COLLEGE OF BASIC AND APPLIED SCIENCES

UNIVERSITY OF GHANA

EFFECT OF SHADE ON ECOPHYSIOLOGY OF COCOA UNDER STRESS CONDITIONS

BY

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY IN

CROP SCIENCE

DEPARTMENT OF CROP SCIENCE

SEPTEMBER 2021

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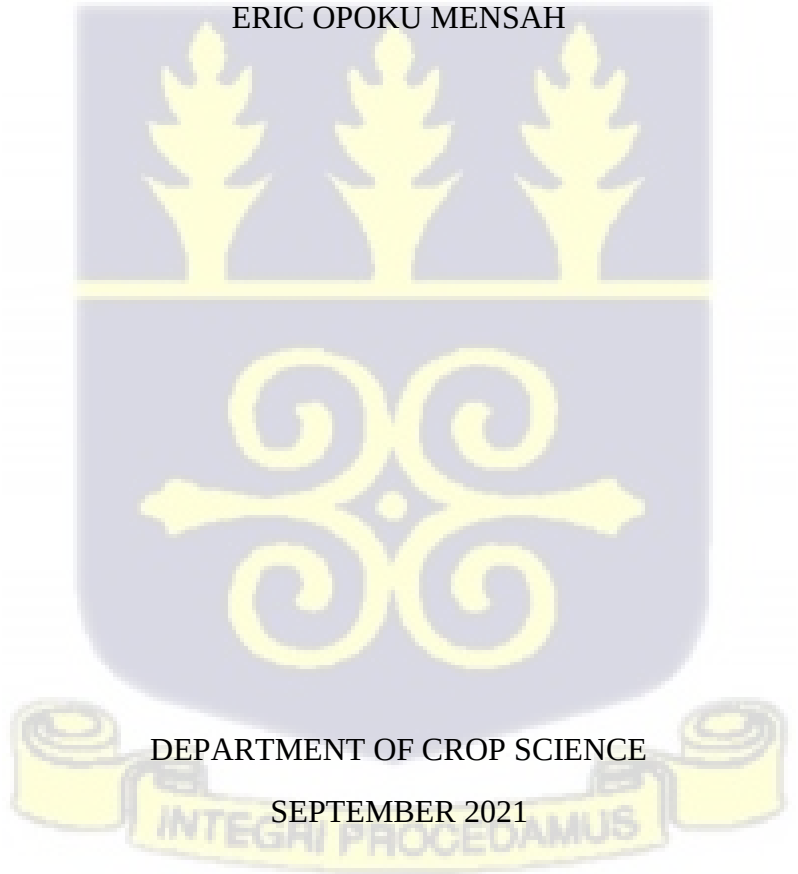

DEPARTMENT OF CROP SCIENCE

SEPTEMBER 2021

DECLARATION

I, hereby declare that this submission is my own work towards the PhD based on original investigations conducted under strict supervision and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Climate models predict increasing air temperature and decreasing rainfall patterns among cocoa growing regions around the world. Both heat and drought stress are known to affect physiology of cocoa plants through reduced rates of photosynthesis, lack of water and generally impaired physiological processes. This in turn leads to decreased yields and in severe cases, increased risks of mortality. Many studies have reported positive effects of shade on cocoa production; however, interest of full sun cocoa farming has been increasing over the last 20 – 40 years due to higher yields under full sun conditions with higher inputs of fertilizer. However, most of the cocoa farms in Ghana are owned by small households who cannot afford to invest into more fertilizer applications. Therefore, providing shade to buffer cocoa against erratic climatic conditions could help sustain the cocoa industry while protecting the environment. Notwithstanding, a strong debate on whether shade can buffer physiological performances of cocoa against climate change thus exists. Reports have indicated shade limiting the effects of bad weather on cocoa, but few on-farm studies have so far been done in tropical conditions to back this claim. The aim of this research was therefore to study the effects of drought and elevated temperature on performances of cocoa as a tropical understory plant and to ascertain whether shade can modify the effects. The research was carried out in two separate experiments. In experiment one, the aim was to evaluate shade on cocoa plants under different levels of water suppression. The study was conducted in a farmer's field with 12-year-old cocoa plants. Water suppression was achieved using plastic sheets to reduce through fall to between 33% and 66%. Shade was provided with 40% black shade net raised 6.5 m over the cocoa plants. Data taken covered a period of 33 months with parameters such as chlorophyll fluorescence, water potential, photosynthesis, stem expansion and yield were monitored. In experiment two, effects of heat on physiological performances of cocoa were studied using 6-month-old cocoa seedlings. Shade was provided using 60% black shade net while air

temperature 2 to 4 ºC above ambient was achieved using infra-red heaters. The experiment took place in September/October 2019 wet months and was repeated in the March/April 2020 dry months. Results from experiment one confirmed the hypothesis that drought can alter physiological functions of cocoa plants and shade can be a promising strategy to modify the effects. Drought had direct effect on water status in the plant affecting plant water potential, stem expansion, chlorophyll fluorescence and photosynthesis. Cocoa plants do not efficiently regulate their stomata to conserve water under drought conditions indicating the need for a constant supply of water to the plants. Canopy density, flower production, cherelles and pods count were higher under shade conditions, however, cherelles and pod damage were a significant problem under shade. Yield in kilograms per hectare depended on season, water availability or shade varying between 90 to 1100 kg/ha/season among treatments. Shade increased yield to about 1100 kg/ha/season irrespective of the levels of water suppression while water suppression proportionally reduced dry weight yield of cocoa plants whatever the shade levels. The 2/3 water suppression plots under full sun conditions had the least yield of 286 kg/ha/season compared with same treatment under shade conditions with yield average of 431 kg/ha/season. In experiment two, shade and heat had additive effects on growth, and physiological performances of cocoa at the seedlings level. However, interactive effects of shade and heat were observed on the immediate climatic conditions of the plants; an indication that shade can modify the immediate harsh conditions around the plants. Shade increased chlorophyll fluorescence, leaf area, chlorophyll pigments of leaves and reduced leaf damage. Shaded plants revealed maximum efficient utilization of limited light available by recording lower light saturation in the range of 325 – 380 µmol m^{-2} s^{-1} and light compensation between 0 – 6 µmol m^{-2} s^{-1}. Full sun plants on the other hand gave slightly higher light saturation between 427 – 520 µmol m^{-2} s^{-1} while light compensation ranged between 11 – 18 µmol m^{-2} s^{-1}. Also, full sun conditions increased leaf density and stomata per unit area and rate of photosynthesis.

Heat further reduced light saturation points and increased light compensation points under shade and full sun conditions. Heat affected chlorophyll fluorescence reflecting damages to photosystem II light harvesting complexes, slowing the rate of photosynthesis. Plants responded to raised heat with increased concentration of heat shock proteins (HSPs), lower light saturation points, reduced growth in height and a shift of optimal temperature for photosynthesis to higher levels to acclimate to or avoid the heat stress. Shade thus, can minimize negative effects of drought and heat on cocoa to improve yield of the plant.

DEDICATION

To God be the glory for His blessings.

To my wife, Sarah Addai, and the 'T' boys (Tobias, Titus, and Teddy) for leaving you all to pursue this programme. May God bless our family.

To my parents, Mr. Johnson Djin and Mad. Mary Obeng, and my in-law, Mad. Christiana Addai, for their advice and prayers that kept the desire burning. Today, I do dedicate this thesis to you to show my appreciation for your support during the days when you sold all your things to take care of us.

My brothers, Victor Asante Djin, Daniel Asamoah Djin, Seth Fosu Djin, Mark Gyasi Djin and a sister, Noami Mensah for the love and support shown on me and my kids during this journey.

ACKNOWLEDGEMENT

I do show much appreciation to my project supervisors, Dr. Mrs. C. A. Amoatey (Crop Science, University of Ghana), Prof. Anders Rabild (IGN, University of Copenhagen, Denmark), Prof. Phillipe Vaast (CIRAD, France) and Dr. Richard Asare (IITA, Ghana) for the time spent to train me for this work. Prof. Kwadwo Owusu (Geography Department, University of Ghana), Dr. Aske Skovmand Bosselmann (University of Copenhagen), Prof. Mette Fog Olwig (Roskilde University, Denmark), and the CLIMCOCOA team are acknowledged for their role on the project.

My acknowledgements also go to;

DANIDA and ORID for the financial support; the team at Danida Fellowship Centre (DFC), Denmark for accommodation during the course work at University of Copenhagen and IITA for the offer of opportunity as a research fellow; Prof. Bo Markussen, Prof. Erik Djahl Kjaer, Athina Koutoulease, Fatou Gning, Oulimata Diatta and all the lecturers and workers at Department of Geoscience and Resource Management, University of Copenhagen, Denmark for the opportunity to work and learn with you.

Dr. Naalamle Amissah (Head of Department), Prof. Vincent Eziah, Prof. Ofosu Budu, Prof. John Ofosu Anin, Rev. Prof. F. Kumaga, Prof. Kwadwo Ofori, Prof. K. Oduro, Prof. K. Afreh Nuamah, Prof. Bruce Sarpong, Dr. T. Nyaku, Dr. G. Esilfie, Dr. E. Cornelius, Mr. B. Boateng, Dr. and Dr. Mrs. Ofori Amoako and all other lecturers at Crop Science Department, School of Agriculture, University of Ghana for the training and the platform offered for this degree.

Dr. Kofi Acheampong (CRIG), Dr. Issaka Abdulai (Georg-August-Universitat Gottingen, Germany) and Mr. Mustapha Dalaa (IITA, Ghana) for their inputs; Drs. Opoku Agyemang and S.Y. Opoku (CRIG) for their role in getting cocoa pods and seedlings for the nursery; Cletus, and Misers Kirk, Otoo, Ampah, Asante, Harunah and Adusei (All of Crop and Soil Science

Departments, University of Ghana) for the role played in supplying shade nets and other research equipment for the experiment; Mr. Fred (CHED, Sefwi Boako) and my own friends, Francis Nwolley Cudjoe and Francis Ackah Mensah for supporting me for a search of cocoa farm suitable for the experimental design.

Nana Francis K Gyabeng (Oluu) for allowing us to use his farm; the people of Sefwi Kunuma (82) for their hospitality during my three years stay especially the families of Mr. Boamah (Fat Joe) and Mad. Asor for offering me an affordable accommodation.

My research assistant, Noah Adjei Owusu; my tricycle drivers, Cudjoe and Eric; my technical team, Amega, Kofi Oduro, Jalah and Emmanuel Dogbeh for their assistances during the field design; Samuel Osafo, Mr. Agyekum, Mr Atsu, Mad Gladys Lohor, Anthony Zipki, Johnny and Agya Osei (All of research farm, University of Ghana) for assisting the campus work.

The teams at Plant Physiology Laboratory, Botany Department; Ecological Laboratory, Geography Department; Biotechnology Laboratory, College of Science; Soil Research Institute, Kumasi; and WACCI Research Farm, University of Ghana for providing the equipment and the directions for the heat experiment. To Ghana COCOBOD for directions on selection of a farm for the research.

Colleagues on the CLIMCOCOA project, Dr. Yaw Agyenim Boafo, Bismark Kwesi Asitoako, and Sylvester Afram Boadi for the mutual support; my friends, Benette Osei Yaw, Chris Owusu Tabiri and Anthony Anang, for assistances offered for field and laboratory analysis; my brothers and a sister for financial support and prayers when emergencies occurred.

Dr. Augustina Frimpong (Immunology Department, Noguchi), when all hopes seemed lost and I had nearly coiled into my shells, you rang the bell and called me out of my slumber. May you continue to soar higher in your pursuance.

All the universities that trained me before this challenge, I say “Thank you”.

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LIST OF ABBREVIATIONS

ENSO	El Niño southern oscillation
PAR	Photosynthetically active radiation
PPFD	Photosynthetic photon flux density
$P_{nPPFDsat}$	Light saturated photosynthetic rate
A or P_n	Rate of photosynthesis
WUE	Water use efficiency
C_i	Sub-Stomatal CO_2 concentration
SLA	Specific leaf area
Chla	Chlorophyll a
Chlb	Chlorophyll b
Chla/b	The ratio between chlorophyll a and chlorophyll b
Chla+b	The sum of chlorophyll a and b
F_m	Maximum fluorescence
F_v	Variable fluorescence
F_o	Minimum fluorescence
F_m/F_v	Maximum fluorescence over variable fluorescence
Φ_{PSII}	Quantum yield of PSII
PSII	Photosystem II
PSI	Photosystem I
NPQ	Non-photochemical quenching of chlorophyll fluorescence
qP	Photochemical quenching
Φ	Apparent quantum efficiency
ETR	Electron transport rate
E	evaporation

g_s	stomatal conductance
A_{max}	Maximum photosynthetic rate
P_{max}	Light saturated photosynthesis
V_{cmax}	Maximum rate of Rubisco carboxylase activity
J_{max}	Maximum rate of photosynthetic electron transport
LSP	Light saturation point
LCP	Light compensation point
LHC's	Light harvesting antenna complexes
LIE	Light interception efficiency
NAR	Net assimilation rate
VPD	Vapour pressure deficit
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced form of NADP
$NADP^+$	oxidized form of NADP
ATP	Adenosine triphosphate
G3P	Triose phosphate
3-PGA	3-phosphoglycerate
ADP	Adenosine diphosphate
RUBP	Ribulose 1, 5 - bisphosphate
RUBISCO	Ribulose-1, 5 - bisphosphate carboxylase/oxygenase
WOC	Water oxidizing complex
ψ_p	Pressure potential
ψ_w	Water potential
ψ_s	Potential energy contribution due to solute concentration
ψ_g	Gravitational potential energy

ψ_m	Matrix potential energy
CAM	Crassulacean acid metabolism
SLA	Specific leaf area
LMA	Leaf mass per area
LMF	Leaf mass fraction
RWC%	Leaf relative water content
WSD	Water saturation deficit
SWC	Soil water content
VWC or Θ	Volumetric water content
PWP	Permanent wilting point
ANPP	Above ground net primary productivity
HSP	Heat shock proteins
ICCO	International Cocoa Organization
IPCC	Intergovernmental Panel on Climate Change
EPA	Environmental Protection Agency
SRID	Statistics and Research Division of MOFA
MOFA	Ministry of Food and Agriculture
NCCAS	Ghana National Climate Change Adaptation Strategy
CRIG	Cocoa Research Institute of Ghana
CHED	Cocoa Health and Extension Division
COCOBOD	Ghana Cocoa Board
IITA	International Institute of Tropical Agriculture
CIRAD	French Agricultural Research Centre for International Development
BCE	Before Common Era

CHAPTER I

INTRODUCTION

1.1 Background of the Study

In the process of photosynthesis, simple inorganic substances such as water and carbon dioxide react in the presence of light and chlorophyll to produce glucose which is later converted into carbohydrate and stored. Plants use the stored product for three main important functions; aerobic maintenance and respiration, conversion into plant's structural parts for growth and development, and as reserved photosynthetic product (Mawunya and Adiku, 2013). From the process of photosynthesis, crop yield output is an outcome of complex interactions of climatic factors as well as other elements such as soil, inherent crop properties and managerial activities. Water availability, temperature, carbon dioxide concentration and light intensity are among the climatic factors and alterations of these climatic conditions can affect yield. High temperature is anticipated to cause enzyme denaturing and phospholipid liquidation (Song et al., 2014) while drought is noted to increase moisture stress during the critical growth periods of plants (Mawunya and Adiku, 2013; Lizumi, 2014). Seasonal and annual variability in the onset, cessation and the duration of the rain characterized by dry spells of unpredictable magnitude provides a major problem to modern day agriculture (Mortimore, 1989; Amos and Thompson, 2015) as water availability becomes uncertain under such fluctuations. Cocoa is noted to be very sensitive to water (Wood and Lass, 1985) and that good production requires uniform distribution of rainfall throughout the year (Adjei-Nsiah and Kermah, 2012). On the other hand, it is projected that temperature will rise by 2.0 ^{o}C while rainfall will decrease by 10.9% in 2050 in Ghana (EPA, 2011). Bunn et al. (2017) anticipated a decreasing climatic suitability of all the cocoa regions under the projected temperature and drought. However, studies on effect of drought on yield and physiological performances of cocoa are few due to the difficulties of

subjecting adult plants to water stress. Most of these studies are based on either interview with farmers, yield records from purchasing clerks, the use of projections and models from young cocoa seedlings or taking the advantage of seasonal changes. Ruf et al. (2015) showed 17% reduction of yield of cocoa due to drought, however, the observations were due to reduction of cocoa farming areas and the shift from cocoa production to other crops. Elsewhere in Ghana, Aneani and Ofori-Frimpong (2013) identified about 82% yield gap between farmer's yield and potential yield of cocoa based on cross-sectional surveys from the major cocoa producing regions. Similar study in Indonesia showed drought reducing yield of cocoa to as high as 62% during the *ENSO* year (Keil et al., 2008) based on data from farmer's records while in Brazil, effect of 2015-16 *El Nino* related drought caused high cocoa mortality to about 15% and a yield reduction of about 89% (Gateau-Rey et al., 2018). These reports are somehow quiet over the effects of drought and elevated temperature on the physiological performances of cocoa as a tropical understory plant. The plant is noted to adapt well in tropical conditions with average temperatures between 18 – 32 ^{o}C and annual rainfall ranging between 1250 mm – 3000 mm (Wood and Lass, 1985). Reports indicate cocoa not efficient enough to limit transpiration in dry air comparing with other tropical plants (Baligar et al., 2008) and therefore the need of continuous water supply to meet transpiration demand. Previous years research indicates that years of increased rainfall correlated positively with yield (Anim-Kwapong and Frimpong, 2004), thus, water availability increases plants physiological functions to improve production of assimilates and partitioning to reproductive organs and then yield. In another development, long drought (about 4 to 6 months of dry season) causes soil water deficit which contributes to seedlings mortality, lower yield and mirid damage (Anim-Kwapong and Frimpong, 2005). Population of mirids (largely *Sahlbergella singularis* H. and *Distantiella theobroma* D.), a problematic pest in cocoa production, are noted to be affected by seasonal fluctuations (Babin et al., 2011) with warm and dry conditions increasing their population. The need of adaptation

strategies is therefore paramount to save cocoa from the unwelcoming conditions of the climate.

Provision of shade through agroforestry is suggested to be a possible strategy to mitigate or to adapt to the eco-physiological changes of the plant under the climate variables (Asare et al., 2017). Cocoa is noted to perform well under moderate shade with evidence of young plants requiring shade to reduce water and nutrient stress (Baligar et al., 2008). Based on earlier reports, shade reduces exposure of cocoa to high solar radiation, increases leaf longevity (Rajab et al., 2016), parades high concentration of chlorophyll to the leaves (Merkel et al., 1994) and makes plants more resilient to drought and heat. Cocoa planted at low densities under shade are noted to store more carbon per unit area of soil than those planted at high density without shade (Ofori-Frimpong et al., 2010). Bisseleua et al. (2013) showed shade trees increasing biodiversity and, in effect, increasing natural enemies of cocoa pests to protect the plant.

Though shade may buffer cocoa against bad climatic conditions, Blaser et al. (2018) reported a decreasing growth and yield under increasing shade-tree cover and then advised that as there is no concrete information of positive effects of agroforestry on soil fertility improvement under increasing shade tree cover, the benefits of agroforestry in cocoa production might not be extensive and adequate to balance short-term costs to production. In their research on effect of shade on microclimate, canopy characteristics and light integrals in dry season field-grown cocoa seedlings, Agele et al. (2016) identified full-sun cocoa farms having better transmitted and photosynthetic active radiation, increased radiation use efficiency especially in the irrigated full-sun treatments, and higher leaf area ratio comparing with moderate and dense shade treatments. In Ghana, Abdulai et al. (2017) suggested that some shade plants compete cocoa plants for available water and therefore may not have a significant benefit to the crop.

The debate continues as there still has not been a substantive agreement on whether shade can buffer performances and yield of cocoa in the wake of climate change. In effect, Bisseleua et al. (2013) identified an average of 56% farmers in five cocoa growing regions in Cameroon removing shade trees from their farm with the view of reducing incidence of pod rot and increasing yield. Though shade removal could increase yield under high fertile soil conditions as claimed by some researchers, this may also have adverse effects on the plants due to excessive irradiance and wind damage (Galyuon et al., 1996). High exposure of cocoa leaves to light is shown to damage chlorophyll to reduce photosynthesis and even cause the production of reactive oxygen species which are injurious to the photosynthetic apparatus (Mathur et al., 2014; Gururani et al., 2015; Murata et al., 2007). Amidst these strong debates in the cocoa industry, more eco-physiological works are instead done on coffee, maize, rice, and wheat in normal field conditions (DaMatta, 2007; Melke, 2014; Chemura et al., 2014; Gourdji et al., 2013; Deryng et al., 2014) and with few on cocoa (Moser, 2010; Zuidema and Leffelaar, 2002; De Almeida, 2007). The few works done are available on shade and yield but not much on the physiological effect of the adult plant *in situ*. This has led to few adaptation-potential models and enhancement of drought resistant varieties of cocoa.

The work looked at effects of varying environmental conditions such as elevated temperature and water availability on leaf physiology, performance, and yield of cocoa and how shade could reduce these effects. The results from the work could lead to a documented knowledge on physiological responses of cocoa to a changing climate and how well cocoa farmers could adapt to the situation. It will help estimate the physiological extent of cocoa failure due to severe weather events and how shade and irrigation could ameliorate the effects thus assisting to clarify the debate on shade and cocoa production.

1.2 General Hypothesis

Negative effects of high air temperature and low rainfall (hence low air humidity and limited soil water availability) on cocoa yield and physiology can be reduced by the provision of shade to the cocoa plants.

1.3 General Objectives

1. To ascertain the impact and contribute to increased understanding of the effects of drought and high temperatures on yield and physiology of cocoa.
2. To clarify whether shade may have a beneficial effect on cocoa under conditions of high temperature, low humidity, and drought.

CHAPTER II

LITERATURE REVIEW

2.1 History of Ghana's Cocoa

The origin of cocoa is connected to South America where cocoa beans were consumed by the Mayans and the Aztecs as far back as 600 – 400 BCE (Ludlow, 2012; Vail, 2009). Use of cocoa is therefore known to have started 8000 years ago where the Native Amazonians picked ripe pods from the forest and consumed them as fruits by sucking the pods and spitting out the seeds (Vaast and Somarriba, 2014).

The name cacao might have come from the early native American word '*kakawa',* which is a *Mayan* word linked to ceramics of cocoa (Vail, 2009). On the other hand, the scientific name of *Theobroma cacao* which means 'food of the gods' was coined by Carolus Linnaeus around the 18th century. The roasted cocoa beans with spices and water were used to make a bitter chocolate drink by the Mayans. They therefore considered cocoa as a gift from the gods (Vail, 2009) as its consumption had a strong religious and medicinal functions (Ludlow, 2012). In 1502, Christopher Columbus was reported to have spotted the Mayans using the seeds of cocoa as money and cherishing it as much as their own eyes (Coe and Coe, 2013).

Cocoa was introduced to Spain and other European countries by the Spanish who picked the plant from Central America through the 15th and 16th century voyage. The Spanish conquistador, Hernando Cortes might be the first European to have observed chocolate in the court of Montezuma in 1519 (Coe and Coe, 2013). To reduce the bitter taste of the beverage, the Spanish added sugar and honey (Fiegl, 2008). A Dutch Chemist Coenraad Johannes van Houten added alkaline salts to chocolate to enhance the taste by reducing its bitterness in 1815 which was called "Dutch Cocoa", but the first modern chocolate bar is credited to Joseph Fry, an English, who made a moldable chocolate paste by adding melted cocoa butter back into

Dutch cocoa in 1847 (Kerr, 2007; Fiegl, 2008). People got interested in cocoa production and started establishing cocoa plantations in other areas of the continent to supply adequate raw materials to the chocolate industry. In the sixteenth century, cocoa plantations were established in Asia and in Africa with Criollo, Amelonado and the Trinitario variety thus led to the dispersal of the seeds from the Americas to the other world (Zhang and Motilal, 2016). Commercial production of cocoa started in Africa after the Portuguese introduced Amelonado cocoa into Principe in 1822 (Zhang and Motilal, 2016). By the 1850s, the cultivation of the Amelonado cocoa had spread to Sao Tome and then finally to Fernando Po (now Equatorial Guinea) (Bartley, 2005). Other cocoa plantations were established in other West African countries such as Liberia, Cameroon, and Cote d'Ivoire (Ould, et al., 2004).

Around 1850s, a missionary from Basel, Rev. Johannes Haas introduced coffee and cocoa seeds to Akropong, Ghana from Surinam, West Indies and wrote a report on his cocoa farm in 1858 (Botchway, 2015; Asamoah-Prah, 2011). Tetteh Quarshie (1842 – 1892), a blacksmith trained by the Basel Missionaries, travelled to Fernando Po, and sent about eleven cocoa pods to Ghana (Okaitey, 2007). Initially, Tetteh Quarshie planted seeds from the pods in his native town at Accra, but the seeds failed on the unsuitable soils and weather conditions of the place. Upon the advice of an agricultural expect, Tetteh Quarshie re-located to Akuapem Mampong where he made a cocoa farm out of the five remaining cocoa pods in 1879 (Okaitey, 2007). The equatorial position of Ghana, as well as the favorable environmental conditions of the country promoted the growth of the crop along the forest belt. Around 1890, Tetteh Quarshie had had almost 300 healthy and productive cocoa trees. The demand of the seeds of cocoa by the Europeans increased interest of farmers to start cocoa farming, and cocoa production spread in Ghana especially in the forest regions.

2.2 Cocoa Production Trends

Today, cocoa is extensively cultivated to produce cocoa butter and powder for the confectionery industry and the global demand is increasing between 2 – 3% (Zhang and Motilal, 2016). About 90% - 95% of the world cocoa production is from small-scale farms in developing countries across Africa, Asia, and Latin America and over 40 – 50 million people's livelihoods depend on cocoa (Zhang and Motilal, 2016; World Cocoa Foundation, 2012).

Cocoa is mostly grown in a narrow belt 20 degrees north and south of the equator as the crop grows well in humid tropical climates with regular rains and short dry seasons. Most of the top 10 cocoa producing countries come from the warm, wet climates like where the plant originated (Mattyasovszky, 2017). Production of cocoa is intensive in West Africa (Cote d'Ivoire, Ghana, Nigeria, and Cameroon), South-East Asia (Indonesia and Papua New Guinea) and Latin America (Brazil, Ecuador, and Colombia) (Lahive et al., 2019). Global distribution stands at 73% from West Africa, 17% from South and Central America and 10% from Asia (Sulaiman, and Boachie-Danquah, 2017). Demand for cocoa outweighs supply with three times faster than population growth over the last 15 years (Sulaiman, and Boachie-Danquah, 2017). Over 40% of the world cocoa beans are consumed in Europe.

2.3 Cocoa Classification and Breeding

Cocoa (*Theobroma cacao* L.) as a perennial dry fruit plant belongs to class Magnoliopsida and family Malvaceae (formerly Sterculiaceae). Over 24 genera in the Malvaceae family exist with about 22 species in the genus *Theobroma* (Cuatrecasas, 1964; ICCO, 2013). Cocoa comes in three main genetic groups based on physical, sensory quality and associated botanical traits: Forastero, Criollo and Trinitario (Cheesman, 1944; Bartley, 2005). Other types reported in literature include Nacional and Refractario which are used for breeding purposes (Ciferri and Ciferri, 1956; Badrie et al., 2015). In another study, it has been reported that ten genetic groups or clusters of cocoa exist rather than the three and these include Maranon, Curaray, Criollo,

Iquitos, Nanay, Contamana, Amelonado, Purus, Nacional, and Guiana (Motamayor et al., 2008). Though the ten genetic groups explain well the genetic diversity of cocoa, Criollo, Forastero and Trinitario varieties are well studied and mostly cultivated.

The origin of Criollo and Forastero is still not certain as whether they originated separately or Forastero is a cultivated form of Criollo. The name *Forastero* is a Spanish word meaning *foreign* (Umaharan, 2018) as it was regarded foreign variety from the Amazonas comparing with Criollo, the original variety. Trinitario originated as a hybrid of Criollo and Forastero (Loor et al., 2009).

Criollo is believed to be the first cocoa to be domesticated with high quality but lower in yield and vigor (Cheesman, 1944; Motamayor et al., 2002). Seed is round with white to pale pink bean colour. The pods have bumpy skin with pointed tips and thin walls. Pods may also have smooth surfaces with ridges which are mostly five-angled (Badrie et al., 2015). It accounts for only 5% of the world's production due to poor adaptability to variable environmental conditions. It is mostly found in Indonesia, Central and South America, Venezuela, Sri Lanka, Samoa, and Madagascar (Umaharan, 2018).

Forastero has diverse populations with different geographic origins (Motamayor et al., 2002). It accounts for over 95% of cocoa cultivated in the world due to its high yielding, vigorous morphology, and less susceptibility to diseases (Umaharan, 2018). Forastero has a round thick-walled pods which turn yellow when ripe. The beans are flat purple with bitter taste. Subspecies of Forastero include the Upper Amazonia, the Lower Amazonia (also called Amelonado), Angoleta, Cundeamor and Calabacillo (Ciferri and Ciferri, 1956; Umaharan, 2018). The early cocoa to Ghana was the old Amelonado type introduced around 1815 by the Dutch, but this was gradually replaced by the Amazonia (Ruf, 2007; Peprah, 2019) owing to susceptibility of

Amelonado to black pod diseases. The two cultivars are, however, close to extinction in Ghana (Thresh et al., 1988).

Trinitario is a cultivated hybrid of Criollo and the lower Amazonia Forastero resulting from natural cross-pollination (Loor et al., 2009). It is said to have been a spontaneous hybrid from the island of Trinidad after a hurricane nearly destroyed the local Criollo around 1727 (Umaharan, 2018). Trinitario has a hybrid vigor with hardiness and high yielding nature of the Forastero and refined taste of the Criollo. They have relatively smooth skin, variable colors, and rounded pod with flat and purple beans. They are mainly cultivated in Asia, Central and South America.

Breeding resilient varieties is considered a way to increasing yield of cocoa especially amidst the future climate scenarios (Vaast and Somarriba, 2014). However, breeding new varieties with increased tolerance to drought, high water use efficiency and yields is slow because of insufficient use of proven breeding methods, long selection cycle of cocoa, and heterozygous nature of hybrid parental clones (Efron et al., 2003). Also, much attention is given to breeding diseases resistance such as to vascular streak dieback, witches broom, black pod, and swollen shoot than to the improvement of drought tolerant traits (Phillips-Mora et al., 2013). The hybrid cocoa (which most of them resulted from crosses between Upper Amazonia genotypes and Trinitario or Amelonado) widely used for planting are results from disease resistance and early maturity breeding programs (Efrom et al., 2003; Laliberte, 2012). Example, one of the hybrid cocoa is the series II bi-parental hybrid which was produced around the 1960s to replace trees killed by swollen shoot diseases (Thresh et al., 1988). *Akokora bedi* hybrid (starts fruiting between 2 - 3 years of age) was also introduced by the Cocoa Research Institute of Ghana (CRIG) around the 1980s to replace the late maturing Amelonada (fruits 7 – 8 years of age) and the Amazonia (fruits around 4 years of age) (Frimpong-Anin et al., 2015). With few studies attempting to breed drought tolerant cultivars, much of the emphasis have been on

morphological, physiological, biochemical and the molecular levels. Morphologically, cocoa breeders use traits such as leaf rolling, presence of hairiness, epicular wax decomposition, leaf dry biomass, reduced leaf area and deep rooting systems as drought tolerant indicators (Faroog et al., 2009; dos Santos et al., 2014). Other substances that modulate plants' ability towards drought include potassium for osmotic adjustments, silicon to improve cell water balance, osmolytes such as glycine betaine, and proline to sustain cellular functions (Faroog et al. 2009). Juby et al. (2021) indicated drought tolerant cocoa plants showing high levels of proline which might have acted as an osmolyte for osmotic adjustment and then to enable the plants to maintain low water potential. Nitrate reductase activity reduced under drought indicating reduced absorption of the nitrates. However, drought tolerant cocoa plants comparatively showed high levels of nitrate activity and Reactive Oxygen Species (ROS) scavenging mechanisms (Judy et al., 2021). Compatible solutes such as glycine betaine were identified to increase in drought tolerant cocoa plants acting as osmoregulators and to stabilize the structures and activities of enzymes. Dos Santos et al. (2014) reported high levels of nitrogen, potassium and phosphorus contents of drought tolerant cocoa plants compared with the other plants and then indicated that the high concentration of N-NO_3^- in the vacuoles maintained cellular turgor to confer tolerance to drought while the P contents increased water use efficiency and stomatal conductance. Other breeding programs have also aimed at building sustainable resistance to the distribution pattern of pests and diseases which are expected to change with changes in rainfall and temperature patterns (Cilas and Bastide, 2020) while at the molecular level, marker-assisted selections are being used to study drought tolerant cultivars and then genes encoding enzymes involved in synthesis of some compounds such as polyamines that function in drought tolerant cultivars are determined (Bae et al., 2008). Generative and vegetative techniques including grafting (using rootstocks from plants adaptable to limited soil water) and the use of prope legitimate seeds (seeds produced from random pollen from one or several

clones in the same block) are also established to select drought tolerant cultivars (Zasari et al., 2020).

2.4. Cocoa Cultivation Systems in Ghana

Cocoa tends to be one of the main cash crops in Ghana. Production of the crop falls within the forest zones with average rainfall around 1000 – 1600 mm, monthly temperature between 30 – 32^{o}C, and day humidity between 70 – 80% (Baah et al., 2016). The forest zone consists of the moist forest (wet evergreen, moist ever green and moist semi-deciduous forests noted of annual rainfall above 1200 mm) and the dry forest (semi-deciduous forest and some portions of transitional zones of annual rainfall between 1000 - 1200) (Asare, 2005). Six regions including Western, Ashanti, Brong Ahafo, Eastern and Volta fall within the four agro-ecological zones and are noted as the cocoa growing regions in Ghana. The Guinea Savannah and the Coastal Savannah agro-ecological zones which encompass the other four regions (Upper East, Upper West, Northern and Greater Accra Regions) do not support cocoa production because of unfavourable environmental conditions such as low rainfall and high temperature. Commercial cocoa stands have 3.0 m x 3.0 m spacing between and within rows with planting density of 1100 – 1200 cocoa trees per hectare (Utomo et al., 2015). A total land area of 14,038,224 hectares representing 58.8% of the country's land area is used for agriculture with 1,600,700 hectares of it used for cocoa production (MOFA, 2016). Average yield of cocoa is 500 kg/ha although achievable yield can reach 1500 kg/ha (Aneani and Ofori-Frimpong, 2013). Agriculture contributes 22.7% to Ghana GDP with which cocoa alone contributes 13.3%.

Somarriba et al. (2018) identified six main shading systems of cocoa from different publications, and these include cocoa without shade; cocoa with a mono-specific 'service' shade such as cocoa with legume trees; cocoa with productive shade tree species such as fruit trees, timber, rubber etc.; cocoa with mixed shade; rustic cocoa; and successional agroforestry. Three of these systems including cocoa without shade, cocoa with productive shade tree species

and cocoa with mixed shade are very common in Ghana. Cropping systems of cocoa in Ghana range between mixed cropping and monoculture where traditionally, forest canopies are thinned out (Asante et al., 2021) to pave way for establishment of new cocoa farms. From nursery where up to 60% of shade is provided to the young plants, mixed cropping system with plantain, cocoyam, cassava, and fruit trees are normally practiced at early stages of cocoa cultivation, but in some parts of Ghana, this is switched to monocropping as the cocoa plants mature and the canopy closes. Interest in cocoa monocropping is mainly due to high yielding resulting from increased rate of photosynthesis owing to improved light interception (Cunningham and Arnold, 1962; Agele et al., 2016). On the other hand, fertilizer inputs need to be high to achieve desirable yield under monocropping (Ahenkorah et al., 1974). Sometimes, farmers maintain few tree species for other benefits such as support for vine crops (such as *Dioscorea spp*). In another note, farmers are encouraged to maintain not less than 12 native desirable trees but not more than 70 trees per hectare to provide at most 40% shade as excessive shading could cause high humidity around cocoa plants to facilitate activities of *Phytophthora spp* (Asare et al., 2017; Bai et al., 2017). In a situation where the native shade trees are inadequate, the government of Ghana, through Forestry Commission, provides seedlings of selected trees to farmers with a recommended planting density of 18 trees per hectare at planting distance of 24 m x 24 m (Asare et al., 2016). Selection of tree species for cocoa agroforestry include reasons such as, height of the trees, area covered by the crown, crown porosity, less competition for resources (Somariba et al., 2018), about 30% shade distribution throughout the year, not serving as an alternative host to pests, and tree species of other economic benefits such as for food, addition of nitrogen to the soil and for timber. Trees such as *Terminalia ivoriensis* (Emeri), *Albizia coriaria* (Awiemfo samina), *Terminalia superba* (Framo), *Alstonia boonei* (Nyamedua), *Milicia excelsa* (Odum) and others of importance (Asare, 2005) are under investigation for their use in cocoa agroforestry in Ghana. Maintaining

the shade plants and their removal are normally monitored along canopy formation of the cocoa plants. Farmers reduce density of the shade trees when canopy formation is complete or near to completion. The agroforestry system is noted to improve farmer's livelihood as income could be generated from the tree species mixed with the cocoa (Sonwa et al., 2019).

2.5 Climate Change

Climate change, according to Intergovernmental Panel on Climate Change (IPCC, 2007; UNFCCC, 2011) is "a change in the state of the climate that can be identified (e.g., by using statistical tests) by changes in the mean and/or the variability of its properties and that persists for an extended period, typically decades or longer". Climate change could be due to natural internal processes, external forces, or persistent anthropogenic changes in the composition of the atmosphere or in land use. These activities contribute to the release of greenhouse gases such as Carbon Dioxide (CO_2), Nitrous Oxide (N_2O) and Methane gas (CH_4) (Hansen, 2015) into the atmosphere. The greenhouse gases, in the atmosphere, form layers and trap heat energy to contribute to global warming. Global warming may facilitate drying of water bodies and death of plants based on excessive and uncontrollable evapotranspiration.

