will be affected (Joslin, 2018; Thornton et al., 2014) thus reducing productivity and household incomes. Though these climate scenarios call for immediate actions, empirical data on physiological responses of cocoa to elevated temperature are lacking and therefore limiting validations of yield forecasts and adaptive measures.

Cocoa is an understory tree crop native to South America (Vail, 2009; De Almeida and Valle, 2007) and cultivated in many tropical semimoiest regions outside its native range. The plant thrives well between 18 and 34 °C (Lahive, 2018). More than six million small-scale farmers globally depend on cocoa for their livelihood, while indirectly, the crop provides employment to millions of people along the value chain (Zhang and Motilal, 2016; World Cocoa Foundation, 2012). Cultivation of cocoa is commonly initiated through planting of seedlings or by direct sowing of viable beans. The seedlings are very sensitive to high temperature and drought (De Almeida and Valle, 2007). The temperature threshold for establishment is approximately 38 °C (Schroth, 2016) above which physiological activities may be altered (Lamaoui et al., 2018; Wisser et al., 2004) causing leaf dehydration (Padi et al., 2013), reduced photosynthetic rates (Balasimha et al., 1991; Schroth et al., 2016), leaf production, plant height and leaf biomass (Lahive, 2018). In severe cases, reduced physiological performance results in decreased production of cocoa beans (Zuidema et al., 2005; De Araujo et al., 2017).

Like other crops, yield of cocoa depends on growing conditions around the plant and on efficient light capture, efficient conversion of intercepted light energy to biomass and partitioning to beans (Long et al., 2006; Asare et al., 2017). Cultivation of cocoa under shade may reduce negative effects of high temperature (Vaast and Somarriba, 2014; Asare and Ræbild, 2016; Tee et al., 2018; Asare et al., 2018) by reducing the radiation load, leaf temperatures and water stress (Wood and Lass, 2001; Medrano et al., 2004). Shade may thus improve photosynthetic efficiency under high temperature. Leaf temperatures under the sun can be 10 °C higher than air temperatures during the day depending on plant species, water status and leaf location. However, under shade conditions, temperatures may drop to a few degrees below air temperature because of evaporative cooling (Vogel, 2009). Likewise, shade significantly reduces daytime soil temperature with differences as high as 15 °C between shade and no-shade treatments (Aguir et al., 2019), also contributing to improved physiological performance and yield of cocoa.

The physiological effects of shade on heat responses of cocoa remain obscure while cocoa farmers continue to experience high temperatures and water deficits (Hutchins et al., 2015). The discussion on how cocoa plants could be protected from rising temperatures is thus highly relevant. Several authors have recommended shade levels of 30–70% for establishment of cocoa seedlings in the field (Alvim et al., 1977; Evans and Murray, 1953; Wood and Lass, 1985). In agroforestry systems, shade levels between 30% and 50% improved yields in mature cocoa stands (Andres et al., 2018; Asare et al., 2018). However, there is limited empirical evidence (Yapp, 1992; Avila-Lovera et al., 2016) indicating the extent to which shade could reduce the stress effects on cocoa imposed by heat. In this paper, we investigate the hypothesis that shade could reduce the negative effects of heat on cocoa physiology.

## 2. Materials and methods

### 2.1. Experimental site and materials

The experiment was conducted in the warm and dry season (February – April 2020) in an open environment on the University of Ghana campus (539°N, 0011°W, 76 m a.s.l.). Four-month-old drought tolerant hybrid cocoa seedlings (Clone 67) were obtained from Cocoa Research Institute of Ghana (CRIG). Upon arrival at the experimental site, seedlings were watered and re-potted into dark plastic nursery bags

(25 cm high x 18 cm wide) perforated at the bottom. The sandy-loam soil type used for re-potting contained 0.09% total nitrogen, 50 ppm available phosphorus, 3.6% organic matter, 0.09 cmol K<sup>+</sup> kg<sup>-1</sup> soil and a pH of 5.0 (Tables A.1). Three grams 15.15.15 NPK (Ofori-Frimpong et al., 2010; FAO, 1989) was applied to each seedling followed by watering every day for the first week and every second day for the rest of the nursery time. Watering was done between 5:00 – 6: 30 pm with a sprinkling can. Initially, seedlings were kept under 60% shade using black shade nets to help reduce transplanting shock. After two weeks under shade nets, half of the seedlings, randomly selected for sun treatments, were gradually acclimatized to sun conditions by placing seedlings under approximately 40% shade for two weeks, and then under approximately 20% shade for another two weeks, before finally being placed under full sun for another two weeks. After two months at the new nursery, seedlings of almost the same height and stem diameter were selected for the treatments.

### 2.2. Experimental design

Using four heaters, a randomized complete block design with two blocks was conducted two times immediately after each other – the first trial was from 13th of February to 30th of March, and the second from 1st of April to 15th of May 2020. The two repetitions of the trial were considered as independent replicates, thus as one single randomized block experiment with four replicates. In each block, we applied two factors: shade level (shade/sun) and heat level (heated/unheated) in combination on four plots (shade/heated, shade/unheated, sun/heated, sun/unheated). Plants under shade treatments were placed in open sheds made of wooden poles with 60% black shade nets, measuring 2 m x 2 m base area and 2 m high. The top and sides of the sheds were covered with shade nets down to 0.5 m to make sure that plants were always shaded while still allowing for aeration. Sun plants were kept under full sun. The heat treatments had a black non-glowing 2000 W infra-red heater (Hortus Patio, NSH NORDIC A/S, Brædstrup, Denmark) of 20 cm × 100 cm suspended vertically at 0.8 m above the top of the cocoa plants. Heaters were on continuously, thus resulting in varying leaf temperatures depending on transpiration and wind speed. Mock heaters made of black wooden boards were raised above the unheated treatments to provide shade effects equal to those of the heaters. Forty cocoa seedlings arranged in 8 × 5 rows at 5 cm apart were placed in each experimental plot. All physiological measurements were taken on the third matured leaves of the middle four plants ( $n = 4$  plants per treatment per replicate totalling  $N = 64$ ) that were placed in a row directly under the infra-red heaters and fringed at the sides with two rows of border plants. Leaves one and two were newly flushed and had not attained physiological maturity and full sizes for measurements. This meant that the first measurements were conducted on leaves that had initiated their development before the start of the treatments, while the last measurements were carried out on leaves developed during the heat exposure. In each repetition of the trial, the duration of exposure was one month. The time of measurement was referenced according to the day-number of the experiment, thus 0 denoting the day before the experiment started and 1 denoting the first day.

### 2.3. Agronomic practices

A week before the start of the experiments, 15:15:15 NPK at a rate of 3 g per plant was applied. At days 2 and 15, Carbendazim (Carbendazim 500 g/kg, Agrimat Limited, Ghana) was sprayed according to manufacturer's recommendations to protect seedlings from fungal infections. Insects were controlled when they appeared on the leaves using Confidor (Imidaclopid 200 g/l, Kumark Company Limited, Ghana) also according to manufacturer's recommendations. Weeds were removed manually. Watering was done first to field capacity by soaking the soil with water and then allowed to drain for 24 h. The weight of the plant plus soil was then assessed. Water status was maintained by adding

water every second day the water corresponding to estimated lost amounts based on weighing the pots.

#### 2.4. Climate and leaf temperature

Relative humidity, temperature, rainfall, radiation, and wind speed were recorded by a weather station (ZL6, UMTS 3 G GSM cellular, Meter Group Inc. USA) 30 m from the experimental site. During the first repetition of the trial in March, temperature was between 22 and 35 °C while relative humidity averaged 80% with total rainfall of 27 mm. During the second repetition of the trial in April, temperatures ranged between 22 and 36 °C with a total rainfall of 39 mm and an average relative humidity of 78% (Table A.2).

Temperature and relative humidity within the plots were recorded at 10 min intervals with radiation-shielded iButtons (DS1923-F5 hygrometer, iButton Link, US), raised 0.5 m over the cocoa seedlings but 0.1 m to the side of the infra-red heaters (in the case of the heat treatments). Predawn leaf temperatures were measured on the third matured leaves of the middle four seedlings on Day 0, 1, 3, 5, 7, 14, 21 and 28, using an infrared thermometer (Laserliner ThermoSpot, Laserliner, Germany) positioned at 5 cm from the leaf surface. Variations over the course of each day were measured at hours 5, 9, 12, 15 and 18 during Day 0, 7, 14, 21 and 28.

#### 2.5. Photosynthesis

Gas exchange of leaves was measured using a CIRAS 3 portable gas analyser equipped with an automated broad leaf cuvette (PP systems, USA). Instantaneous rates of photosynthesis ( $P_n$ ), transpiration ( $E$ ), stomatal conductance ( $g_s$ ), and water use efficiency (WUE) were assessed between 10:00–11:00 AM and at 12:00 – 1:00 during Day 0, 7, 14, 21 and 28, using natural light conditions with  $[CO_2]$  set at  $400 \pm 10 \mu\text{mol mol}^{-1}$ , humidity at  $50 \pm 5\%$  of the ambient air and air temperature at  $28 \pm 1 \text{ }^\circ\text{C}$ . Water use efficiency was determined as the ratio of rate of photosynthesis to rate of transpiration (Hatfield and Dold, 2019).

Response curves of  $P_n$  to photosynthetic active radiation (PAR) and to air temperature ( $T$ ) were measured during the last week of heat imposition on leaves that had developed during the experiment. Constant parameters included  $[CO_2]$  at  $400 \pm 10 \mu\text{mol mol}^{-1}$ , humidity at  $50 \pm 5\%$ ,  $T$  ambient at  $28 \pm 1 \text{ }^\circ\text{C}$  for light response curves, and PAR at  $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  for temperature response curves.

Light responses were measured following protocols from Qui et al. (2019) and Cabrera-Bosquet et al. (2009) at fifteen light levels (in the PAR order of 500, 550, 600, 700, 800, 1000, 1500, 2000, 500, 450, 400, 300, 200, 100, 50 and  $0 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) with 4 min acclimation time for the lower levels and 2 min acclimation after  $700 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , optimized after trial runs. Two measurements were taken on three selected plants per plot giving a total of 24 response curves per block and 96 light response curves for the entire set-up. All measurements were taken on the third and fourth leaves from the top phyllotaxy, as leaves one and two were still young and had not attained full maturity for physiological measurements. Light response curves were fitted using the non-rectangular hyperbola model (Prioul and Chartier, 1977) as it proved to have the best fit, and parameters were extracted using the Excel calculator by Lobo et al. (2013):

$$P_n = \frac{(f_{(I_0)} * I + P_{gmax} - ((f_{(I_0)} * I + P_{gmax})^2 - 4\theta * f_{(I_0)} * I * P_{gmax})^{0.5}}{2\theta} - R_d$$

Where  $P_n$  = net photosynthesis [ $\mu\text{mol (CO}_2\text{) m}^{-2} \text{ s}^{-1}$ ];  $f_{(I_0)}$  = quantum yield at  $I = 0$  [ $\mu\text{mol (CO}_2\text{) mmol}^{-1}$  (photons)];  $I$  = photosynthetic photon flux density [ $\mu\text{mol (photons) m}^{-2} \text{ s}^{-1}$ ];  $P_{gmax}$  = maximum gross photosynthesis [ $\mu\text{mol (CO}_2\text{) m}^{-2} \text{ s}^{-1}$ ];  $\theta$  = convexity (dimensionless);  $R_d$  = dark respiration [ $\mu\text{mol (CO}_2\text{) m}^{-2} \text{ s}^{-1}$ ].

The parameters quantum yield at the initial slope ( $f_{(I_0)}$ ), maximum net photosynthetic rate at light saturation ( $P_{n(max)}$ ), dark respiration rate ( $R_d$ ), convexity ( $\theta$ ), light compensation point (LCP), and light saturation point (LSP) were obtained from the fitted curves.

Responses of photosynthesis ( $P_n$ ) to temperature were measured at eight temperature levels from 28 to 42 °C, starting at the low temperature with a stepwise 2 °C addition until the highest temperature. The acclimation time at each level was 5 min, which proved sufficient to adjust the cuvette to the next temperature level and to reach a steady-state photosynthesis. Fifteen plants per treatment were measured giving a total of sixty individual temperature response curves. Fitting of temperature responses was done with second order polynomials (Cavieres et al., 2000). Optimum temperatures ( $T_{opt}$ ) and maximum photosynthetic rates ( $P_{max}$ ) were determined according to:

$$T_{opt} = \frac{-b}{2 * a}$$

$$\text{and } P_{n(max)} = a(T_{opt})^2 + b(T_{opt}) + c$$

Where  $a$ , the coefficient from the quadratic term,  $b$ , the coefficient from the linear term and  $c$ , the intercept, were obtained from the fitted curves.

#### 2.6. Chlorophyll fluorescence

Chlorophyll fluorescence ( $F_v/F_m$ ) of the leaves was measured using a mini-PAM photosynthesis yield analyzer (Heinz Walz GmbH, Germany). Predawn measurements were done in darkness at Day 0, 1, 3, 5, 14, 21 and 28. Additionally, at Day 7, 14, 21 and 28, diurnal measurements were taken at 5-, 9-, 12-, 15- and 18-hours. Measurements were conducted after a minimum of 30 min dark adaptation, and under the natural light using the mini-PAM leaf clip. Variable fluorescence was recorded on dark-adapted samples ( $F_v/F_m$ ) and in the light ( $F_q'/F_m'$ ) and electron transport rate (ETR) on light adapted samples was calculated as  $ETC = F_q'/F_m' * PAR * 0.5 * 0.84$  (Walz, 1999; Toomey, 2013; Motohashi and Myouga, 2015).

#### 2.7. Leaf chlorophyll contents

Relative chlorophyll contents of the cocoa leaves were assessed at Day 0 and Day 28 with a SPAD (Chlorophyll meter SPAD-502 Plus, Konica Minolta, Japan). We used the same leaves as for  $F_v/F_m$  measurements, thus measuring on leaves that had developed before and during the experiment, respectively. Five measurements were taken on each leaf between 10 am and 11 am and then averaged. After the completion of experiments, pigment concentrations were determined spectrophotometrically. A part of the leaf used for fluorescence measurements was excised, pooled as a unit per treatment and immediately transported in plastic bags to the laboratory for further analysis. One gram of the homogenized pooled leaf samples per treatment was weighed and 10 ml of 80% acetone added, ground, and centrifuged at 10000 rpm (Lichtenthaler and Bushchmann, 2001). Absorbances of the supernatant were read at 663 nm and 645 nm using a UV/VIS spectrophotometer (Spectroquant pharo 300, Merck KGaA, Darmstadt, Germany). Contents of Chlorophyll a and b were determined following the equations of Lichtenthaler and Buschmann (2001).

#### 2.8. Stomatal density

Stomata were counted on the same leaves used for physiological measurements using the nail varnish approach (Schroeder and Stimart, 2005). Adaxial samples were taken from each of the leaves from the middle four plants between 10 and 11 am and were viewed under a compound microscope (Leica Application suite, version 1.8.1, Leica Microsystems Limited, Switzerland). Digital images of about  $310 \times 223 \mu\text{m}$  were used for the count of stomata as outlined by Schroeder and Stimart (2005).