The sub-Saharan countries could be the hardest hit regions of the world in the wake of climate change because of high reliance on agriculture and their low levels of coping capabilities (Kotir, 2011; Enete and Amusa, 2010). About 97% of agricultural land in sub-Saharan countries is rainfed (Rockstrom et al., 2004). It is estimated that crop yield in sub-Saharan Africa will decline by more than 10% by 2055 mainly due to elevated temperature, interaction of elevated temperature with rainfall, reduced soil nutrients status, enhanced vulnerability to weed competition, and increased pests' infestations (Gachene et al., 2014; Muller et al., 2014). IPCC (2021) report indicates 1.5 ^{o}C rise in Global warming above the pre-industrial levels with expected increase of 2.0 ^{o}C during the 21st century if emissions of CO_2 and other greenhouse gases are not reduced. The expected increase may result in reduced soil moisture and more

frequent heat waves (IPCC, 2021). Increased heat waves may induce persistent heat stress on plants causing weakened photo-protection systems and reducing yield potentials (Joslin, 2018). The effect may also increase evaporative demand to cause a rise in crop water requirement and an increased rate of water uptake from the soil. When soil water is limited in supply, the situation could slow crop production and damage the quality and quantity of crop yield (Joslin, 2018; Thornton et al., 2014).

Annual average temperature in Ghana varies between 27 ^{o}C around the coastal areas (Asare-Nuamah and Botchway, 2019) to 28.7 ^{o}C in the savannah regions (Asamoah and Ansah-Mensah, 2020) while daytime temperature may rise above 40 ^{o}C. Compiling historical data from 1961 to 2015 show a progressive rise in temperature and a decrease in mean annual rainfall in all the six ecological zones in Ghana (Mawunya and Adiku, 2013; Asare-Nuamah and Botchway, 2019). Average temperature is estimated to increase between 0.8 and 5.4 ^{o}C while annual rainfall is predicted to decline by 1.1 to 20.5% between 2020 and 2080, (NCCAS, 2012). It is projected that by the year 2050, temperature in the three Northern regions of Ghana will rise by 2.1 – 2.4 ^{o}C and the other regions by 1.3 – 2.0 ^{o}C (Asante and Amuakwa-Mensah, 2015). This is confirmed by Asamoah and Ansah-Mensah (2020) who reported a significant rise of annual temperature over the 40-year period in the Bawku area. In effect, production of cocoa is threatened by the erratic and unpredictable patterns of rainfall and temperature as the plant is very sensitive to water stress and elevated temperature (Car and Lockwoods, 2011; Laderach et al., 2013). Any change in climatic conditions may affect performances of cocoa as well as farmer's coping strategies. The sections that follow will talk more about cocoa physiology and threats of climate change to cocoa production.

2.6 Cocoa Physiology

This section deals with fundamental processes of cocoa such as photosynthesis, transpiration, and other leaf functions. Understanding how cocoa trees live and function in a complex

interaction with environmental conditions such as soil and climatic factors is a gateway of devising strategies to improving yield of cocoa and preparing against climate change.

In oxygenic photosynthesis, carbon dioxide reacts with water to produce glucose in a general equation as follows.

$$6H_2O + 6CO_2 \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 6O_2$$

Water + Carbon Dioxide → Glucose + Oxygen

From the equation, four main factors are necessary for photosynthesis in green plants, and these include light, chlorophyll, water, and carbon dioxide.

2.6.1 Light

Light energy is needed to drive the processes of photosynthesis (Ruban, 2009). Photosynthetically active radiation of light is in the wavelength range of 700 nm to 400 nm. Energy levels higher than the range can cause pigment bleaching while lower levels are not strong enough to raise an orbital electron to excited state. The light energy absorbed is used to split water molecule into oxygen and protons to remove electrons. The generated electron moves through electron carriers where it is used to reduce NADP+ to NADPH to drive the Calvin-Benson cycle of photosynthesis (Lodish et al., 2000).

Light also plays a signalling and regulatory role in developmental and metabolic processes in the plant. Flowering is induced when ratio of red and far-red light decreases, also through photoperiodic pathway where light signals are transmitted to the circadian clock by the help of phytochromes and cryptochromes to activate a gene expression cascade needed to induce flowering (Eckstein et al., 2012; Jaeger et al., 2006). Light can also serve as a limiting factor to photosynthetic activities, a condition known as photoinhibition (Vass et al., 2007). This condition results when the metabolic processes cannot keep up with the electron flow produced

by the primary photoreactions – the primary site for photoinhibition is the photosystem II complex (Vass et al., 2007). Increasing light intensity causes the reaction centres to increasingly saturate with energy which leads to the reduction of the fraction of energy utilized in photosynthesis and therefore building up of "unused" potentially harmful excitation energy in the photosynthetic membrane (Ruban, 2009; Ruban, 2016). Some of the adaptive measures by the plant to reduce photoinhibition include leaf blade movement, chloroplast movement and the presence of leaf hairs (Eckstein et al., 2012).

2.6.2 Chlorophyll

The main pigments for photosynthesis include chlorophylls (five important chlorophylls exist which include chlorophyll a, b, c, d, and e), carotenoids (e.g., lycopene; zeaxanthin and β-carotene) and xanthophylls (Koning, 1994). Chlorophylls are specifically assembled along carotenoids and lipids in a protein matrix to form light-harvesting antenna complexes (LHC's) (Ruban, 2009), the main sites for light interception.

Chlorophyll (Chl) determines the photosynthetic capacity of plants (Li et al., 2018). Chlorophyll *a* and *b* are essential for the primary reaction. Chlorophyll *a* is the main primary pigment and mostly absorbs energy from the blue-violet and orange-red spectrum at 675 nm while chlorophyll *b* is an accessory pigment to chlorophyll *a*. It absorbs light in the blue spectrum around 640 nm. Ratio of occurrences of chlorophyll *a* and *b* has been reported to be around 3:1 in fruits and vegetables (Erge et al., 2008). Under low light conditions, plants produce more chlorophyll *b* than *a* to increase their photosynthetic efficiency. Plants use this strategy as chlorophyll *b* is efficient in the use of diffused solar radiation than chlorophyll *a* (Kume et al., 2018).

2.6.3 Water

Water is one of the most important substances for plant growth and is the main constituent of plant cells (Nobel, 2009). Processes such as photosynthesis, respiration and transpiration are affected by water availability (Holding and Streich, 2013). More than 95% of the water absorbed by plants are used for transpiration while the rest remain in the plant for growth (Sterling, 2004). Difference in water vapour concentration (ΔC_{wv}) between leaf and air is responsible for the diffusion of water vapour from leaf to air; difference in pressure potential ($\Delta\psi_p$) drive the bulk flow of water through the xylem conduits and differences in water potential ($\Delta\psi$) is responsible for the movement of water across the living cells in the root (Taiz et al., 2015). Thus, water has a ubiquitous function in the physiological and metabolic activities of plants. The hydrogen bond of water gives unique physical properties of water. Water performs the following functions in plants; a raw material for the manufacture of carbohydrates in plants, temperature regulation, a solvent to dissolve many molecules, for gaseous exchange between air and the inner surface of leaf cells due to its cohesive and adhesive properties, a transporting agent for both simple and complex materials in the plant, a means for the equilibrium of salt and other dissolved substances maintained in the cell, and as a structural agent for the maintenance of turgidity of plant cells (Holding and Streich, 2013; Nobel, 2009; Lakrim, 2013; Ferguson, 1959). During photosynthesis, water molecules split to produce electrons to replace the lost electrons of photosystem II in the light dependent reaction. Water also provides H+ ions to reduce NADP to NADPH and, also, a source of the oxygen that evolve from photosynthesis (Park, 2009). The O_2 evolved is used by other organisms for respiration. Production of ATP involves extraction of the component of water from ADP plus phosphate (Nobel, 2009). The ATP and the NADPH drive the Calvin cycle to produce glucose.

2.6.4 Carbon Dioxide

Carbon dioxide is reduced to organic carbon (glucose) during photosynthesis. According to Whitmarsh and Govindjee (1995), production of carbohydrate from inorganic carbon involves a sequence of biochemical reactions (through three basic stages of fixation, reduction and regeneration) that reduce carbon and rearranges to produce the organic carbon. It starts with the addition of CO_2 to a five-carbon compound known as ribulose 1, 5- bisphosphate (RUBP). The reaction is catalyzed by Rubisco (an enzyme). The six-carbon produced splits to produce two molecules of a three-carbon compound called 3-phosphoglycerate (3-PGA). The 3-PGA is then broken down into two molecules of triose phosphate (glyceraldehyde-3-phosphate (G3P)). Various rounds of Calvin cycle lead to the formation of 6-G3Ps. The ATPs and NADPHs made during light dependent reactions energize one of the 6-G3Ps for a further conversion into glucose while the remaining 5-G3Ps are used for RUBPs regeneration. The new RUBPs replace the used RUBPs to continue with the circle. More carbon dioxide is also prepared to be fixed. Each molecule of CO_2 requires 2 molecules of NADPH and 3 molecules of ATP as well as 13 enzymes in the Calvin cycle to reduce to a sugar (Whitmarsh and Govindjee, 1995). Photorespiration is a situation where Rubisco binds to O_2 instead of CO_2. This probably does not serve any useful purpose for the plant and cause complications in photosynthesis. Some plants such as C4 and CAM plants have evolved specialized structures and biochemical pathways that concentrate CO_2 near Rubisco (Whitmarsh and Govindjee, 1995), avoiding photorespiration.

2.7 Determinants of Plant Physiological Functions

Many processes occur in plants to ensure survival, growth, development, and reproduction. These processes are studied with specific determinants (parameters). This part of the review seeks to describe some of the determinants with a focus on cocoa production.

2.7.1 Specific Leaf Area ($m^2 kg^{-1}$) and Leaf Mass per Area (kg m^{-2})

Specific Leaf Area (SLA) as the ratio of leaf area to leaf dry mass indicates the amount of leaf area a plant builds with a given amount of leaf biomass.

$SLA = \frac{AL}{\mathrm{DML}}$ ($m^2 kg^{-1}$)

Where A_L – Area of the given leaf and D_{ML} = dry mass of the given leaf.

The other version of this equation is the leaf mass per area (LMA) which is the inverse of SLA. Thus;

LMA = $\frac{DML}{AL}$ (kg m^{-2})

SLA and LMA are used often in research to analyse the investments plants make into their structural components such as number of cells per unit area of leaf, amount of carbon and nutrients per area, and the reproductivity of the plant. SLA and the fraction of plant biomass allocated to leaves (leaf mass fraction, LMF) determine the total amount of leaf area that is displayed per unit biomass, an important parameter to determine a plant's relative growth rate (Poorter et al., 2009). SLA and LMA could also give an indication on the stress status of water and temperature. Under drought stress, leaf expansion rate decreases. The cells are therefore smaller and more tightly packed with lower fraction of air spaces and the thickness of cell walls are also greater giving higher leaf density (Poorter et al., 2009). Presence of large volume of air spaces could reduce the leaf density enhancing conductivity within the leaf which may facilitate photosynthesis while a higher leaf density indicates a high proportion of lignified tissues contributing to leaf toughness and plant survival (Niinemets, 1999; Alvarez-Clare and Kitajima, 2007; Poorter et al., 2009). Also, low temperature leads to limited cell expansion giving many small cells per unit area and more cell wall materials per unit leaf volume. This implies more cell layers, higher protein content per unit area and increased secondary compounds such as proline making LMA high (Poorter et al., 2009). High temperature and

drought could affect LMA or SLA at a rate depending on the species (Laureano et al., 2008; Marron et al., 2003; Poorter et al., 2009).

SLA is in the range of 2 – 27 $m^2 kg^{-1}$ for woody plants while LMA is in the range of 35 – 470 kgm^{-2} depending on the tree species (Poorter et al., 2009). Using the leaf disc method, Daymond et al. (2011) observed that specific leaf area (SLA) of eight genotypes of cocoa ranged from 17.3 to 23.9 $m^2 kg^{-1}$. Working on 7-year-old cocoa plants, Jaimez et al. (2018) recorded SLA in the range of 11.1 and 14.8 $m^2 kg^{-1}$. In Brazil Da Matta (2001) recorded average cocoa SLA of 25.2 $m^2 kg^{-1}$ when working on 13 months old cocoa plants. In Colombia, Cocoa plants under high photosynthetically active radiation (PAR) showed SLA of 14.94 $m^2 kg^{-1}$, medium PAR of 16.83 $m^2 kg^{-1}$ and low PAR of 18.59 $m^2 kg^{-1}$ (Salazar et al., 2018) indicating SLA increasing when PAR decreases. High SLA exhibited by cocoa plants under low PAR is an adaptation strategy to cope with the low light condition (Salazar et al., 2018). Thus, it might be a mechanism to maximize photon capture efficiency which may improve photosynthetic capacity and carbon gain in addition to concentrating resources invested in the construction of photosynthetic tissue (Salazar et al., 2018). Forastero cocoa tree of over 50 years had SLA of 17.28 m^2 kg^{-1} while its Criollo counterpart averagely had 14.1 m^2 $kg^{-1.}$ in Venezuela (Tezara et al., 2016). The higher value from Forastero were noted to be due to lower content of mechanical tissue such as cellulose and lignin. Prihastanti and Nurchayati (2018) showed that multiple shade trees in a cocoa farm affect cocoa growth and productivity. The researchers recorded average LMA values of cocoa planted with many types of trees, cocoa with one-type shade tree and cocoa with no shade trees as 5.90, 7.30 and 8.20 mg/cm^2 respectively. Their explanation was that leaves of cocoa plants under many protective trees had relatively broader and thin leaf morphology while leaves of cocoa plants under full sun had thicker and brighter green leaves.

2.7.2 Leaf Relative Water Content (RWC%)

Leaf relative water content (RWC%) estimates the water content of leaves relative to the maximum amount of water it can hold at full turgidity (Heerden and Villiers, 1996). It is a useful indicator of water balance in the plant (Yamasaki and Dillenburg, 1999). RWC% in drought tolerant plants are mostly higher than in drought sensitive plants. It is defined as the percentage of water present in the leaf at the time of sampling relative to the amount of water when saturated (Tanentzap et al., 2015). RWC% quickly responds to environmental conditions such as temperature and water supply, and it correlates strongly with plants' physiological activities and soil water status (Tanentzap et al., 2015). Values of leave relative water content is used to assess the water status of a plant to, sometimes, reflect the balance between water supply to the leaf tissue, its transpiration rate and cell volume (Yamasaki and Dillenburg, 1999; Lugojan and Ciula, 2011). At low relative water content, stomata may close affecting photosynthesis and therefore yield. Plants may have osmotic adjustments by producing more solutes to attract water. Values of RWC% range between 40% in severely stressed leaves to about 98% in well turgid leaves with most of the crops recording 60% to 70% at wilting though exceptions exist (Lugojan and Ciula, 2011). Water saturation deficit which is opposite to RWC% indicates the condition of water deficiency compared to saturated condition (Zakariyya et al., 2017).

Many researchers have reported leaf RWC% in cocoa worldwide. For five consecutive months of dry season in Indonesia, Zakariyya et al. (2017) noted cocoa RWC% in the range of 70 – 80% while in Ghana, Djan et al. (2017) indicated RWC% between 69% - 75% for cocoa seedlings with enough water while the stressed seedlings rather gave values in the range of 39 – 44%. Another study in Ghana showed parallel results with RWC between 77 – 92% for cocoa seedlings but 42.8 – 65.5% after water stress (Dzanku, 2016). A similar report was given in Malaysia by Mohd (1992) with values ranging from 95% at day zero to 55% at day 12 when

cocoa plants were suppressed with water for 12 days. When plants can maintain RWC% levels for a longer period, then it may mean the plants have higher cell wall strength, ability to minimize mechanical damage or have a higher level of osmoregulatory capacity (Heerden and Villiers, 1996).

2.7.3 Water Potential (-MPa)

Water movement into plants is influenced by water potential (ψ_w) which is defined as the potential energy per unit volume of water (Sane and Singh, 2011). It measures the relative tendency of water to move from one point to another. Water potential indicates the sum of osmotic potential (ψ_s), matric or capillary potential (ψ_m), pressure potential or turgor pressure (ψ_p), and gravitational potential (ψ_g) (Sane and Singh, 2011).

$$\Psi_w = \psi_s + \psi_m + \psi_p + \psi_g$$

where Ψ_w is water potential; ψ_s is potential energy contribution due to solute concentration (osmotic potential, cRT, c- solute concentration, R – the gas constant, T – temperature in Kelvin); ψ_m – the contribution due to the cohesive and tensile interactions of water with the matrix within which it is transported, ψ_p – the pressure potential due to pressure difference and ψ_g – the gravitational potential energy (equal to ρgh; ρ – density of water; g- acceleration due to gravity and h – height of the water column). Water potential of pure water is zero and water moves from less negative potential to more negative potential (Sane and Singh, 2011). The movement of water up the plant is because of the differences of water potential between the soil and the atmosphere surrounding the plant and these differences create a gradient forcing water to move towards areas with less water (Sterling, 2004). Chone et al. (2001) indicated that plant water transport follows four distinct steps: soil to root; root to shoot xylem; shoot to leaf through the petiole; and leaf to atmosphere through the stomata. Therefore, stem water potential could be measured from the leaf when the water potential in the leaf is at equilibrium with that

of the stem. Figures around 0.0 MPa to - 0.2 MPa indicate no water deficit while - 0.8MPa and below usually indicate high water deficit (Deloire and Heyns, 2011).

In their research on drought response of *Theobroma cacao* and the regulations of genes involved in polyamine biosynthesis by drought and other stress, Bae et al. (2008), determined cocoa leaf water potential using the excised leaf disc method in the Wescor HP 115 water potential system and reported that leaf water potential decreased when drought stress was increased for 10 to 13 days recording as low as - 4.5 MPa at midday on the 13th day after drought stress. Carr and Lockwood (2011) reported that at a leaf water potential of about -1.5 Mpa, partial stomatal closure of cocoa leaves begins. Moreover, Moser et al. (2010) had recorded a declining water potential of cocoa coarse roots from - 0.12 MPa to - 0.95 MPa at the sixth month of desiccation of a through fall displacement experiment in adult cocoa in Indonesia. When the predawn leaf water potential was determined, Zanetti et al. (2016) noted a leaf water potential of - 0.85 MPa on non-irrigated plants as against - 0.36 MPa on irrigated plants. In an experiment conducted on young cocoa plants of physiological response to air humidity in Brazil, leaf water potential was affected by humidity and was consistently more negative at low relative humidity. Also, stomatal regulation reflected direct effects of humidity on guard cells rather than responses to changes in bulk leaf water potential (Gomes et al., 1987b). Avila-Lovera et al. (2016) recorded lower average leaf water potential of - 0.41 ± 0.05 MPa on twelve cultivars of young cocoa plants studied during dry season as compared to an average of - 0.21 ± 0.02 MPa during the rainy season but this did not affect the relative water content (RWC) of the plants. Avila-Lovera et al. (2016) elaborated that this low effect of leaf water potential on RWC of the cocoa could be explained by osmotic adjustments of the cocoa tissue through accumulation of solutes and changes of the cell wall module of elasticity during water stress.

2.7.4 Stomatal Conductance - Leaf Gas Exchange (mmol m^{-2} s^{-1})

According to Pask et al., (2012) "Stomatal conductance is an estimation of the potential rate of gas exchange and transpiration through the leaf stomata as determined by the degree of stomatal aperture". Stomatal conductance regulates both transpirational water loss and CO_2 diffusion into the leaves of a plant (Barbour, 2016). The uptake of carbon dioxide is associated with a loss of water by the leaves (Daszkowska-Golec and Szarejko, 2013) and this occurs in the stomata. Two guard cells and sometimes neighbouring cells called subsidiary cells (the function is to support the guard cells) are responsible for opening and closing the stomata (Chavarria and dos Santos, 2012). When turgid, the stomatal pores open and when flaccid due to water loss they close (Chavarria and dos Santos, 2012).

Many models such as ATP model (Farquhar and Wong, 1984); Jarvis and Ball-Berry families of models (Buckley, 2017); OnGuard Model (Chen et al., 2012); and mesophyll-driven, light sensing and sucrose signalling models (Lawson et al., 2014) have tried explaining stomatal movements for uptake of CO_2 for photosynthesis and transpirational water loss as well as their effects on plant water use efficiency. One of these models covering the osmoregulation processes of guard cells is the influx of potassium and chloride through proton pump activation (Chavarria and dos Santos, 2012). From the model, the change in the turgor (hydrostatic pressure) of the guard cells controls the stomatal aperture. The decrease in osmotic pressure in the guard cells is caused by an uptake of potassium ions. A phototropin pigment in the leaves absorbs blue light in the spectrum to activate a proton pump (-ATPase) in the plasma membrane of the guard cells. This pumps protons (H^+) out of the guard cells making the cell more negative and therefore attracts more potassium ions (K^+) into the cell to decrease the osmotic pressure. Water is therefore moved into the cells because of osmotic gradient to make the cells turgid. The gaining of turgor causes the thin outer walls of the guard cells to bulge out while forcing

the inner walls to attain a curve shape to open the stomata. The closing of the stomata is normally triggered by abscisic acid usually when soil water is limited. This leads to many processes leading to the loss of K^+ and anions such as NO^- and Cl^- from the cell. Stomata then closes in response to reduced turgor of the guard cells.

Stomatal conductance has a relationship with plant water potential. Especially, for the isohydric species (species of plants that close their stomata after sensing a drop in soil water potential or an increase in atmospheric water demand), a decline in water potential decreases stomatal conductance (Urban et al., 2017). Average values of stomatal conductance vary between species and sites and are strongly affected by soil moisture, plant conditions, vapour pressure deficit, leaf age and positions, and other prevailing climatic conditions. Also, stomatal conductance is influenced by stomatal size, density, distribution between the leaf adaxial and abaxial sides and the pore dimensions (Fanourakis et al., 2015). Brodribb et al. (2009) measured different species of land plants including higher angiosperms, conifers, ferns and lycopods in Costa Rica at 380 µmol mol^{-1} CO_2 and had stomatal conductance (g_s) ranging from 60 - 280 mmol m^{-2} s^{-1}. Stomata conductance values of 140 – 1260 mmol m^{-2} s^{-1} for selected tropical trees and 50 - 160 mmol m^{-2} s^{-1} for temperate forest trees were also compiled by Mulkey et al. (1996) from three different publications. With their experiment on combining ability, heritability, and genotypic relations of different physiological traits in cacao hybrids, Pereira et al. (2017) recorded cocoa stomatal conductance ranging from 12 – 23 mmol m^{-2} s^{-1}. Water deficit has a greater impact on plant productivity by reducing stomatal conductance, leaf turgor, cell expansion and photosynthesis while increasing abscisic acid (ABA) and solute concentration in the tissue (Lauer and Boyer, 1992). Plants avoid drought damage by reducing transpiration rate through the closure of their stomata.

2.7.5 Sub-Stomatal CO_2 Concentration (µmol mol^{-1})

Sub-stomatal CO_2 (C_i) includes diffused CO_2 through intercellular air spaces, across cell walls and liquid phases to sites of carboxylation; and CO_2 produced after respiration (Evans and von Caemmerer, 1996). CO_2 consumption and production could alter CO_2 concentration in the leaves though atmospheric CO_2 may be constant. Plants responses to atmospheric CO_2 will therefore depend on the effect of changes on C_i concentrations and these responses may have effects on photosynthesis (P_n) and stomatal conductance (g_s) (Mott, 1990). Hence, it is noted that CO_2 has a signalling role to regulate stomata size and other physiological functions (Mott, 1990). Several environmental factors such as water availability and temperature affect C_i concentrations. Plants under stress showed a decreased photosynthetic rate, stomatal conductance, and mesophyll conductance and an associated increase in C_i in wheat cultivars studied (Siddique et al., 1999). Marino et al. (2018) identified a curvilinear relationship between C_i concentration and gs under water deficit experiments. At g_s lower than 150 mmol m^{-2} s^{-1}, Marino et al. (2018) observed increasing C_i to indicate non-stomatal limitations to photosynthesis in Olive (*Olea europea* L.). Sub-stomatal CO_2 concentration has been reported to increase at severe stress conditions (Lawlor, 1995) as a response to loss of chlorophyll fluorescence (Brodribb, 1996) and damages to photosynthetic apparatus at C_i inflexion point (minimum C_i) (Flexas et al., 2002; Mariona et al., 2018). The situation has been confirmed by Haworth et al. (2018) who reported damages to photosystem II in plants subjected to water deficit. Damages may be associated to limitation to CO_2 assimilation and stomatal closure and therefore reducing CO_2 flux across the mesophyll membranes (Tholen et al., 2012; Haworth et al., 2018). Renou et al. (1990), however, indicated that Ci concentration was not affected by water stress though CO_2 concentration in the chloroplast decreased. The study of sub-stomatal CO_2 concentration, as elaborated by Evans and von Caemmerer, (1996), is important because CO_2 gradient within the leaf affects the efficiency of Rubisco and the nitrogen use efficiency.

Limited information is available on the effect of environmental conditions on sub-stomatal CO_2 concentration on cocoa.

2.7.6 Transpiration Rate (mm d^{-1} ; mmol m^{-2} s^{-1})

Transpiration is simply the movement of water vapour out of the aerial parts of the plant mostly through the stomata of the leaves**.** It is noted that when water is available, plants can have a considerable ability to adapt to their growth conditions to function even at extremely high temperature (Kirschbaum, 2012). This indicates the necessity of water to plants for physiological functions. Most of the water absorbed by plants are transpired into the atmosphere and the rate of transpiration is affected by the differences in the vapour pressure deficit in the atmosphere and the interior of the leaf as well as by stomatal conductance. Increasing temperature could increase vapour pressure deficit of the air which would lead to a concomitant increase in transpiration rate. As elaborated by Bareja (2013), plants respiration is also affected by root-shoot ratio, leaf area, number of stomata, leaf structure and stomatal movement. Plants with higher root-shoot ratio tend to transpire faster. Thus, plants with large root surface area will absorb more water than plants with small root surface area. Also, structural features on the leaves such as thick cuticles, thick cell walls, sunken stomata and hairs on the leaves reduce the rate of transpiration. Plants also regulate transpiration by stomata closure, but this could also lead to a reduction of the entry of CO_2 into photosynthetic cells (Kirschbaum, 2012). Thus, rate of transpiration is affected by the stomata aperture and density. There is almost always a correlation between transpiration and stomatal conductance and, that plants normally regulate their stomatal conductance to maintain a specific transpiration rate over a different ranges of vapour pressure deficit (VDP) (Urban et al., 2017), except, maybe, when the ambient air is fully saturated with water.

De Almeida and Valle (2007) estimated the daily transpiration rate of a 7-year-old cocoa tree to be 90L per tree (2.4 L $m^{-2}d^{-1}$) for unshaded trees and 40 L per tree (1.2 L $m^{-2}d^{-1}$) for shaded trees when 100% of the leaf area was exposed to incident irradiance on a sunny day. On a cloudy day, the rate of transpiration was reduced to 45 L per tree (1.2 L $m^{-2}d^{-1}$) for non-shaded cocoa tree and 26 L per tree (0.8 L $m^{-2}d^{-1}$) for shaded cocoa trees. Using the Penman-Monteith equation, the daily transpiration rate of cocoa was computed to be between 3.0- and 6.1-mm d^{-1} during the wet season and from 1.0 to 1.9 mm d^{-1} during the dry season in Cote d'Ivoire when annual rainfall was 1500 mm (Radersma and Ridder, 1996; Carr, 2012). Total evapotranspiration was estimated to be 584 mm for the wet season and 294 mm for the dry season (Carr, 2012) to indicate higher transpiration when water is non-limiting but stomatal regulation when water is limited. With the sap-flow technique, transpiration in 8 years cocoa plant was reported to fluctuate between 2.5 to 3.0 L dm^{-2} h^{-1} on dry days but relatively stable in wet days (Carr, 2012). Kohler et al. (2009) also reported monthly transpiration rate in the range of 0.46 – 0.58 mm d^{-1} for cocoa trees. In this research, transpiration was rather expressed in mmol m^{-2} s^{-1} instead of the different reported units (such as mm d^{-1} or L $m^{-2}d^{-1}$) in the review.

2.7.7 Respiration Rate (µmol m^{-2} s^{-1})

Respiration involves the breakdown of sugar produced after photosynthesis, to release energy. Through the process, energy conserved and stored in organic molecules is released to produce ATP which is the universal currency of biological energy transformations (Gonzalelez-Meler et al., 2004). Net leaf photosynthesis is equal to gross photosynthesis minus leaf respiration (Lin et al., 2012). Bruhn (2002) noted that it is through respiration that the plant creates the necessary ATP, reducing equivalents, intermediary C-skeletons for e.g., amino acid synthesis, and in some instances heat. Energy and carbon skeletons produced by mitochondrial respiration are used in various processes essential for growth, maintenance, nutrient uptake, and transport

within plants. Bruhn (2002) further stated that respiration in plants can be divided into two components, respiration associated with growth of new plant mass and ion uptake as well as respiration linked to maintenance of existing plant mass. Respiration in plants is like that in animals where oxygen is used to break down glucose to release carbon dioxide at the site of the mitochondria. One-third and, under stressful conditions, as much as two-thirds of a plants' daily fixed carbon dioxide can be respired. According to Van der Werf (1994), plants can release up to 70% of their daily fixed Carbon through respiration depending on the environmental conditions and the species.

2.7.8 Net Photosynthetic Rate (µmol m^{-2} s^{-1})

Net photosynthetic rate (P_n) indicates the difference between rate of carbon fixation during photosynthesis and the rate of carbon dioxide loss during respiration. It has been identified to be high during periods of low evaporative demand while water deficit reduces rate of photosynthetic. Optimum temperature for photosynthesis is reported to range between 31 – 33 ^oC with light saturation occurring at 400 µmol m^{-2} s^{-1} (Balasimha et al., 1991). On the other hand, higher P_n values coincides with higher water potential values on diurnal readings (Balasimha et al., 1991).

2.7.9 Water Stress and Rate of Photosynthesis

Plant cells need aqueous medium to function, therefore, photosynthesis is impaired when plant water status falls below critical values (Kirschbaum, 2004). At lower moisture content of the soil, stomata are closed to conserve water, but this decreases rate of photosynthesis and builds up O_2 in the leaf. This might lead to low [CO_2]: [O_2]. In this way, Rubisco (Ribulose- 1,5-bisphosphate oxygenase-carboxylase) binds to oxygen instead of carbon dioxide and adds it to RuBP (ribulose – 1, 5 - bisphosphate) especially under high temperature (Hajiboland, 2014).

The addition of oxygen to RuBP leads to Photorespiration. The process wastes energy, reduces the rate of carbon assimilation and energy efficiency of photosynthesis (Lopez and Barclay, 2017; Dokulil and Kaiblinger, 2009).

Stomatal control of water loss is noted to be an early event in plant response to water deficit that leads to a limitation of carbon uptake by the plant leaves. Rate of photosynthesis is reduced because of the restriction of diffusion of CO_2 into the leaf caused by stomata closure and inhibition of CO_2 metabolism (Tezara et al., 1999). This is supported by Ort et al. (1994) who reported a decreased rate of net photosynthesis at low CO_2 concentrations. Siddique et al. (1999) noted a decreasing rate of photosynthetic rate with decreasing stomatal conductance because of water stress, however, a weak relationship between photosynthetic rate and stomatal conductance implies the influence of non-stomatal limitations to photosynthesis. Stomatal response is often more closely linked to soil moisture content due to a response to chemical signals (e.g., ABA) produced by dehydrating roots (Chaves et al., 2002).

The response to drought stress by plants depends on the duration, intensity, and rate of exposure as well as stage of growth of plants (Brar et al., 1990). When drought stress is very severe, photodamage of PSII may result. Although photorespiration reduces net rate of photosynthesis, the photosynthetic reduction of O_2 through photorespiration increases and serves as a sink for excess excitation energy to prevent damages to PSII reaction centres. Under severe drought conditions, electron transport to O_2 and increased quenching of excitation energy in the PSII antennae may be unable to dissipate the excess excitation energy in the PSII antennae and this may result in photodamage of PSII (Nogues and Baker, 2000).

2.7.10 Temperature and Rate of Photosynthesis

Temperature restricts the location in which cocoa can be grown (Daymond and Hadley, 2004). Temperature has effect on the light independent reactions of photosynthesis as it affects

enzymatic activities. High temperature denatures enzymes, reduces turgidity of mesophyll cells, and decreases rate of photosynthesis. An experiment on the effect of low and high temperature on the photosynthetic performance of *Lantana camara* L. leaves in darkness showed a temperature range below -5.0^{o}C and above 47.50^{o}C causing permanent damages to the photosynthetic apparatus and the leaf tissues (Carrion-Tacuri et al., 2013). At least, three major stress-sensitive sites of the photosynthetic machinery are noted, and these include the PSII, the ATPase and the carbon assimilation process (Mathur et al., 2010). In their review, Song et al. (2014) mentioned that among the photosynthetic apparatus, photosystem II is noted to be the most heat-sensitive component. This is confirmed by the earlier work of Chen et al. (2012) on fingered citron (*Citrus medica var. sarcodactylis* Swingle) indicating a high temperature above 40^{o}C causing a reduction of CO_2 assimilation, and inactivation of photosystem II and photosynthetic electron transport. The two factors making PSII electron transport highly susceptible to heat stress include (1) increase in fluidity of thylakoid membranes at high temperature causes dislodging of PSII harvesting complexes from thylakoid membrane and (2) dependence of PSII integrity on electron dynamics (Mathur et al., 2014). Photoinhibition mainly occurs in PS II under heat stress and over reduction of electron transport chain results in the generation of large amounts of reactive oxygen species which are responsible for the damage (Essemine et al., 2012). High temperature increases Rubisco's affinity for O_2 and leads to photorespiration. This results in the production of reactive oxygen species (ROS) such as H_2O_2 that are poisonous to the plant, causes thylakoid structural disorganization and negatively affect chloroplasts and mitochondria (Chen et al., 2012). Also, high temperature inactivates the enzyme system which changes sugars to starch and then builds up sugar in the cell to slow down the rate of photosynthesis (Bajera, 2011). High temperature is also noted to initially disrupt the water oxidizing complex (WOC), PSII reaction centres and the light harvesting complexes (LHC) (Salvucci et al., 2001; Mathur et al., 2014).

Plants respond to high temperature and excessive exposure of light by increasing photooxidation capacity and reducing CO_2 assimilation capacity (Chen et al., 2012). Plants also overcome heat and light stress by reducing the rate of electron transport as they convert the excessively absorbed light into thermal energy (Guruani et al., 2015). The excess thermal energy is dissipated as heat in a process called non-photochemical quenching of Chl fluorescence (NPQ) (Essemine et al., 2012; Guruani et al., 2015). NPQ reduces the concentration of chlorophyll excited states in PSII by activating a heat dissipation channel (Guruani et al., 2015). Some plants avoid heat stress through changing their leaf orientation, transpirational cooling, stomatal closure, leaf rolling and early maturation. Other species tolerate the heat stress through alteration of lipid membrane composition, protein synthesis and transcriptional control, expressions of stress proteins, accumulation of osmoprotectants, and induction of antioxidant defense systems (Mathur et al., 2014).

2.7.11 Water Use Efficiency (mmol mol^{-1})

Water Use Efficiency (WUE), a concept introduced 100 years ago, is the ratio of water used for photosynthesis to water lost through transpiration at the same period (Hatfield and Dold, 2019; Tambussi et al., 2007; Water, 2004). The concept has three definitions which include instantaneous water use efficiency, integrated water use efficiency and intrinsic water use efficiency. The instantaneous water use efficiency is the ratio of the rate of photosynthesis to the rate of transpiration while integrated water use efficiency indicates the ratio of produced biomass per the rate of transpiration at the same period (Water, 2004; Tambussi et al., 2007; Lambers et al., 2008). Intrinsic water use efficiency, on the other hand, compares the ratio between the rate of photosynthesis to stomatal conductance (Tambussi et al., 2007). WUE in a simple term measures carbon gain relative to water lost (leakay et al., 2019). It is a parameter used to select clones for drought tolerance. When crop productivity is improved under limited

water availability, it contributes to global food security and sustainability (Balyan et al., 2017). Ayegboyin and Akinrinde (2016) recorded instantaneous water use efficiency between 3.0 and 4.9 mmol mol^{-1} among four genotypes of cocoa studied while intrinsic water use efficiency was in the range of 42.3 – 67.4 µmol mol^{-1} for all the genotypes under the various treatments. Four main factors affecting plant water use efficiency include carbon dioxide concentration, temperature, precipitation, and humidity (Hatfield and Dold, 2019). The effects of temperature and precipitation are being considered here.

Increasing temperature beyond a threshold for plant growth, such as above 33 ^{o}C for cocoa, reduces water use efficiency due to increased rates of evapo-transpiration (Bhattacharya, 2019). Higher temperature, under low relative humidity, increases transpiration with the change of leaf surface vapour pressure deficit (VPD). Water loss by transpiration therefore increases due to the driving forces for the exchange of water vapour from the leaf surface to the surrounding atmosphere (De Geijn and Gourdriaan, 1996).