**Table 1**

Mean values of environmental conditions within the treatments, and P-values from tests of significance. Values represent means  $\pm$  standard error. Means in a row with different letters are significantly different at  $P < 0.05$  according to Tukey HSD. Air temperatures and relative humidity were measured with shielded ibuttons (see methods). Leaf temperature ( $T_{\text{leaf}}$ ) was measured at predawn between the hours of 4:30 am to 5:30 am, ( $n = 4$ ).

| Conditions                                  | Shade           |                 | Sun             |                    | P-values    |            |             |
|---|-----------------|-----------------|-----------------|--------------------|-------------|------------|-------------|
|   | Heated          | Unheated        | Heated          | Unheated           | Shade level | Heat level | Shade* Heat |
| <b>Microclimate</b>                         |                 |                 |                 |                    |             |            |             |
| RH (%)                                      | 68.7 $\pm$ 5.3c | 78.4 $\pm$ 4.7a | 74.0 $\pm$ 4.8b | 76.6 $\pm$ 4.4a    | 0.051       | 0.015      | 0.025       |
| $T_{\text{max}}$ ( $^{\circ}$ C)            | 38.2 $\pm$ 1.9b | 37.0 $\pm$ 2.1c | 39.2 $\pm$ 2.4a | 38.4 $\pm$ 2.4b    | 0.005       | 0.006      | 0.032       |
| $T_{\text{min}}$ ( $^{\circ}$ C)            | 28.3 $\pm$ 2.1a | 25.9 $\pm$ 1.4b | 26.0 $\pm$ 1.4b | 25.6 $\pm$ 1.8b    | 0.024       | 0.022      | 0.032       |
| $T_{\text{mean}}$ ( $^{\circ}$ C)           | 32.1 $\pm$ 1.4a | 29.9 $\pm$ 1.2c | 30.7 $\pm$ 1.4b | 30.2 $\pm$ 1.3 BCE | 0.036       | 0.013      | 0.022       |
| <b>PAR and leaf temperature</b>             |                 |                 |                 |                    |             |            |             |
| $T_{\text{leaf}}$ ( $^{\circ}$ C)           | 33.1 $\pm$ 3.3a | 24.6 $\pm$ 1.5c | 30.7 $\pm$ 3.0b | 23.8 $\pm$ 1.5c    | 0.001       | < 0.001    | 0.049       |
| PAR ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) | 446 $\pm$ 7b    | 520 $\pm$ 7b    | 1514 $\pm$ 20a  | 1485 $\pm$ 32a     | < 0.001     | 0.516      | 0.117       |

## 2.9. Specific leaf area (SLA)

Specific leaf areas of individual plants were determined at the end of the heat imposition. Five leaf discs (2.01 cm<sup>2</sup>) excluding the mid-vein were sampled from the leaves used for fluorescence measurements using a core sampler. Leaf discs were dried to a constant mass at 70  $^{\circ}$ C and SLA was determined as the ratio of leaf disc area and the respective dry mass.

## 2.10. Plant growth and leaf area

Plant growth in height and in diameter were measured at start and at the end of the experiment following the protocols of Najihah et al. (2018), and the number of leaves were counted. Areas of the third, fourth and fifth fully matured leaves of the middle four plants were recorded using a Portable Leaf Area Meter (Li-3000 C, Licor, USA) after the end of the treatments and averaged.

## 2.11. Leaf damage

Number of leaves were counted at the start and at the end of the experiments, and the numbers of brown (Necrotic) or pale (Chlorotic) leaves were assessed using a five-point scale, 0 score indicating no damage or completely green; 1 - leaf appearing speckled; 2 - less than 50% damaged, 3 - more than 50% damaged; and 4 - fully damaged (Waters, 2015). Since very few leaves showed chlorosis and this could not be assigned to a specific group of plants, they were not referenced further.

## 2.12. Plant biomass

At the end of the experiment, seedlings were harvested, roots carefully washed, and parts separated into leaves, stems, and roots. These were dried at 70  $^{\circ}$ C to constant weight.

## 2.13. Data analyses

The repeated measurements of chlorophyll fluorescence and gas exchange were modelled using a linear mixed effects model allowing for temporal correlation of the error terms within the individual plants. The model included the fixed effects of shade, heat, and time as well as their interactions, and the random effects of plant, plot, and block (which includes repetitions). Parameters taken at the end of the experiment such as leaf area, specific leaf area, stomatal density, biomass, and chlorophyll content of leaves were modelled with linear mixed effect models with fixed effects of shade and heat as well as their interactions, and random effects of plant, plot, and block. Assumptions of homoscedasticity and normality of residuals were investigated by residual and normal quantile plots, and transformations of response variables were done if the model assumptions were not valid. Selection of data transformations was based on the level of skewness of the data. Because of

this chlorophyll fluorescence was transformed with the arcsine transformation, leaf temperature, light and growth measurements were squared root transformed, and stomatal conductance, transpiration and water use efficiency were log transformed. The statistical analysis was done using the R software (v4.1.1: R Core Team, 2021) using the nlme package (Pinheiro et al., 2014).

Leaf damage rated on a Likert scale was weighted together with the number of leaves damaged per plant to have an ordinal damage score per plant. The damage score was modelled by a proportional odds model (Agresti, 2019) with fixed effects of shade and heat and their interaction, and random effects of plant and plot. This was done using the ordinal package (Christensen, 2019).

In all models, the significances of fixed effects were assessed by the backward selection method (Pope and Webster, 1972) at a significance level of  $P < 0.05$ . Post hoc comparisons of the levels within the selected fixed effects were done using the emmeans package (Lenth, 2021), which entails correction for multiple comparisons.

## 3. Results

### 3.1. Environmental conditions

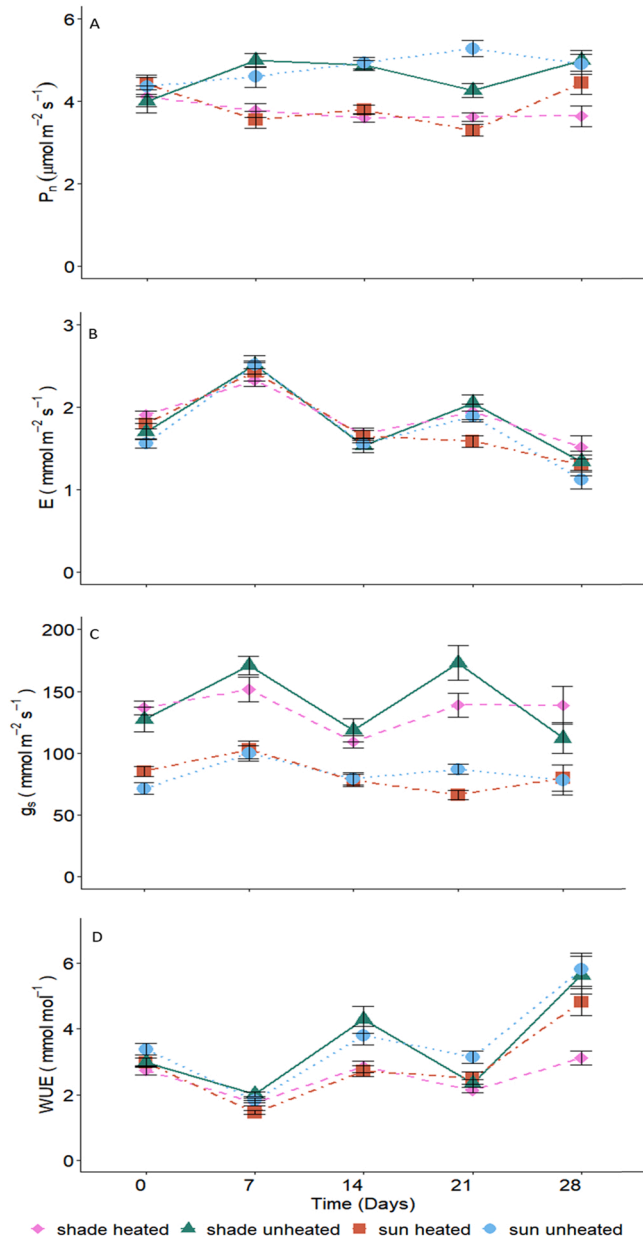
Average air temperatures during the experiment were approximately 30  $^{\circ}$ C and showed significant interactions between shade levels and heat levels (Table 1). While air temperatures in full sun appeared almost unaffected by the heat treatment, heat under shade resulted in an increase of 2  $^{\circ}$ C compared to the unheated conditions. This also meant that average relative humidity (RH%) in the shade heated plots was 69% compared with 78% in the shade unheated plots, and sun unheated treatments being intermediate.

Differences in leaf temperatures were higher and there were significant interactions between shade levels and heat levels (Table 1). While unheated treatments had averages around 24  $^{\circ}$ C in both shade and sun, corresponding values for heated treatments were 31  $^{\circ}$ C in the sun and 33  $^{\circ}$ C in the shade. Analysing the diurnal variation, heated treatments in the shade had the highest predawn leaf temperatures but the values were lower than for heated treatments in the sun during the day (Fig. A.1).

As expected, the levels of photosynthetically active radiation (PAR) reaching the surfaces of the leaves indicated significant differences among the shade levels with the sun treatments having average levels around 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  (Table 1). The shade nets intercepted between 60% and 70% of the photons directed to the plants.

### 3.2. Photosynthesis under ambient conditions

After initially being at the same level for all treatments, rates of photosynthesis were reduced by heat compared to unheated treatments. Surprisingly, despite some variations over time, plants under shade had similar levels of photosynthesis as plants under full sun. While stomatal conductance was always higher under shade than in the sun,



**Fig. 1.** Gas exchange as affected by duration of treatments; A- rate of photosynthesis ( $P_n$ ); B - transpiration ( $E$ ); C - stomatal conductance ( $g_s$ ); D - water use efficiency ( $WUE$ ). Bars indicate  $\pm$  standard error, ( $n = 4$ ).

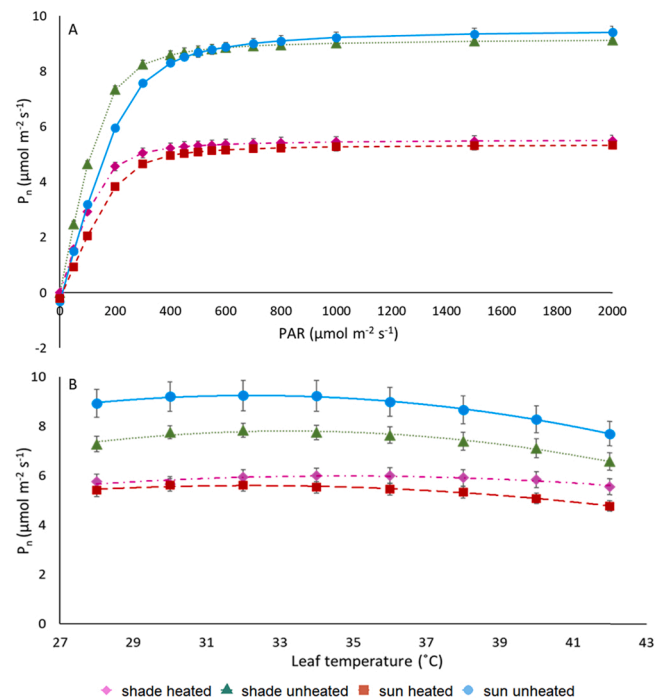
transpiration was close to similar across all treatments (Fig. 1). Water use efficiency tended to be higher in the unheated treatments compared to heated treatments (Fig. 1 D).

### 3.3. Photosynthetic response curves

Photosynthetic light response curves were different between treatments (Fig. 2a). Differences appeared to be primarily due to low maximum rates in heated plants, whereas differences between shade and sun plants were seen primarily at low light levels.

Analysis of the parameters from the light curve models indicated that heat decreased  $P_{n(max)}$  both in shade and in sun. Quantum yield ( $f_{(I_0)}$ ) was higher in shade than in sun and in unheated plots than in heated plots (Table 2).

Convexity and rates of dark respiration were not affected. Light saturation points (LSP) and light compensation points (LCP) were,



**Fig. 2.** Mean rates of photosynthesis at different levels of light (A) and leaf temperature (B). PAR - photosynthetically active radiation;  $P_n$  - rate of photosynthesis. Bars indicate  $\pm$  standard error, ( $n = 4$ ).

however, significantly different for both shade and heat levels. Rates of photosynthesis for shade grown plants saturated between 325 and 453  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Table 2) while those of sun grown plants had saturation points between 427 and 523  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Heat treatments tended to have lower rates of photosynthesis at saturation points compared to the unheated plants. As expected, light compensation points were lower for shade plants, with values ranging between 0.8 and 14.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for shade grown plants while they ranged between 11.2 and 17.5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for sun grown plants. Heated plants showed higher levels of LCP than unheated plants. Sun heated plants had LCP at 17.5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  compared with sun unheated at 11.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , while shade heated plants had average values of 5.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  compared with 0.8  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in shade unheated treatments.

Response curves of  $P_n$  to temperature showed a flat optimum within the tested range with borderline significant interactions between shade levels and heat levels (Fig. 2 B, Table 2).  $P_n$  increased from 28 °C to approximately 33 °C (Fig. 2B) after which the rate declined. Optimum temperatures varied between 31.0 °C to 33.1 °C, being lowest in shade unheated plots and highest in the shade heated plots (Table 2). Rate of photosynthesis ( $P_n$ ) at the optimum confirmed results from the light response curves, being low for the heated plants.

### 3.4. Chlorophyll fluorescence

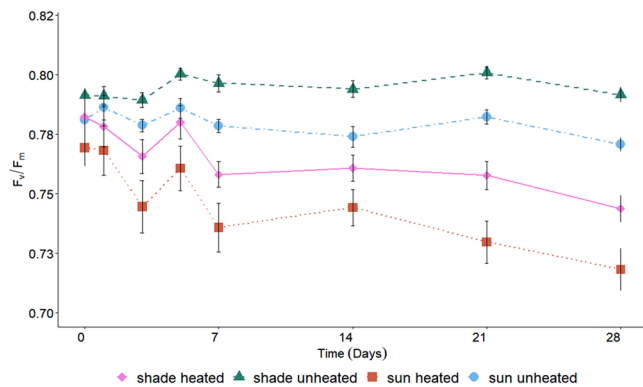
Shade and heat levels had significant impacts on fluorescence parameters with shade grown plants having higher predawn yields of fluorescence than full sun plants, and heated plots showing lower yields than unheated plots (Fig. 3, Table 3). The sun grown plants from the start of the experiment had lower values of predawn  $F_v/F_m$  than plants grown in shade. After the initiation of heat treatments, differences tended to increase over time, with high levels recorded in the shade unheated plots, slightly lower levels in the sun unheated plots, and low and decreasing values in the heat treatments, especially under sun.

Diurnal variations in leaf activity in terms of dark acclimated fluorescence ( $F_v/F_m$ ), light adapted fluorescence ( $F_q'/F_m'$ ), electron transfer and leaf temperature were also studied (Fig. 4; Fig.A.1). Both  $F_v/F_m$  and

**Table 2**

Parameters from photosynthetic response curves to light and temperature. Values represent means of parameters across treatments, and P-values from tests of significance. Means in a row with different letters are significantly different at  $P < 0.05$  according to Tukey HSD. Means are  $\pm$  standard error, ( $n = 4$ ).