Plant water use efficiency is negatively correlated with annual precipitation (Zhan et al., 2015) and therefore increases under water stress conditions (Rada et al., 2005). Reduction in precipitation increases the physiological stress of plants. WUE is increased under such conditions to mitigate the impacts of moisture deficiency (Zhan et al., 2015). Under high water stress, stomatal conductance is reduced and thereby improving WUE. WUE of four-year Guasare cocoa plants increased from 0.98 mmol mol^{-1} when plants were stressed for three days to 1.19 mmol mol^{-1} when stressed for 25 days (Rada et al., 2005). Under water deficit, more CO_2 is taken up at the leaf surface per unit water transpired. Thus, intrinsic, and instantaneous water use efficiencies are often improved in water deficit plants (Lahive et al., 2018). Hebbar et al. (2019) identified water use efficiency increasing from 2.2 mmol mol^{-1} under 50% field capacity to 3.4 mmol mol^{-1} under 100% field capacity for their cocoa trial in India. The improvement of water use efficiency, as they indicated, might be due to higher declining rate

of transpiration and stomatal conductance than photosynthesis during the stress period. The researchers indicated root growth and stomatal regulation as the key mechanisms for water conservation in cocoa during water deficit. Deductions from these researchers suggest that WUE increases under water stress due to higher rate of decline of transpiration than photosynthesis. Other researchers, however, have had different views. With effect of elevated CO_2 and water stress on cocoa plants, Lahive et al. (2018) reported cocoa instantaneous water use efficiency ranging between 4 and 10 mmol mol^{-1} while intrinsic water use efficiency was noted to be between 100 to 180 umol mol^{-1}. Although CO_2 treatments resulted in significant differences, water deficit had no significant effect on the plants. The researchers argued that the absence of effects might be due to a strong reduction in both stomatal conductance(g_s) and light saturated photosynthesis (P_{max}). Thus, WUE during drought may be controlled by severe stress conditions which impacted on photosynthetic efficiency owing to changes in photosynthetic metabolism or the occurrence of oxidative stress. De Almeida et al. (2015) noted differences in response of various cocoa cultivars to WUE under water deficit. Some cultivars recorded increasing WUE while other cultivars recorded a declining rate. The researchers further suggested that there is no general response of WUE to drought in cocoa and that high leaf water status rather than optimizing water use is the priority in the cocoa physiology.

2.7.12 Chlorophyll Fluorescence (F_v/F_m)

Photosynthesis involves many components such as CO_2 reduction pathways, photosynthetic pigments and photosystems, and the electron transport systems (Ashraf and Harries, 2013). The primary target of heat injury in plants is the Photosystem II oxygen-evolving complex with the associated cofactors (Allakhverdiev, 2008). Chlorophyll fluorescence reflects the photochemical activity of photosystem II (PSII) and is therefore used as a parameter to detect

and quantify temperature-induced changes in the photosynthetic apparatus (Chen et al., 2012). Chlorophyll fluorescence is the light re-emitted by chlorophyll molecules when excited electrons are returning to the ground state. According to Maxwell and Johnson (2000), when light energy is absorbed by the leaf of the plant, it has three main destinies – i) to drive photosynthesis (photochemistry), ii) excess energy is emitted as heat, iii) and it can be re-emitted as light (chlorophyll fluorescence). When chlorophyll molecule absorbs light energy, it becomes excited and moves from its ground state to the excited state. The energy is either passed on to the next chlorophyll molecule to be used for photosynthesis or the excited chlorophyll could return to the ground state by emitting the energy as heat or as fluorescence (Muller et al., 2001). The increase in the efficiency of any one of them would lead to the decrease of yield of the other two. These are the main principles behind chlorophyll fluorescence analysis. It is a commonly used technique to measure photosystem II (PSII) activity as an indicator to plants response to environmental changes including sensitivity of PSII to biotic and abiotic factors (Murchie and Lawson, 2013). Analyzing the fluorescence signals provide information on the status and function of PSII reaction centres, light harvesting antenna complexes and donor/acceptor sides of PSII (Kalaji et al., 2016).

Fluorescence yield can be quantified by exposing a leaf to a light with a defined wavelength and then measuring the amount of light re-emitted at longer wavelengths (Maxwell and Johnson, 2000). The light system is switched on and off at a high frequency and a detector is tuned to detect only fluorescence excited by the light. The measurements could be dark adapted (keeping the leaf in a dark for some minutes e. g. 30 minutes) or light adapted. Dark adapted measurements involve parameters such as F_o – minimum fluorescence yield that occurs when a measuring light is switched on when there is virtually no electron transport, F_m – maximum fluorescence yield which is a fluorescent yield equivalent to that which would be attained in the absence of photochemical quenching during the flash of light, and F_v- variable fluorescence

yield which is the difference between F_o and F_m. Similarly, light adapted measurements involve parameters such as F'_o – minimum fluorescence yield, F'_m – maximum fluorescence yield F'_v – variable fluorescence yield. The other parameter is F_s or F_t – steady state fluorescence yield. From these parameters, a number of deductions include;

2 Maximum quantum yield of PSII $\mathbf{F_v/F_m = (F_m - F_o)/F_m}$ - this is the ratio of variable to maximum fluorescence yield. It indicates the quantum efficiency of open photosystem II centres (Maxwell and Johnson, 2000; Murchie and Lawson, 2013). For a healthy or an unstressed leaf, the F_v/F_m value is around 0.83 and it correlates to the maximum quantum yield of photosynthesis (Murchie and Lawson, 2013; Demmig and Bjorkman, 1987). Values lower than 0.83 is an indication of stress conditions (Maxwell and Johnson, 2000). The prevailing environmental conditions such as light or temperature causes a greater or lesser proportions of reactions centres to close and closure will cause a decline in quantum efficiency of PSII (Murchie and Lawson, 2013).

3 Quantum yield of PSII $\mathbf{\Phi_{PSII} = (F'_m - F_t)/F'_m}$ – This measure the proportion of light absorbed by chlorophyll associated with PSII that is used in photochemistry (Maxwell and Johnson, 2000). Apparent quantum efficiency (Φ) is one of the important photosynthetic parameters in shade conditions to indicate how efficiently available light is utilized (Lahive et al., 2018). As indicated by Pallady, (2008), the efficiency of the photosynthetic apparatus under light limiting conditions is indicated by the quantum efficiency, the initial slope of the absorbed light versus photosynthesis curve.

Using this formula $\mathbf{J = \Phi_{PSII} \times PFDa \times (0.5)}$, where PFDa is absorbed light (µmol photon m^{-2} s^{-1}) and 0.5 a correction factor for the partitioning of energy between PSII and PSI, the linear electron transport rate (J) could be calculated to deduce overall photosynthetic capacity in vivo (Maxwell and Johnson, 2000; Genty et al., 1989).

4 Photochemical quenching **qP = (F'$_m$ - F$_t$)/ (F'$_m$- F'$_o$) -** This measures the proportion of open PSII or the proportion of PSII centres that are opened (Murchie and Lawson, 2013). The proportion of PSII centres that are closed (excitation pressures) could be calculated from this formula by **1 – qP.**

5 Non-photochemical quenching **NPQ = (F^o_m- F'm)/F'm -** Non-photochemical quenching is related to the amount of heat dissipated during photochemistry. At saturating light intensities, values might range between 0.5 – 3.5 but values may vary depending on species and plant history (Maxwell and Johnson, 2000).

Three main types of quenching are deduced from the fluorescence measurements and these are fluorescence quenching (the falling off of fluorescence level over a time scale after earlier rise when a leaf is transferred from darkness to light), photochemical quenching (the increase in the rate at which electrons are transported away from PSII due to light induced activation of enzymes involved in carbon metabolism as well as the opening of the stomata) and non-photochemical quenching (the increase in efficiency at which energy is converted into heat) (Maxwell and Johnson, 2000).

In their research on the effect of irradiance level on cocoa (*Theobroma cacao* L.): II. Gas exchange and chlorophyll fluorescence, Galyuon et al. (1996) indicated that chlorophyll fluorescence showed a reduction in the photochemical efficiency of PSII and the rate of electron transport under full sunlight. Notwithstanding, net photosynthesis increased under full sun even though half time fluorescence rose and the F_v/F_m was reduced. Increased leaf fluorescence was observed in the blue and green spectral ranges when cocoa leaves were stressed for 10 to 13 days, and fluorescence emission was lower in the stressed leaves than the control (Bae et al., 2008). For F_v/F_m, values ranging between 0.72 – 0.77 were recorded during the rainy season and 0.65 – 0.73 during the dry season of five cultivars of criollo cocoa. Araque et al. (2012) observed a decreased maximum quantum yield of PSII (F_v/F_m) for all the cultivars

during the dry season compared to the wet season for two years established criollo cocoa plants. Further observations indicated qN values ranging between O. 83 – 0.88 during the rainy season and 0.81 – 0.89 during the dry season indicating no rise of non-photochemical quenching during the water deficit period which might be, perhaps, because the cultivars developed under relatively low light conditions. F_v/F_m values of 0.83 could be translated as 83% efficiency of the photosystems, which indicates full functioning of the photosystem II (Falkowski and Raven, 2007). Feller and Vaseva, (2014) found fluorescence yield of 0.80 for healthy leaves with values below as an indication of irreversible damage to Photosystem II apparatus. A work by Acheampong et al. (2013) on influence of shade regimes on the photosynthetic activities of different clones of cocoa in Ghana showed that F_v/F_m was consistently higher under heavier shade and that F_v/F_m values between clones varied between 0.55 and 0.80. In a study from Brazil on influence of low light intensity and soil flooding on cacao physiology, Branco et al. (2017) noted that F_v/F_m were influenced by genotypes of cocoa and water regimes and that significant decrease of F_v/F_m was observed in flooded plants for the genotypes studied.

2.7.13 Electron Transfer Rate during Photosynthesis (µmol m^{-2} s^{-1})

Electron transfer rate describes the rate at which electrons move from one molecule to another and the parameters that control the movement. In the process of photosynthesis, the photosynthetic pigment such as chlorophyll a absorbs light energy. This causes the excitation of the electrons in the pigment thus the electrons jump to a higher orbital position. The excited electrons can either return to the lower orbit and then emit the energy as heat (Non-photochemical Quenching (NPQ), or as photons of light, (the re-emission of light as fluorescence); or the electrons return to the lower orbit again and pass the energy to other neighbouring molecules (energy acceptor molecules); or the excited electron itself moves to an electron acceptor molecule (Graham et al., 2003). During photosynthesis, linear and cyclic

electron flow occur. For the linear mode, the transfer of electrons is from water to the NADP via photosystem II (PSII), cytochrome b_6f (cyt bf), and photosystem I (PSI) which are the three major complexes of the photosynthetic pathway (Joliot and Joliot, 2002). At PSII (P680), light energy is used to split water molecules to generate electrons and protons. Oxygen is a by-product.

$$2H_2O \longrightarrow 4H^+ + 4e^- + O_2$$

The electrons are carried by plastoquinone (a mobile electron carrier) to cytochrome b_6f (a proton pump) and then by plastocyanin molecules to PSI (P700) (Figure 2.1). From PSI, the electrons are either transferred back to plastoquinone to start a cyclic electron flow or are transferred to Ferrodoxin.

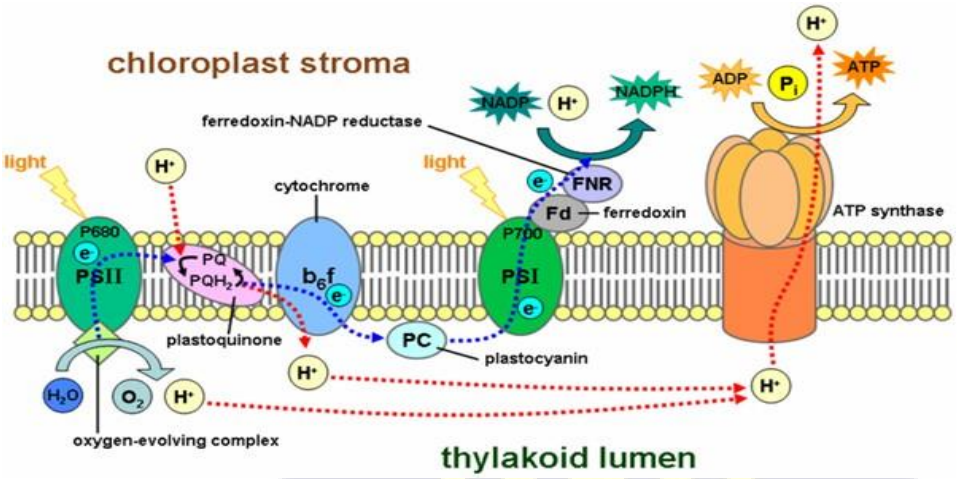

Figure 2.1 Diagram Showing Light Harvesting Complexes, Electron Generation and Transfer through the Photosystems and the Electron Carriers. Source: https://slideplayer.com/slide/4183502/

This process leads to conversion of $NADP^+$ to NADPH, an energy carrier, by an enzyme $NAPD^+$ reductase. The protons (H^+) produced along the path are used to produce ATP by the activities of ATP synthase (an enzyme). The cyclic mode happens in PSI, where excited electrons from reaction centres of PSI move back to plastoquinone to start the process again to

generate more ATP. Here, no water is split, no oxygen is released, and no NADPH is made. Only the production of ATP is increased (Graham et al., 2003). ATP is an energy carrying molecule that provides energy to drive the Calvin cycle while NADPH is an electron donor, providing electrons and hydrogen to combine carbon dioxide to produce glucose in the Calvin cycle.

When net CO_2 assimilation proceeds at lower quantum yield of PSII, the excess photon energy may damage PSII by stimulating the production of reactive oxygen species. To reduce this effect excess photon energy are dissipated as heat serving as a protective mechanism (known as non-photochemical quenching (NPQ) of chlorophyll fluorescence (Miyake et al., 2005). The researchers further identified a strong NPQ of chlorophyll fluorescence in high light plants than in low light plants in Tobacco. In a study on photosynthesis limitations in cacao leaves under different agroforestry systems in the Colombian Amazon, Salazar et al. (2018) recorded electron transport rates (ETR) in the range of 0 – 80 µmol m^{-2} s^{-1} for all the treatments depending on the amount of PAR and it increased under high light treatments than under medium and low light treatments. The same pattern was observed with NPQ. When cocoa plants were subjected to open sun for about 17 weeks, electron transport rate and PSII efficiency declined indicating occurrence of photodamage and photoinhibition (Galyuon et al., 1996; Lahive et al., 2019). Notwithstanding, higher rates of transpiration, stomatal densities and leaf thickness were recorded with treatments under open sun. Jaimez et al., (2017) identified high ETR and NPQ under high photosynthetic photon flux density for all the cultivars of cocoa studied with values ranging between 40 to 90 µmol m^{-2} s^{-1}. The researchers further indicated that high ETR under high PPFD leads to high rate of photosynthesis due to provision of amount of ATP and NADPH needed to drive photosynthesis. They, however indicated that very high PPFD may cause intercepted light energy to exceed the capacity of

photosynthetic machinery and could cause photoinhibition resulting in a decrease in maximum quantum efficiency of PSII (F_v/F_m) and photosynthesis.

Araque et al. (2012) recorded ETR values ranging between 47.9 ± 5.2 – 63.3 ± 8.7 µmol m^{-2} s^{-1} during the raining season and 43.3 ± 5.0 – 53.2 ± 5.0 µmol m^{-2} s^{-1} during the dry season for the four cultivars of cocoa studied. No significant differences existed between the two seasons for all the cultivars though reduction occurred during the dry season for all the cultivars.

2.8 Soil Moisture (m^3/m^3)

Soil moisture is the amount of water stored in the soil or held in soil surfaces (Acker et al., 2003). The indicators of water availability in the soil include hydraulic conductivity, infiltration, soil moisture, water filled pore spaces, ponding patterns and water holding capacity (NRCS, 2011). Soil infiltration as the downward entry of water into the soil is an indicator of the soil's ability to allow water movement into and through the soil profile (Lowery et al., 1996). Restricted infiltration leads to poor soil aeration causing poor root functions, reduced nutrient availability, and recycling by soil organisms (Lowery et al., 1996). Soil water content (SWC), the amount of water present in the soil, influences plant growth, soil temperature, transport of chemicals and groundwater recharge (Datta et al., 2018). Volumetric water content (VWC) and Soil matric potential (SMP) are normally used to assess SWC. VWC is the ratio of the volume of water to the unit volume of soil while SMP (also called soil suction or soil water tension) is the force that binds water molecules to solid particles in soil pores (Datta et al., 2018). When the movement of water through the soil matrix is restricted because of SMP, plants must apply a stronger force to extract water from the soil. Once this situation continues for a longer duration, leaves may start drooping to eventually reach permanent wilting point (PWP). PWP is the threshold where plants are not able to extract water at a rate fast enough to keep up with their water demand (Datta et al., 2018) and from which the plants cannot recover. The water molecules are held so strongly to the soil particles to the extent that plant roots are

not able to extract them making transpiration and other processes vital for plants' survival come to a near stop (Datta et al., 2018). This situation may lead to stunted growth and death of the plants. For example, young cocoa plants are more sensitive to changes in soil moisture content and will not recover if stressed beyond critical levels (Lahive et al., 2019).

Rise in temperature and a decrease in relative humidity increase evapotranspiration and therefore decrease the soil water content whereas drought causes a decrease in water infiltration, storage, and plant water supply (Karmakar et al., 2016). This, also, affects organic matter turnover. It is noted that warm temperature and adequate soil moisture are necessary for the decomposition of organic matter indicating that the physical properties of the soil, also, have effect on the nutrient distribution in the soil. As nutrients are carried to the roots by water, drought decreases nutrient diffusion over short distances; mass flow of water-soluble nutrients such as nitrate, sulphates, Ca, Mg and Si also decrease over long distances (Karmakar et al., 2016).

Cocoa has most of the roots found within the 0.4 – 0.8 m of the soil profile with over 86% of it within the 0.4 m zone and therefore not adapted for deep water extractions (Moser et al., 2010; Lahive et al., 2019). It is also noted that the stem xylem vessel has larger diameter than the root xylem vessel in cocoa and this may contribute to the plant's sensitivity to cavitation under drought conditions (Kotowska et al., 2015). Many researchers have contributed to the knowledge on soil moisture content under cocoa production and their findings give a guideline on the water distribution within the soils under cocoa production. In Indonesia, volumetric water content (Θ) was around 0.38 – 0.48 m^3/m^3 at a depth of 10 and 75 cm but around 0.30 – 0.37 m^3/m^3 at a depth of 150 cm in cocoa control plots indicating lower water content in the deep horizons which might be due to higher bulk density at such horizons. This was confirmed by Schwendenmann et al. (2010) who recorded a bulk density ranging from 1.25 g/cm^3 to 1.60 g/cm^3 as the soil depth increased from 0.05 m to 2.50 m. During the dry periods, roofed plots

showed a progressive decrease in volumetric water content with values 0.12 – 0.15 m^3/m^3 lower than the control plots in Indonesia (Moser et al., 2010).

In Ghana, it was detected that soil water was much depleted in the top 60 cm of the soil horizon especially during dry seasons indicating competition for soil moisture between cocoa roots and selected shade trees under study (Issaka et al., 2017) (Figure, 2.2). Volumetric water content for all the treatments ranged between 0.1 – 0.4 m^3/m^3 showing similarities with what was recorded in Indonesia by Moser et al. (2010). However, in Ghana, soil water content at the deep horizons even during the wet season was higher than the upper horizons as against what Moser et al. (2010) reported in Indonesia.

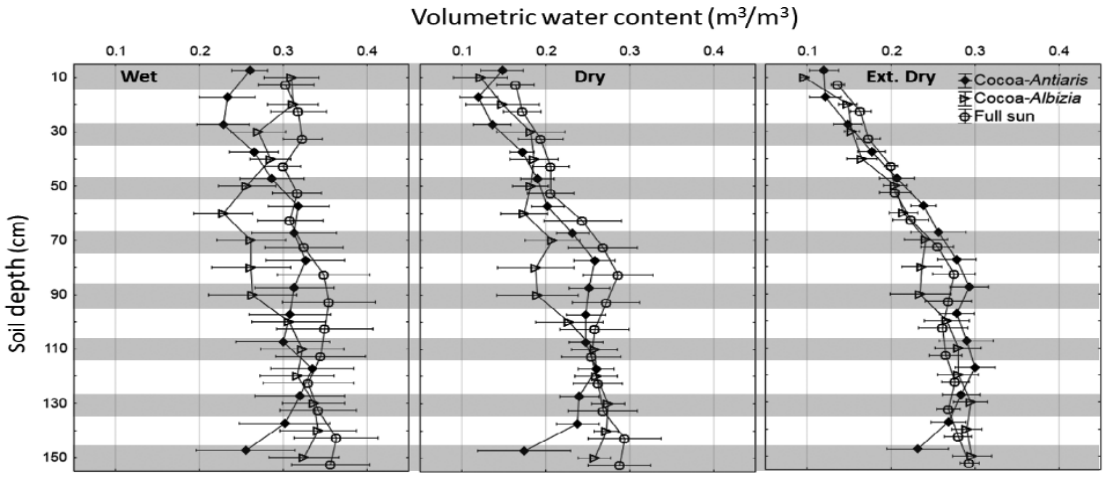

Figure 2.2 Variation in Mean Volumetric Soil Water Content within the Soil Profile of Three Cocoa Agroforestry Systems During (a) the Wet Month (October) (b) the Dry Month (January 2015) and (c) Extremely Dry Months (January – February 2016) in Ghana. Source: Abdulai et al. (2017). Cocoa agroforestry is less resilient to sub-optimal and extreme climate than cocoa in full sun.

For their work on rubber plantation, Wu et al. (2016) in South-Western China identified that soil water decreases gradually from topsoil to deep soil layers. For the rubber intercropped with cocoa, the researchers noted that cocoa trees absorb about 73.5% of their water from the topsoil

while seasonal changes occurred only in the 5 – 15 cm soil layer (Wu et al., 2016). At volumetric soil water content between 0.08 to 0.14 m^3/m^3 in loam and sandy loam soils, plants normally experience permanent wilting point (Saxton and Rawls, 2006). Schwendenmann et al. (2010), however, noted that cocoa plants could still thrive when soil water levels are lower than 0.2 m^3/m^3 in the top 75 cm. Issaka et al. (2017) argued that higher relative humidity and lower vapour pressure deficit during the study period of Schwendenmann et al. (2010) might have resulted in lower evaporative demand making the cocoa survive under lower soil moisture content especially under shade as compared with full-sun cocoa.

Under cocoa monoculture, soil moisture mainly decreases in the uppermost layer during the dry season but are high in the lower layers throughout the year (Niether et al., 2017a). On the other hand, Niether et al. (2017a) recorded higher moisture content at the upper layer of the soils under agroforestry than under the monoculture system implying the role shade trees play in conserving soil moisture. Therefore, cocoa agroforestry with compatible trees may help buffer cocoa plants against drought. Alternatively, maintaining field capacity at the top 0.3 m of the soil profile through supplementary irrigation could increase pod yield by 40% (Hutcheon, 1973).

2.9 Leaf Litter Fall (Mg ha^{-1})

Litterfall represents the total mass of plant materials such as leaves that falls to the ground normally per unit area (Vogt et al., 1986). Litter is an important way for energy and organic matter transfer from plant canopy to decomposer organisms of the soil surface (Carnevale and Lewis, 2001). It is a quantitative parameter for stand vitality and provides information on the canopy conditions of the plants (Pitman et al., 2010). Litter biomass is used to quantify the annual return of organic matter and nutrients into the soil (Pitman et al., 2010). The analysis of litter contributes to organic matter production rate, nutrients availability and its recycling capacity. It is a habitat for soil organisms, an effective interface between vegetation and soil, a

means of returning carbon to the soil, a protective layer to limit erosion, and a technique to reduce soil compaction and losses by drainage (Averti et al., 2019).

Factors such as temperature and soil moisture availability influence litter production of a plant. Drought and lower night temperature stimulate abscisic acid synthesis in the plant foliage leading to leaf senescence and litter fall (Yang et al., 2003; Dawoe et al., 2010). Reduced soil moisture increases canopy water stress upon which plants shed their leaves to reduce the effect. Net primary productivity (The rate at which energy is stored as biomass = gross primary productivity – rate of energy loss to metabolism and maintenance) may decline with low soil moisture, and its relative allocation among leaves, stems and roots may be affected (Brando et al., 2008). It is increased when there is enough photosynthetically active radiation with adequate soil moisture to speed photosynthesis. Low soil moisture, light and high temperature may affect this connection and therefore its effect on leaf functions. Brando et al. (2008) observed a declining litter production in the water stressed plots as compared with the control plots as exclusion progressed, but litter production was higher in the stressed plots during the first years of exclusion (Figure 2.3). With these observations the researchers concluded that allocation of above-ground net primary productivity (ANPP) to wood and litter was highly sensitive to soil moisture availability.

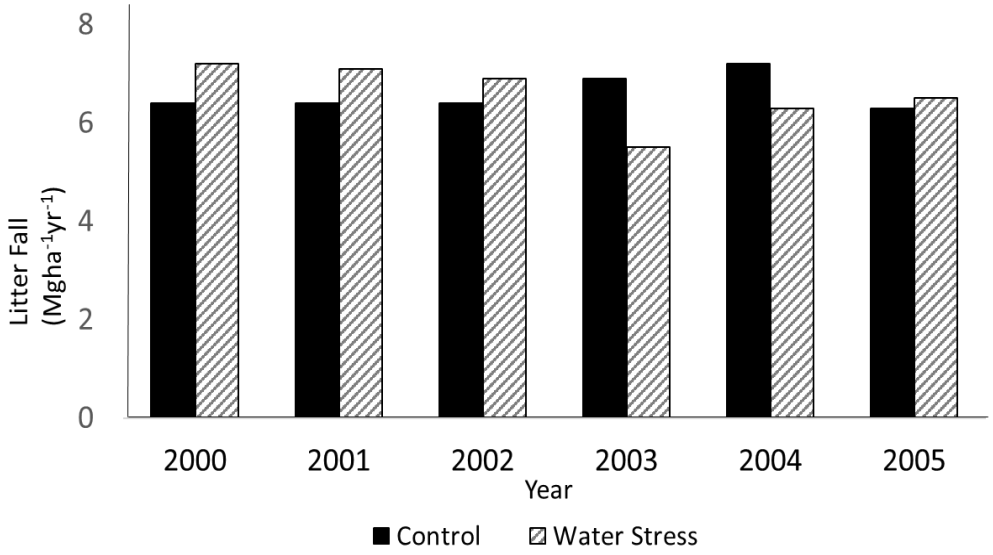

Figure 2.3 Effect of Water Stress on Litter Production in Amazon Forest

Source: Brando et al. (2008). Drought effects on litterfall, wood production and belowground carbon cycling in an Amazon Forest: results of a throughfall reduction experiment.

Dawoe et al. (2015) studied litter production under cocoa agroforestry systems in the Ashanti region of Ghana. The region falls within the wet semi-equatorial rain forest climate zone and the cocoa agroforestry was the mixed stands of cocoa and variable proportions of naturally generated upper canopy shade trees such as trees for timber and fruit trees. The results showed that litter production peaked during the dry season and reduced during the rainy season (Figure 2.4) and that the older cocoa plants (15 and 30 – year old treatments) produced much litter as compared with the 3-year-old system. Total litterfall productions were reported to have shown a significant increase with time after forest conversion and ranged between 5.0 – 10.4 Mg DM $ha^{-1}yr^{-1}$.

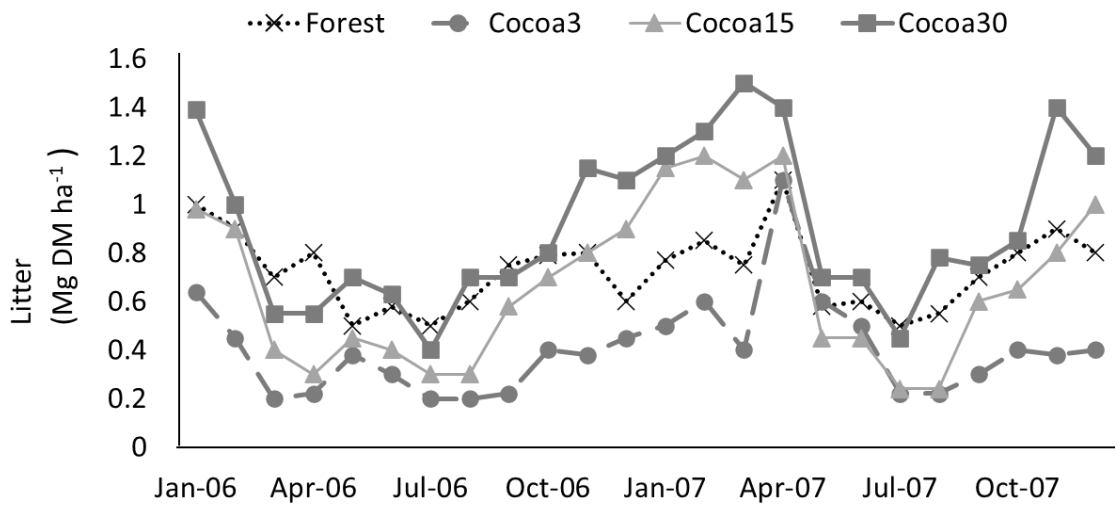

Figure 2.4 Monthly Litterfall Production (Mg DM ha^{-1}) at the Cocoa Agroforestry System in the Wet Semi-equatorial Rain-forest Climate Zone Ghana. Source: Dawoe et al. (2010). Litterfall and litter nutrient dynamics under cocoa ecosystem in lowland humid in Ghana

The earlier research by Owusu-Sekyere et al. (2006) showed a litter production of secondary forest ranging between 6.5 - 9.1 t ha^{-1} and that litter fall was higher in the drier months than in the rainy months. This is in line with what Dawoe et al. (2010) reported as the researchers worked in the same agro-ecological zones. In Asante Mampong, Ghana, Asiedu et al. (2013) recorded leaf litter fall ranging between 1.9 – 12.7 Mg ha^{-1} using a 1 m x 1 m wooden quadrant for 3 – 5 years cocoa plants. The very old cocoa plants (5 years) produced the highest litter among the three treatments studied. Pure cocoa stand litter (without shade trees) production was on the average of 6.9 t ha^{-1} year^{-1} around the Tinte-Bepo Forest Reserve, Ashanti Region with the dry months recording higher litter fall than the wet months (Owusu-Sekyere et al., 2006b). Dawoe et al. (2010) explained the seasonal variation as litterfall might be affected by physical factors such as wind and rain. Falling of leaves during the dry season might be a strategy to reduce the surface area for transpiration and therefore conserving moisture within the plants. Plants (especially deciduous species) reduce their leaf area through shedding most of their leaves to minimize leaf water loss (Da Silva et al., 2013). The leaves remaining on the

plant during the dry season can then strongly influence the water balance by adjusting transpiration as a function of hydraulic limitation due to an increase atmospheric vapour pressure deficit and soil surface desiccation (Da Silva et al., 2013; Prado et al., 2004).

For their research on shaded verses un-shaded cocoa: implications on litter fall, decomposition, soil fertility and cocoa pod development, Ofori- Frimpong et al. (2007) noted that unshaded cocoa farms produced higher litter than the shaded farms with litter ranging between 3.10 and 5.11 Mg ha^{-1} in the Eastern part of Ghana. On the other hand, the rate of decomposition of cocoa leaf litter under shaded farms was higher than the unshaded farms making nutrient release such as NKP higher under the shaded farms. The importance of this faunal activity was known to increase with increasing total precipitation and minimum temperature (Paudel et al., 2015a).

The reported works indicate that litter production has a linkage with the plant stand status. Very healthy plants will produce many leaves as compared with unhealthy plants and the fall of the litter might be higher. Drought and high temperature have effects on litterfall as leave senescence is facilitated by these factors. However, shade could reduce the effects and then facilitate the decomposition of the litter to make nutrients available to the plants.

2.10 Effect of Climate Change on Cocoa Physiology

Climate change can affect several physiological and biochemical activities in plants such as reduced photosynthetic activities; altered metabolism and enzymatic activity; tissue thermal injury and reduced pollination. It has effect on pests and diseases incidence, host-pathogen interactions, distribution, and ecology of insects (Kondinya et al., 2014).

2.10.1 Drought and Heat Stress

According to Lamaoui et al. (2018), drought stress occurs when soil and atmospheric humidity are low while ambient air temperature is noted high creating an imbalance between the evapotranspiration flux and water intake from the soil. A period during which plant can no longer extract sufficient water for normal life processes due to a reduced soil moisture content denotes drought (Coder, 1999). Heat stress on the other hand was defined by Lemaoui et al. (2018) as "the rise in soil and air temperature beyond a threshold level for a minimum amount of time such that permanent harm to plant growth and development occur". Plants are said to be under drought stress when either the root water supply is limited or transpiration water loss is very high (Anjum et al., 2011).

Studies indicate that drought and heat stress have severe effects on cocoa physiology. Water relations within and between plants and soil are influenced by factors such as leaf water potential, leaf and canopy temperature, rate of transpiration and stomatal conductance (Fahad et al., 2017). A reduction in leaf water potential and transpiration rate increases leaf and canopy temperature (Turner et al., 2001). Increased temperature of the leaves above optimum for photosynthesis affects thermo-tolerance adjustment of the photosystem II and it is partially terminated under the stress (Fahad et al., 2017). It is shown that reduction in net photosynthetic rate due to stress is linked to stomatal closure, and this leads to increased water use efficiency (net CO_2 assimilation rate/transpiration) (Lamaoui et al., 2018). Improvement in water use

efficiency is mainly due to the accumulation of dry matter using less water (Fahad et al., 2017). In their research on influence of environmental factors on photosynthesis in cocoa trees in India, Balasimha et al., (1991) illustrated that net photosynthesis and stomatal conductance were highest during the early hours (at 10:30 h) but declined towards midday. Plant water potential recorded more negative values during the midday and coincided with lower photosynthetic rate but PAR and VPD peak at the same hours. Further analysis of the researchers indicated that both diurnal and monthly variations of climatic conditions have significant effects on rate of photosynthesis, water potential and stomatal conductance (g_s) with lower g_s values in the months where both soil and atmospheric moisture were low. Avila-Lovera et al. (2016) recorded maximum photosynthetic rate (A_{max}) between 2 and 6 µmol m^{-2} s^{-1} and stomatal conductance (g_s) between 90 to 300 mmol m^{-2} s^{-1} with A_{max} and g_s recording higher values in the rainy season than in the dry season although highest A_{max} values did not fully correspond with the highest g_s values in the 12 cultivars studied. Water Use Efficiency (WUE) values ranged between 2 and 6 mmol mol^{-1} but with no significant effect of season on WUE (Avila-Lovera et al., 2016). Temperature range of 31 – 33 ^oC was noted to be optimum for photosynthetic rate though Yapp (1992) had given a range of 33 – 35 ^oC as optimum. A similar worked conducted by Acheampong et al. (2013) indicated that photosynthetic rates were higher during the first half of the day and that highest rate of photosynthesis were around 10: 00 and 12: 00 after which the rates declined. Chlorophyll fluorescence (F_v/F_m) rather declined towards the midday and then increased during the second half of the day. For seasonal variations, the researchers noted that photosynthetic rates, chlorophyll fluorescence and stomatal conductance were higher in the rainy seasons than in the dry seasons. As plants were watered throughout and soil water was not limiting, their explanation of the seasonal variation was likely because of changes in vapour pressure deficit (VPD. In another work from Brazil, net photosynthetic rate (P_n), Chl a/b and net assimilation rate (NAR) reduced significantly after

plants were withheld with water up to -2.5 MPa leaf water potential (Baligar et al., 2017). The reduction in P_n were projected to be due to reduced total leaf area (LA) and lower leaf water potential.

Scholarly articles further maintain that stomatal opening in cacao relates to air relative humidity and that stomata are kept more open at higher relative humidity instead of lower relative humidity (Sena et al., 1987). In this view, De Almeida and Valle (2007) notified that control of water loss is not efficiently controlled by stomatal closure and the issue could be due to higher cuticular transpiration. Abo-Hamed et al. (1983) had identified the surfaces of young red pigmented cocoa leaves to possess numerous hairs of four different kinds that form a relatively dense cover which helps reduce transpiration rate. They estimated low cuticular resistance during the early stages of leaf growth, but the value went higher as the leaf expands. In this wise, it was further explained that changes in the rate of water uptake which reflects rate of water loss from the surfaces of leaves, correlates better with changes in stomatal diffusion resistances instead of expansion of leaf area. Acheampong et al. (2015) noted an average of 7.5 times higher stomatal conductance in rainy season than in the dry season and this reflected in the rate of photosynthesis where plants recorded higher rate (around 71.8% higher) in the rainy season. The researchers further noted that the mean water use efficiency of the plants during the dry season was 3.2 µmol mol^{-1} and it was over 100% higher than in the rainy season.

2.10.2 Stomatal Density and Regulation

Literature indicates that stomata of cocoa leaves are present only on the lower leave surfaces (Abo-hamed et al., 1983; Hardwick et al., 1981). Galyuon et al. (1996) reported that treatments under 50% exposure of sunlight had lower stomata than those grown under full sun. The explanation was that increased stomatal density under full sunlight could be associated with reduced stomatal resistance that would be expected to increase rates of transpiration and leaf

cooling. Similar observation was reported by Alvin (1960) that stomatal opening increased with increasing light intensities. In Ghana, Hutcheon (1977) observed partial stomata opening at low light intensities. As reviewed by Carr and Lockwood (2011), average stomatal density of 700 mm^{-2} in USA under greenhouse experiment has been reported in cocoa. Mixed hybrid cocoa plants recorded 820 mm^{-2} stomata for irrigated seedlings and as many as 1110 mm^{-2} for unirrigated seedlings in Malaysia with smaller leaves having higher densities (Huan et al., 1986; Carr and Lockwood, 2011). Similarly, in Europe (UK), for the eight genotypes of cocoa studied in greenhouse, stomatal densities between 788 – 1081 mm^{-2} were observed as the guard cells length ranged between 12.6 to 15.1µm (Daymond et al., 2011; Carr and Lockwood, 2011). Water deficit treatments significantly increased Stomata density from 776 mm^{-2} in well-watered plants to an average of 896 mm^{-2} in water stressed plants (Lahive et al., 2018).