|  | Shade              |                    | Sun                |                     | P-values    |            |             |
|--|--------------------|--------------------|--------------------|---------------------|-------------|------------|-------------|
|  | Heated             | Unheated           | Heated             | Unheated            | Shade level | Heat level | Shade* Heat |
| <b>Light response</b>  |                    |                    |                    |                     |             |            |             |
| $f_{(IO)}$ ( $\mu\text{mol mmol}^{-1}$ )                     | 0.037 $\pm$ 0.003b | 0.049 $\pm$ 0.003a | 0.028 $\pm$ 0.002c | 0.039 $\pm$ 0.003ab | 0.001       | < 0.001    | 0.680       |
| $P_{n(\text{max})}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | 5.2 $\pm$ 0.3b     | 8.4 $\pm$ 0.4a     | 5.0 $\pm$ 0.3b     | 8.9 $\pm$ 0.3a      | 0.564       | < 0.001    | 0.283       |
| $R_d$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )               | 0.3 $\pm$ 0.1      | 0.1 $\pm$ 0.1      | 0.6 $\pm$ 0.1      | 0.4 $\pm$ 0.1       | 0.550       | 0.700      | 0.440       |
| Convexity  | 0.8 $\pm$ 0.1      | 0.8 $\pm$ 0.4      | 0.9 $\pm$ 0.1      | 0.9 $\pm$ 0.1       | 0.360       | 0.220      | 0.253       |
| LSP ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )                 | 325 $\pm$ 14c      | 379 $\pm$ 29 BCE   | 428 $\pm$ 24b      | 521 $\pm$ 30a       | < 0.001     | < 0.001    | 0.117       |
| LCP ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )                 | 5.7 $\pm$ 1.7c     | 0.8 $\pm$ 0.5d     | 17.5 $\pm$ 2.8a    | 11.2 $\pm$ 2.3b     | < 0.001     | 0.004      | 0.432       |
| <b>Temperature response</b>                                  |                    |                    |                    |                     |             |            |             |
| Optimum T ( $^{\circ}\text{C}$ )                             | 33.1 $\pm$ 0.7     | 31.00 $\pm$ 0.8    | 32.1 $\pm$ 0.6     | 32.0 $\pm$ 0.3      | 0.719       | 0.100      | 0.052       |
| $P_n$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )               | 6.0 $\pm$ 0.3c     | 7.8 $\pm$ 0.3b     | 5.8 $\pm$ 0.3c     | 9.3 $\pm$ 0.6a      | 0.146       | 0.040      | 0.040       |



**Fig. 3.** Chlorophyll fluorescence of cocoa seedlings as affected by shade and heat levels. Bars indicate  $\pm$  standard error, ( $n = 4$ ).

$F_q/F_m$  for all treatments were high at dawn and sunset but low at middays (Fig. 4 B and C). Shade grown plants had higher chlorophyll fluorescence for both light and dark acclimated leaves. The electron transfer rate (ETR), on the other hand, was as high as  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  on sun grown plants but below  $35 \mu\text{mol m}^{-2} \text{s}^{-1}$  for shade grown plants during the midday. Transfer of electrons followed diurnal patterns of light distribution with higher values during the morning and midday.

### 3.5. Plant growth and chlorophyll pigments

Height growth ( $\Delta\text{Height}$ ) was affected by an interaction between shade level and heat treatment with the unheated plants in shade growing faster than heated plants. Plant growth in height in heated treatments was on average 35 – 50% lower than in unheated treatments

**Table 3**

Results from tests of significance of predawn fluorescence, leaf temperature, diurnal measurements of chlorophyll fluorescence, temperature, weekly measurements of stomatal conductance, photosynthesis, transpiration, and water use efficiency.

| P Values  | Shade   | Heat    | Time    | Shade* Heat | Shade* Time | Heat* Time | Shade*Heat*Time |
|---|---------|---------|---------|-------------|-------------|------------|-----------------|
| <b>Gas exchange</b>                             |         |         |         |             |             |            |                 |
| $P_n$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )  | < 0.058 | < 0.001 | < 0.001 | 0.818       | < 0.001     | < 0.001    | < 0.001         |
| $E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )      | < 0.402 | 0.727   | < 0.001 | 0.883       | < 0.001     | < 0.001    | < 0.001         |
| $g_s$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )    | 0.001   | 0.833   | < 0.001 | 0.838       | 0.001       | < 0.001    | 0.034           |
| WUE ( $\text{mmol mol}^{-1}$ )                  | 0.295   | 0.007   | < 0.001 | 0.677       | < 0.001     | < 0.001    | < 0.001         |
| <b>Predawn measurements</b>                     |         |         |         |             |             |            |                 |
| $F_v/F_m$                                       | < 0.028 | 0.001   | < 0.001 | 0.817       | 0.112       | < 0.001    | 0.736           |
| Leaf surface temperature ( $^{\circ}\text{C}$ ) | < 0.001 | < 0.001 | < 0.001 | 0.001       | < 0.001     | < 0.001    | < 0.002         |
| <b>Diurnal variations</b>                       |         |         |         |             |             |            |                 |
| PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )    | < 0.001 | 0.817   | < 0.001 | 0.881       | < 0.001     | 0.955      | 0.977           |
| $F_v/F_m$                                       | < 0.001 | < 0.001 | < 0.001 | 0.468       | < 0.001     | 0.001      | 0.323           |
| $F_v/F_m'$                                      | < 0.001 | 0.001   | < 0.001 | 0.881       | < 0.001     | 0.045      | 0.742           |
| ETR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )    | < 0.001 | < 0.001 | < 0.001 | 0.343       | < 0.001     | < 0.001    | 0.045           |
| Leaf surface temperature ( $^{\circ}\text{C}$ ) | < 0.001 | 0.005   | < 0.001 | 0.002       | < 0.001     | < 0.001    | 0.004           |

while stem expansion and leaf numbers showed no significant differences (Table 4).

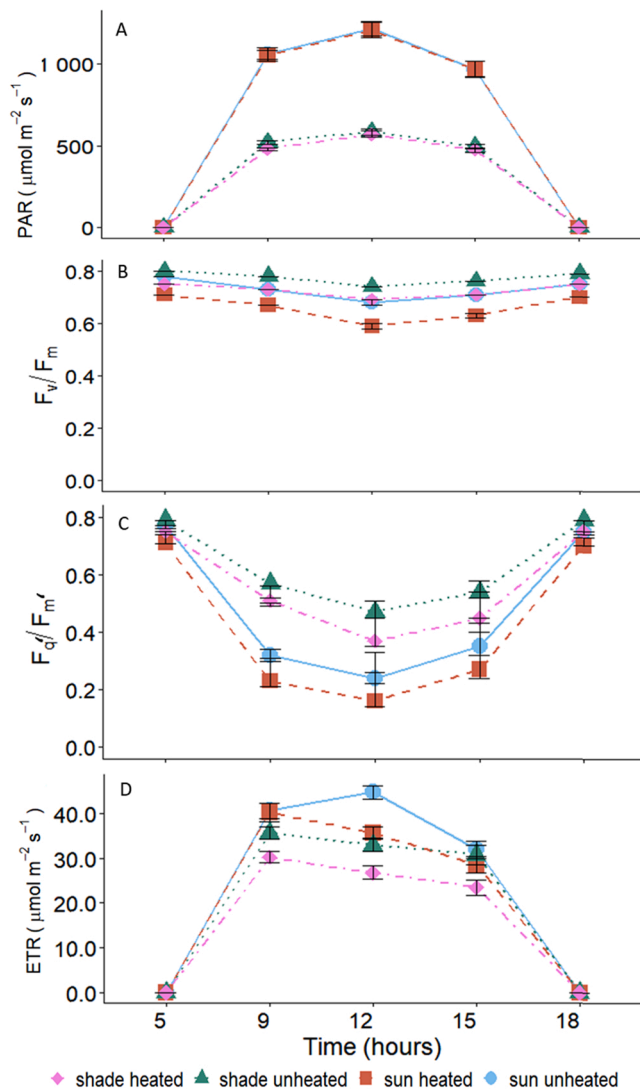
Sun grown plants had lower values of total chlorophyll contents before the heat imposition (Table 4), possibly because of the acclimatization period where the plants were kept under sun for some weeks. Heat caused further loss of chlorophyll especially in the sun heated plants with a quantified decrease of about  $10.1 \pm 0.5 \text{ nmol/cm}^2$  compared with shade heated plants with a decrease of about  $5.4 \pm 0.4 \text{ nmol/cm}^2$ . The shade unheated plants showed the smallest decrease of  $1.0 \pm 0.1 \text{ nmol/cm}^2$ .

Both shade and heat levels were observed to have significant effects on the pigment composition as expressed by chlorophyll a and b concentrations (Table 4). Shade grown plants had higher contents of Chl a and Chl b than plants under full sun. Likewise, heat reduced both Chl a and Chl b contents compared to plants grown under shade. Heat also affected the Chl a/b ratios with unheated plants showing higher ratios than heat treated plants.

### 3.6. Leaf status

Leaf numbers after the heat imposition ranged between 7 and 9 (Fig. 5) with no significant differences among treatments. Necrosis was more severe under heated conditions than in unheated treatments but not significantly affected by the shade levels. Twice as many leaf damages were recorded in the heat treatments compared to the unheated treatments.

Most of the brown leaves recorded were speckled (score 1) and less than 50% damaged (score 2) for all the treatments. Score two was more frequent in the sun heated plants than shade heated plants. Few leaves were noted to be more than 50% damaged irrespective of treatment.



**Fig. 4.** Diurnal variation of chlorophyll fluorescence and electron transfer rate under natural light conditions. A –Photosynthetically active radiation (PAR); B–Chlorophyll fluorescence after dark adaption; C – Chlorophyll fluorescence in the light, D - Electron transfer rate (ETR); Values represent means of measurements done at the hours of 5, 9, 12, 15 and 18 on weekly intervals for 4 weeks. Bars indicate  $\pm$  standard error, (n = 4).

### 3.7. Leaf area, stomatal density, and biomass

Shade grown plants produced leaves with significantly larger surface areas and with higher specific leaf area (SLA) compared with sun grown plants (Table 5). Leaves of heated plants expanded less resulting in 43% reduction in leaf size compared to unheated, and with lower values of specific leaf area (SLA), indicating denser leaves than unheated plants.

Stomatal densities were highest for sun heated plants, with low values recorded for the shaded unheated plants. Leaf dry weight was significantly lower in heated plants but showed no differences between the shade levels. Stem dry weight was not affected by shade nor heat although root and total dry weights were significantly different between the heat levels, being highest in the unheated plants. In terms of biomass distribution, shade interacted with heat to affect leaf/stem and leaf/root ratios but independently affected stem/root ratios. Plants under heat showed lower ratios of leaf/stem and leaf/root but higher ratios of stem/

## 4. Discussion

Despite mentioned frequently as a potential threat to cocoa (Schroth et al., 2016; Daymond and Hadley, 2004; Sena Gomes and Kozłowski, 1987), scientific evidence on effects of heat stress is scarce. Our simple setup thus represents one of the first attempts to characterize physiological reactions of cocoa to heat stress. The experiment overall confirms that heat stress will challenge cocoa cultivation and suggests that shade may have only limited mitigating effects at very high temperatures. Infra-red heaters increased leaf temperatures by 7–8 °C compared to unheated plants but with slightly higher differences during early morning when stomata were closed, and transpiration was low. Air temperatures were less affected than leaf temperatures, and relative humidity thus showed significant but limited differences between treatments. Plants exposed to infrared radiation hence experienced high midday temperatures (between 37 and 40 °C), values well above what has been considered the optimum of 34 °C (Lahive, 2018) and over the threshold of 38 °C for growth (FAO, 2007; Schroth, 2016). Our treatments were thus sufficient to bring cocoa into the supposed danger zone. It should be noted, however, that the heat load decreases with the distance from the heater, and exposure of lower leaves would have been less. Therefore, we emphasize leaf results in our discussion, as most of the data were recorded on leaves experiencing the same degree of exposure.

Results from the light response curves show that four weeks exposure to heat caused a substantial reduction in photosynthesis when exposed to different light levels, and results of dark-adapted chlorophyll fluorescence ( $F_v/F_m$ ) and  $f_{(IO)}$  indicate that this is partly due to lower photosystem II efficiency. Photosystem II complexes with the associated cofactors are known to be a primary target for heat injury (Chen et al., 2012). As a result of photoinhibition under heat and high photosynthetic photon flux density (PPFD), the D1-protein becomes irreversibly aggregated making turnover impossible and removal by proteases difficult (Yamamoto, 2016), partly because high temperature inhibits the repair of PSII (Allakhverdiev et al., 2008). The observed high PPFD of almost 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in sun may cause intercepted light energy to exceed the capacity of the photosynthetic machinery (Jaimez et al., 2018) and subsequently reduce maximum quantum efficiency of PSII (Kyle et al., 1984). Oxidation of water by PSII activity to release electrons is disrupted by heat, reducing rates of electron transport along the chain. In turn carboxylation and RuBP regeneration capacities directed by electron transport may be affected (Warren, 2008; Greer and Weedon, 2012). At high temperature, Farquhar et al. (1989) noted that the decline of electron transfer (ETR) diminishes the rate of photosynthesis at increasing light conditions.

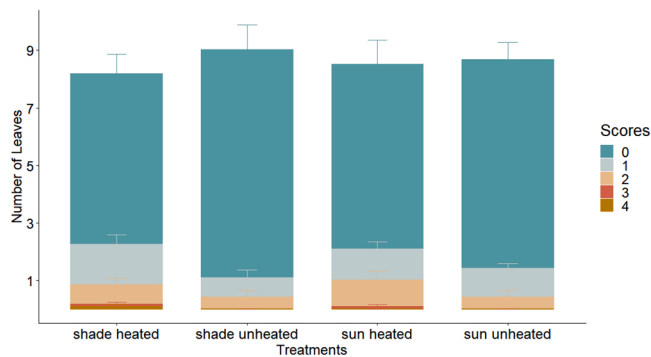
$P_{n(\text{max})}$  values were also affected, being around 5  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in heated plots but as high as 8.9  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the absence of heat. Reduced  $P_{n(\text{max})}$  in heated plants may partially be explained by the lower activity of Rubisco at elevated temperature and higher light exposure, as a response to a limitation of some of the processes involved in RuBP regeneration rate (Yang-Ping and Sage, 2005). Also, high temperatures may cause denaturation of Rubisco Activase, thus decreasing Rubisco activity (Salvucci et al., 2001).

When leaf temperature was increased from 28 to 42 °C in the temperature response measurements, we identified divergent levels of temperature responses between the treatments, but the response curves were quite similar. Optimum temperatures for photosynthesis were observed to be between 31 and 33 °C like the mean air temperature of 29.9 – 32.1 °C around the treatments. Similarly, Balasimha et al. (1991) reported optimum levels of photosynthesis between 31 and 33 °C, while Yapp (1992) found optimum at 33 – 35 °C. Having optimum temperature close to the ambient air temperature to which plants are exposed enhance assimilation rates (Slot and Winter, 2017), and many plant species acclimatize to the prevailing temperatures (Berry and Bjorkman, 1980; Medlyn et al., 2002). Still, we found only very limited signs of this in cocoa, as average temperature optima in the treatments were within

**Table 4**

Mean values for parameters of plant growth and leaf chlorophyll contents, and associated tests of significance. Means in a row with different letters are significantly different at  $P < 0.05$  according to Tukey HSD. Means are  $\pm$  standard error, ( $n = 4$ ).