Stomatal density and aperture are important features on the leaf of cocoa to control CO_2 movement in and out of the plant. The higher the stomata density, the higher the potential surface area for movement of CO_2 into the leaf of the plant. Also, the degree of opening of the stomata (stomatal aperture) determines the actual flow of CO_2 into the leaf. Lower stomatal aperture could be compensated for by higher stomata density (Camargo and Marenco, 2011). Shaded leaves show higher relative water content and fewer stomata per unit leaf area than unshaded leaves (Almeaida and Valle, 2007). Lamaoui et al. (2018), however, reported that leaves that develop under drought are reported to have smaller cells and higher stomata density. The same observation has been reported by researchers such as Martinez et al. (2007) with beans (*Phaseolus vulgaris*), Quarie and Jones (1977) with spring wheat (*Triticum aestivum*) and Spence et al. (1986) with the broad bean plant (*Vicia faba)*

2.10.3 Chlorophyll Content

Chlorophyll production is affected by environmental conditions such as temperature, light availability, and water. Green color loss occurs when magnesium in the chlorophyll ring is replaced by hydrogen ions resulting in an olive brown pheophytins (Erge et al., 2008). When plants are exposed to heat stress, it results in impaired chlorophyll biosynthesis due to down-regulation of gene expression and protein abundance of several enzymes involved in tetrapyrrole metabolism (Dutta et al., 2009). The situation reduces photosynthesis beyond the optimum temperature, resulting in substantial loss in plant productivity (Duta et al., 2009). The problem is attributed to inhibition of electron transport and the activity of Rubisco (the photosynthetic enzyme), perturbation of thylakoid membrane fluidity and a reduction in photophosphorylation and CO_2 assimilation (Dutta et al., 2009). Chloroplast ultrastructure alteration such as lamella structure loosening, matrix zone expanding and chloroplast swelling under high heat stress was also reported. Other abiotic stress effects include stunted growth and accelerated leaf senescence (Sohrabi et al., 2012).

In cocoa production, Baker and Hardwick (1973) reported an increase content of chlorophyll from 14 – 16 ug cm^{-2} at the flushing termination to a maximum of 28 ug cm^{-2} in the older leaves. In another development, Salazar et al. (2018) indicated that cocoa plants under low photosynthetically active radiation (PAR), due to higher agroforestry, recorded higher total leaf chlorophyll content than those under medium and high PAR. The researchers explained the observations as an adaptation strategy by the plants to survive under shade conditions. It is also indicated that high or low temperature inhibits chlorophyll synthesis and that optimum temperature for chlorophyll synthesis is 30 ºC (Li et al., 2018). The researchers further reported water as a medium for the transport of minerals to the sites for chlorophyll synthesis and therefore lack of water promotes chlorophyll decomposition to speed yellowing of leaves.

2.10.4 Cocoa Production and Yield

It is reported that increased temperature and drought can reduce yield of crops to as much as 50% (Lamaoui et al., 2018). Drought and heat induce negative changes in photosynthetic pigments and the thylakoid membranes, resulting in improper functioning of the photosynthetic machinery and impairing the performances of important enzymes to cause considerable loses in plant growth and yield (Wiser et al., 2004; Fahad et al., 2017). Male reproductive development is known to be very sensitive to drought during meiosis in the microspore mother cells (Saini, 1997) and that water deficit at this stage causes inhibition of microspore development or pollen grains viability. This happens when water stress causes disturbances in carbohydrate metabolism and distribution within anthers. Decreased sugar delivery to the reproductive tissue because of inhibition of photosynthesis may trigger metabolic lesions to cause failure of male gamete development (Saini, 1997).

2.10.5 Plant Growth

Cell division, enlargement and differentiation are the main mechanisms for plant growth. Drought is noted to impair the process of mitosis and cell elongation due to loss of turgor within the cells (Fahad et al., 2017). The situation can lead to decreased rate of diameter and height growth because of cell initiation shortages, cell enlargement problems and inefficient supply of food to the growing parts (Coder, 1999). Droughted seedlings are therefore identified by premature leaf fall, yellowing of basal leaves, wilting and small leaf sizes (Carr and Lockwood, 2011).

Maximova et al. (2008) indicated average stem diameter of 15 mm of cocoa plants six months after planting and this increased to 60 mm fifty-seven months after planting showing average diameter expansion of 0.88mm per month. Also, after measuring the height of cocoa stem from

the soil surface to the shoot apex of young plants or to the first jorquette of adult plants, the researchers identified maximum height averaging between 59 and 90 cm to indicate a growth rate between 4.9 cm and 7.5 cm per month during the first year. For the rest of years that the research lasted (4.5 years in total for the growth), growth of the plants in terms of height was slow. Depending on the type of growth media and the size of the polybags for cocoa seedlings, Sosu, (2014) showed average plant height reaching between 33.2 cm and 63.9 cm, a stem diameter of 0.7 to 1.3 mm and leaf number ranging between 10 and 24 six months after planting. Niether et al. (2017b) revealed a decreasing stem diameter with increasing stem density from full sun cocoa monoculture to cocoa- agroforestry systems. With the seven cocoa accessions studied, dos Santos et al. (2018) showed significant reduction of plant growth in terms of height, stem diameter, leaf number and leaf area under drought. Generally, plants under soil water limitation recorded slower growth rate as compared with the control. Coder (1999) indicated that plants go through temporal and permanent wilting. Temporal wilting occurs during the day as the leaves are visibly drooping but this is followed by rehydration and recovery during the night. Temporal wilting results in permanent wilting during long periods of drought when wilted plants do not recover and die. Total dry biomass, leaf area and stem diameter of different genotypes of cocoa have been reported to reduce due to drought and heat stress (Baligar et al., 2017). Agele et al. (2018) noted that growth parameters such as plant height, stem girth, number of leaves, root and shoot weight were enhanced by 100 and 60% field capacity (1.5 and 0.9 litres of water per plot) watering regime as compared with 40% field capacity (0.6 litres of water per plot) treatments.

2.10.6 Stem Expansion

Growth of plant stem diameter could be affected by plant environmental conditions such as available water. On daily time scale, tree stems swell and shrink due to their stem water status (Vandegehuchte et al., 2014) therefore measurement of expansion of stem diameter could be related to plant water status. It is noted that trees attain their maximum circumference just before dawn, and then when the stomata open, trees start losing water to the atmosphere more rapidly than taking it up from the soil (Herrmann et al., 2016). Xylem water tension dynamics and water storage in the bark and phloem are factors for the shrinkage reaching minimum at the early hours of the day and then swelling to a new maximum before the next dawn (Herrmann et al., 2016). Zhang et al. (2006) had indicated that in the afternoon, the plant swells again because stomata close and transpiration rate decreases, root water uptake therefore exceeds plant water loss.

Seasonally, the bark of the plant swells during the onset of the season due to hydration and then shrinks during the dry periods (Fabian et al., 2016). In the dry period, soil water content declines and hence affecting the amount of water stored in the stem. There is, therefore, less water to keep the stomata open for most of the day (Dias and Marenco, 2016). This mostly occurs when the amount of water lost by transpiration exceeds the uptake capacity of the tree (Cermak et al., 2007; Dias and Marenco, 2016). Dias and Marenco (2016) observed 26 out of the 28 species of the trees studied having higher water content of the wood in the rainy season than in the dry season. However, water content in the bark had a different pattern in the dry season than in the rainy season. During dry season, transpiration increases which might result in the wood having lower water content and therefore the shrinkage of the pole. For higher water in bark during the dry season, the researchers notified it was a result of accumulation of osmotic active products that seemed to draw water from neighbouring cells. Grogan and

Schulze (2012) observed stem expansion of *Swietenia* sp during successive wet seasons and contraction during successive dry seasons when measured with Vernier dendrometer in Brazil. The expansion rate of stem diameter was from 0.07 to 1.41 cm/yr over the five years data was taken (Grogan and Schulze, 2012). Also, it was noted that stems contract as crowns shed leaves and expand again as crowns flush with new leaves. Following the observation, Dias and Marenco (2016) indicated an interrelationship between production of new leaves and tree bark water content with a postulation that stem expansion is associated with flushing of tropical dry forests trees (Borchert, 1994; Dias and Marenco, 2016).

2.10.7 Flowers and Pods Development

Flowering in cocoa starts 18 months after planting for some early yielding varieties while for most of the varieties, it starts between 3 to 5 years after planting (de Almeida and Valle, 2007). Of the flowers that produce, only 0.5 – 5% develop into a mature pod (Carr and Lockwood, 2011). It is noted that flowering intensity, pod formation and sizes are affected by drought and heat (Coder, 1999). Maximova et al. (2008) identified highest flowering of cocoa during spring months as compared with the winter months and that flowering occurred mainly at the end of the dry season and at the beginning of the rainy season in Eastern Caribbean. The reproductive processes such as pollen and stigma viability, anthesis, pollen tube growth and early embryo development are noted to be vulnerable to heat and drought (Lamaoui et al., 2018; Giorno et al., 2013). Handley (2016) identified higher effects of long drought on number of flowers of cocoa produced. In effect, low flower production was noted during the dry season, but this coincided with higher pod production (Wuriandani et al., 2018). Omolaja et al. (2009), in Nigeria, also reported higher number of flowers in the rainy months such as May as compared with the dry months such as January. For the eight clones of cocoa studied, an average of 105 flowers were produced around the main trunk between 60 cm to 120 cm above ground level

and on the first, second and third branches in January comparing 275.0 flowers of the same clones counted in May (Omolaja et al., 2009). Similar observation was reported by Gordon (1976) that flower production is higher within April to May. Alvin (1966) had observed increased rainfall promoting flushing and flower initiation in cocoa to indicate the rainy months promoting flowering. In Ghana, Frimpong-Anin et al. (2014) observed higher number of unpollinated flowers dropping in the dry season than it was, in the rainy season. The same pattern was observed with the pollinated flowers where higher stability of around 95% was observed even after fifth day of pollination in the rainy season while stability reduced to 65% on the third day after pollination in the dry season. The higher number of flowers that dropped in the dry season could be due to water stress while the few that dropped during the rainy season could be due to mechanism of cherelle wilt (Frimpong-Anin et al., 2014)

A pod takes about 5 to 6 months (about 150 – 180 days) to mature and many environmental factors happen before maturity (Baah et al., 2016). Cherelle wilt or fruit abortion is one of the inherent factors to manage resources available for the parent plants to carry the numerous cherelles to matured pods (Handley, 2016). Fruit abortion is common in cocoa as it is in other plants such as apple, citrus, and oak (Stephenson, 1981; Handley, 2016). Mckelvie (1956) reported that cocoa pods are liable to wilt at two stages in their development and therefore called the stages as first wilt and second wilt. The first wilt is high at seven weeks after pollination and ceases as cell walls are laid down on the endosperm while the second wilt reaches its peak at ten weeks from pollination and declines in response to greatly increased pod metabolism. The researcher further noted that both wilts arise because of lack of hormones produced by the endosperm, causing a decline in the uptake of water and food materials to the cherrelles. Other factors, as elaborated by Handley (2016), include the distance between the cherelles and the source of assimilates as basal pods that develop earlier may have resource advantage over the terminal pods that develop late; and the nature of pollination where cross

pollinated flowers may have a genetic advantage over self-pollinated flowers. With the effect of cherelle wilt on yield of cocoa, Mckelvie (1959) indicated that yield is dependent on the nutrient status of cocoa and independent of fluctuations in cherelle wilt. Carr and Lockwood (2011) observed more cherelle wilt occurring when there is heavy flushing but when all the cherelles are at the same developmental stage, wilt is reduced. Thus, manual pollination helps to synchronize development of flowers to cherelles and reduce wilt.

Though pod yield of as high as 108,183/ha is reported for some cultivars (Goenaga et al., 2015) water stress could induce a higher volume of fruit wilt due to lower rate of photosynthesis. Handley (2016) observed a significant 22.6% reduction in pod size with treatments under stressed conditions. Wuriandani, et al. (2018) noted seasonal effect on number of pods per tree as well as pod length on the types of cocoa varieties studied. The cocoa pods showed their best performances in the dry season, but these were the pods that developed in the rainy season and harvested in the dry season. Evidently, the soil moisture availability increased rate of photosynthesis and therefore the distribution of assimilates towards pods formation during the rainy season. As pods take about six to seven months to be harvested, these pods that developed in the rainy season were rather harvested during the dry season giving the observation. However, Lehive et al. (2019) identified from literature that rainfall is beneficial to yield in the initial stages of pod development but are less influential in the later stage as it can have negative effect on pod at maturity and, increases diseases incidence.

2.10.8 Dry Bean Yield

In 2017, the Chief Executive of Cocobod in Ghana pledged the government's resolution to increase average cocoa yield to 25 bags per acre thus harvesting 200 to 300 pods per tree through artificial pollination and planned irrigation (Quaynor and Akuffo-Asante, 2017). Yield of cocoa is 0.5 t/ha though there is an indication of 1 to 1.5 t/ha potential yield in Ghana

(MOFA, 2016). Theoretically, potential yield of cocoa is reported to be around 6 t/ha (Gockowski et al., 2013) indicating a yield gap of about 4.5 t/ha even when recorded potential yield in Ghana is achieved. In 2016, there was a reported yield of 1229 kg/ha/yr in Indonesia while Ghana produced 794 kg/ha/yr (Daymond et al., 2017). Though yield seemed improved over the national average, a very low estimated ratio between highest and lowest yield in Ghana as compared with that of Indonesia showed few farms in Ghana contributing to the reported yield. Yield of more than 1000 kg/ha and around 2500 kg/ha have been reported before in Ghana on farmers field and on-station trials, however, national average is low (Aneani and Padi, 2017; Mendes, 2017) due to a major percentage of the cocoa farms producing yield between 350 – 400 kg/ha (Strauss, 2018). Aneani and Ofori-Frimpong (2013) identified farmer-based yield gap of 1537.2 kg/ha (about 82.0% yield gab) of the farmer yield potential when a cross-sectional socio-economic survey was carried out in six cocoa growing districts in Ghana. Laven and Boomsma (2012) reported around 50 to 60% of cocoa farmers producing an average of 0.4 t/ha while between 20 to 40% produced 0.65 t/ha and then very few that use the appropriate technicalities produced around 1.4 t/ha.

Rainfall distribution has effect on yield of cocoa. Extended drought has been noted to reduce yield and that drought for six months before harvest decreases productivity by 4.92 – 42.54% (Santosa et al., 2018). In Brazil, Gateau-Rey et al. (2018) noted an average of 89% reduction of pod yield because of drought. Abdulai et al. (2018) identified lower yield of cocoa of around 288 kg ha/yr/ in the dry regions of Ghana and it was significantly lower than yields recorded in the mid and wet regions of 712 and 849 kg ha/yr/ respectively: an estimated 50% yield reduction due to drought. In Brazil Gateau-Rey et al. (2018) recorded higher potential pod yield of 242 ± 25 kg/ha during the rainy season in 31 cocoa farms but the yield reduced to 26 ± 9 kg/ha representing 83% loss during the dry season. The reports confirm the sensitivity of cocoa plant to available soil moisture. On the other hand, monthly rainfall between 125 – 200 mm is

reported to increase yield of cocoa (Santosa et al., 2018) and that values above 200 mm correlate negatively with yield (Santosa et al., 2018; Lawal and Ommoma, 2014).

2.10.9 Heat Shock Proteins (HSP)

When the temperature of the environment is constantly raised above the normal, plants put out adaptive mechanisms to the changes of the environmental temperature. One of the mechanisms is the production of heat shock proteins. These proteins were originally discovered around the 1960s by Ferruccio Ritossa on the fruit fly *Drosophila melanogaster* (Ritossa and Mitteilungen, 1962) and the expression of HSPs was identified to be induced after exposure to stress such as heat stress. Although, the precise functions of the HSPs are not well defined, there are considerable evidence that HSPs are essential for survival at both normal and elevated temperature (Kregel, 2001).

HSP 70, as one of the members of HSP 70 family is highly inducible and its synthesis is increased in response to multiple stressors (Kregel, 2001). Its transcription was identified to increase under high temperature stress in *P. lactiflora* (Zhao et al., 2019). Also, a transgenic *A. thaliana* with HSP70 was noted to confer tolerance to high heat stress by exhibiting higher chlorophyll fluorescence; more intact cell membranes, chloroplasts, and starch grains; lower accumulation of hydrogen peroxide (H_2O_2); lower superoxide anion free radicals (O_2^-); and lower relative electric conductivity than the wild type (Zhao et al., 2019). Many works on HSP 70 done in cucumber (*Cucumis sativus* L.), pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.) indicate upregulation of the protein when exposed to heat ranging between 32 – 42 ^oC for 12 – 168 hours (Zhao et al., 2019; Chen et al., 2017; Guo et al., 2016; Xu et al., 2016; Han et al., 2016). When comparing three different genotypes of cocoa under different levels of exposure to light, De Araujo et al. (2017) identified repression of HSP 70 at the leaf level for two of the genotypes under 100% light comparing

with the 50% and 5% light exposure, but a slight increase of expression was observed in the third genotype.

Heat shock proteins of lower molecular unit (sHSPs) such as HSPs 17 - 32 are also mostly located in the cytosol, nucleus and mitochondria and they function as microfilament stabilization, antiapoptotic, proapoptotic (apoptosis is programmed cell death), refold proteins and prevent aggregation of denatured proteins (Kregel, 2001). Though HSP 70 is known to produce during heat stress, Elthon, et al. (2003) reported that HSP 70 homologs are not effective indicators of plant's ability to tolerate heat stress. The researchers maintained that the small HSPs (sHsps) are the effective indicators of heat resistance. One notable sHSPs known as HSP 22 was reported in Pea leaf to conditionally express only at high temperatures of around 40 ^{o}C for 3 continuous hours and declined slowly after the leaves were transferred to 25 ^{o}C (Lenne and Douce, 1994). In another development, when two cultivars of wheat were subjected to a temperature of 34 ^{o}C and 37 ^{o}C, there was elevated synthesis of HSP 22 in the heat tolerant cultivar (Krsihnan et al., 1989). Benzet et al. (1998) identified that mitochondrial HSP 22 are accumulated in tomato cells when subjected to oxidative stress and, thus, giving an indication that plants cells are protected against oxidative injury by the sHSP.

These reviews indicate that the two HSPs families, HSP 70 family such as HSP 70 and small sHSPs such as HSPs 22 may be of subject of interest in their role to help reduce thermal injury in plants such as cocoa.

2.11 Shade and Cocoa in the Wake of Climate Change

Shade in cocoa production has been one of the topics talked at length but has still not been concluded. The problem of what percentage of shade is needed at what growing age of cocoa plant has had varying answers from researchers with percentages varying from 80 to full sun.

Also, whether shade is needed by adult cocoa plants swings between two schools of thought, shade, or no-shade (full sun).

No-shade cocoa production is noted to increase yield more than shaded cocoa. In 1962, Cunningham and Arnold, reported that removal of shade from cocoa plants resulted in higher yield and thus shade removal increased positive interaction between increased light and nutrients. The idea was supported by Agele et al. (2016) who maintained that no shade treatments gave the best solar radiation transmittance, photosynthetic active radiation, higher radiation use efficiency and leaf area ratio. Some shade trees are also reported to compete with cocoa plants for light and nutrients and thus reduce yield of cocoa (Blaser et al., 2017). In Nigeria, Agele et al. (2016) recorded high PAR differences between no-shade, moderate shade, and dense shade. PAR values were as high as 1031 µmol m^{-2} s^{-1} during the dry season for the no-shade treatments but around 893 µmol m^{-2} s^{-1} for moderate shade and 603 µmol m^{-2} s^{-1} for dense shade treatments. During the major rainy season, the no-shade treatments recorded 755 µmol m^{-2} s^{-1} PAR as against 459 µmol $m^{-2}s^{-1}$ and 285 µmol m^{-2} s^{-1} for moderate and dense shade treatments, respectively. The researchers observed that high PAR in the no-shade treatments translated into growth vigour and canopy formation as well as available radiation energy for dry matter accumulation usage. De Araujo et al. (2017) in Brazil compared cocoa plants under 100% light, 50% light and 5% light. The result indicated that plants (more especially the *Catongo* genotype) under 100% light produced greatest CO_2 assimilation, stomatal conductance, and transpiration than those under medium and low light. On the other hand, plants under low light recorded the highest F_v/F_m values as compared with those at high light. *Catongo* genotype, for example, recorded P_n of 10.39 µmol $m^{-2}s^{-1}$ at 100% light, 8.40 µmol $m^{-2}s^{-1}$ at 50% light and 7.89 µmol $m^{-2}s^{-1}$ at 5% light but F_v/F_m values ranged from 0.65 at 100% light, 0.77 at 50% light and 0.81 at 5% light (De Araujo et al., 2017). Similar work by Salazar et al. (2018) in Colombian Amazon, indicated treatments under high PAR showing the

greatest photosynthetic efficiency although leaves from Low PAR gave higher leaf content of total chlorophyll.

On the other hand, Tee et al. (2018) reported enhanced growth of cocoa under temporal shade and that survival rate of cocoa in terms of growth and physiological performances under hot and dry period was more efficient in shade. Shade treatments were noted to reduce effects of vapour pressure deficit on cocoa plants in dry seasons (Acheampong et al. 2013) and served as an alternative strategy to ensure species diversity, year-round soil cover and high level of stored carbon in soil and in wood (Lobao et al., 2007; Somarriba et al., 2018). Hoogendijk (2012) had noted that shade trees in cocoa reduced wind velocity, excessive evapotranspiration, low soil-fertility, and other unfavourable ecological factors. Photosynthetic rate, growth, and yield of cocoa have been reported to improve under shade (De Almeida and Valle., 2008). Ofori-Frimpong et al. (2007) identified no-shade cocoa trees producing higher number of pods, however, incidence of cherelle wilt and nutrient deficiency was higher on the no-shade farms due to high temperature and poor rate of litter decomposition

Ahenkorah et al. (1974) provided a graphical model to explain yield of cocoa under shade and no-shade treatments after 17 years of study (Figure 2.5). The four treatments from the graph indicated cocoa plants under no-shade but with fertilizer (NSF) performing better than the same treatment under shade (SF). With the no-fertilizer treatments, the same yield patterns were recorded where no-shade-no-fertilizer (NSNF) treatments on average performed better than shade-no-fertilizer treatments (SNF). However, decline in strength of the trees under no-shade-but-fertilizer was rather faster partly due to higher loss of exchangeable bases and the greater stress caused by the higher yield. Also, the no-shade regime influenced faster distribution of mistletoe and mosses than the shade regime. More so, the NSF treatment was projected to decline in yield with time while the SF treatment showed a positive yield effect with time.

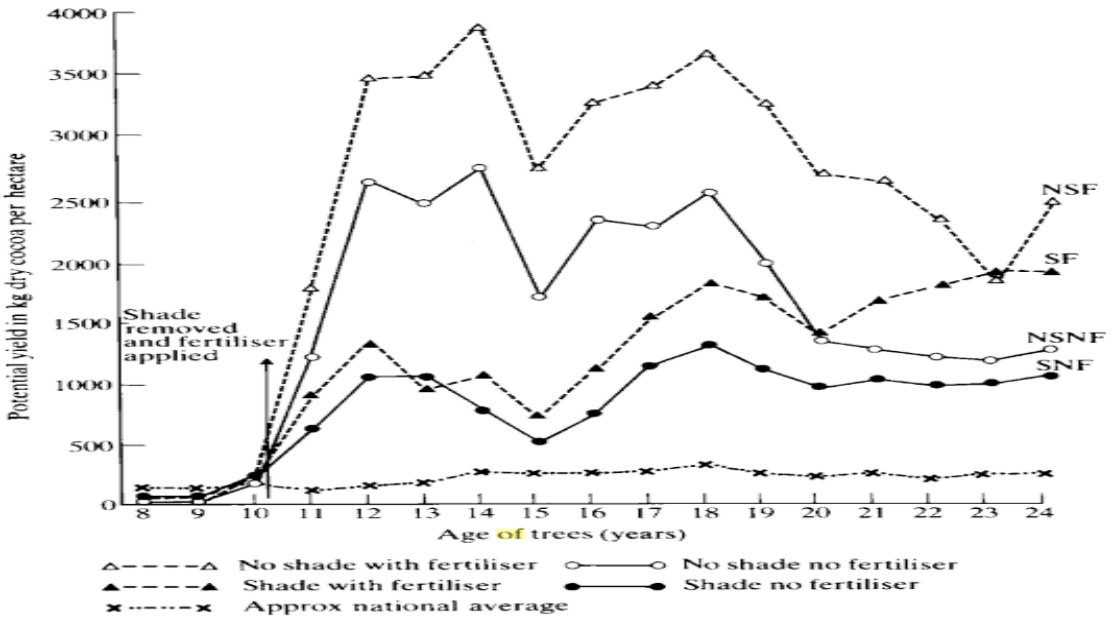

Figure 2.5 Effect of Shade Removal and Fertilizer Applications on Yield of Amelonado Cocoa in Ghana. Source: Ahenkorah et al. (1974). The end of the first cocoa shade and manurial experiment at the Cocoa Research Institute of Ghana

Kyere (2017) specifically worked on the effects of different shade trees on cocoa and reported that the seven species of trees studied recorded moisture effect between -0.28 – 0.19 (Table 2.1) with *M. lucida* recording the highest effect and PAR transmittance ranging between 2.95% and 69.22%; and with E. *angolense* providing the highest transmittance. The plants with the highest moisture effect were assumed to reduce evapotranspiration leading to an increased soil moisture content around the cocoa.

Table 2.1 Effect of Tree Species on Soil Moisture Content and PAR Transmittance in Cocoa Agroforestry System During the Dry Season

Tree Species	% Soil Moisture Content		Moisture Effect	Transmitted PAR (%)
	Sub-Canopy	Open Area		
M. lucida	8.06±2.25	5.33±1.23	0.19±0.08	23.33±3.01
S. campanulata	9.8±2.38	7.56±2.12	0.16±0.08	18.23±5.13
F. capensis	12.69±2.29	9.24±1.22	0.13±0.06	33.75±3.77
T. superba	1079±0.61	10.24±0.95	0.03±0.03	67.14±5.24
M. indica	6.43±0.65	6.06±0.30	0.02±0.07	2.95±0.81
E. angolense	12.64±2.47	13.47±2.77	-0.03±0.02	69.22±5.63
C. sinensis	4.75±1.67	7.31±1.77	-0.28±0.12	5.63±1.70

Source: Kyereh, (2017). Shade trees in cocoa agroforestry systems in Ghana: Influence on water and light availability in dry seasons

Trees with higher PAR transmittance also increased yield of the cocoa pods because of PAR availability to the sub-canopy cocoa trees. However, shallow rooted trees competed with cocoa for soil moisture and nutrients. This work seems to provide a solution to the argument that shade trees compete with cocoa for water and available nutrients (Abdulai et al. 2017). The root zone of the shade trees serve as a factor to this argument since deep rooted plants will have a different soil horizon for water absorption and will not compete for available nutrients and water.

2.11.1 Light Response Curves in Cocoa – Effects of Shade and Stress

When plants are exposed to the natural conditions, they receive light from the sun and the amount, quality and duration mostly depend on the season of the year, hour of the day, geographical location, and weather (Lopez, 2018). According to Pallardy (2008), plants that can grow under shade have ability to adapt their photosynthetic apparatus to low light intensity and that such plants must have the capacity to efficiently trap the available light and convert

them into chemical energy, maintain a low rate of respiration and partition a large fraction of assimilates into leaf growth. Shade tolerant cocoa exhibits saturated rates of net carbon assimilation at low photosynthetically active ratio (PAR) levels between 200 and 750 µmol m^{-2} s^{-1} and light compensation point between 5 to 57 µmol m^{-2} s^{-1} with a maximum photosynthetic rate (P_n) in the range of 1 and 8 µmol m^{-2} s^{-1} (Salazar et al., 2018). Low light compensation points of 400 µmol m^{-2} s^{-1} and low maximum photosynthetic rate of around 7 umol m^{-2} s^{-1} at light saturation is reported in cocoa (Anim-Kwapong and Frimpong, 2004; Hutcheon, 1981). More so, rate of photosynthesis is reduced at light exposure above 1800 µmol m^{-2} s^{-1} (Galyuon et al., 1996). Sterck et al. (2013) defined shade tolerance as the light level at which plants can survive and possibly grow and this light level is the light compensation point (LCP) of the plant. Light interception efficiency (LIE) on the other hand is an index of self-shading. Plants may achieve a low LCP by a low leaf compensation point (LCP_{leaf}) which depends on the light response curve of leaf photosynthesis and low self-shading among leaves (Sterck et al., 2013). When plants are kept under shade, they may receive a higher ratio of far red than red (Red light is important for the regulation of flowering and fruiting while far red light are important for plant elongation) light and may tend to grow taller to reach more light (Lopez, 2018). The LCP starts when light energy is enough for the activity of photosynthesis to start (Lopez, 2018). This is indicated on the graph as zero point of net photosynthesis (Figure 2.6). If the light intensity is below the light compensation point, the plant is starved as the rate of photosynthesis is less than the rate of respiration. Shaded plants have lower light compensation point, lower maximum photosynthetic rate and lower light saturation range making them to grow well at low light intensities.

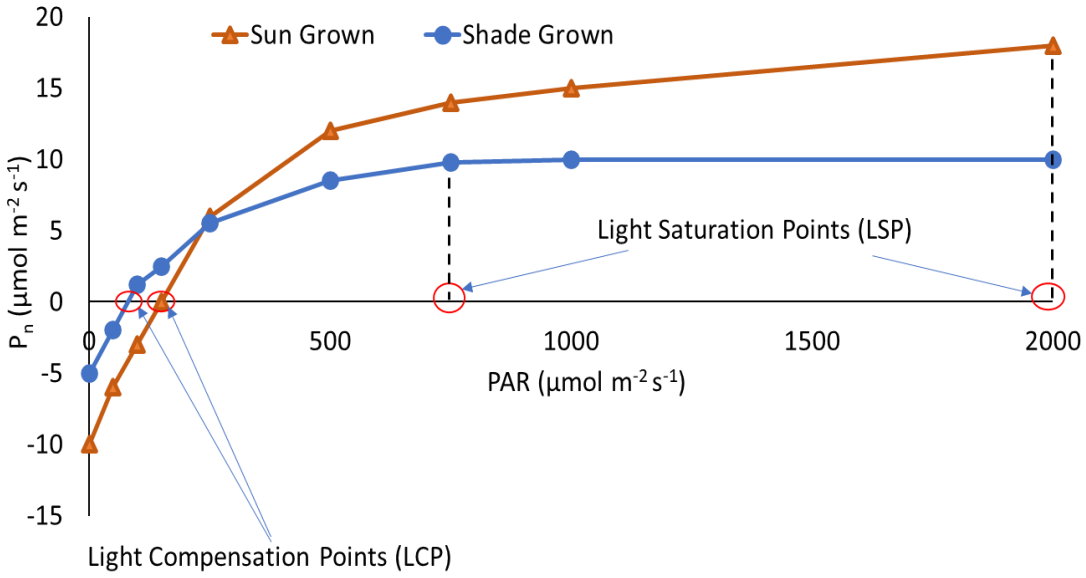

Figure 2.6 A Diagram Illustrating Effects of Different Levels of Light (PAR) on Rate of Photosynthesis (P_n) by Shade and Sun Plants.
Modified from http://www.profitcoachinginternational.com/index.php?id=940

Avila-Lovera et al. (2016) reported values of saturating PPFD in the range of 400 – 500 µmol m^{-2} s^{-1} and LCP of 11.1 µmol m^{-2} s^{-1} of three genotypes of cocoa in Venezuela. Daymond et al. (2011) indicated light saturated photosynthetic rate ($P_{nPPFDsat}$) in the range of 3.4 to 5.7 µmol m^{-2} s^{-1}, transpiration rate in the range of 1.06 to 1.56 mmol m^{-2} s^{-1} and instantaneous water use efficiency (wue) ranging between 3.1 to 4.2 mmol mol^{-1} for the eight cocoa genotypes studied in greenhouse in Readings. Da Matta et al. (2007) indicated that cocoa seedlings show increasing P_n as PAR increases to values in the range of 400 to 750 µmol m^{-2} s^{-1}. Water deficit treatments caused a significant increase in LCP compared to well-watered plants, from 7.1 µmol m^{-2} s^{-1} to 9.9 µmol m^{-2} s^{-1} (Lahive et al., 2018). Likewise, there was a slight reduction (-17%) in LSP in the plants grown under water deficit. Low light can lead to decreased CO_2 assimilation rate resulting in low production of carbohydrates. On the other hand, high exposure to light can cause photoinhibition (De Araujo et al., 2017). Photoinhibition may be divided into two types; dynamic and chronic photoinhibition (De Araujo et al., 2017). Dynamic

photoinhibition indicates a reduction in quantum efficiency of the photosystem II and it is reversible but Chronic photoinhibition occurs when excess light generates a series of highly reactive oxygen intermediates that could damage lipid membrane constituents and destroy pigments and cofactors critical for protein subunits (De Araujo et al., 2017).

2.11.2 Temperature Response Curves in Cocoa – Effects of Shade and Stress

Temperature is one of the main limiting factors for cacao production (Almeida and Valle, 2008). At least, three major stress - sensitive sites in photosynthesis include the photosystem II, the ATP generating, and the carbon assimilation processes (Allakhverdiev et al., 2008). Moderately high temperature is noted to inhibit the repair of PSII (Allakhverdiev et al., 2008). However, the extent of the damage depends on the balance between damage and repair processes during the stress (Allakhverdiev et al., 2008). Although much has not been done in cocoa on temperature and photosynthetic response curve, there are more works on other plants. Warren and Dreyer (2006) studied temperature response of photosynthesis on deciduous forest tress species, *Quercus canariensis* (Oak tree). The observations indicated that rate of net photosynthesis peaked at 24 ^{o}C (Figure 2.7) while internal CO_2 (Ci) averaged 39 μmol m^{-2} s^{-1} at 25 ^{o}C.

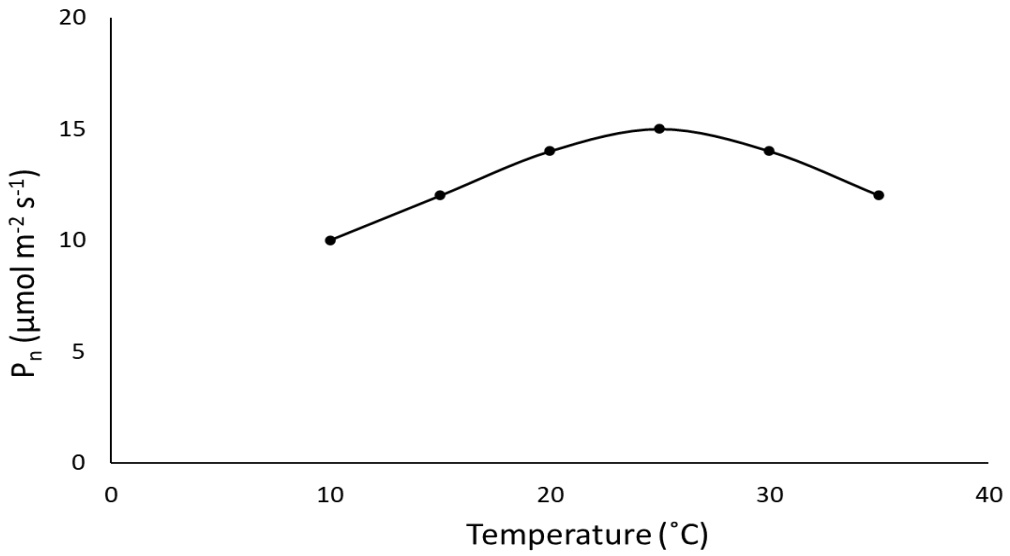

Figure 2.7 Temperature Response of Maximum Rate of Net Photosynthesis (Amax) in *Quercus canariensis*. Source: Warren and Dreyer (2006). Temperature response of photosynthesis and internal conductance to CO_2: results from two independent approaches

Similar work conducted on sweet orange showed highest P_n value at 35 to 40 ^oC (Ribeiro et al., 2006) (Figure 2.8). Optimum temperature of 35 ^oC was finally recommended by the researchers indicating that the effects of heat damage to the metabolism of sweet orange were more evident when considering the balance between photosynthesis and respiration.

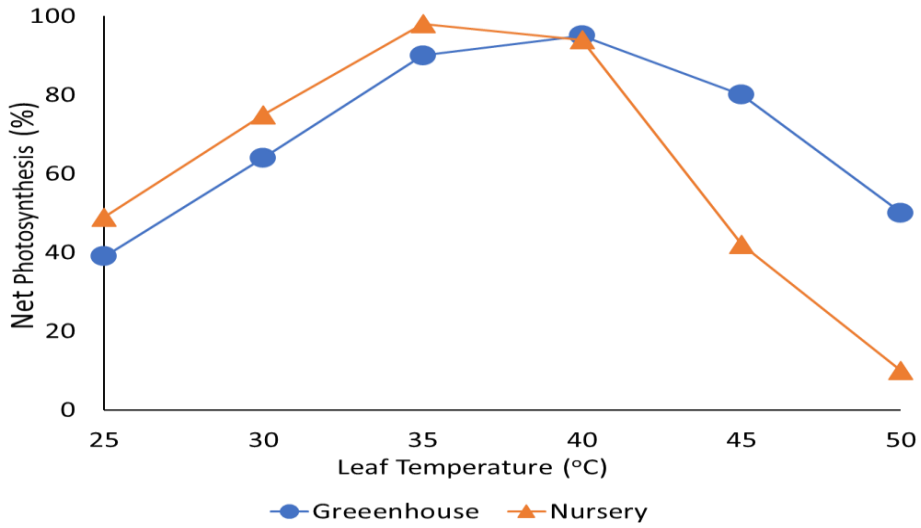

Figure 2.8 Net Photosynthesis as a Function of Leaf Temperature in Sweet Orange Leaf Disc in Greenhouse and Nursery Conditions. Source: Ribeiro et al. (2006). Temperature response of photosynthesis and its interaction with light intensity in sweet orange leaf discs under non-photorespiratory condition

High temperature probably damages the thylakoid membrane due to increased strength of hydrophobic bonds and decreased strength of hydrophilic bonds (Ribeiro et al., 2006). At high temperature, photosynthesis reduces, also, because high temperature reduces the leaf mesophyll conductance to CO_2. Based on biochemical models of photosynthesis, change in photosynthesis verses temperature curve is attributed to four factors, namely, intercellular CO_2 concentration, activation energy of the maximum rate of RuBP, carboxylation (V_{cmax}), activation energy of the rate of RuBP regeneration (J_{max}) and the ratio of J_{max} to $V_{cmax.}$ (Hotosaka et al., 2006). Generally, the researchers identified that every species increases the activation energy of V_{cmax} with increasing growth temperature. But enzymes work within a temperature limit of not more than 45 ^{o}C and that higher temperature decreases photosynthetic pigments due to inhibition of enzyme activities in the biosynthetic pathway (Hasanuzzaman et al., 2013).