|                                      | Shade            |                 | Sun             |                  | P values    |            |            |
|--------------------------------------|------------------|-----------------|-----------------|------------------|-------------|------------|------------|
|                                      | Heated           | Unheated        | Heated          | Unheated         | Shade level | Heat level | Shade*Heat |
| <b>Growth</b>                        |                  |                 |                 |                  |             |            |            |
| Height (cm)                          | 60.8 $\pm$ 0.8b  | 65.3 $\pm$ 1.1a | 60.5 $\pm$ 0.7b | 62.6 $\pm$ 0.6ab | 0.166       | 0.008      | 0.225      |
| Diameter (cm)                        | 9.5 $\pm$ 0.2    | 9.8 $\pm$ 0.3   | 9.4 $\pm$ 0.2   | 9.8 $\pm$ 0.2    | 0.803       | 0.098      | 0.803      |
| Leaves                               | 8.0 $\pm$ 1.0    | 10.0 $\pm$ 1.0  | 9.0 $\pm$ 1.0   | 9.0 $\pm$ 1.0    | 0.618       | 0.196      | 0.618      |
| $\Delta$ Height (cm)                 | 3.1 $\pm$ 0.2b   | 6.4 $\pm$ 0.5a  | 2.7 $\pm$ 0.2c  | 4.2 $\pm$ 0.3b   | 0.002       | < 0.001    | 0.010      |
| $\Delta$ Diameter (cm)               | 1.0 $\pm$ 0.1    | 1.1 $\pm$ 0.2   | 0.8 $\pm$ 0.1   | 1.1 $\pm$ 0.1    | 0.395       | 0.218      | 0.444      |
| $\Delta$ Leaves                      | 2.0 $\pm$ 1.0    | 3.0 $\pm$ 1.0   | 2.0 $\pm$ 1.0   | 3.0 $\pm$ 1.0    | 0.876       | 0.184      | 0.364      |
| <b>Chlorophyll content</b>           |                  |                 |                 |                  |             |            |            |
| SPAD                                 |                  |                 |                 |                  |             |            |            |
| Chl before (nmol/cm <sup>2</sup> )   | 40.6 $\pm$ 0.9ab | 41.9 $\pm$ 1.3a | 39.0 $\pm$ 0.7b | 39.4 $\pm$ 0.7b  | 0.001       | 0.137      | 0.491      |
| Chl after (nmol/cm <sup>2</sup> )    | 35.2 $\pm$ 0.7b  | 40.9 $\pm$ 1.3a | 28.9 $\pm$ 1.0c | 34.4 $\pm$ 0.5b  | < 0.001     | < 0.001    | 0.676      |
| $\Delta$ Chl (nmol/cm <sup>2</sup> ) | 5.4 $\pm$ 0.4b   | 1.0 $\pm$ 0.1c  | 10.1 $\pm$ 0.5a | 5.0 $\pm$ 0.5b   | < 0.001     | < 0.001    | 0.085      |
| Spectrophotometry                    |                  |                 |                 |                  |             |            |            |
| Chl a (ug/g)                         | 13.2 $\pm$ 0.8b  | 20.8 $\pm$ 2.7a | 8.6 $\pm$ 1.6c  | 14.8 $\pm$ 1.6b  | 0.012       | 0.002      | 0.712      |
| Chl b (ug/g)                         | 10.5 $\pm$ 1.0b  | 12.6 $\pm$ 1.6a | 6.6 $\pm$ 0.6d  | 8.7 $\pm$ 0.8c   | 0.003       | 0.022      | 0.984      |
| Chl a/Chl b                          | 1.3 $\pm$ 0.2b   | 1.7 $\pm$ 0.2a  | 1.3 $\pm$ 0.2b  | 1.8 $\pm$ 0.3a   | 0.821       | 0.038      | 0.830      |



**Fig. 5.** Average number of leaves and severity of leaf damage (necrosis). 0 denotes a fully green leaf, while 4 represents a fully damaged leaf, ( $n = 4$ ).

one-two degrees from each other and with only borderline significant differences. Unlike temperature response curves from other plants which show large decreases in assimilation at elevated temperatures (Slot and Winter, 2017; Vargas and Cordero, 2013) photosynthesis of cocoa showed only modest decreases at temperatures exceeding 35 °C. However, because of the overall decrease in photosynthetic capacity in response to heat treatments, net CO<sub>2</sub> assimilation, SLA and biomass was reduced compared to the unheated plants. Thicker leaves (lower SLA) may result in low rates of photosynthesis due to reduced CO<sub>2</sub> diffusion, smaller intercellular air spaces and low internal conductance in the spongy mesophyll cells (Nobel, 1977; Vargas and Cordero, 2013).

**Table 5**

Mean values of leaf morphology and biomass parameters, followed by P-values from tests of significance. Means in a row with different letters are significantly different at  $P < 0.05$  according to Tukey HSD. Means are  $\pm$  standard error, ( $n = 4$ ).

|  | Shade              |                 | Sun             |                   | P values    |            |             |
|--|--------------------|-----------------|-----------------|-------------------|-------------|------------|-------------|
|  | Heated             | Unheated        | Heated          | Unheated          | Shade level | Heat level | Shade *Heat |
| <b>Leaf morphology</b>                 |                    |                 |                 |                   |             |            |             |
| Average leaf area (cm <sup>2</sup> )   | 85 $\pm$ 6b        | 130 $\pm$ 9a    | 44 $\pm$ 3c     | 78 $\pm$ 5b       | < 0.001     | < 0.001    | 0.178       |
| SLA (m <sup>2</sup> kg <sup>-1</sup> ) | 24.0 $\pm$ 0.6 BCE | 26.1 $\pm$ 0.5a | 20.1 $\pm$ 0.7c | 23.4 $\pm$ 0.5b   | < 0.001     | < 0.001    | 0.495       |
| Stomata density (mm <sup>2</sup> )     | 834 $\pm$ 21c      | 614 $\pm$ 20d   | 1077 $\pm$ 23a  | 922 $\pm$ 19b     | < 0.001     | < 0.001    | 0.040       |
| <b>Biomass yield (dry weight)</b>      |                    |                 |                 |                   |             |            |             |
| Leaves (g)                             | 2.7 $\pm$ 0.4b     | 4.9 $\pm$ 0.4a  | 2.6 $\pm$ 0.2b  | 3.6 $\pm$ 0.4ab   | 0.124       | 0.004      | 0.131       |
| Stem (g)                               | 5.7 $\pm$ 0.2      | 6.7 $\pm$ 0.4   | 5.2 $\pm$ 0.2   | 6.3 $\pm$ 0.4     | 0.427       | 0.102      | 0.956       |
| Roots (g)                              | 2.6 $\pm$ 0.1b     | 3.5 $\pm$ 0.3a  | 2.3 $\pm$ 0.1b  | 3.0 $\pm$ 0.2a    | 0.163       | 0.024      | 0.980       |
| Total dry weight (g)                   | 10.9 $\pm$ 0.4b    | 15.1 $\pm$ 0.9a | 10.1 $\pm$ 0.4b | 12.9 $\pm$ 0.9ab  | 0.226       | 0.016      | 0.552       |
| Leaf/stem                              | 0.5 $\pm$ 0.1c     | 0.7 $\pm$ 0.1ab | 0.5 $\pm$ 0.1c  | 0.6 $\pm$ 0.1 BCE | 0.057       | 0.001      | 0.011       |
| Leaf/root                              | 1.0 $\pm$ 0.1b     | 1.5 $\pm$ 0.2a  | 1.1 $\pm$ 0.1ab | 1.2 $\pm$ 0.1ab   | 0.519       | 0.028      | 0.041       |
| Stem/root                              | 2.2 $\pm$ 0.1ab    | 2.0 $\pm$ 0.1b  | 2.4 $\pm$ 0.1a  | 2.1 $\pm$ 0.1b    | 0.045       | 0.007      | 0.925       |

Partial stomatal closure in response to higher air temperature noted in the heated plants also contributed to lower CO<sub>2</sub> conductance in the leaves causing lower photosynthetic rates. We recommend further research on temperature responses of cocoa to provide further information on the mechanisms involved.

Heat treated plants lost between 5 and 11 nmol chlorophyll cm<sup>-2</sup> after the heat imposition. Many enzymes are involved in the synthesis of chlorophyll and these enzymes may denature at higher temperatures (Li et al., 2018). Under heat, plants produce more Chlorophyll b, which is considered a thermally stable pigment (Erge et al., 2008) for light interception, but higher temperatures can also severely and irreversibly decrease leaf chlorophyll contents (Ribeiro et al., 2006). Vascular cell damage or death of leaves may occur at prolonged heat exposure (Qaderi et al., 2019) and appears to have occurred in the sun heated plants showing higher leaf damage than the unheated treatments. Higher chlorophyll contents recorded under the shade treatments (Sortentino et al., 1997; Salazar et al., 2018) is likely an adaptation to efficiently capture the limited light available.

Shade enhanced plant performances and increased physiological functions compared to plants in the sun. No observable differences were shown on rate of photosynthesis between the two unheated treatments and between the two heat treatments in sun and in shade although earlier reports (Agele et al., 2016; Avila-Lovera et al., 2016; Salazar et al., 2018) indicate higher rates under sun. The location of the experiment or the level of shade used might be a factor to the differences in observations. However, similar shade-sun effects on photosynthesis of three genotypes of cocoa were reported by De Araujo et al. (2017). Photosynthesis, as assessed by the light curves, saturated at relatively



**Table A1**

Soil chemical composition for the re-potting of the seedlings. Analysis was conducted at the Ecological Laboratory of University of Ghana.

| Parameter             | Value        | Recommended    | Reference                | Status   |
|-----------------------|--------------|----------------|--------------------------|----------|
| pH                    | 5.0          | 5.1 – 7.0      | Snoeck et al. (2016)     | Acidic   |
| Ec ds/cm              | 50.7         | 70 – 90        | Brito-Vega et al. (2018) | Low      |
| Available P           | 50.44        | > 20           | Horneck et al. (2011)    | Adequate |
| %C                    | 2.07         | 1.7 – 3.2%     | Snoeck et al. (2016)     | Adequate |
| % Total Nitrogen      | 0.09         | > 0.2          | Singh et al., 2019       | Low      |
| Ca cmol+ /kg          | 2.341        | 4–18           | Snoeck et al. (2016)     | Low      |
| Mg cmol+ /kg          | 1.324        | 0.9–4          | Snoeck et al. (2016)     | Adequate |
| K cmol+ /kg           | 0.089        | 0.2 – 1.2      | Snoeck et al. (2016)     | Adequate |
| Na cmol+ /kg          | 0.0043       | < 4.5          | Apori et al. (2020)      | Adequate |
| Cu mg/kg              | < 0.01       | 0.4 – 1.8      | Snoeck et al. (2016)     | Low      |
| Mn mg/kg              | 9.0          | 3–12           | Snoeck et al. (2016)     | Adequate |
| <b>Soil particles</b> | <b>%</b>     | <b>Texture</b> |                          |          |
|                       | <b>Comp.</b> |                |                          |          |
| Sand                  | 71%          | Sandy Loam     |                          |          |
| Silt                  | 19%          |                |                          |          |
| Clay                  | 10%          |                |                          |          |

low levels of PAR, meaning that saturating rates were reached even under the shade nets. Similar low light saturation points (LSP) of  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  and low maximum photosynthetic rates of around  $7 \mu\text{mol m}^{-2} \text{s}^{-1}$  at light saturation were reported in cocoa by Anim-Kwapong and Frimpong (2004) and Hutcheon (1981). Plants growing under shade acclimate their photosynthetic apparatus to low light intensity and are capable of efficiently trapping available light and converting it into chemical energy, while maintaining a low rate of respiration and partitioning a large fraction of assimilates into leaf growth (Pallardy, 2008). Salazar et al. (2018) reported LCP around  $5 - 57 \mu\text{mol m}^{-2} \text{s}^{-1}$  for shade tolerant cocoa plants grown in the Colombian Amazon while in Venezuela, Avila-Lovera et al. (2016) observed LCP of  $11.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Shade acclimated plants in our study adjusted LCP to lower light levels and confirm cocoa as a shade tolerant plant.

Shade grown plants overall had better growth and larger biomass production than sun grown plants. They had broader leaves, higher chlorophyll contents (Sorrentino et al., 1997; Salazar et al., 2018) and low specific density. Similarly, leaf conductance for  $\text{CO}_2$  and  $\text{H}_2\text{O}$  increased, facilitating photosynthesis under shade conditions, and compensating for smaller availability of light. Though part of the biomass was allocated before the start of the experiment, we detected changes in the allocation at the end of the heat imposition. Partitioning of the biomass favoured leaf production instead of stems and roots for shade grown plants, while heated plants showed higher biomass allocation to stems and roots. In contrast, earlier reports by Sena Gomes and Kozłowski (1987) found increased partitioning to the leaves at higher temperature. As the source of heat was right above the heated plants, development of new leaves may have declined, limiting exposure to heat but also the capacity for production of assimilates. Similarly, Bhattacharya, 2019 showed that increased partitioning of biomass to roots and stems (rather than to leaves) meant reduced radiation use efficiency and biomass production per unit of light intercepted.

This study showed for the first time the combined effects of shade and heat on cocoa physiology and provide a first glimpse of policies needed to protect cocoa plants from erratic climatic conditions. Our

**Table A2**

Climatic conditions at the experimental site in 2020. Measurements were taken from a weather station 30 m from the experimental plots.

| Month | Precipitation (mm) | Max. temp. (°C) | Min. temp (°C) | Ave. temp (°C) | Rel. Hum (-) | Wind-speed (m/s) | Radiation (W/ $\text{m}^2$ ) | Atmos. pressure (kPa) | Wind gusts (m/s) |
|-------|--------------------|-----------------|----------------|----------------|--------------|------------------|------------------------------|-----------------------|------------------|
| Feb.  | 3.62               | 36.60           | 22.90          | 29.66          | 0.74         | 1.13             | 245.85                       | 100.18                | 2.45             |
| Mar.  | 27.00              | 34.80           | 22.20          | 29.35          | 0.80         | 1.76             | 273.63                       | 100.11                | 3.68             |
| Apr.  | 39.09              | 35.60           | 22.10          | 29.30          | 0.78         | 1.48             | 320.41                       | 100.23                | 3.13             |
| May   | 179.17             | 35.20           | 22.10          | 28.04          | 0.87         | 1.12             | 216.11                       | 100.37                | 2.29             |

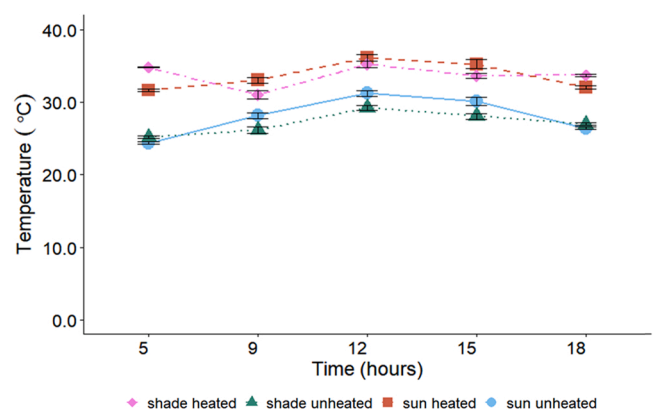
analysis showed only few interactions between shade and heat indicating that the effects are mostly additive, and that shade does not strongly modify the responses to heat. For example,  $F_v/F_m$ , ETR, LSP, LCP, and leaf area were significantly affected by shade and heat but with no interactive effects, while  $P_n$ , WUE and total biomass responded only to heat. Only plant height showed interactive effects of shade and heat. Still, while shade in our experiment seems not to have reduced the impact of heat to any large extent, under natural conditions shade will reduce the maximum temperatures the plants experience through the reduction of heat load from the solar radiation, warranting further research on the topic. In addition, the enhanced microclimatic conditions caused by shade led to overall better performance, showing that shade under both heated and non-heated conditions results in better plant health. However, these preliminary findings on cocoa seedlings need to be followed up by studies in real cocoa agroforestry systems, where the use of shade trees should also consider the competition for water and other resources that may have negative effects on cocoa production.

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## CRedit authorship contribution statements

The contribution of the authors to the paper is as follows: study conception and design: EOM, AR, PV, RA, CAA, and KO; data collection: EOM, and BKA; analysis and interpretation of results: EOM, BM, AR, PV, and RA; manuscript preparation: EOM, AR, PV, RA, CAA, and BKA; supervision: AR, PV, RA, CAA, BM, and KO; project management: KO, AR, and RA. All authors reviewed the results and approved the final version of the manuscript.



**Fig. A1.** Diurnal variations of leaf temperature measured at ca. 3-hour intervals from 5 am until 6 pm. The first measurement was taken at predawn (5 am) instead of 6 am to ensure minimal interference of light.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix

See Tables A1, A2 and Fig. A1 here.

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