CHAPTER III

ECOPHYSIOLOGICAL RESPONSES OF 12-YEAR COCOA PLANTS TO SHADE AND WATER SUPPRESSION

3.1 Introduction

Water availability is considered a critical factor to plant production and that many physiological processes in plants are depended on plant water status. Electrons are generated from water to replace lost electrons of photosystem II and drive the light stage of photosynthesis. Water also provides H+ ions needed to reduce NADP to NADPH for Calvin-Benson cycle of photosynthesis (Park, 2009). Any factor that tends to affect water availability to plants affects plant growth and other physiological processes. Drought occurs when soil water is reduced to the extent that plants can no longer extract sufficient water for normal life processes (Coder, 1999). High transpiration but limited root water supply creates an imbalance mechanism between evapotranspiration flux and water intake from soil water (Anjun et al., 2011; Lamaoui et al., 2018). The situation could result in water stress which is very severe during the periods of drought. Flow of water from xylem to nearby cells is reduced under drought. Cell turgor declines to impair the process of mitosis and cell elongation, (Fahad et al., 2017). Leaf water potential decreases, and transpiration rate increases affecting canopy temperature (Turner et al., 2001). Stomata closure occurs to limit water loss, but the situation also reduces CO_2 uptake (Baligar et al., 2017). This facilitates the degradation of photosynthetic pigments which leads to loss of activity of some enzymes such as Rubisco and therefore, damages to or even death of the plant (Feller and Vaseva, 2014).

Cocoa (*Theobroma cacao* L.) is a water loving plant that grows well in humid tropical climates with regular rains. About 10 of the top producing countries are found within the warm and wet climate regions (Mattayasovszky, 2017) indicating the specificity of the plant to wet

environments. The plant requires not less than 1200 mm of water per annum to survive (Zuidema et al., 2005; Ameyaw et al., 2018). It is therefore not surprising that annual total rainfall for most of the cocoa growing regions falls within the range of 1300 to 2800 mm (Wood 1985; Carr and Lockwoods, 2011). The plant thrives well when distribution of rains is uniform across the year (Carr and Lockwoods, 2011). In West Africa where about 70% of cocoa is produced (Wessel et al., 2015), most of the cocoa farms are rain-fed (Lahive et al., 2019) and changes in the pattern or the distribution of the rain could significantly affect performance of the crop. Furthermore, about 4 -5 months in a year of the cocoa growing regions are noted to be dry with monthly rainfall below 100 mm (Ruf et al., 2015). Climatic trends over the 1960 – 1998 period indicated a sharp decline in rate of precipitation at 3 – 4% per decade in the northern tropical Africa (Malhi and Wright, 2004). In West Africa, where cocoa production is high, a wider range of around -30 – 30% precipitation uncertainties are projected with continuous warming of about 1.5 – 6.5 ^{o}C rise above ambient (Sylla et al., 2016). Drought was suggested to be more severe threat to cocoa production than temperature (Car and Lockwood, 2011). Studies have revealed about 27% loss of cocoa yield due to drought in West Africa in the 1980s (Schroth et al., 2016). During the 2015 - 16 ENSO year, field experiments in Brazil indicated not less than 15% cocoa tree mortality and 89% yield reduction due to drought (Gateau-Rey et al., 2018).

With a future projection of unpredictable nature of rainfall, shading in a form of cocoa agroforestry, is considered a possible solution to mitigate the effects of drought (Ahenkora et al., 1974; Baligar et al., 2008; Asare et al., 2016). A work by Acheampong et al. (2013) on influence of different shade regimes on the photosynthetic activities of different clones of cocoa in Ghana showed that F_v/F_m was consistently improved under shade. On the other hand, there was a reduction in photochemical efficiency of PSII and rate of electron transport under full sunlight (Galyuon et al., 1996). Notwithstanding, net photosynthesis increased under full sun

even though half time fluorescence increased. Thus, though shading is noted to improve the microclimatic conditions around cocoa plants (Wood and Lass, 2001) interest in full sun cocoa farming has been increasing over the last 20 – 40 years due to many complex factors such as enhanced rate of photosynthesis and increased returns (Ruf, 2011; Laderach et al., 2013). Under trees, there are confounding effects such as competition for root space, water and nutrients at the same time as there may be benefits or limitations from shade. In view of these compounding factors, there is a sure movement towards eliminating shade trees from most of the cocoa farms in West Africa (Padi and Owusu, 1998) since the mechanism behind shading is poorly understood which factors could be due to limited research in the area (Gateau-Rey et al., 2018; Moser et al., 2010). Under these complexities and with the emerging trend of climate change, more eco-physiological studies are needed to estimate the repercussive effects of shade removal on cocoa plants. It is based on these factors that the effects of drought on cocoa physiological processes and how shade could mitigate the effects was tested. The main aim of the study was to examine the effects of water availability on cocoa physiology and how shade could reduce the effects. The general aim is expressed in the following specific objectives a) to study the effects of rainwater suppression on the physiology of cocoa including gas exchange, chlorophyll fluorescence and water use efficiency and b) to study the effects of shade on reducing the impacts of rainwater suppression on cocoa physiology. Results from the experiment is anticipated to serve as a possible guide to find possible means to either adapt or mitigate the effects of bad weather conditions on cocoa production.

3.2 Methodology

The experiment was conducted between April 2018 and March 2021 at a cocoa farming community in the Western North Region of Ghana (Sefwi Kunuma, popularly known as 82). Administratively, the community is under Sefwi Boako district in the Sefwi Wiawso Municipality (Figure 3.2.1). Wiawso Municipality falls within the moist semi-deciduous forest

zone with average monthly temperature ranging between 25 ^{o}C to 35 ^{o}C and monthly relative humidity around 90% at night and 75% during the day (Gyapong, 2015; Nyarko, 2014). Rainfall patterns come as a double maximum peaking at May-July and September – October with an average between 1524 mm and 1780 mm (Gyapong 2015; Nyarko, 2014). About 74.1% of all households in the municipality are into agriculture with almost 98.8% of the section in agriculture, engaging in crop production (Nyarko, 2014). The soil type falls within forest Ochrosols and Oxysols. Forest Ochrosol covers the widest area (Nyarko, 2014). Common tree species in the area include Wawa (*Triplochiton scleroxylon*), Onyina (*Ceiba petandra*), Odum (*Milicia excelsa*), Mahogany (*Khaya ivoriensis*), Sapele (*Entandropragma angolense*), Emire (*Terminalia ivoriensis*), Framo (*Terminalia superba*), Asamfina (*Aningeria robusta*), and other less populated species (Nyarko, 2014).

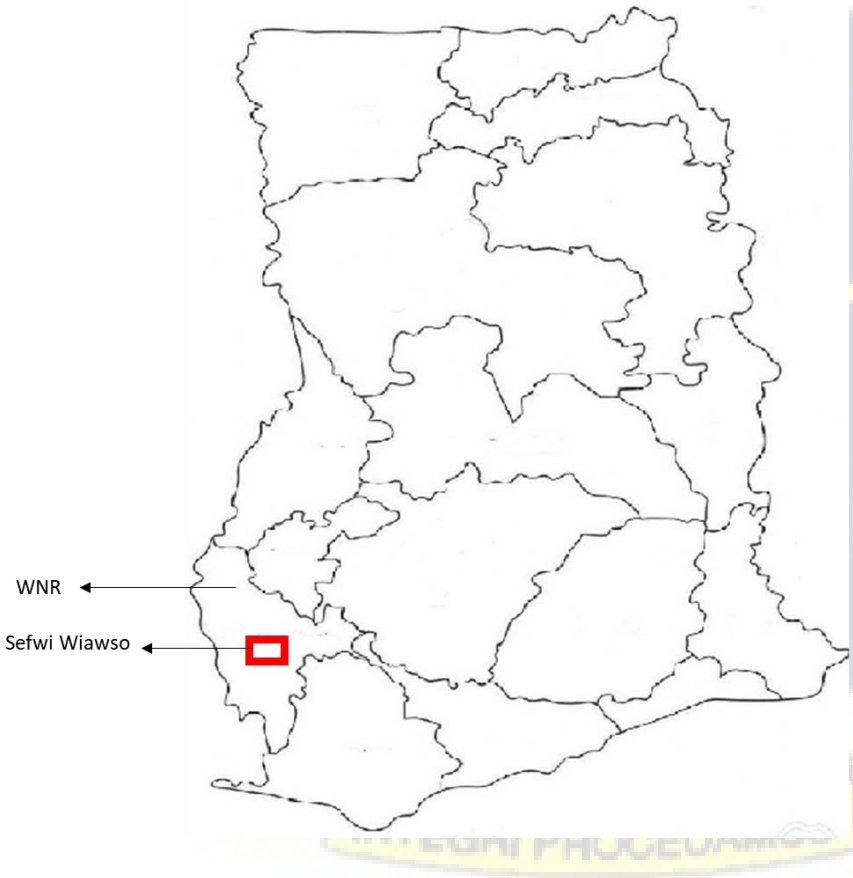

Figure 3.2.1 Ghana Map Showing the Regional Demarcations and the Site for the Experiment (Western North Region – WNR)

Sefwi Kunuma (Figure 3.2.2), is a farming community of about 900 inhabitants. The village is located at latitude 6°23'N and longitude 2°33'W at an elevation of 165 m (± 8.00 m). Main occupation of the indigenes is farming with cocoa as the major cash crop. Other cultivated crops include plantain, cassava, maize, rice, cocoyam, and some vegetables in a small scale. The selected farm was about two kilometers from the village and the cocoa trees at the start of the experiment were about 12 years of age with average height of 4.5 m and average DBH of 8.5 cm.

Figure 3.2.2 Image Showing Sefwi Kunuma and the Cocoa Farm Selected for the Research

3.2.1 Experimental Design

A two-factor split plot design was adopted with two main plots (factor A) and three subplots (factor B) in three blocks.

Factor A – Shade effect

Levels of factor A:

- Shaded cocoa plants with 40% shade nets.
- Full sun cocoa plants.

Factor B – Water stress

Levels of factor B (subplots of main plots):

1. 2/3 water suppression plots (2/3 of rows between cocoa plants roofed – to reduce about 66.6%. of rainwater between rows, stem flow was not accounted for in this design)
2. 1/3 water suppressions plots (1/3 of rows between cocoa roofed – to reduce about 33.3% of rainwater between rows of plants)
3. Control plots (no roofing of rows)

Each block had six subplots including shade 2/3 water suppression (Shade.2/3W), shade 1/3 water suppression (Shade.1/3W), shade control (Shade.0/3W), sun 2/3 water suppression (Sun.2/3W), sun 1/3 water suppression (Sun.1/3W), and sun control (Sun.0/3W). In all, data was taken from 18 subplots of the various treatments. Each subplot contained 25 cocoa plants on an area of 144 m^2. The area calculation per subplot excludes the peripheries. A total of 600 cocoa trees on a land size of 5000 m^2 (0.5ha) were used for the whole drought stress experiment. The middle 9 plants of each subplot were tagged for data taking, but physiological data were taken from four selected plants from the nine cores. Two rows of plants remained between replications to reduce border effects (Figure 3.2.3).

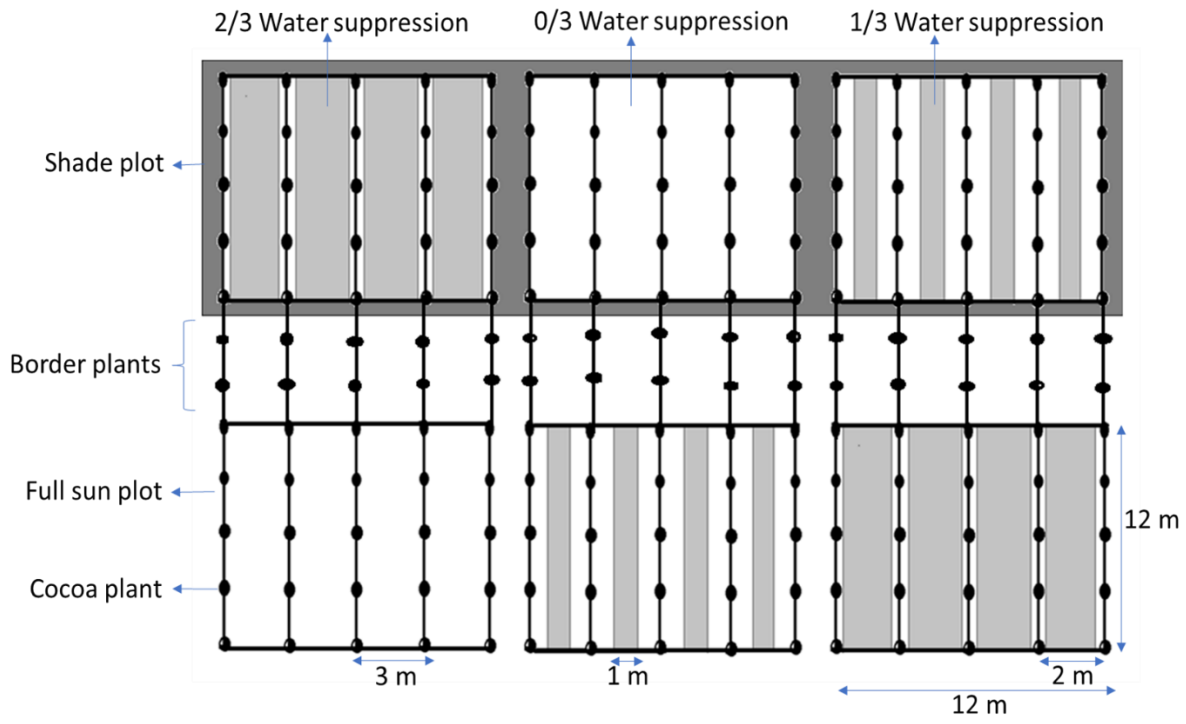

Figure 3.2.3 The Water Stress Field Lay-Out. Dots represent cocoa plants; deep shade represents shade nets while light shade represents the plastic sheet platforms for water suppression. The figure represents one of the three blocks

3.2.2 Experimental Set-up

The design of the set-up started from 16th of May 2018 to October 3, 2018, but continuous work for maintenance and stabilization of shade and water platform structures was executed throughout the experimental period.

3.2.3 Water Suppression Platforms

Plastic sheet platforms were raised using bamboo sticks and transparent plastic sheets (350 microns plane plastic sheets, Poly-products, Ghana). The platforms (Plate 3.2.1) were raised 1 m high at one side and 0.5 m high at the other side covering the inter-rows to facilitate water movement out of the open spaces between the rows of the cocoa plants.

Plate 3.2.1 Plastic Sheet Platforms to Collect Water from Water Suppression Plots

Stem flow was not accounted for in this design. The 2/3 water suppression treatments had the plastic platforms covering 2/3rds of the spaces within the inter-rows while the 1/3 water suppressions plots had 1/3rd of inter-rows covered. Trenches were created using aluminium sheets around each subplot to collect water from the plastic sheet platforms (Appendix 6). The trenches were extended 10 m away from the treated plots (Appendix 7) to reduce contamination on the neighbouring plots. In all, 48 plastic sheet platforms were raised for the water suppression.

3.2.4 Shade Structures

To ensure uniform shading on all the treatments for unbiased interpretation of results, 40% shading was provided using black shade nets. Three main plots each measuring 30 m x 48 m (1440 m^2) were demarcated for the replications. Half of each of the plots measuring 12 m by 48 m (576 m^2) were covered with shade nets. The shade nets were raised 6.5 m from the ground and 2 m above the cocoa plants with the help of wooden poles (Figure 3.2.4; Appendix 6). Two

rows of cocoa plants were allowed between shade plots and full sun plots to reduce shading effects to the full sun treatments.

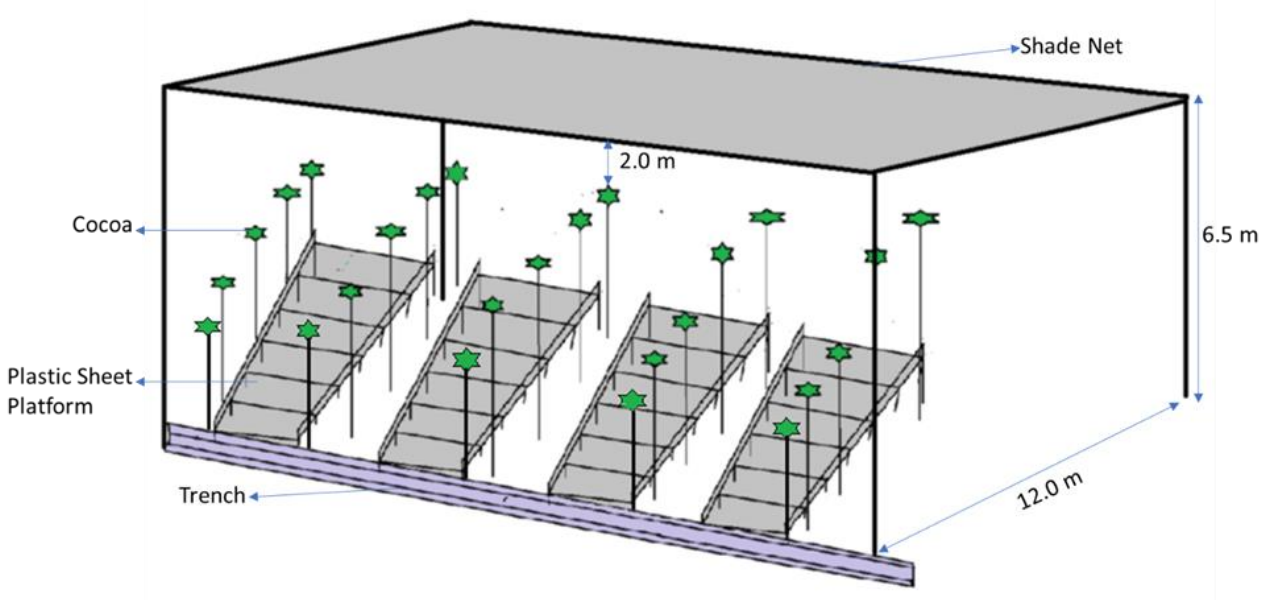

Figure 3.2.4 Annotated Diagram to show Plastic Sheet Platforms, Trenches to Collect Water from Plastic Sheet Platforms, Cocoa Plants of 4.5 m Average Height and 40% Shade Net Raised 6.5 m over the Cocoa Plants to Provide Uniform Shade

3.2.5 Pruning

The plants were pruned in April 2020 prior to the start of the experiment under a government-initiated program to support cocoa farmers. Removal of chupons were subsequently done and as when spotted on the plants.

3.2.6 Weeding

Weed control was done by slashing, every two months during the rainy seasons (April – October) and every three months during the dry season (November – March). Weeds were mostly slashed to the ground when about 30 cm high using a cutlass.

3.3.7 Insects and Diseases Control

Control of mirids and other insects were mostly done three times during the year; February, July and sometimes September (Bymolt et al., 2018) using confidor (active ingredient: Imidaclopid) and Akatsi master (active ingredient: Bifenthrin) at a recommended rate of 150 ml/ha (30 ml per 11 litres of water) and 500 ml/ha (100 ml per 11 litres of water) respectively (Baah et al., 2016). Control of black pod diseases was done in July and repeated in September every year. Ridomil Gold 66 WP at a rate of 50 g per 15 litres of water was used to control black pods. Regular removal of damaged pods as spotted ensured the reduction of sporulation of the fungus. Parasitic plants such as mistletoe were also removed with cutlasses during the experimental season.

3.3.8 Fertilizer Application

Application of Asaasewura (NKP 0-22-18+9CaO+75+MgO) was done through broadcasting in May 2018 at a rate of 3 bags per acre (150 kg/acre, about 400 kg/ha). Ammonium sulphate was placed in shallow basins around the trees at a rate of 70 g per tree and about 40 cm from the base of the trees in May 2019. In 2020 season, Asaasewura was applied again at the first week of June to increase the fertility of the soil. Sidalco (a liquid fertilizer) at 300 ml per acre was applied by foliar in March and in September each year during the experiment to enhance tree vigor and growth.

3.2.9 Climatic Parameters

A weather station containing photosynthetic active radiation (PAR) sensor (S-LIA-M003), temperature/RH smart sensor (S-THB-MOO8), rain gauge sensor (S-RGx-M002) and HOBO data logger (H21-USB) (Onset Computer Corporation, USA) was built in the community, two kilometers from the farm to monitor temperature, relative humidity, solar radiation, and rainfall

at the experimental area. Data were taken every ten minutes with the data logger. Below canopy temperature and relative humidity of the cocoa trees were monitored every 10 minutes using tinytags (Plus 2 tinytags TGP-4017, Gemini Data Loggers (UK) Ltd) and ibuttons ((DS1923-F5 hygrochron, ibutton Link, United States). One Tinytag and one ibutton per block, were hung below the cocoa canopy 1.5 m above the soil surface to monitor below canopy temperature and relative humidity. Tinytags and ibuttons were shielded under plastic bowls plated with aluminum foil to reduce heat absorption. Below-shade photosynthetic active radiation (PAR) was monitored with the light sensors on CIRAS3 (PP systems, USA). Three leaves from the top branches of four cocoa plants per plot were selected for measurements. Measurements were taken in January, April, July, September, and December each year. All measurements were taken between the hours of 9: 30 am to 11: 30 am.

3.2.10 Soil Analysis

Both physical and chemical analysis of the soil were carried out prior to the set-up. Physical analysis carried out included soil texture, bulk density, and soil moisture content.

3.2.11 Soil Texture and Bulk Density

Three pits at 50 meters distance from each other were dug to a depth of 1.6 m (Plate 3.3 C and D). Soil samples were taken at each 10 cm zone from each of the pit and composited for soil textural analysis. Analysis of soil texture followed the protocols from Soil Science Department, University of Ghana as illustrated by Bouyoucos (1962) and Barretta et al. (2014). Samples were also taken using core samplers for soil bulk density determination.

2.2.12 Soil Moisture Content

Soil water content was monitored with Diviner soil moisture probe (Diviner 2000 Series II, Sentek Soil Moisture Sensors, Sentek Technologies, South Australia). Three PVC pipes (NJPLAST GH uPVC 2" (600 cm), class O) of length 160 cm covered with access and end-cups were inserted into the soil of each plot (Plate 3.2.2. A and B). In total 54 pipes were inserted into the experimental field for soil moisture determination. Spaces around the pipes and the soil were filled with slurry (prepared from 50% sandy and 50% of the soil-type at the farm) to ensure strong contact between soil and the pipes. For each measurement, the probe was inserted into the pipes to measure soil moisture at 10 cm, 20 cm….160 cm except treatments with rocky pans which included the full sun control plots of the first block and the full sun 1/3 water suppression plots also of the first block. For the hard pan plots, measurements were taken up to the 120 cm level. Data was taken every second week from September 2019 to March 2021.

Plate 3.2.2 Monitoring Soil Moisture Content Using Diviner Soil Moisture Sensor (A and B) and a Core Sampler (C and D)

3.2.13 Root Profiling

Root profiling was done on six cocoa plants selected from the control plots using auger. The auger was driven into the soil at a 10 cm distance from the cocoa trees. Soil samples were taken at every 10 cm to a depth of 50 cm. The soil samples were placed on fine wire mesh (0.125 mm mesh) and the roots washed clean with water. Roots were then sorted into small (< 0.3mm diameter), medium (0.3 – 0.8 mm diameter) and large (> 0.8 mm) sizes (Bedeneau and

Auclair, 1989; Moser et al., 2010). Root fragments from the soil samples were oven dried at 70 ^{o}C to constant weight and tabulated as indicator of root biomass per unit soil volume.

3.2.14 Leaf Water Potential

Leaf water potential was measured using pump-up chamber (Pump-Up Chamber Instrument - PMS Instrument Company, USA). Measurements were done on monthly basis starting from November 2018 to December 2020. All measurements were taken at predawn (4: 00 am to 5:30 am) (Avila-Lovera et al., 2016). A twig (small stems of about 1mm thick) per tree was selected from the upper or the peripheral branches from three randomly selected plants per plot, eighteen plots in total. The three plants were selected from the middle nine plants and were tagged for the monthly data. The twigs were inserted into the pressure chamber and pressure was pumped into the chamber until water appeared from the cut end. The pressure in bars (later converted into MPa) at which the water appeared was recorded as the water potential of the leaves.

Diurnal measurements were also studied in January, April, July, September, and December every year during the experimental period at five different time points (5, 9, 12, 15 and 18 hrs) to monitor variations of leaf water status during the day. Selected twigs for the daily measurements were sealed in aluminium foil for 30 minutes before measurements (Plate 3.2.3). Measurements took place over three-day period with one block per day.

Plate 3.2.3 Measuring Leaf Water Potential of Plants Using Pump-Up Chamber. A – leaf covered with Aluminum foil for 30 minutes dark adaptation ; B – Reading water potential values after measurement

3.2.15 Chlorophyll Fluorescence

Dark adapted chlorophyll florescence was studied using mini-PAM photosynthesis yield analyzer (Portable Chlorophyll Fluorometer - Heinz Walz GmbH, Germany). Measurements were taken at predawn between the hours of 4:30 - 5: 30 am monthly from November 2018 to December 2020. Three matured leaves selected from top branches from four plants per plot were used for the measurement.

Diurnal measurements were also taken in January, April, July, September, and December at 4, 9, 12, 15 and 18 hours. Measurements during the day were taken after 30 minutes dark adaptation using light exclusion clips (Plate 3.2.4).

Plate 3.2.4 Measuring Chlorophyll Fluorescence Using Mini-PAM. A – Cocoa leaf with light exclusion clips; B – Taking readings from mini-PAM after measurement of chlorophyll fluorescence.

3.2.16 Gas Exchange and Photosynthesis

Measurements of rate of photosynthesis, stomatal conductance, sub-stomatal CO_2 concentration, transpiration, and water use efficiency were taken with CIRAS 3 (PP systems, USA) in September 2019, December 2019, February 2020, April 2020, July 2020 and September 2020 between 10 am – 11 am using the same leaves selected for chlorophyll fluorescence. Three matured leaves from the top branches per plant from four plants per subplots were selected for the measurements. Like water potential, measurements lasted for three days with a block per day. Data were taken at the natural light conditions with CO_2 set at 400.0 ± 10.0 µmol $mol^{-1,}$ temperature at 28.0 ± 1.0 °C, cuvette flow at 300.0 cc min^{-1} and analyzer flow at 100.0 cc min^{-1}.

3.2.17 Litter Fall

Plant litter fall was monitored from October 2018 to December 2020. Four wooden boxes of 0.25 m^2 base area and 0.5 m high (Brando et al., 2008; Triadiati et al., 2011; Ofori-Frimpong

et al., 2006) were placed on each subplot around the middle nine plants (Paudel et al., 2015b; Dawoe et al., 2010; Triadiati et al., 2011). Each litter box was placed on bamboo sticks 5 cm above the soil surface to prevent decay. In all, 72 wooden boxes were used for the litter fall. The boxes were emptied monthly. Litter from the four boxes per subplot were pulled together, oven dried at 70 ^{o}C for 48 h (Schwendenmann et al., 2010) and weighed to measure biomass leaf yield per treatment.

3.2.18 Stem Growth and Expansion

Dendrometer bands (DBM80 manual band dendrometer, ICT International) were placed around the circumference of the stem (Plate 3.2.5) of two selected plants from each subplot to monitor stem expansion as affected by shade and water suppression levels. The bands were inserted 90 cm from the base of the stem to monitor growth of stem. The sliding spring scale on the dendrometer bands extends along a fixed scale and increased in diameter in response to growth or water status in a plant. This was monitored on monthly basis from November 2018 to December 2020 between 10 am to 11 am. Growth differences between months were plotted against time to monitor trends of growth as affected by shade, water suppression and time.

Plate 3.2.5 Dendrometer Bands Wrapped Around the Circumference of a Cocoa Tree

3.2.19 Data Analyses

Monthly mean values of temperature (maximum, minimum and average), relative humidity, and photosynthetic active radiation (maximum and daily average) were tabulated for graphical presentations. Total rainfall per month and yearly averages were calculated to show monthly trends of rainfall distribution within the experimental area. Averages were calculated for below canopy temperature and relative humidity per month. Analysis of chlorophyll fluorescence, water potential, stem expansion, photosynthesis and other physiological data followed repeated measures analysis in a split design using the nonlinear mixed effect model (nlme) as proposed by Lindstrom and Bates (1990) with the formular below;

$$Y_{ij} = \mu + \alpha_j + \beta_j + (\alpha\beta)_j + n(\text{treatments})_{ij} + \varepsilon_{ij}$$

Where;

Y_{ij} = response variable of experimental unit i receiving treatment j.

μ - intercept

α_i - j^{th} levels of the shade factor (whole plot) – fixed variable

β_i - j^{th} levels of the water suppression factor (subplots) – fixed variable

$(\alpha\beta)_i$ - interaction between the j^{th} levels of shade factor and water suppression factor

$n(\text{treatments})_{ij}$ - random variables in the treatments which included the effects of the number of blocks and plants within subplots.

ε_{ij} - the error factors to estimate variations between main plots and within subplots

Model validity was tested with normal distribution plots of residuals and qqnorm plots in the R statistical package, version 4.1.1. Data on diurnal measurements of water potential, transpiration, sub-stomatal CO_2 concentration, stomatal conductance, water use efficiency,

stem expansion and litter fall were log-transformed to meet data homogeneity. Significant differences were tested with backward elimination using the drop1 function in the R statistical package. Mean separation was done with Tukey Honest Significant Difference test (Tukey HSD). Extracted means and standard errors were used for graphical presentations.

3.3 Results

3.3.1 Climatic Conditions at the Experimental Site

Average yearly rainfall during the 2019 and the 2020 year at the experimental site ranged between 1110 – 1250 mm. The months of January and August recorded the least rainfall within the year with total monthly rainfall below 5 mm (Figure 3.3.1 C). Rainfall was frequent between March to July, a short dry spell in August and then strong rains in September and October with the later recording the highest monthly total of 250 mm**.** Monthly average temperature ranged between 25 – 30 ^{o}C throughout the year (Figure 4.1 B). Maximum temperature was between 35 to 40 ^{o}C with February, March and April being the hottest months in the year. Minimum temperatures were between 15 to 21 ^{o}C while January, February and August were the coolest months of average temperatures between 15 – 18 ^{o}C. Relative humidity was high between May to July but low in January and February. The maximum photosynthetic active radiation recorded indicate that months with high rainfall come with high solar radiations with maximum PAR as high as 2191 µmol m^{-2} s^{-1} in October and 2221 µmol m^{-2} s^{-1} in May. January showed a low maximum PAR of 1541 µmol m^{-2} s^{-1}. Monthly average PAR for the hours with light (Day length was virtually equal at the experimental site) on the other hand indicated the months of February, March, and April as showing higher PAR averaging between 522 - 720 µmol m^{-2} s^{-1}. July gave the least average PAR of 235 µmol m^{-2} s^{-1} in a year.

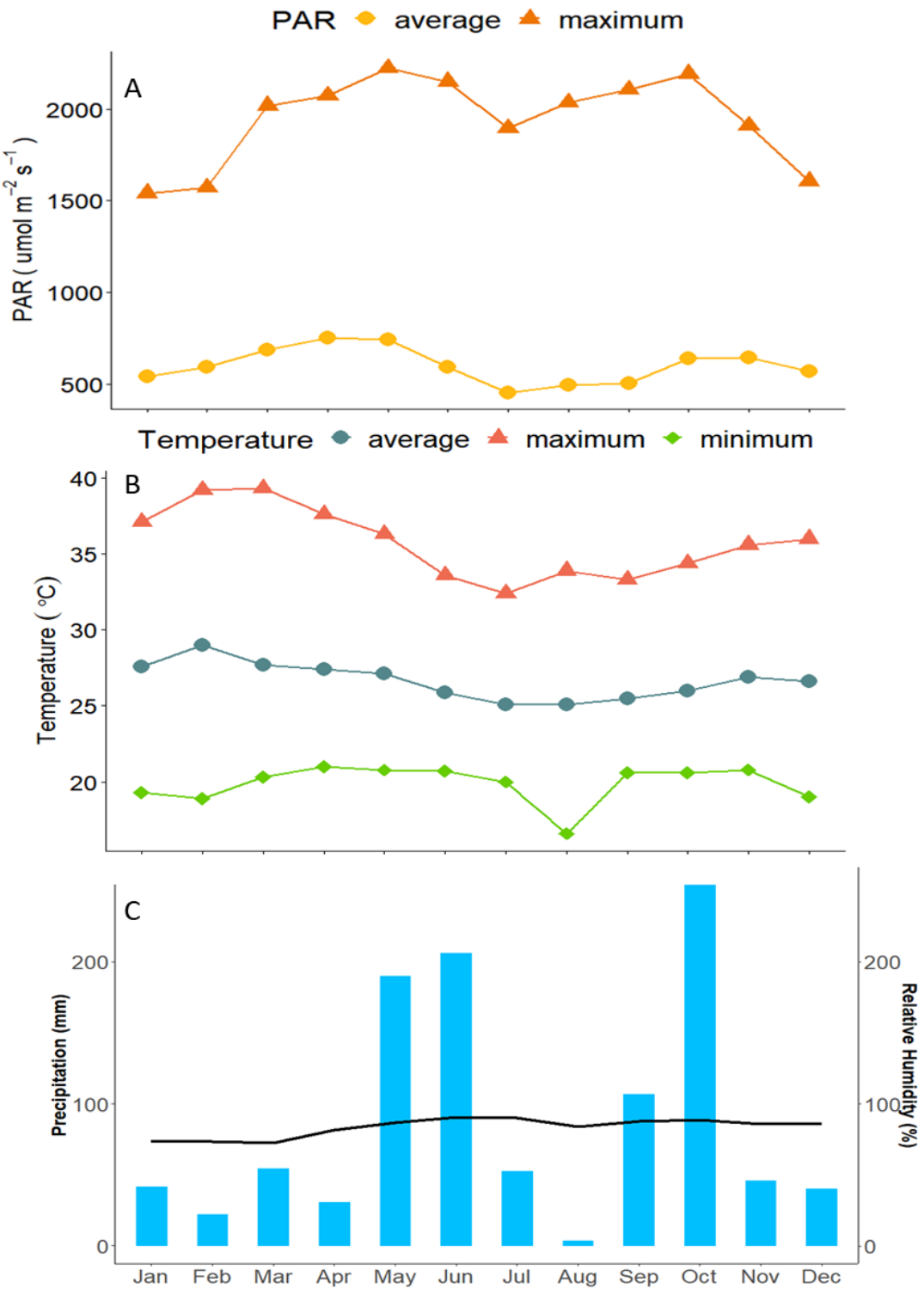

Figure 3.3.1 Monthly Means of Photosynthetic Active Radiation (PAR) (A); Average, Maximum and Minimum Air Temperature (B); Rainfall and Relative Humidity (C) at the Meteorological Station Near the Experimental Site (Sefwi Kunuma). For Figure 3.3.1 C, blue bars indicate monthly rainfall while black line indicates relative humidity

3.3.2 Climatic Conditions in the Farm

Temperature and relative humidity below the canopy of the cocoa plants varied between shade and full sun conditions (Figures 3.3.2). On average relative humidity increased under shade conditions with monthly averages between 75 to 95% while the highest relative humidity under full sun conditions was 85% in April and in July (Figure 3.3.2 A). On the other hand, high temperature was noted under full sun conditions (Figure 3.3.2 B and C) with the months of February and March showing average monthly maximum temperature in the range of 40 – 42 ^{o}C comparing with shade which was at maximum around 38 - 39 ^{o}C. Minimum temperature showed no significant difference between shade and full sun conditions (Figure 3.3.2D).

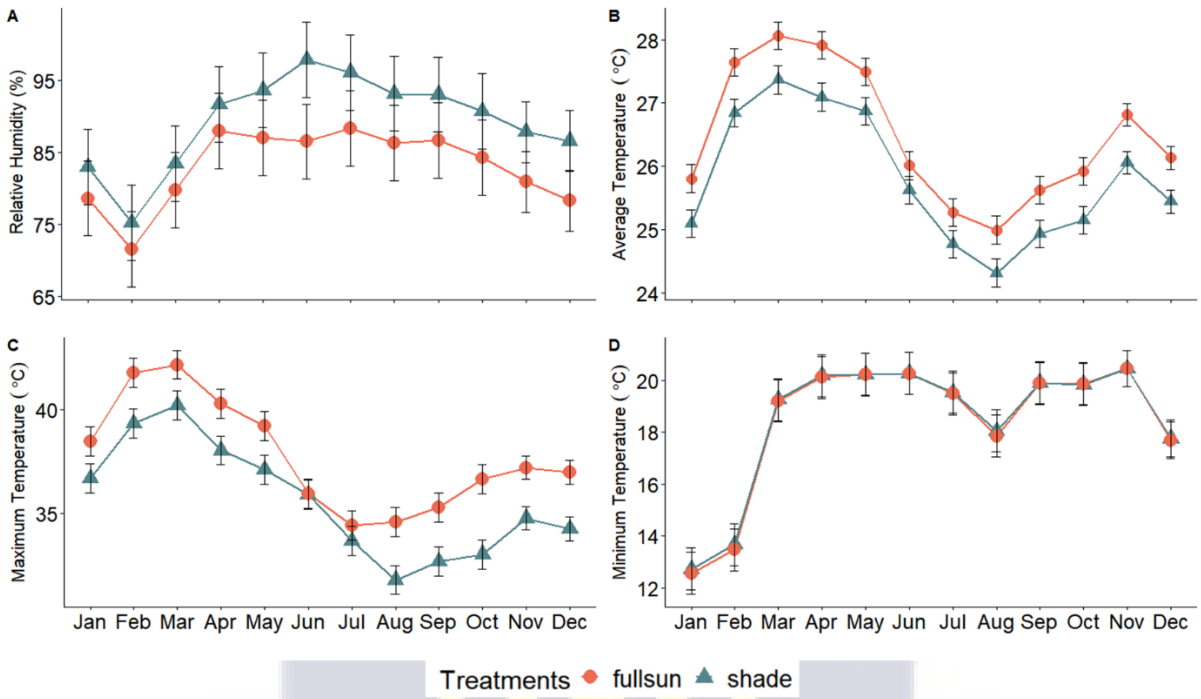

Figure 3.3.2 Monthly Variations of Relative Humidity, Maximum, Minimum and Average Temperature Below the Cocoa Plants Measured Under Shade and Under Full Sun Conditions. Bars indicate standard error

Light availability measured above the cocoa canopy but below the shade nets was highly variable among the shade levels. Full sun plots showed values as high as 1500 µmol m^{-2} s^{-1} (Figure 3.3.3). On the other hand, shade treatments had limited access to light between 300 – 500 µmol m^{-2} s^{-1} which interaction between shadow effects from neighbouring plants and the shade net could contribute to the low light distribution.

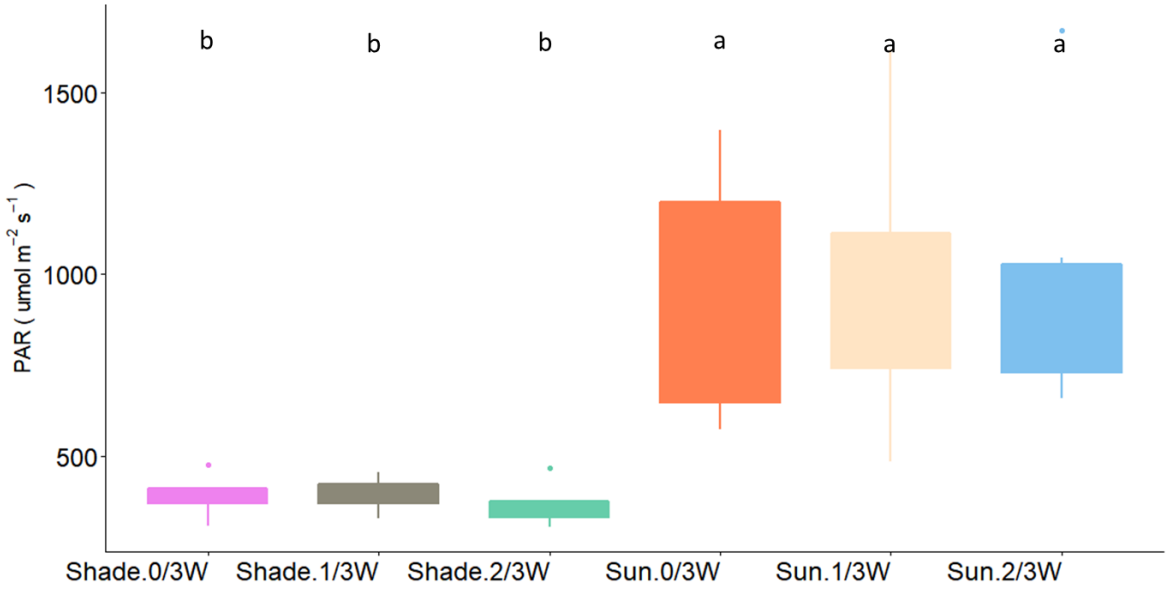

Figure 3.3.3 Light Distribution Among Treatments Under Shade and Under Full Sun Conditions

3.3.3 Soil Texture and Bulk Density

Soil textural classes at every 10 cm for the 160 cm depth indicated sandy-clay soil at the top 10 cm but clay soils from 20 cm to 160 cm (Figure 3.3.4). The similar textural classes could show comparable soil characteristics including water movements, wilting points and chemical compositions along the soil profile. Soil bulk density, however, differed along the soil profile with the top 10 cm soil showing compaction of 1.6 g/cm^3 bulk density while the subsoil (10 –

20 cm depth) was more loose having values below 1.4 g/cm^3. Soil bulk density from 30 cm depth increased along the profile ranging between 1.4 g/cm^3 to 1.7 g/cm^3.

Soil Profile	Soil Depth (cm)	%Sand	%Silt	%Clay	Textural Class
	10	48	12	41	Sandy Clay
	20	43	7	51	Clay
	30	32	5	63	Clay
	40	23	7	71	Clay
	50	27	6	68	Clay
	60	23	4	73	Clay
	70	24	3	73	Clay
	80	23	4	73	Clay
	90	21	4	75	Clay
	100	22	3	75	Clay
	110	24	3	73	Clay
	120	27	2	71	Clay
	130	26	8	66	Clay
	140	26	11	63	Clay
	150	34	8	58	Clay
	160	30	9	61	Clay

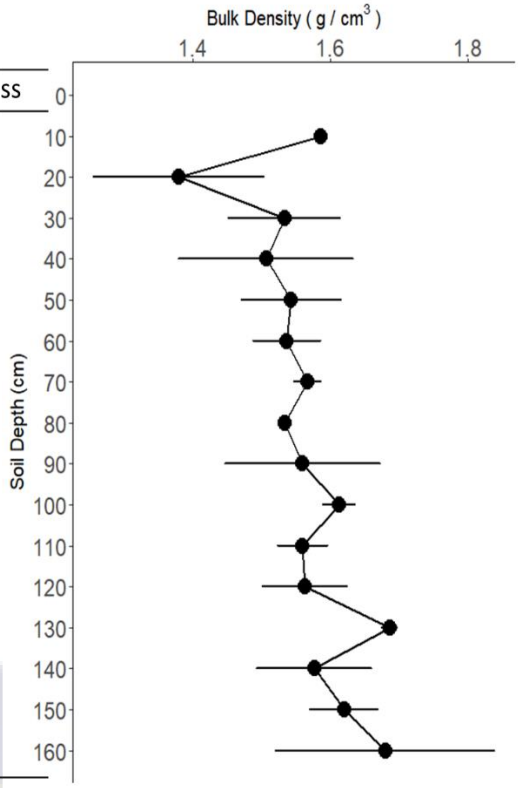

Figure 3.3. 4 Soil Profile, Soil Texture and Bulk Density at Different Soil Depths in the Cocoa Farm at Sefwi Kunuma. November 2019 Dry Season, n=3. Soil profile shows change of soil color at each 10 cm level along the profile to the 160 cm depth. Bars on bulk density graph indicate standard error

3.3.4 Soil Moisture and Root Profile

The cocoa plants in the study area had high root distribution within the 10 cm soil zone (Figure 3.3.5 A) with a decreasing effect along the soil profile. Volumetric soil moisture contents were between 0.20 – 0.45 m^3/m^3 along the soil profile (Figure 3.3.5 B). The top 10 cm soil was comparatively drier among the water suppression levels under shade and full sun conditions. Soil moisture within the 20 cm zone was high among all the treatments. The soil bulk density

within the profile and the drainage rate from the topsoil could be contributing factors to this effect. Shade, water suppression and soil depth interacted to affect soil moisture distributions (Table 3.3.1). Control plots had the highest moisture content under shade and under full sun conditions along the soil profile with values between 0.30 to 0.40 m^3/m^3 for shade control plants and between 0.3 – 0.35 m^3/m^3 for full sun plants. Water suppression treatments under full sun conditions gave the least soil moisture with between 0.20 – 0.30 m^3/m^3 and decreased as soil depth increased.

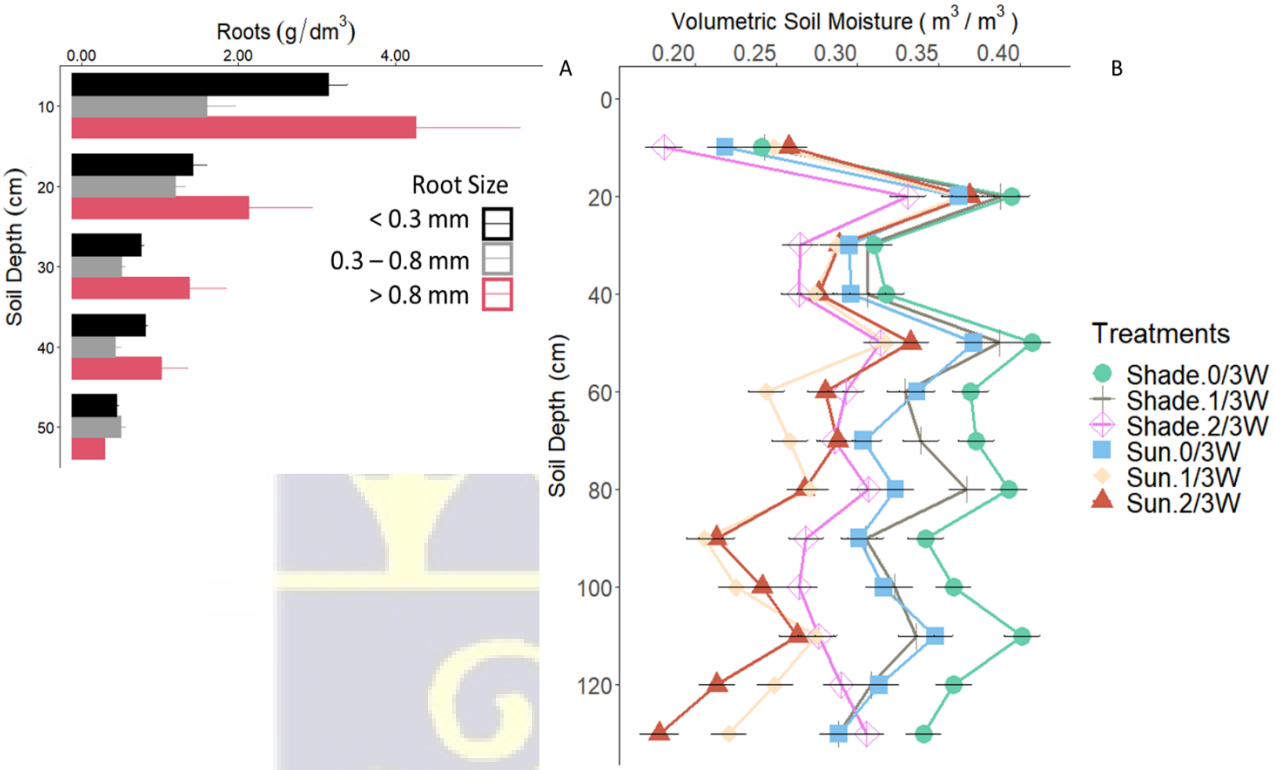

Figure 3.3.5 Roots Profile (A) and Effect of Shade and Water Suppression Levels on Volumetric Soil Moisture Content (B) at Different Depths of Soil. Soil moisture data were monitored every two weeks from June 2019 to December 2020. Bars indicate standard error

3.3.5 Stem Water Potential

Shade, water suppression and time independently affected predawn stem water potential (Table 3.3.1). Plants kept under shade showed increased water potential than plants kept under full sun conditions (Figure 3.3.6) while water suppression reduced stem water potential to as low as -1.0 MPa for the full sun 2/3 water suppressions plants.

Table 3.3.1 Probability Values for Effects of Shade and Water Suppression on Below Canopy Temperature, Below Canopy Relative Humidity, Predawn Chlorophyll Fluorescence, Diurnal Variation of Chlorophyll Fluorescence, Predawn Water Potential, Diurnal Changes of Water Potential, Stem Expansion and Litter Fall

	Ave. Below Canopy Tem.	Below Canopy Hum.	Soil Moisture	C.Flu-Predawn	Cflu-diurnal	Water Potential-Predawn	Water Potential-Diurnal
Sources of Variation							
Shade	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Suppression	na	na	<0.001	<0.001	<0.001	<0.001	<0.001
Time/Soil Depth	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Shade*Suppression	na	na	<0.001	0.003	0.007	0.136	<0.001
Shade*Time/Soil Depth	0.999	0.997	<0.001	<0.001	0.003	0.053	<0.001
Suppression*Time/Soil Depth	na	na	<0.001	0.811	0.265	0.723	<0.001
Shade*Suppression*Time/Soil Depth	na	na	<0.001	0.996	0.011	0.979	0.125

*na – not applicable

Between May to July in both 2019 and 2020 where there were frequent rains, plants under shade had stem water potential as high as -0.2 MPa while full sun suppressed plants gave values between -0.3 to -0.4 MPa. Between January to March 2020, all treatments gave lower values coinciding with the dry months.

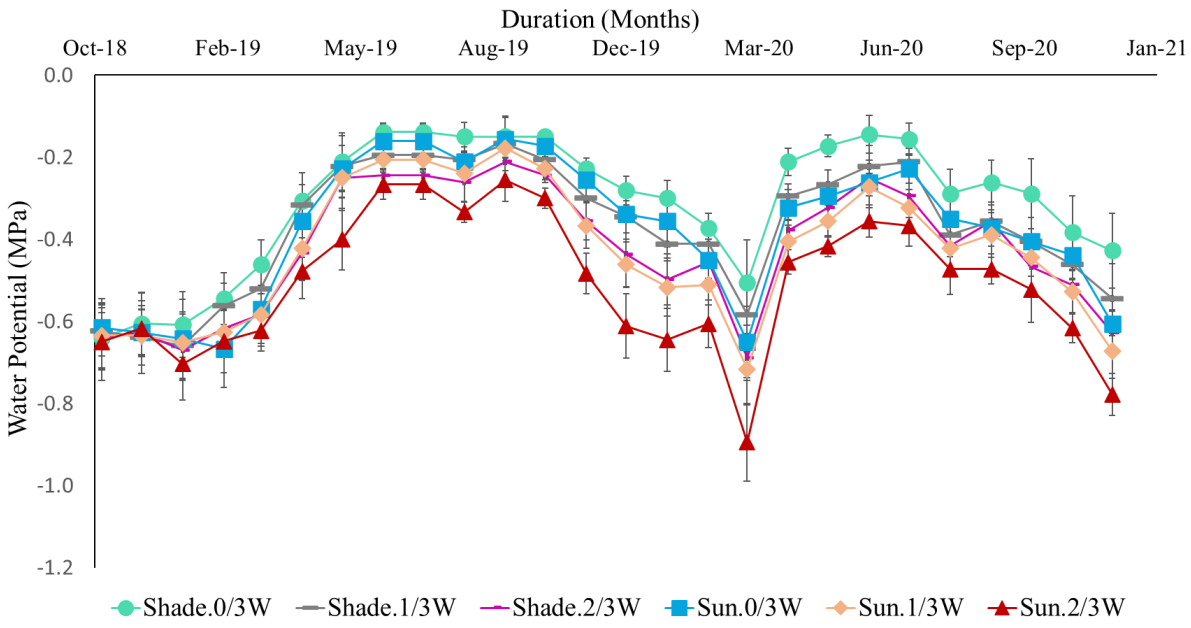

Figure 3.3.6 Monthly Variations of Predawn Water Potential as Affected by Shade and Water Suppression Levels. Bars indicate standard error

3.3.6 Diurnal Variations of Water Potential

Shade and water suppression statistically interacted to affect water potential across the day while each of the factors (shade or water suppression) also depended on the time of measurements to vary water potential over the day (Table 3.3.1). Generally, stem water potential improved at predawn and later parts of the day but impaired at the midday (Figure 3.3.7). Full sun treatments during the rainy and dry seasons comparatively showed reduced water potential at all time points data were taken. Plants under shade showed improved values at both seasons ranging between -0.1 to -0.6 MPa while values under full sun averaged between -0.2 to -1.5 MPa at both seasons. The 2/3 water suppression treatments under full sun had the least stem water potential values at the various time points and was as low as -1.5 MPa during the midday in the dry season.

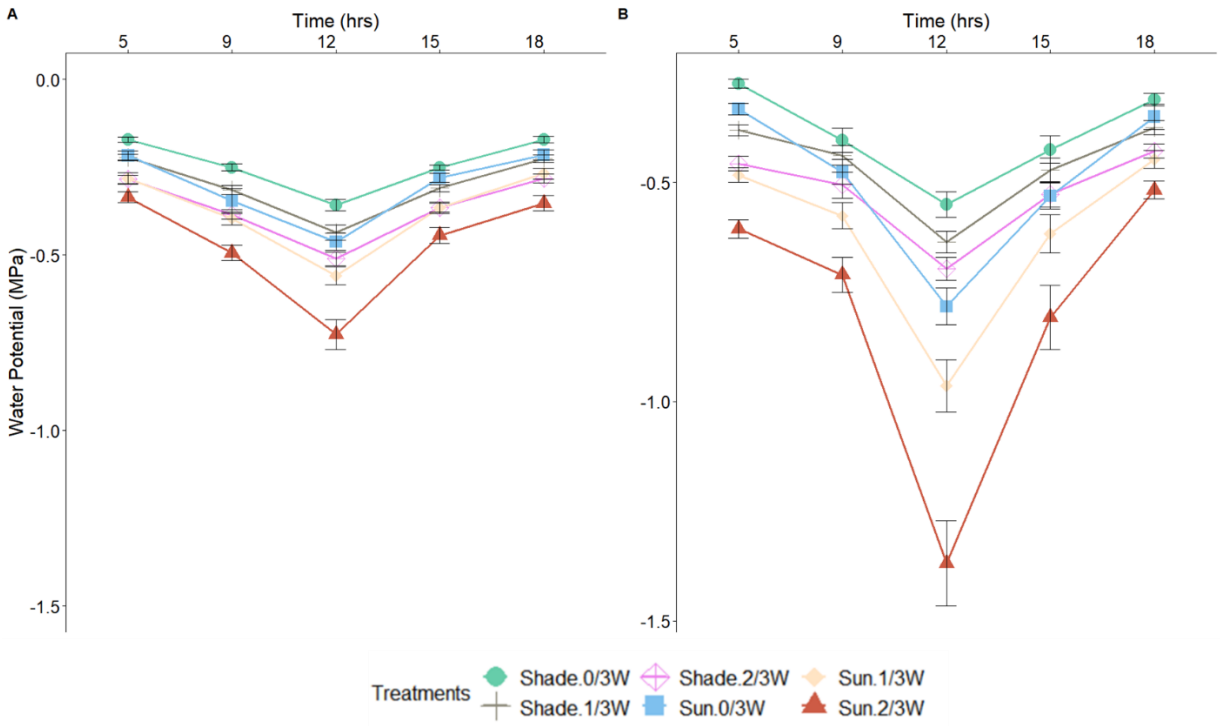

Figure 3.3.7 Effect of Shade and Water Suppression on Diurnal Variations of Water Potential During the Wet (A) and the Dry (B) Months. Data for wet months were taken in April, July, and September every year while data for dry months were taken in December and February every year. Bars indicate standard error

3.3.7 Chlorophyll Fluorescence

Shade interacted with water suppression levels and with time (in months) to affect response to fluorescence during the experiment (Table 3.3.1). Chlorophyll fluorescence was low between November 2018 to March 2019 (Figure 3.3.8) showing values as low as 0.68 in the Sun.2/3W treatments. The same pattern was seen in the November 19 to March 2020 dry season among the full sun treatments.

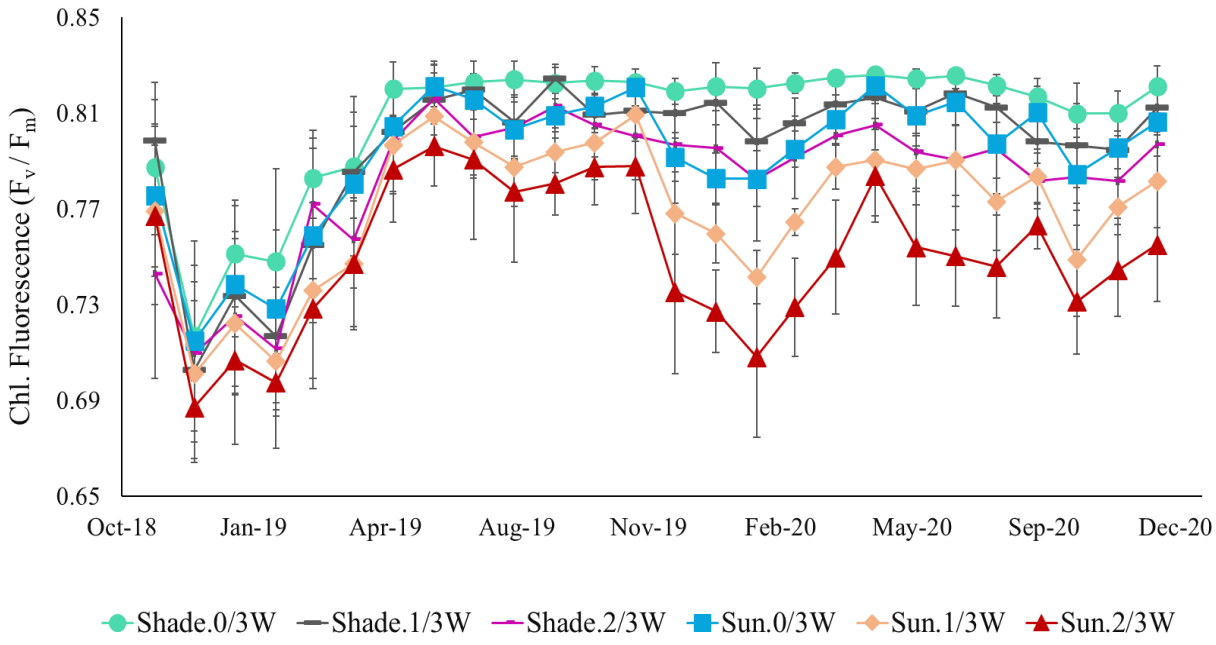

Figure 3.3.8 Monthly Variations of Predawn Chlorophyll Fluorescence as Affected by Shade Levels and Water Suppression Levels. Bars indicate standard error

Plants under shade were less affected by water suppression on chlorophyll fluorescence comparing with plants under full sun conditions, but the shade effect also depended on the weather conditions within the month. During months with frequent rains (Figure 3.3.8) increased response to fluorescence was noted. Chlorophyll fluorescence was also observed to have a high positive correlation (0.83, $P < 0.001$) with soil moisture with increased soil moisture enhancing fluorescence.

3.3.8 Diurnal Variations of Chlorophyll Fluorescence

Response to fluorescence averaged between 0.65 and 0.8 during the day in both wet and dry seasons (Figure 3.3.9). Time of the day interacted with shade and water suppression levels to affect leaf chlorophyll fluorescence. Like water potential, chlorophyll fluorescence was generally low during the midday with plants under full sun showing comparatively lower

values. Two third (2/3) water suppressed plants under full sun produced the least response to fluorescence at each time of measurement in a day.

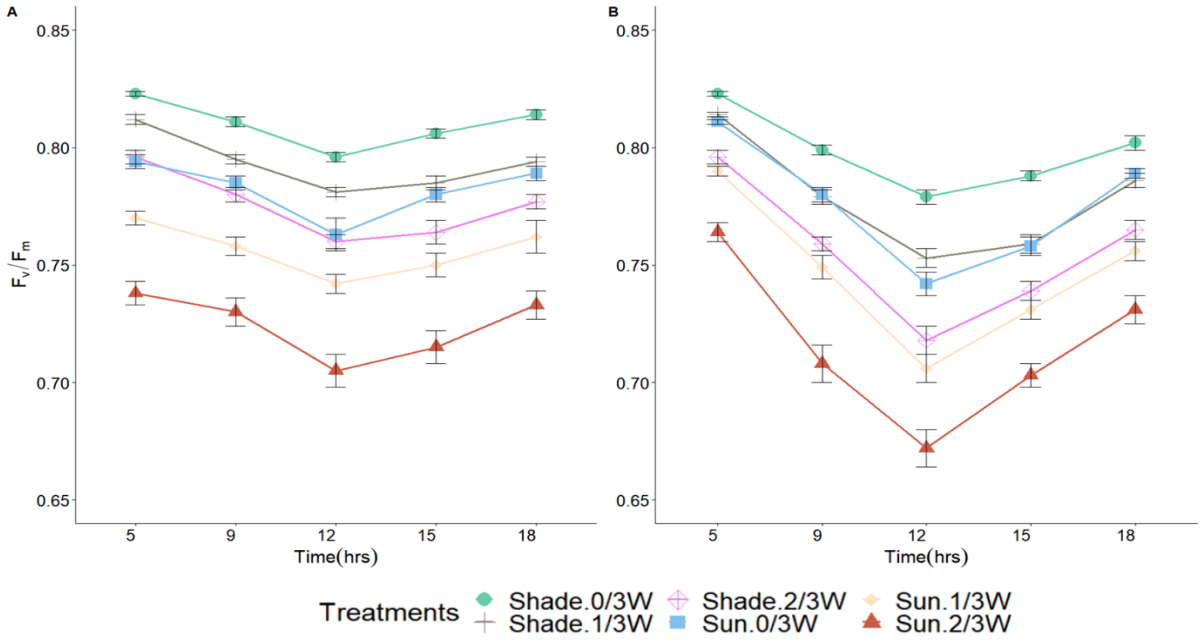

Figure 3.3.9 Effect of Shade and Water Suppression on Diurnal Variations of Chlorophyll Fluorescence During the Wet (A) and the Dry (B) Months. C.Fluorescence = Chlorophyll Fluorescence measured after leaves were dark adapted for 30 minutes. Bars indicate standard error

3.3.9 Gas Exchange and Photosynthesis

Leaf stomatal conductance (gs) was affected by shade and time interactions, but not the water suppression levels (Table 3.3.2). Stomatal conductance values were between 268 – 310 mmol m^{-2} s^{-1} under shade while full sun grown plants had values between 179 – 194 mmol m^{-2} s^{-1} with increased values during the wet months (Figure 3.3.10). Thus, full sun grown plants had partially closed stomata compared with shade grown plants, reflected in the rate of transpiration where full sun grown plants showed reduced rates than shaded plants depending on the month of measurements.

Table 3.3.2 Probability Values for Effects of Shade and Water Suppression Levels on Stomatal Conductance (g_s), Photosynthesis (P_n); Transpiration; (E) and Water Use Efficiency (WUE) and Sub-Stomatal CO_2 Concentration (C_i)

	g_s	P_n	E	WUE	C_i	PAR	Stem Exp.	Litter Fall
Sources of Variation								
Shade	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.328	0.001
Suppression	<0.553	<0.001	0.434	<0.001	<0.001	0.228	<0.001	<0.001
Month	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Shade*Suppression	0.122	0.303	0.148	0.273	<0.119	<0.144	0.546	0.723
Shade*Month	0.008	<0.001	0.013	0.002	0.004	<0.001	0.802	<0.001
Suppression*Month	0.755	0.001	0.508	<0.001	0.950	0.552	0.647	0.378
Shade*Suppression*Month	0.084	0.078	0.022	0.004	0.914	0.410	0.777	0.733

Rate of photosynthesis and water use efficiency were affected by the interactions between shade and month of measurement or between water suppression and month of measurements while sub-stomatal CO_2 concentration of the leaves depended on interactive effects of only shade and month of measurements. Sub-stomatal CO_2 concentration and transpiration statistically increased under shade conditions than under full sun conditions depending on month of measurements. Among the water suppression levels, transpiration statistically remained unaffected by the suppression levels (Figure 3.3.10). On the other hand, rate of photosynthesis and water use efficiency increased under full sun conditions especially during the wet months (September and July). Full sun control plants had the highest rate of photosynthesis of 9.04 umol m^{-2} s^{-1} noted in September 2020 while 2/3 water suppression treatments under shade gave the least of 5.30 umol m^{-2} s^{-1} observed in December 2019. Control plants under shade and under full sun conditions showed increased water use efficiency than the suppressed plants due to the observed higher rates of photosynthesis for control plants but

with similar rates of transpiration among the water suppression levels under both shade and full sun conditions.

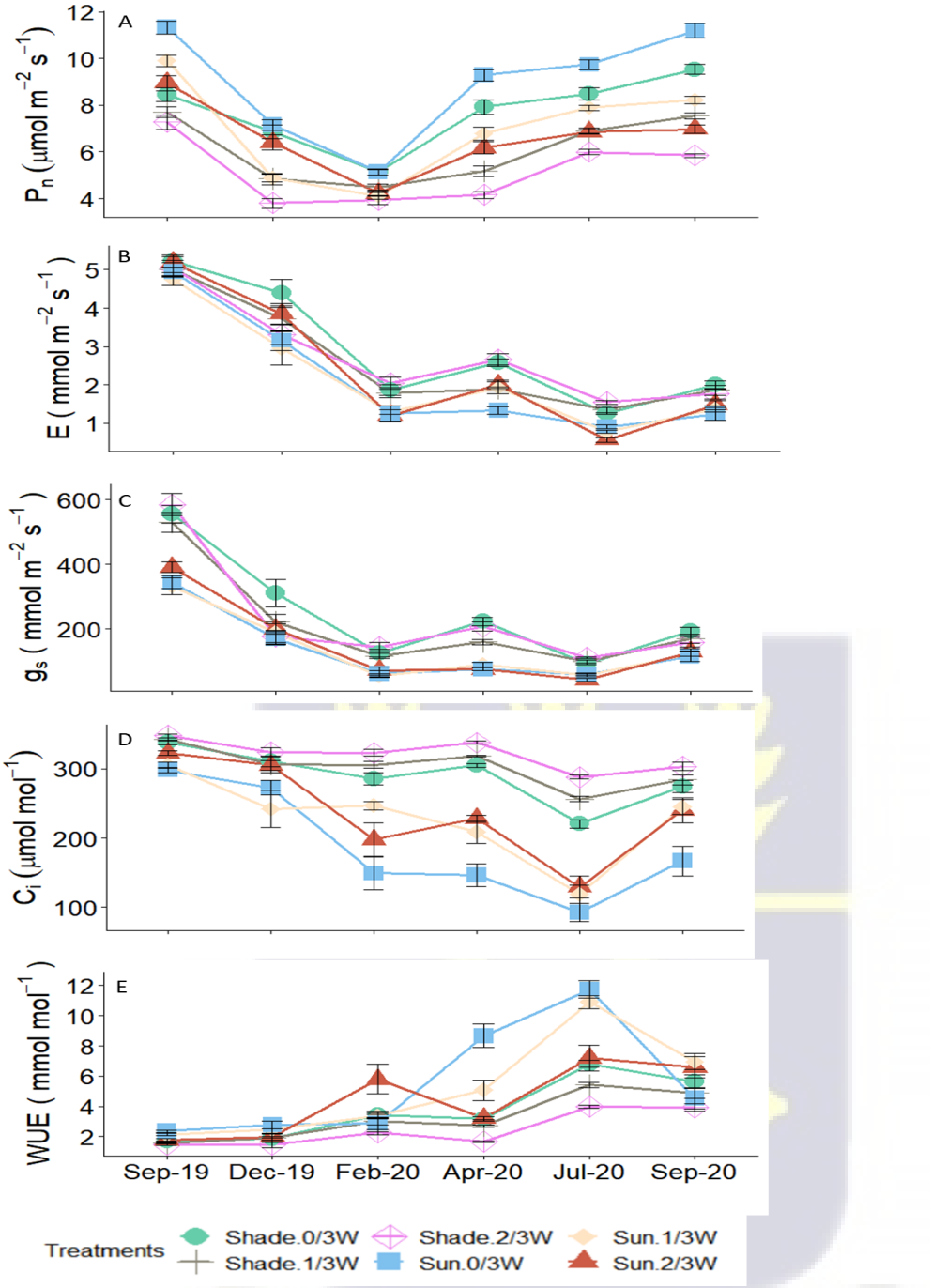

Figure 3.3.10 Effect of Different Levels of Shade and Water Suppression on A) Photosynthesis (P_n); B) Transpiration (E); C) Stomatal Conductance (g_s); D) Sub-Stomatal CO_2 Concentration (C_i) and E) Water Use Efficiency (WUE). Bars indicate standard error

3.3.10 Litter Fall and Stem Expansion

Interaction of shade and month of measurement affected yield of litter significantly (Table 3.3.2). Litter production increased more in full sun during the dry months than in shade. Two third (2/3) water suppressed plants under shade and full sun conditions had the highest litter fall among the treatments (Figure 3.3.11A).

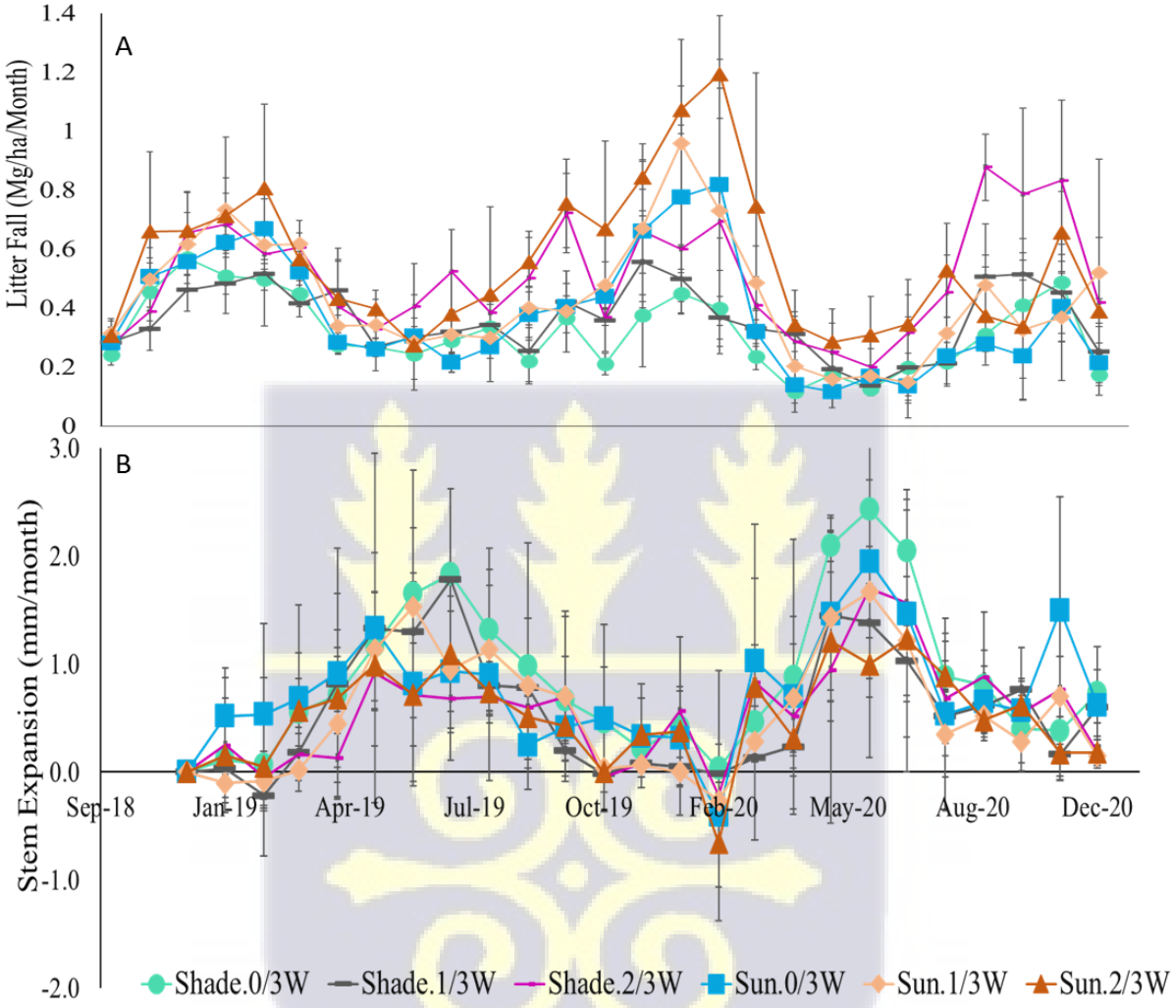

Figure 3.3.11 Effect of Different Levels of Shade and Water Suppression on Stem Expansion and Litter Fall of Cocoa Plants Monitored for Two Years. Bars indicate standard error

The dry months such as December to February increased plant's litter fall. Monthly variations of leaf litter were between 0.2 to 1.2 Mg/ha with the 2/3 water suppression treatments under full sun conditions giving the highest monthly litter fall of 1.2 Mg/ha in February 2020. Annual litter fall under shade conditions showed values in the range of 3.6 – 6.2 Mg/ha among the water suppression levels while values under full sun conditions were between 4.4 – 6.7 Mg/ha with 2/3 water suppression levels producing much litter per year under the two conditions.

Growth in terms of stem expansion increased between December to July but reduced afterwards towards the end of the year among all the treatments (Figure 3.3.11). On average, yearly total stem expansion showed values between 3.9 to 6.1 mm among the treatments. Plants from control treatments had the fastest growth peaking at 5.3 mm/year under full sun and 6.1 mm/year under shade conditions. No interactive effects were detected between shade, water suppression and time of measurement (Table 3.3.2). Stem expansion impaired with decreasing water availability to the plants with the 2/3 water suppression plants having the least expansion. Like litter fall, stem expansion varied between -1.0 to 2.5 mm/month. Months with low rainfall such as December, January and February caused almost cessation of growth under all the treatments comparing with the wet months. Months with low stem expansion corresponded with months of high leaf litter fall (-0.70, $P < 0.001$) among all the treatments. Stem expansion also correlated strongly with water potential (-0.73, $P < 0.001$) and soil moisture (0.54, $P<0.001$).

3.4 Discussion

The results from the experiment confirmed the hypothesis that drought can alter the physiological functions of cocoa plants and shade could be a promising strategy to modify the effects. The work confirms the earlier reports by Baligar et al. (2008) and Asare et al. (2016)

that shade has a positive effect on cocoa production. The results suggest that a 40% shade could sustain some of the plant's physiological functions such as chlorophyll fluorescence, stomatal conductance, and sub-stomatal CO_2 concentrations under drought.

Immediate climatic conditions of the plants were improved under shade with relative humidity as high as 95% comparing with full sun conditions of maximum of 85%. Temperature and solar radiation were high in some months of the year, but temperature was about 4 – 10 ºC lower under shade while solar radiation reduced to about 50% to 60% of values observed in full sun depending on month of measurement. Between February and March, below canopy maximum temperature conditions ranged between 40 – 42 ºC which might affect some physiological activities of cocoa especially plants in full sun conditions. Optimum temperature for growth of cocoa is reported to be between 24 ºC to 34 ºC (Gomes and Kozlowski, 1987a; Najihah et al., 2018) while optimum temperature for photosynthesis is in the range of 31 to 35 ºC (Balasimba et al., 1990; Yapp, 1992). Though plants can survive under extreme temperature of about 40 ºC (Valle et al., 1990) many physiological activities such as PSII activation, ATPase activity and the carbon assimilation process are impaired under extreme temperatures posed by limited water availability (Mathur et al., 2010; Chen et al., 2012; Carrion-Tacuri et al., 2013). The reduction of temperatures under shade may reduce the likelihood of adverse effects. In addition, high temperature, low relative humidity, and high solar radiation under full sun conditions might increase leaf surface temperature which would increase transpiration rate and water demand for transpiration. On the other hand, shade had the potential to maintain high humidity in an optimum temperature to reduce evapotranspiration and conserve soil moisture.

Root number per unit volume was high at the upper 30 cm indicating increased absorption of available water at the upper zone as marked by Moser et al. (2010) and Lahive et al. (2019) for cocoa. Moisture contents for the suppressed treatments under shade were maintained within the 0.25 – 0.40 m^3/m^3 range and showed limited effects on chlorophyll fluorescence and stem

water potential. As stated by Wood and Lass (2001), shade can reduce stress conditions such as radiation load and heat thereby conserving soil moisture. High soil moisture content under shade was identified at all the soil levels compared with the treatments under full sun plots. Shading therefore may have a positive effect on cocoa production and could reduce effects of drought thereby protecting the plants from poor climatic conditions. Stakeholders who rely on cocoa cultivation may thus prepare for future climatic waves when optimum shade is provided for the plants.

At the site of photosystem II, water splits down by light energy to produce electrons and hydrogen ions. The efficiency of the photosystem II complexes is very important for such processes. High chlorophyll fluorescence (f_v/f_m) indicates improved photosystem II activity while low values indicate otherwise (Galyuon et al., 1996). A strong pattern of similarity existed between chlorophyll fluorescence, leaf water potential and soil moisture, showing parallel variations in response to rainfall. Values during the wet months were high compared with values during the dry months as also observed by Araque et al. (2012) with values in the range of 0.72 – 0.77 during the rainy months but 0.65 – 0.73 during the dry months in cocoa. Drought stress has been identified to increase non-photochemical quenching of chlorophyll fluorescence, a strategy that might be used by the plant to dissipate heat due to energy excess and then protect the PSII reaction centres from damage (Janusz et al., 2006). Increased non-photochemical quenching is an indication of light energy absorption exceeding utilization capacity of plants under full sun and under drought. Solar radiation for full sun conditions was high ranging between 1200 to 2000 µmol m^{-2} s^{-1}, much above the light saturation point of around 200 to 750 µmol m^{-2} s^{-1} for cocoa (Salazar et al., 2018; Da Matta et al., 2001). To protect the photosystem II antennae from stress effects of high light intensity, heat and drought, plants needed to dissipate heat by grounding excited electrons thus suggesting increasing non-photochemical quenching under full sun conditions. Water is noted to rapidly quench $^1O_2$

generated via ^{3}P680* and thereby protecting the thylakoid or the light harvesting complexes from damage (Asada, 2006). When there is not enough soil moisture, stomata closure occurs to decrease CO_2 conductance and therefore causing a diversion of electrons from the photosynthetic electron transport chain to molecular oxygen (Miller et al., 2010; Dalal and Tripathy, 2018). The situation builds up reactive oxygen species and causes damage to the light harvesting complexes and the thylakoid membranes (Dalal and Tripathy, 2018). It is anticipated that when the drought stress was high, plants were unable to dissipate enough heat to protect the photosystem II light harvesting complexes due to damages. This was evident at the midday measurement of chlorophyll fluorescence of values below 0.70 for full sun conditions but above 0.75 for shade conditions. Values ranging between 0.65 to 0.77 have been reported under full sun cocoa while values around 0.8 have been noted under shade conditions showing intact PSII system under shade conditions as confirmed by De Araujo et al. (2017).

Rate of photosynthesis has been shown to decrease alongside leaf water potential (Hirasawa and Ishihara, 1978) and that a strong relationship exists between plant water potential and gas exchange (g_s) (Marino et al., 2018). However, Ishihara and Saito (1983) reported that photosynthetic activity is not actually affected by reduction of leaf water potential, but the effect is entirely due to a decreased CO_2 supply to the leaves because of stomata closure. Evidently, plants under full sun conditions recorded lower stem water potential and gas exchange (g_s) than those under shade conditions although rate of photosynthesis (P_n) improved. More so, improved P_n under full sun conditions might be associated with abundant solar radiation for photosynthetic rate to reach early saturation point comparing with limited light available to shaded plants. As earlier reported by Cunningham and Arnold (1962), full sun conditions increase positive interaction between increased light and nutrients. Solar radiation transmittance, photosynthetic active radiation and higher radiation use efficiency are enhanced under full sun conditions (Agele et al., 2016) but plant's energy balance may be at

stake at higher radiation level which would mean photosynthesis would be high under full sun conditions at the cost of the life span of the plant (Ahenkorah et al., 1974). The situation was, however, not the same among the water suppression levels; sub-stomatal CO_2 concentration decreased proportionally with water suppression though g_s was not affected. This agrees with Marino et al. (2018) which indicated that non-stomatal factors are predominantly high at elevated stress levels suggesting impaired photosynthetic metabolism in stressed plants. Rate of photosynthesis (P_n) reduced proportionally with water suppression levels to reflect the impairment of photosynthetic metabolism at high water suppression. The condition has been linked to a loss of chlorophyll fluorescence (Brodribb, 1996) giving evidence of damage to photosynthetic machinery under drought conditions. Under severe drought, quenching of excitation energy in the PSII antennae may be unable to dissipate excess energy resulting photodamage to PSII (Nogues and Baker, 2000). Gas exchange was only affected by shade levels instead of water suppression levels. Thus, though soil moisture content around the roots reduced on the stressed plots, the suppression was not so severe to have caused stomata closure. Research indicates that the relationship between stomata conductance and leaf water potential differs among growth forms and that stomata conductance and transpiration are incentive to progressive larger changes in water potential from herbaceous annuals to woody plants (Lambers et al., 2008).

At high temperature and low relative humidity, plants, especially under full sun conditions, might have experienced partial stomatal closure to reduce transpiration, but this was a cost to CO_2 supply to the plants (Dazkowska-Golec and Szarejko, 2013). In effect carbon gain relative to water loss during photosynthesis was low (Evans and von Caemmerer, 1996). This may explain increased water use efficiency recorded in the full sun conditions though photosynthesis was not affected. It is noted that stomata closure does not efficiently control water loss in cocoa and thus cuticular transpiration may be a contributing factor (De Almeida

and Valle, 2007). In another development, cocoa leaves do not show high stomatal resistance under water stress (De Almeida and Valle, 2007). In these results, transpiration rate did not differ among water suppression levels indicating a possibility of poor control of stomata to water loss. This could be the reason why cocoa plants normally perform well at lower belt between 20^{o} North and South where rainfall ranges between 1200 to 3000 mm per annum. Further work, however, is needed to prove the claim.

Drought has direct effect on water status in the plant affecting stem expansion, chlorophyll fluorescence and photosynthesis. Cocoa plants do not efficiently regulate their stomata to conserve water under drought conditions indicating the need of a constant supply of water to the plants. Shading could be a strategy to enhance physiological performances of cocoa under harsh climates such as drought. The experiment has proven that shading increases plants microclimates to increase plant's water status enhancing photosystem II efficiency to protect photosynthetic machinery. Continuous survival of cocoa plants for many years is more positive under shade conditions than under full sun conditions. The results indicate the need for further physiological studies on cocoa plants to understand the sensitivity of the plants under drought conditions and how policies could be deployed to enhance survival at the expected climate change.

CHAPTER IV

SHADE INCREASES YIELD OF COCOA UNDER WATER STRESS

4.1 Introduction

Global cocoa demand is growing at an annual rate of 2% with an estimated quantity of about 4 671000 tons consumed in 2019/2020 (Meyers and Gillett, 2021). Over 4 726 000 tons of cocoa beans were produced in 2019/2020 cropping year, 75.4% was contributed by Africa (ICCO, 2021). Cocoa is notably important to the livelihoods of over 800 000 Ghanaian households that depend on it for survival and contributes to about 70 – 100% of their annual household incomes (Anim-Kwapong and Frimpong, 2004; Najihah et al., 2018). Despite the significant contribution of the crop to household income, cocoa farmers face many challenges including low tree productivity because of limited extension delivery, increasing soil degradation, poor quality of planting materials, diseases, and pests (Wessel, 2015; Maguire-Rajpaul et al., 2020). Yields of cocoa are notably sensitive to rainfall distributions and extended drought periods (Wood 1985; Anim-Kwapong and Frimpong, 2004). Long drought periods impact flower production (Wuriandani et al., 2018; Handley, 2016; Omolaja et al., 2009) and contribute to increased number of flower abortion, reducing pollinated flower stability to about 65% (Frimpong-Anin et al., 2014).

Several models have predicted increase heat waves (Schroth et al., 2016) which can negatively affect soil moisture in most cocoa growing areas (Lahive et al., 2019). The unpredictable nature of rains in terms of space, amount and time coupled with dry spells continue to be a major challenge in the cocoa sector under the expected heat waves. As a result, sustainability of cocoa production as well as farmer's income, food security and livelihood are likely to be affected by rainfall variability through changes in distribution and seasonal patterns (Okoffo et al., 2016).

It is therefore anticipated that declining precipitation trends combined with increasing temperature could reduce output of cocoa (Gateau-Rey et al., 2018).

Shade grown cocoa (agroforestry) has been suggested as a means of improving cocoa tree productivity as it buffers cocoa against harsh climatic conditions such as drought (Vaast and Somarriba, 2014). It is also established that removal of shade trees from cocoa farms can result in higher yield, especially in favorable soil and ecological conditions (Jagoret et al., 2017; Ahenkorah, 1987; Cunningham and Arnold, 1962). However, both Jagoret et al., (2017), and Ahenkorah (1974) agree that shade provides conditions for a longer life span and consistent long-term yield benefits through reduced, wind velocity, excessive evapotranspiration (Kyereh, 2017), and other unfavourable ecological factors. Asare et al. (2016) noted higher yield under shaded trees at plot levels and recommended 30 – 40% shade for cocoa plants. Thus, selection of tree species to provide optimum shade for cocoa has the potential to conserve soil moisture and improve cocoa physiology when rainfall is limited which is becoming more frequent with climate change.

The debate on whether shade could reduce the harsh effects of climatic conditions, especially drought to improve yield of cocoa is still on going. According to Jones and Thornton, (2003), crop yields in Africa and Latin America could decline more than 10% by 2055 due to rainfall and other climatic conditions. Other authors like Wheeler and Braun (2013) have indicated that areas vulnerable to hunger and undernutrition could be the hardest hit by climate effects. Hence, there is the need for adequate information to develop a better cocoa system based on the interactive nature of shade and drought on yield.

The objective of the present study is to assess the effects of water limitation and shade on flower production, cherelles, pods and general yield of cocoa plants. It aims at providing

scientific evidence to policy makers to promote environmentally sound cocoa production to improve yields of cocoa without compromising on the environmental integrity.

4.2 Methodology

4.2.1. Study Site

A twelve-year-old cocoa farm in Sefwi Kunuma of Western North Region in Ghana was used for the experiment. Sefwi Kunuma (6º23'N, 2º33'W) at 165 m above sea level, is a farming community of about 900 inhabitants and falls within the moist semi-deciduous forest zone of average temperature between 25 ºC to 35 ºC. Relative air humidity ranges between 75% and 90%. Rainfall patterns peak in May-July and September – October with annual range between 1524 mm to 1780 mm (Gyapong, 2015; Nyarko, 2014). Soil in the vicinity is classified as Ochrosol with some few places occupied by Oxysol (Nyarko, 2014). Cocoa is the main cultivated cash crop aside food crops such as plantain, maize, rice, cassava, and cocoyam.

4.2.2 Experimental Design

The experimental design followed a two-factor split plot in three replications. Shade was used as the main factor and water suppression as sub-factor. Shade had two levels including shade and full sun. Shade was provided with 40% artificial shade net raised at a height of 6.5 m over the cocoa trees using wooden poles as support (Appendix 8). The water suppression factor included control, 1/3 water suppression (about 33% through-fall suppression) and 2/3 water suppression (about 66% through- fall suppression). In total, six treatment combinations were repeated 3 times for a total of 18 subplots**;** where Shade.0/3W – shade control; Shade.1/3W – shade 1/3 water suppression; Shade.2/3W – shade 2/3 water suppression; Sun.0/3W – sun control; Sun.1/3W – sun 1/3 water suppression; and Sun.2/3W – sun 2/3 water suppression. Each subplot contained 25 cocoa trees of 4.5 m average height and 8.5 cm average diameter at

breast height and on a subplot land area of 144 m^2. Measurements were taken on the middle nine plants per subplot. Water suppression was achieved with the use of plastic sheets. Plastic sheet platforms measuring 1 m by 12 m for the 1/3 water suppression subplots and 2 m by 12 m for the 2/3 water suppression subplots were raised between the rows of the cocoa subplots using bamboo sticks. Platforms were tilted at one end to allow water to drain into trenches made with aluminium sheets. Trenches were extended 10 m away from water-suppressed subplots to prevent water-contamination to the neighbouring plots. The research lasted for 33 months starting in June 2018 and ending in March 2021. Actual yield data collection started in September 2018 and ended in March 2021 to cover two major seasons and two minor seasons of cocoa production. Data for major harvest seasons lasted from September to March while those of minor seasons were taken between April and August (Appendix 5) as recommended by earlier reports (Baah et al., 2016; Wood et al., 1985; 1CCO, 2014).

4.2.3 Soil Chemical Analysis

Soil samples were taken randomly at six different spots in each subplot at 2 depths, namely 30 cm depth as topsoil and 31 – 50 cm depth as sub-soil, 50 cm away from cocoa trees (Balasimha et al., 1990; Vanhove et al., 2016). Five kilograms of each composite sample was sent in sealed plastic bags to Soil Research Institute, Kumasi for analysis. The main parameters analyzed for included pH, organic carbon, total N, P, K, Mg, Ca, Na, % base saturation and effective cation ion exchange capacity (ECEC). Soil pH was determined with electronic method using 1:2.5 sample solutions (Page et al., 1982). Organic carbon was determined with Walkley and Black method (Walkley and Black, 1934; Meersmans et al., 2009), total nitrogen with Kjeldahl method (Kjeldahl, 1883; Bremmer and Mulvaney, 1982) while available phosphorus was determined with Bray No. 1 method (Bray and Kurtz, 1945; Lumbanraja et al., 2017). The

other chemical analyses were based on the available protocols at Soil Research Institute, Kumasi.

4.2.4 Data on Climate

Below canopy temperature (Plus 2 tinyta$_{gs}$ TGP - 4017, Gemini Data Loggers Ltd, UK) was monitored every 10 minutes for two-year duration. Dataloggers were shielded with plastic bowls covered with aluminium foil. Four dataloggers were hanged" below the canopy of the cocoa at breast height with two under shade and under full sun conditions respectively. Rain Gauge (Rain Gauge Sensor, S-RGx-Moo2, Hobo Onset Computer Corporations) was installed at an open place around 2 km away from the experimental site to monitor daily rainfall.

4.2.5 Soil Moisture

Monthly variations in volumetric soil moisture content were measured with Diviner soil moisture probe (Diviner 2000 Series II, Sentek Soil Moisture Sensors, Sentek Technologies, South Australia). Three PVC pipes (NJPLAST GH uPVC 2" (600 cm), class O) of 160 cm long covered with access and end cups were inserted 5 m apart into the soil profile on each subplot to monitor monthly variations in soil moisture. Measurements were taken every two weeks from September 2019 to March 2021.

4.2.6 Flower Intensity and Canopy Density per Plant

Flower production and canopy density were assessed on a visual scale from 0 to 5 (Plate 3.8); Score zero indicating no flower or no canopy and score 5 indicating maximum flowering intensity or dense canopy. Flower production was quantified using average values of flower cushions and number of flowers per cushion produced at the section of the tree below diameter at breast height (DBH). Before taking data on flowers, ten plants were selected under each

flowering score during the peak flowering month (July 2018). Number of flower cushions below DBH (DBH = 1.5 m) of selected plants were counted and averaged for each level of score. Again, number of individual flower parts of a flower cushion of one hundred randomly selected cushions were counted and averaged (Average number of individual flowers per cushion = 16). Total number of flowers below DBH per tree, were then calculated by taking the product of number of flower cushions per score and average number of individual flowers per cushion. Flower production was monitored monthly for two years.

Plate 4.2.1 Scale Used for Flower Intensity and Canopy Density

4.2.7 Number of Cherelles and Pods per Tree

Number of healthy and dead cherelles (below DBH) per tree from the middle nine trees per subplot were counted on monthly basis to assess transition of flowers to pods formation. Dead pods and cherelles were removed after each monthly count. Young pods measuring less than or equal to 6 cm long by 3 cm wide were considered as cherelles. Total number of pods on the whole tree were also counted monthly to monitor trends in pod density as affected by rainfall patterns. Ripe and healthy pods from each whole tree were harvested monthly and counted.

4.2.8 Seeds per Pod and Pod Weight

Measurements of pod weight, length and width were taken from ten randomly selected pods per subplot after each harvest. Measurements were taken during the peak harvesting months (September, October, and November) every year. Number of beans per pod were counted and weighed. Then all beans from a subplot were pulled together and 100 beans were randomly selected, and fresh weight taken. The beans per subplots were covered with plantain leaves, labelled, and then kept under a heaped cocoa beans of about 500 kg for six days fermentation. Fermentation was recommended to produce the chocolate flavor (Baah et al., 2016). After six days of fermentation, beans per subplots were sun dried for seven days as farmers normally do and then weighed to compute dry bean yield per hectare and season.

Yield calculations;

$$Y = \frac{m \ x \ NPH \ x \ NBP \ x \ MDB(g)}{n \ \ x \ 1000g}$$

Where;

Y = Yield/ hectare/season (kg/ha/season)

m – total number of trees per hectare (1110 trees per hectare with 3 m x 3 m planting distance)

n – number of trees used for yield measurements per treatment (n= 9)

NPH – Average number of healthy pods harvested per season per treatment

NBP – Average number of beans per pod per treatment

MDB – Average mass per dry bean per treatment

1/1000g – a factor to convert yield in g to yield in kg

Formulated with modifications from Wibaux *et al. (*2017) and Lachenaud (1984).

Plate 4.2.2 A – Calibration of Scale, B - Pod Weight, C – Counting Number of Beans per Pod, and D – Beans Weight after Sun Dry

4.2.9 Data Analyses

Data from the middle nine plants selected from each subplot were averaged at tree level and then at subplot level for all the 18 subplots of the 6 treatments. Canopy density, flower intensity, cherelles and pods production followed repeated measures analysis using the nonlinear mixed effect model (nlme). Shade and water suppression levels were used as fixed factors while time, replications, and number of plants selected for data were used as random factors. For monthly trends of pod density and seasonal variations in yield, shade, water suppression levels, and time were used as fixed factors while tree number was nested into replicate as random factors. Analysis of residuals were done by plotting residuals against fitted values in a normal Q-Q plots to check data normality and variance homogeneity. Log transformation of number of pods and cherelles was performed for data normality. Significant differences were tested using the F-test-based backward selection method (Pope and Webster, 1972) and means separated with multiple comparison using Tukey Honest Significant Difference test (Tukey HSD).

4.3. Results

4.3.1 Soil Chemical Constituents

Before the treatments in July 2018, soil samples were taken and analyzed for the chemical status. Soil pH in the upper 30 cm zone was 5.7 indicating slightly acidic soil which is good for cocoa growth (Table 4.3.1).

Table 4.3.1 Chemical Composition of the Cocoa Soils at the Research Site

Parameters	Top (0-30cm)	Sub (31-50cm)	Recom.	References	Top	Sub
pH 1: 2.5	5.69	4.93	5.10 -7.00	Snoeck et al. (2016)	Adequately Acidic	Acidic
%O.C	0.57	0.35	1.70 – 3.20 %	Snoeck et al. (2016)	Low	Low
%Total Nitrogen	0.05	0.02	>0.20	Singh et el. (2019)	Low	Low
P ppm	43.05	9.09	>20.00	Horneck et al. (2011)	Adequate	Low
K/cmol/Kg	0.33	0.37	0.20 – 1.20	Snoeck et al. (2016)	Adequate	Adequate
Other Exchangeable Cations/cmol/Kg						
Ca	5.34	2.94	4.00 – 18.00	Snoeck et al. (2016)	Adequate	Low
Mg	1.60	1.34	0.90 – 40	Snoeck et al. (2016)	Adequate	Adequate
Na	0.13	0.20	<3.00	Botta (2016)	Adequate	Adequate
ECEC/cmol/Kg	8.15	5.80	>12.00	Botta (2016)	Low	Low
%Base Sat.	90.80	83.63	>60.00%	Eponom et al. (2019)	Adequate	Adequate

The soil between 30 to 50 cm zone was, on the other hand, more acidic than the upper 30 cm zone. Soil organic carbon, and total nitrogen content were below the recommended limits. Plant-available phosphorus content was adequate at the top 30 cm zone though low at the sub-soil. Exchangeable cations such as calcium, magnesium and potassium were adequate. Effective cation exchange capacity (ECEC) as the sum of the exchangeable bases and the exchangeable acidity such as Al^{3+}, Mn^{2+} and H^{+} of the sampled zones was fairly low. Percentage base saturation was, however, adequate to indicate most of the nutrient holding sites being occupied by the base cations such as Ca^{2+}, $Mg,^{2+}$, Na^{+} and K^{+}.

4.3.2 Climate

Rainfall distribution and below canopy temperature varied between months (Figures 4.3.1 and 4.3.2). May, June, July, September, and October were the wet months while December, January, February, and August showed limited rainfall. Average temperature in the minor season (April – August) was over 25.9 ± 1.1 °C while the major season (September – March) was slightly warmer with monthly average around 26.2 ± 1.0 °C. Total rainfall distribution

among seasons was on average 500 mm for the minor season and 700 mm for the major season calculated over the 2020/2021 seasons.

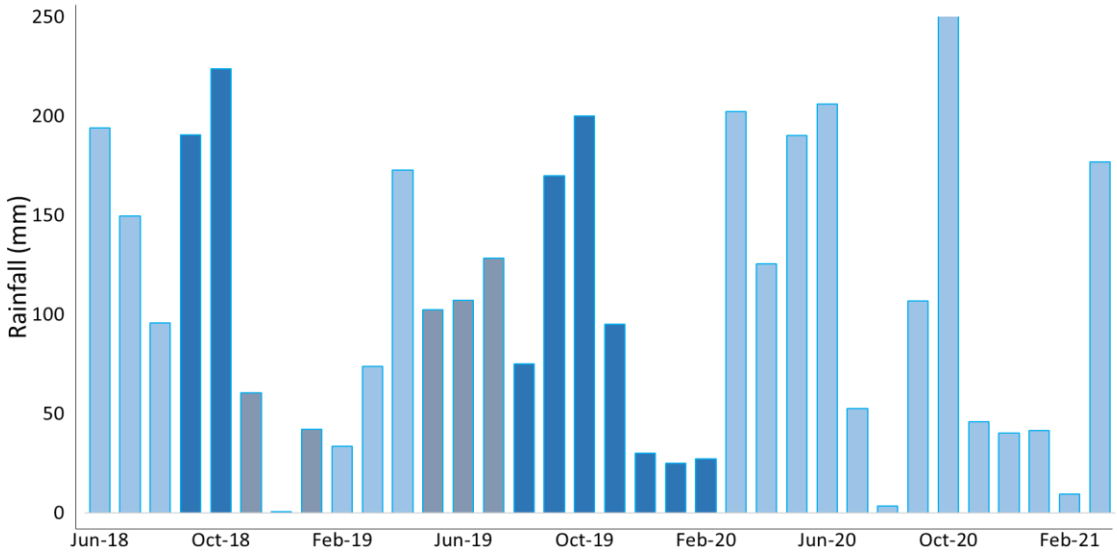

Figure 4.3.1 Monthly Rainfall Patterns at Sefwi Kunuma (The Experimental Site). Light blue colors represent data frown own built weather station; deep blue colors represent data taken from regional weather station and grey colors represent data taken from Olam weather station in the same town where the experiment took place.

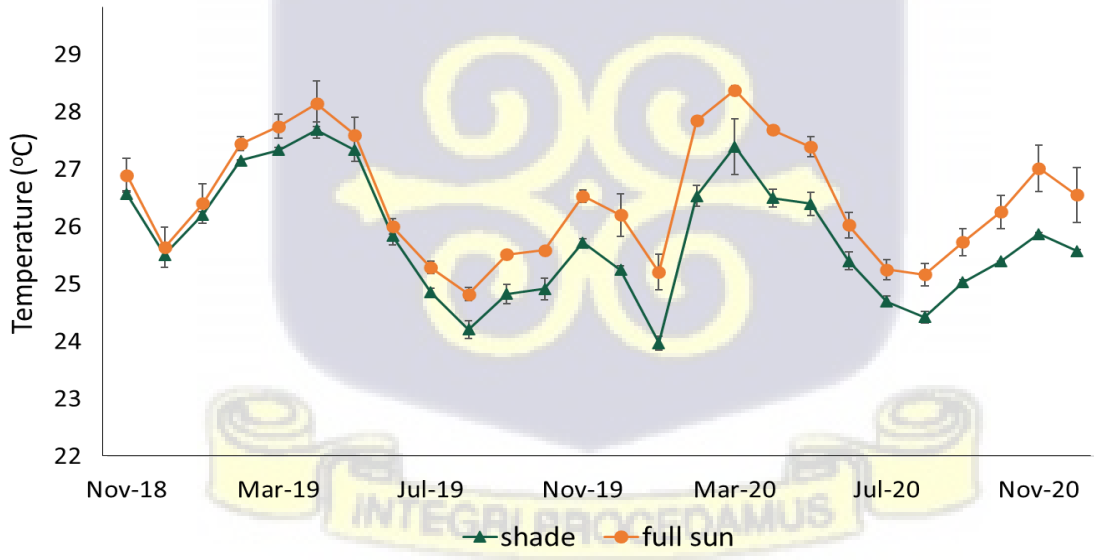

Figure 4.3.2. Monthly Trend of Below Canopy Temperature Variations Under Shade and Full Sun Conditions. Bars indicate standard error

4.3.2 Soil Water Status

Variations in volumetric soil water status within months indicated soil moisture between 0.2 to 0.5 m^3/m^3 (Figure 4.3.3). Soil moisture was high in the rainy months such as May to July and September to October.

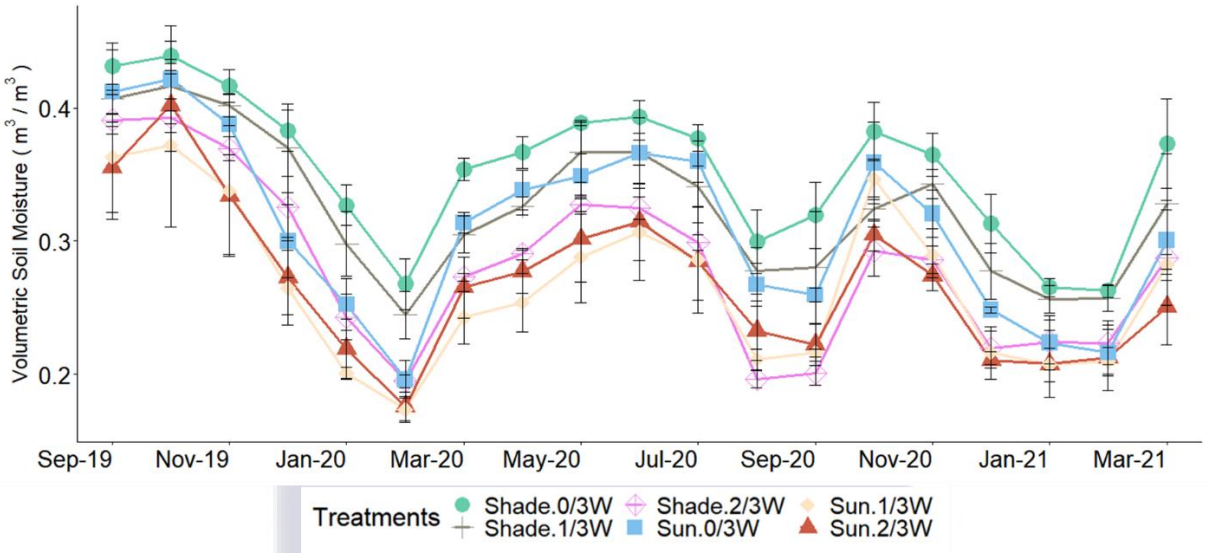

Figure 4.3.3 Monthly Variations of Volumetric Soil Moisture Content from September 2019 to March 2021 as Affected by the Treatments. Bars indicate standard error

Two-third (2/3) water suppressions treatments under both shade and full sun conditions showed low soil moisture contents with the full sun treatments significantly ($p < 0.001$) affected by the suppression level. Between September and November 2019, when rainfall was high with monthly average around 150 mm, soil moisture for all treatments was above 0.30 m^3/m^3 with shade control treatments having values as high as 0.45 m^3/m^3. A sharp decline of soil moisture was noted between January to March 2020 where monthly rainfall was as low as 5 mm. The three levels of water suppression under full sun were at their lowest below the 0.25 m^3/m^3 threshold for clay soils. Soil moisture increased after the dry months with values between 0.25

to 0.35 m^3/m^3 in April to July but a reduction again in August as a response to the short dry spell in the month.

4.3.3 Canopy Density, Flowers, Cherelles and Pods Production

Plant canopy density differed ($p < 0.001$) among shade levels and water suppression ($p < 0.001$) levels (Figure 4.3.4 A). Plants under shade produced denser canopy than full sun plants. Canopy density decreased with decreasing soil moisture under full sun. Production of flowers below diameter at breast height (DBH) increased under shade producing as high as 3000 flowers for the shade control treatments. Shade, water suppression and interactions affected flower production (Appendix 3). Two-third (2/3) water suppression treatment under full sun conditions produced around 1500 flowers at DBH which is about 30% reduction from the full sun control treatments and about 50% reduction from the shade control treatments. Though flower production was high ranging between 1500 to 3000, cherelles production was generally low between 10 to 40 below DBH, an indication of about only 2% of the flowers produced developing into cherelles and about 1% developing into mature pods (Figure 4.3.4 C). Shade and water suppression levels independently had significant effects on cherelles production. Shaded plants showed improved number of cherelles comparing with full sun plants. On the other hand, trees under 2/3 water suppression produced very low number of cherelles below DBH per tree.

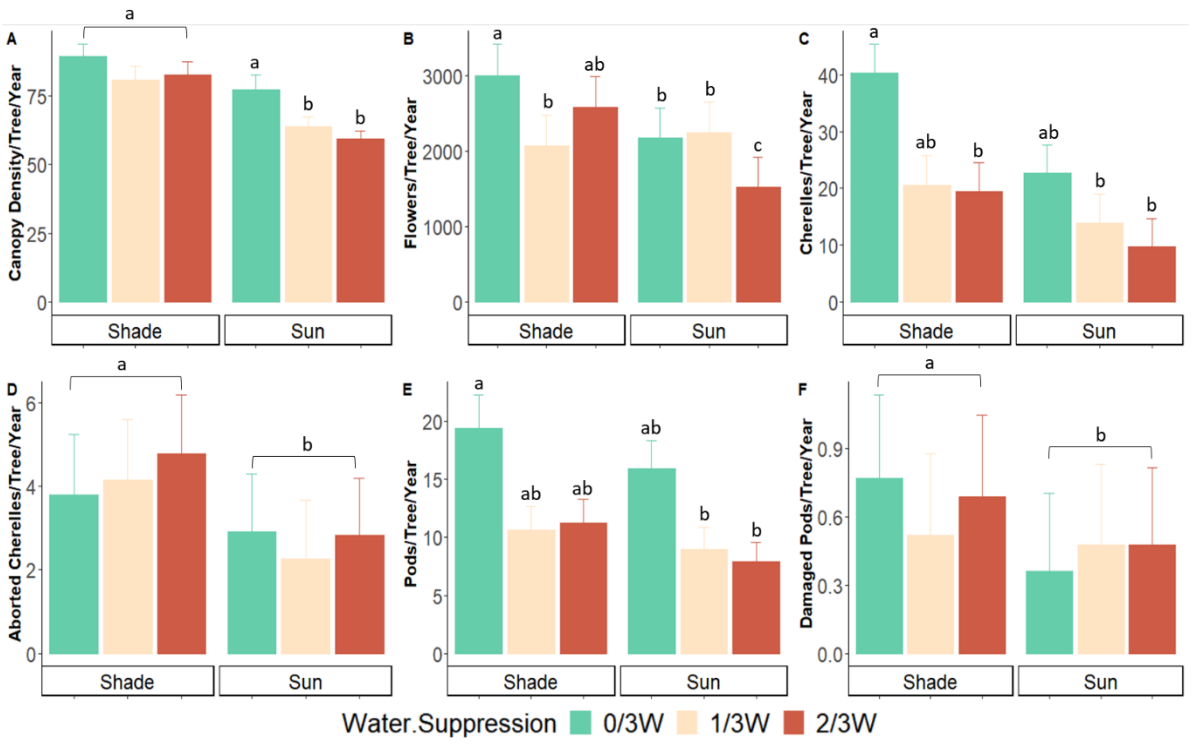

Figure 4.3.4 Effects of Shade and Water Suppression on Canopy Density (A), Flowers (B), Cherelles Production (C) Aborted Cherelles (D), Pods Production (E) and Damaged Pods (F). Bars indicate standard error; means with different letters are significantly different at $p < 0.05$ according to Tukey HSD; measurements of pods, flowers, and cherelles production were taken from 0.50 m to breast height (BH, 1.5 m of the plant height) on monthly basis for two years (2019 - 2020)

Regardless of water suppression levels, shade significantly increased the number of aborted cherelles and damaged pods yet, number of pods per tree improved comparing with full sun (Table 4.3.2). Pods number varied between 7 and 20 for all the treatments indicating about 50% of cherelles maturing into pods (Figure 4.3.4 E). Aborted cherelles and damaged pods were not significantly affected at the water suppression levels.

4.3.4. Monthly Variations of Pods Density per Tree

Pods density per tree varied among shade levels ($p < 0.001$), water suppression levels ($p < 0.005$) and months ($p < 0.001$). Interactions between treatments did not show any significant

effects. Plants under shade gave higher pods density per tree especially during the wet months (Figure 4.3.5). Water suppression significantly reduced pods density and this was more pronounced during the dry months. Both 2/3 water suppression and 1/3 water suppression treatments under full sun showed the least pods density per full tree across the months. Generally, pods per tree was high between July to November and low between December to May, irrespective of the year. Pods density per tree and hence per treatment peaked in October every year. In general, number of pods harvested from a full tree per season were between 0 to 60. On average, shade control plants gave the highest number of pods harvested per tree per year with an average of 29 ± 17 followed by full sun control plots with 27 ± 15 pods. Pods count per tree from 2/3 water suppression treatment was on an average of 20 ± 14 under shade conditions and 18 ± 9 under full sun conditions indicating average reduction of 31% from shade control treatments and 34% from the full sun control treatments. Generally, pod production under shade conditions was about 7% more than values observed in full sun conditions.

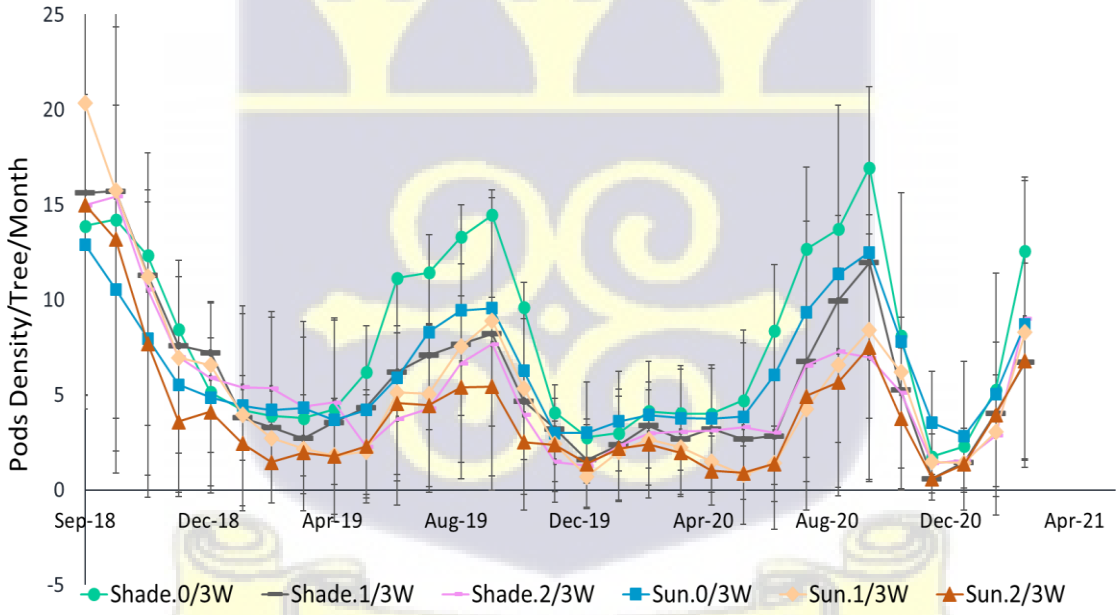

Figure 4.3.5 Effects of Shade and Water Suppression Levels on Monthly Variations of Number of Pods on the Full Cocoa Trees. Bars indicate standard error

4.3.5 Pods and Beans Physical Characteristics

Pods weight, length and diameter varied significantly among shade and water suppression levels (Appendix 4). Average pod weight ranged between 431.0 g to 516.0 g among all the treatments (Table 4.32). Pods from shade conditions were heavier than those in full sun conditions. Water suppression decreased pods weight by about 10% in the full sun conditions and by about 8% in the shade conditions. Average pod length was between 14.5 cm to 15.6 cm while average pod diameter varied between 8.0 cm to 8.5 cm.

Table 4.3.2 Effect of Shade and Water Suppression on Pods and Beans Physical Characteristics

Treatments	Shade.0/3W	Shade.1/3W	Shade.2/3W	Sun.0/3W	Sun.1/3W	Sun.2/3W
Pods Physical Appearance						
Pod Weight (g/pod)	516±122a	475±151abc	486±158ab	479±104ab	455±115bc	431±117c
Pod Length (cm/pd)	15.6±1.6a	15.0±1.2ab	15.2±0.5ab	15.2±1.7ab	15.1±0.2ab	14.5±0.4b
Pod Diameter (cm/pod)	8.5±0.3a	8.2±0.1b	8.2±0.3b	8.1±0.4b	8.0±0.9b	8.1±0.2b
Length/Diameter	1.8±0.2b	1.8±0.2b	1.8±0.2b	1.9±0.1a	1.9±0.2a	1.8±0.2b
Beans Quantity and Weight						
Beans/Pod	36.5±1.2a	35.9±2.2a	36.7±3.7a	34.4±5.6b	34.3±2.6b	34.8±3.6b
Tot. Bean Weight (g/pod)	123.5±14.7a	113.8±12.1abc	117.4±9.1ab	114.9±44.0abc	103.2±15.2c	107.0±18.2bc
% Beans Content/Pod	24.5±4.1ab	24.6±4.8ab	24.4±3.8ab	23.7±3.9ab	22.9±3.6b	25.1±4.2a
Fresh Weight (g/bean)	3.5±0.4a	3.2±0.1ab	2.8±0.2c	3.0±0.2bc	2.7±0.5cd	2.4±0.2d
Dry Weight (g/bean)	1.3±0.7a	1.2±0.1ab	1.2±0.1ab	1.3±0.1ab	1.2±0.1ab	1.1±0.1b
Moisture (g/bean)	2.2±0.4a	1.9±0.1ab	1.6±0.1c	1.7±0.1bc	1.5±0.4cd	1.3±0.2d

Means are ± Standard Error; Means in a row with different letters are not significantly different at p < 0.05 according to Tukey HSD

Plants from shade conditions produced bigger pods than from full sun conditions. Number of beans per pod were between 34.0 to 37.0 under all treatments. Only shade had effect on number

of beans produced per pod with shaded plants comparatively giving more beans per pod than full sun plants. Total bean weight per pod ranged between 10.0 g to 124.0 g for shade control plants producing heavier bean weight than in full sun. Pods from water suppression treatments produced significantly lighter beans than control treatments. On the other hand, shade significantly ($p < 0.027$) interacted with water suppression levels to affect percentage bean contents per pod while individual effects of shade and water suppression was not significant. Strong effect of water suppression was observed on fresh and dry weight per bean. Fresh weight per bean was 2.4 to 3.4 g while dry weight was 1.1 g to 1.3 g. On the other hand, shade resulted in improved bean water content than full sun conditions.

4.3.6. Dry Bean Yield in Kilograms per Hectare

Dry bean yield (in kilograms per hectare per season) was affected positively by shade ($p < 0.035$), negatively by water suppression ($p < 0.008$), season ($p < 0.001$) and season by water suppression interactions ($p < 0.006$). Significantly, higher yield was recorded under shade conditions irrespective of the water suppression levels while water suppression proportionally decreased yield regardless of the shade conditions. Water suppression decreased bean yield to as low as 286 kg/ha/season, about 60% reduction from the control treatments under full sun conditions and to as low as 431 kg/ha/season, about 55% reduction under shade conditions during the 2019 major season (Figure 4.3.6). The same pattern of yield was also noted in the 2020 major season. No significant difference was observed between the 1/3 and the 2/3 water suppression treatments under shade and full sun.

Yield pattern was affected by rainfall distribution as well as by below canopy temperature variations. No significant differences were noted among treatments during the minor seasons. Average yield of 87 to 291 kg/ha/treatment was recorded in the minor season compared with 287 to 960 kg/ha/treatment in the major seasons.

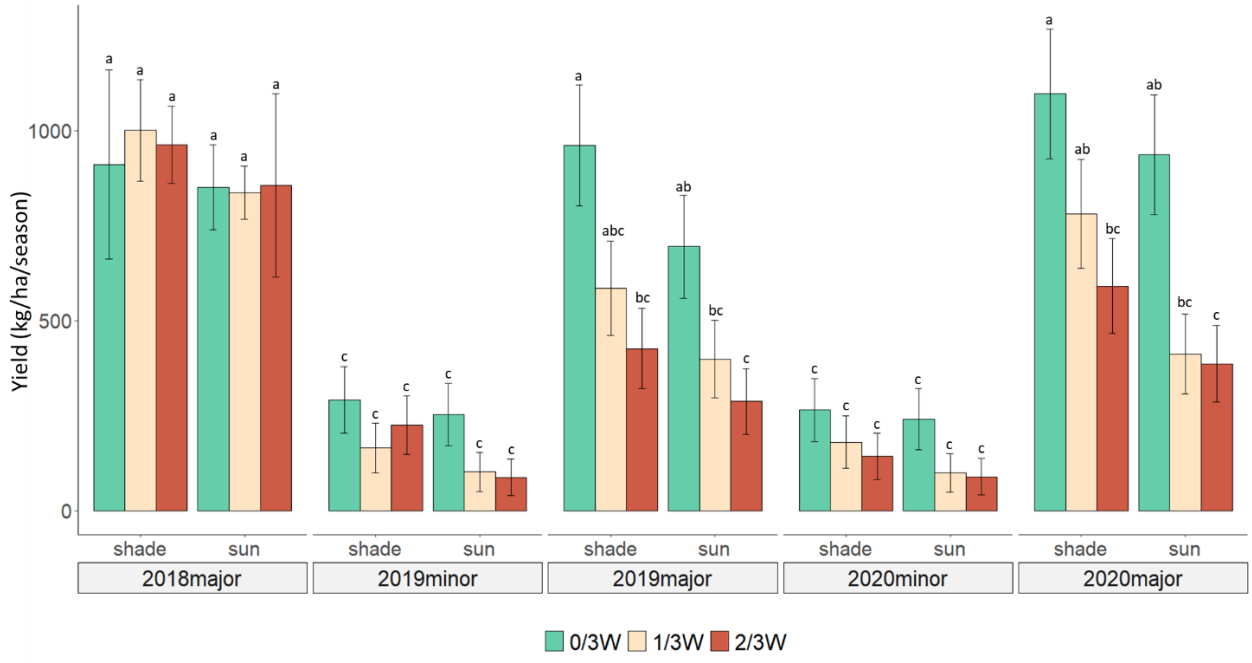

Figure 4.3.6. Effect of Shade and Water Suppression on Yield of Cocoa Plants Between September 2018 – March 2021. Bars indicate standard error

4.4. Discussion

Yield analysis of cocoa under contrasting shade/sun levels and three water suppression levels indicated yield sensitivity to low soil moisture, which can be reduced by shade. Shade has been reported to modify abiotic stress by serving as a physical barrier between plants and direct solar radiations (Aguiar et al., 2019) and therefore improving air temperature and relative humidity around the shaded plants. In effect, vegetative growth and reproductive organs development including canopy density, flower intensity and pods density were enhanced under shade.

Lower temperature between 24 - 26 ^{o}C and higher volumetric moisture content around 0.25 – 0.40 m^3/m^3 recorded in the shade treatments increased canopy density to as high as 90% leading to improved leaf functions and longevity. The enhanced leaf canopy density resulted in an increased leaf light interception maximizing assimilates production (Adjaloo et al., 2012) to contribute to yield. On the other hand, water suppression under full sun conditions increased

leaf losses to reduce transpiration but altering canopy gross light interception for photosynthesis. Loss of canopy density has been noted to increase wind and solar energy reaching bare soil to increase evaporation (Adams et al., 2012). These effects may alter soil moisture causing a cascade of plant drought effects as noted in the severe water stress treatments.

Based on data from this work, higher flower and cherelle production under shade conditions might be a response to optimum temperature and increased soil moisture content observed. Though flowering is all year round, peak flowering production is seen between May to July every year when there is frequent rain (Omolaja et al., 2009; Adjaloo et al., 2012). Less than 2% of the flowers produced developed into cherelles among all the treatments thus confirming earlier studies (de Almeida and Valle, 2007; Groeneveld et al., 2010; Carr and Lockwood, 2011). Though cherelle wilt is an inherent trait to manage resource allocation (Mckelvie, 1956; Handley, 2016). Below canopy temperature in full sun conditions was noted to be higher than shade conditions affecting flower development to cherelles, especially in the full sun water suppressed plots. Stigma viability, pollination, pollen tube growth and early embryo development are noted to be vulnerable to heat stress (Lamaoui et al., 2018; Giorno et al., 2013). Water suppression during anthesis and early cherelle development can cause abnormalities in floral organs interfering into pollination and inducing abscission of newly formed embryo (Saini, 1997). In effect, Frimpong-Anin et al. (2014) observed an increased proportion of un-pollinated flowers drop in the dry seasons than in the rainy season. Cherelle abortion and pods damage were more pronounced under shade conditions than under full sun conditions contrary to previous studies (Ofori-Frimpong et al., 2007). The findings corroborate those of Bos et al. (2006) with both early and pathogenic fruit losses being more manifested under shade. On this note, it is assumed that low temperature under the shade conditions could have provided favourable environment for *Phytophthora spp* and therefore causing much

damage to cherelles and pods (Delgado-Ospina et al., 2021). Pruning plants, however, could increase uniform light distribution within the canopy to allow good airflow and reduce trapped moisture that may provide a medium for the growth of the fungi (Riedel et al., 2019; Delgado-Ospina et al., 2021). The number of pods suspected to be damaged by black pod under shade did not, however, affect overall cocoa yield as yield still improved under shade conditions compared with full sun conditions.

Pods density was most affected by water availability and shade. Months with high rainfall coincided with increased soil moisture content promoting vigorous growth of leaves which increased leaf surface area for photosynthesis and thus enhanced distribution of assimilates to reproductive organs to increase number of pods per plant during the wet months as also reported by Valle et al. (1999). Effects of shade and water suppression on number of pods per plant was manifested more during the wet months than in the dry months. Therefore, low pods density per plant observed in the dry months could be attributed to genetic and physiological timing rather than environmental influences. This observation is in line with Adjaloo et al. (2012) who indicated that improved reproductive phase in the rainy months might be due to intrinsic factors such as genetic and physiological elements that play important role in the plant phenology.

In this present study, yield (in kg/ha/season) increased under shade conditions as in contrast with earlier reports (Cunningham et al., 1962; Ahenkorah et al., 1974; Agele et al., 2016) where higher yields were observed under full sun conditions. On the other hand, uniform artificial shade netting was used and competition between cocoa plants and shade trees for water and nutrients was zero. However, yield was more sensitive to water availability than to shade. As reported by Issaka et al. (2018), yield of cocoa in the mid and wet regions of Ghana is higher than in the dry regions to show effects of spatial distribution of water on cocoa production. As a result, several reports (Geonaga et al., 2015; Gateau-Rey et al., 2018) have indicated lower

yield of cocoa under drought conditions compared with moist areas. In effect, a greater number of pods that are normally harvested in the dry season are usually pods that developed during the rainy months (Wuriandani et al., 2018). Yield data from this study was above the national average of 600kg/ha which factors might be due to good agronomic practices such as row planted trees, removal of shoots and parasitic plants; regular slashing of weeds; control of pests and diseases; fertilization, removal of dead pods and cherelles and regular harvesting of ripe pods. Yield between 1000kg/ha and 2500kg/ha have been reported on farmers and experimental fields (Aneani and Padi, 2017; Mendes, 2017) in Ghana which goes parallel with the result from this work.

The present study has proven that agroforestry could be a useful strategy for climate smart agriculture in cocoa production. Trees that are used for shade could also provide alternative source of income for households to improve the economic gains of countries that depend on cocoa. Due to the high humidity of Sefwi Kunuma, low shade cocoa agroforestry is recommended in the Western North Region of Ghana. Therefore, as indicated by Asare et al. (2016), percentage shade of not more than 40% could be provided for cocoa production in the region. Levels of shade provided for cocoa agroforestry in the other regions may depend on the environmental conditions such as temperature, relative humidity, and rainfall patterns. Further research is, however, needed to select shade trees that will give uniform shade and at the same time will provide less competition for soil moisture and nutrients.

CHAPTER V

EFFECTS OF SHADE AND HEAT ON PHYSIOLOGICAL PERFORMANCES OF COCOA

5.1 Introduction

Over the years, emissions of green-house gases have necessitated a rise in global mean temperature by 1.5 ^{o}C above averages from pre-industrial levels (Masson-Delmotte et al., 2018). High temperature is noted to increase evaporative demand of crops to increase crop water requirement and uptake from soil moisture. Crop production, yield, quality, and quantity are affected in the process (Joslin, 2018; Thornton et al., 2014). High temperature also causes impairment of chlorophyll biosynthesis due to down-regulation of gene expression and protein abundance of several enzymes involved in the tetrapyrrole metabolism because of increased temperature (Dutta et al., 2009). The situation reduces photosynthesis to cause substantial loss in plant productivity (Dutta et al., 2009). In another development, high temperature affects light independent reactions of photosynthesis, decreases enzymatic activities, disrupts assimilates transports, reduces chlorophyll content and the turgidity of mesophyll cells (Lamaoui et al., 2018; Wiser et al., 2004). Photosynthetic pigments, activities of photosystem II and the regeneration capacity of Ribulose 1,5-bisphosphate (RuBP) are also affected (Wiser et al., 2004; Fahad et al., 2017). At least, three major stress-sensitive sites of the photosynthetic machinery are noted, and these include the PSII, the ATPase and the carbon assimilation process (Mathur et al., 2010). The PSII tends to be the most heat-sensitive component among the three (Song et al., 2014). Temperatures above 40 ^{o}C reduces CO_2 assimilation, and slows photosynthetic electron transport (Chen et al., 2012). Factors making electron transport highly susceptible to elevated temperature include increased fluidity of thylakoid membranes at high temperature that causes dislodging of PSII harvesting complexes, and dependence of PSII

integrity on electron dynamics (Mathur et al., 2014). Heat stress is again noted to increase photoinhibition and this generates large amounts of reactive oxygen species responsible for the damage of the photosynthetic machinery (Essemine et al., 2012). At high temperature sugar is built in the cell to slow photosynthesis because enzymes activity to convert sugar to starch is affected (Bajera, 2018). High temperature is observed to disrupt the water oxidizing complex (WOC) and the light harvesting complexes (LHC) (Salvucci et al., 2001; Mathur et al., 2014) to slow down carboxylation. Water loss by transpiration increases at high temperature (De Geijn and Gourdriaan,1996) to increase water use efficiency especially in warm-dry environments (Zhang et al., 2015).

Cocoa (*Theobroma cacao* L) is an understory tree crop mostly found in tropical regions. More than six million small-scale farmers depend on cocoa for livelihood while indirectly, the crop provides employment to over 50 million people across the globe (Zhang and Motilal, 2016; World Cocoa Foundation, 2012). Cultivation of cocoa is commonly by seedlings or by direct planting of viable beans. Seedlings are very sensitive to climate change and success of establishment on the field is usually low because of bad climatic conditions. Growth, and development depend on temperature (Valle, 2007) and water availability. Seedling's mortality due to extreme temperature is high in most of these cocoa regions due to extreme temperature. Temperature between 18 to 34 ^{o}C are reported to support growth of cocoa (Lahive, 2018) but means above 34 ^{o}C can reduce leaf photosynthesis (Balasimba et al., 1991), rate of leaf production, plant height and leaf biomass (Lahive, 2018). Extreme temperatures such as 10 ^{o}C and 44 ^{o}C (Alvin, 1977; Asare et al., 2017; Abdulai et al., 2017) are reported in different regions of cocoa production but knowledge on the effects of these temperatures on growth and physiological functions of cocoa are limited. It is noted that high temperature increases leaf dehydration because of cuticular transpiration nature in cocoa seedlings (Padi et al., 2013). However, most of the research on environmental effects on cocoa production at seedling stage

are mainly on water stress, fertilization, and other agronomic activities (Frimpong et al., 1994; Oppong et al., 2007). Limited knowledge therefore exists on how elevated temperature affect the eco-physiological performances of cocoa, especially in tropical conditions. As the plant is cultivated in a narrow belt North and South of the Equator (Bhattacharjee and Kumar, 2007), most of the top 10 cocoa producing countries are within the warm, wet climates (Mattyasovszky, 2017) where any additional rise in temperature could endanger the plant. Several researchers have reported that production and yield of cocoa could drastically be affected by climate change (Cilas and Bastide, 2020; Schroth et al., 2016; Lahive et al., 2019). Shading has been suggested as a possible solution to solving the limiting effects of high temperature on cocoa production (Asare et al., 2016; Tee et al., 2018; Asare et al., 2018), reducing radiation load, heat, and water stress (Wood and Lass, 2001). Shading is noted to maintain plants in the most productive way by cooling the surfaces of the leaves and maintaining the best conditions for leaf transpiration (Medrano et al., 2004). Vogel (2009) indicated that leaf temperature under the sun can be a few degrees higher than air temperature depending on the plant and the leaf location, but that under shade conditions, temperature may drop to a few degrees below air temperature. Shading has been reported to have significant reduction on soil temperature with differences as high as 15 ^{o}C between shaded and unshaded treatments (Aguiar et al., 2019).

Controversies, however, exist on whether shading really reduces the effects of climate change on cocoa if the plants are watered regularly. Full sun conditions are noted to increase positive interactions between light and nutrients (Cunningham and Arnold, 1962) making plants to have higher radiation use efficiency and leaf area ratios (Agele et al., 2016). The problem of some shade producing thermal emissions and consequently increasing leaf surface temperature as against reducing it is also reported (Abdel-Ghany et al., 2019). Despite the controversy, maximum to minimum levels of shade between 30-70% are invariably recommended for the

establishment of cocoa seedlings in the field (Alvim, 1977; Evans and Murray, 1953; Wood and Lass, 1985) and improvement of yields in mature cocoa trees (Andres et al., 2018; Asare et al., 2018).

However, in the discourse to unravel the effect of shade, there seem to be limited empirical evidence to indicate the extent to which shading could reduce the stress effects of cocoa imposed by heat. A lot of work about temperature responses of crop species and temperate species is done but little information about tropical trees. Here, shade tolerant understory species is taken and exposed to shade and heat to study reactions at the physiological level. It is hypothesized that high temperatures will negatively affect cocoa physiology, but this can be modified by shade. The aim is to contribute to increased understanding of the effects of high temperature on the eco-physiological performance of cocoa as a tropical understory tree and how shade could help reduce the effects to improve seedling survival and establishment in the field.

5.2 Methodology

5.2.1 Site and Materials

Cocoa seedlings of four months old from Clone 67 were collected from Cocoa Research Institute of Ghana. On arrival at the experimental site, seedlings were watered and repotted into 2 kg nursery bags (25 cm * 18 cm) perforated at the bottom. Soil used for the repotting had 0.09 % total nitrogen, 50.44 ppm available phosphorus, 3.57% organic matter, 0.09 cmol+/kg potassium and 5.0 pH level (See appendix 2 for details). Three grams 15.15.15 NPK (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 at the later part of the day with sprinkling can. 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 (selected for full

sun treatments) were gradually acclimatized to full sun 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. Six-month old seedlings of almost the same height and stem diameter were then selected for the treatments.

5.2.2 Experimental Design

The experiment was conducted in the wet and cool season (September – November 2019) and repeated in the warm and dry season (February – April 2020) in an open environment at the school farm of University of Ghana (05º39'N, 00º11'W, 76 m a.s.l). Since only four heaters were available, a randomized complete block design (Appendix 1) with two blocks was conducted twice immediately after each other. Here, the two repetitions of the trial were considered as independent replicates, thus as one single randomized block experiment with four replicates. In each block, two levels of light (shade/sun) and two levels of heating (heat/control) were applied in combination on four plots (heat/sun, heat/shade, control/sun, control/shade). Plants under shade treatments were placed in greenhouses made with wooden poles and 60% black shade nets, measuring 2 m x 2 m and being 2 m high (Figure 5.2.1, See Appendix 9 for pictures). The top and sides were covered with shade nets down to the 0.5 m level to allow aeration. The heated treatments had black non-glowing 2000 W infra-red heaters (Hortus Patio, NSH NORDIC A/S, Braedstrup, Denmark, made in PRC) of 20 cm x 100 cm placed vertically at 0.6 m above the cocoa plants. Heaters were on continuously, thus resulting in varying leaf temperatures depending on transpiration. Mock heaters made with black wooden boards were raised above the control treatments to provide equal shade effects to those of the heaters. Forty cocoa seedlings arranged 8 x 5 rows at 10 cm apart were placed in each experimental plot (a total of 160 seedlings for each treatment and 640 seedlings for the entire experiment). All measurements were made on the middle four plants that were placed in a row

directly under the infra-red heaters and fringed at the sides with two rows of border plants. 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.

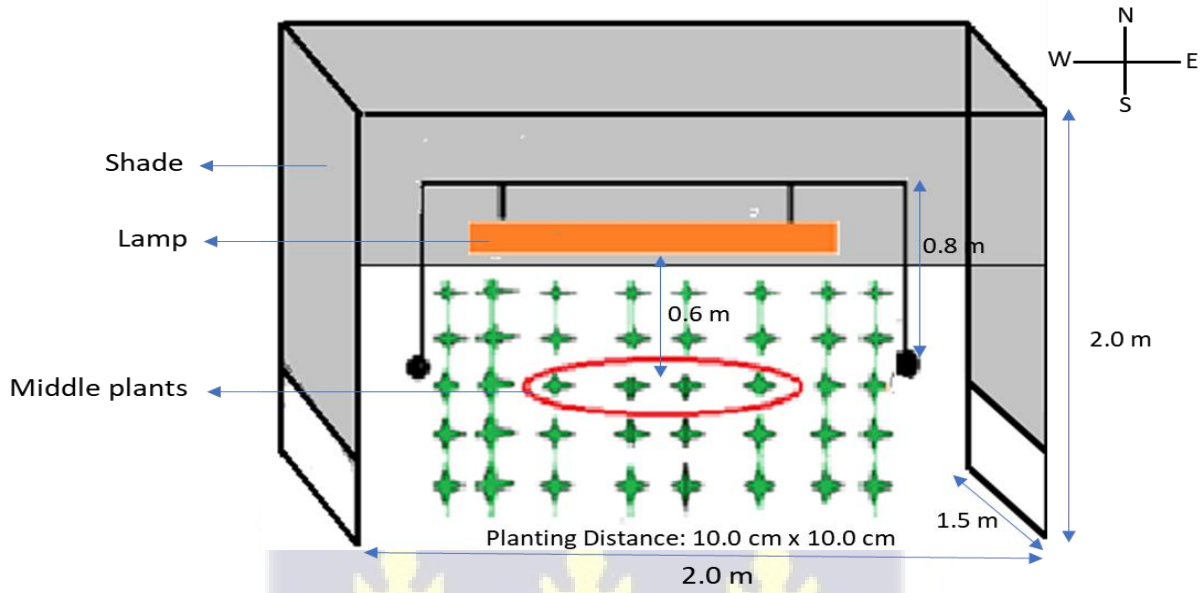

Figure 5.2.1 An Annotated Diagram showing the Improvised Greenhouse used for the Shade Trial

5.2.3 Agronomic Practices

A week before the start of the experiment, 15:15:15 NPK at a rate of 3 g per plant was applied. At Day-2 and -15, a mixture of Carbendazol and Mancozeb (carbamates) 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 200g/l) also according to manufacturer's recommendations. Weeds were removed manually. Watering was done first to field capacity, and the weight of plant plus soil was assessed. Water status was then maintained every second day by adding differences in water loss through weighing the pots.

5.2.4 Climate and Leaf Temperature

Meteorological data including relative humidity, temperature, rainfall, radiation, and wind gusts were recorded by a weather station (ZL6, UMTS 3G GSM cellular, Meter Group Inc. USA) 30 m from the experimental site. September and October were humid and cold. Monthly maximum temperature was between 32.00 – 33.00 ^{o}C while monthly minimum averaged around 21.80 ^{o}C (Table 5.2.1). Total rainfall of 278.83 mm was recorded in the months of October/September 2019. Relative humidity was as high as 91% with radiation and wind gust about 170 W/m^2 and 2.70 m/s, respectively. March and April were comparatively hot and dry. Monthly maximum temperature during the two months were high with maximum day averaging between 34.00 – 35.00 ^{o}C, 2 – 3 ^{o}C above the maximum in September/October. Minimum temperatures were on average 0.40 ^{o}C higher. Total precipitation for the two months was 66.09 mm, about 212.74 mm less the total recorded in September/October. Relative humidity was quite low though averaging over 80 – 85%.

Table 5.2.1 Climatic Conditions at the Experimental Site

Year	Month	Precipitation (mm)	Max tem (oC)	Min temp (oC)	Ave temp (oC)	Rel. Hum (-)	Windspeed (m/s)	Radiation (W/m2)	Atm.pressure (kPa)	Windgusts (m/s)
2019	Aug	13.06	31.60	21.60	25.29	0.87	1.72	159.46	100.57	3.61
	Sep	91.43	32.70	21.80	25.77	0.90	1.51	161.88	100.42	3.12
	Oct	187.40	33.10	21.70	25.89	0.91	1.26	174.12	100.23	2.59
	Nov	17.08	34.60	22.10	29.24	0.79	1.05	329.17	100.12	2.25
2020	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

Measurements were taken from a weather station 30 meters from the experimental plots

Additionally, temperature and relative humidity in the plots were recorded at 10 min intervals with radiation-shielded ibuttons (DS1923-F5 hygrochron, ibutton Link, US). Predawn leaf temperatures were measured on Day 0, 1, 3, 5, 7, 14, 21 and 28, using an infrared thermometer (Laserliner ThermoSpot, Germany) positioned at 5 cm from the leaf surface. Variations over the course of each day were measured at 5-, 9-, 12-, 15- and 18-hour during Day 0, 7, 14, 21 and 28.

5.2.5 Chlorophyll Fluorescence

Chlorophyll florescence of the leaves per each treatment was studied 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 during the heat imposition. Additionally, at Day 7, 14, 21 and 28, diurnal measurements were taken at 5-, 9-, 12-, 15- and 18-hour. Measurements were conducted after a minimum of 30 minutes dark adaptation and under the natural light using the miniPAM leaf clip. Variable fluorescence (F_v/F_m) was recorded on dark adapted samples and electron transport rate (ETR) on light adapted samples (calculated as ETC = (F_v'/F_m' x PAR x 0.5 x 0.84) (Walz, 1999; Toomey, 2013; Motohashi and Myouga, 2015).

5.2.6 Photosynthesis

Gas exchange of leaves was measured using a CIRAS 3 analyzer (PP systems, USA). Instantaneous rates of photosynthesis and transpiration were assessed between 10:00 - 11:00 AM and at 12:00 – 1:00 PM during Day 0, 7, 14, 21 and 28, using natural light conditions with CO_2 set at 400 ± 10 µmol mol^{-1}, and temperature at 28 ± 1 °C.

Response curves of rate of photosynthesis (P_n) to photosynthetic active radiation (P_n/PAR) and to temperature (P_n/T) were measured during the last week of heat imposition. Constant

parameters included CO_2 at 400 ± 10 umol mol^{-1}, T at 28 ± 1 °C for light response curves, and PAR at 1000 µmol m^{-2} s^{-1} for temperature response curves.

Light responses were measured starting at a PAR of 500 µmol m^{-2} s^{-1} and increasing stepwise to 2000 µmol m^{-2} s^{-1} (in the PAR order of 500, 550, 600, 700, 800, 1000, 1500, 2000, 500, 450, 400, 300, 200, 100, 50 and 0 µmol m^{-2} s^{-1}) with 4 minutes acclimation time for the lower levels and 2 minutes acclimation after 700 µmol m^{-2} s^{-1}, optimized after trial runs. In total, ninety-six response curves were measured with twenty-four per treatment.

Light response curves were fitted using the nonrectangular 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).

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

Where;

P_n = net photosynthesis rate [µmol (CO_2) m^{-2} s^{-1}]

$f_{(Io)}$ = quantum yield at I = 0 [µmol (CO_2) $mmol^{-1}$ (photons)]

I = photosynthetic photon flux density [µmol (photons) m^{-2} s^{-1}]

P_{gmax} = maximum gross photosynthesis rate [µmol (CO_2) m^{-2} s^{-1}]

θ = convexity (dimensionless)

R_d = dark respiration rate [µmol (CO_2) m^{-2} s^{-1}]

Parameters such as light compensation point (LCP), light saturation point (LSP), Maximum gross photosynthetic rate for light saturation ($P_{g(max)}$), convexity (θ), Maximum net photosynthetic rate for light saturation, ($P_{n(max)}$) and dark respiration (R_d) were obtained from the fitted curves.

Responses of photosynthesis to temperature were measured at eight temperature levels from 28 to 42 ^{o}C, starting at a low temperature with a stepwise addition of 2 ^{o}C to each level until the highest temperature was reached. Fitting of temperature response curves were done with second order polynomial (Cavieres et al., 2000). Optimum temperatures (T_{opt}) and maximum photosynthetic rates (P_{nmax}) were determined according to:

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

$$P_{nmax} = a(T_{opt})^2 + b(T_{opt}) + c$$

Where a is the coefficient from the quadratic term, b is the coefficient from the linear term and c is the intercept.

5.2.7 Biochemical Analysis (Heat Shock Proteins)

The presence of two main Heat Shock Proteins (HSP), HSP 22 and HSP 70 (De Araujo et al., 2017; Elthon, et al., 2003), were analyzed at the Biotechnology Center, University of Ghana. The young leaves of all plants in each treatment were pooled and transported on ice to the laboratory. Ribonucleic acids (RNAs) coding for HSP 22 and 70 were extracted using Quick-RNA^{TM} Plant/Seed Miniprep Kit (Zymo Research, USA). Quality of RNAs coding for the two HSPs were checked on a 1.2% agarose gel stained with ethidium bromide running at 80V for 1 hour. The RNAs were then converted to complementary Deoxyribonucleic acid (cDNA) with DNA reverse transcriptase kits (ProtoScript II, first Strand cDNA Synthesis Kit, New England Biolabs Inc.). Following the conversion, cDNAs coding for HSP 22 and 70 were ran through reverse transcriptase polymerase chain reaction (RT-PCR) using Heat Shock Specific Primers to identify the presence of HSPs 22 and 70 as affected by the treatments imposed. HSP bands were amplified on 1.2% agarose gel stained with ethidium bromide and ran for 1hour 30

minutes. The process was repeated two more times on different leaf samples for confirmation of results.

Table 5.2.2 Heat Shock Specific Primers

Primer Name	Sequence		Reference
HSP 70	Forward	5' CGATGGCTGCCCTCAATC 3'	(De Araujo et al., 2017)
	Reverse	5' GATGAATCTGAAGGCCCACTTT 3'	
HSP 22 (At4g10250)	Forward	5' CGGTTCCCTGATCCATTCAAGAT 3'	(Banti et al., 2008).
	Reverse	5' ACAGAGCCACGCTTGTGT 3'	

5.2.8 Leaf Chlorophyll Contents

Relative chlorophyll content of the cocoa leaves was assessed at Day 0 and Day 28 with a SPAD (Chlorophyll Meter SPAD-502 Plus, Konica Minolta, Japan), using the fully developed leaves of each plant to assess loss of chlorophyll content due to elevated temperature. Five measurements were taken on each leaf between 10 am and 11 am and then averaged for each leaf.

After the treatments, components of chlorophyll distribution within the cocoa leaves were determined spectrophotometrically. Leaf samples taken from fully matured leaves from the middle four plants were pooled as a unit per treatment and blocked and immediately transported in plastic bags to the laboratory. One gram of fresh leaves was weighed and 10 ml of 80% acetone added, ground, and centrifuged at 10000 rpm (Lichtenthaler and Buschmann, 2001). Absorbances of the supernatant was 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);

C_a (µg/ml) = $12.25A_{663} - 2.79\ A_{645}$

C_b (µg/ml) = $21.50A_{645} - 5.10\ A_{663}$

Where C_a = Chlorophyll a

C_b = Chlorophyll b

A_{663} – Absorbance at 663

A_{645} – Absorbance at 645

5.2.9 Stomatal Density

Stomata cell counts were made on the same leaves used for physiological measurements using nail varnish approach (Schroeder and Stimart, 2005). Adaxial samples were taken from each of the leaves from the middle four plants between the hours of 10 – 11 and were sent to the laboratory for stomatal count. Stomata in-prints were viewed under a compound microscope (Leica Application suite, version 1.8.1, Leica Microsystems Limited, Switzerland) equipped with imaging. Digital images of about 310 x 223 µm were taken and used for the stomata count as outlined by Schroeder and Stimart, (2005).

$$\text{Stomata Density} = \frac{\text{Number of Stomata@40x}}{\text{Area of View (mm}^2\text{)@40x}}$$

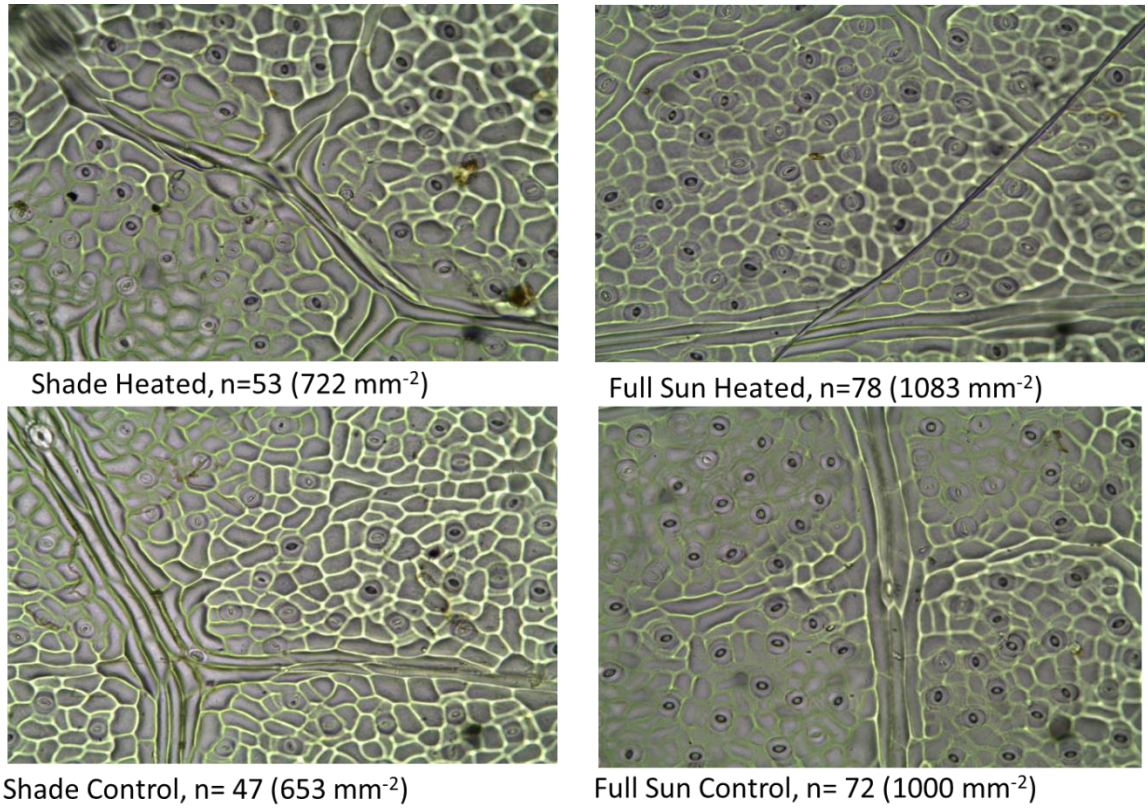

Figure 5.2.2 Stomatal Density of Sampled Leaves from Shade, Full Sun, Heat, and No-heat Treatments

5.2.10 Specific Leaf Area (SLA)

Specific leaf area of individual plants was determined after the heat imposition. Five leaf discs (2.01 cm^2) excluding the mid-vein were sampled from the fully developed leaves of each plant using a core sampler. Leaf discs were dried to a constant mass at 70 ^oC and SLA was determined as the ratio of leaf disc area and the respective dry mass.

$$SLA\ (cm^2/g) = \frac{\pi r^2\,(m^2)}{M(kg)}$$

Where SLA = Specific leaf area; r = radius of leaf disc in m^2; M= dry mass of leaf disc in kg

5.2.11 Plant Growth

Plant growth in height and in diameter were measured at start and at the end of the experiment. Plant height was measured from the base (2 cm from the soil surface) of the stem to the tip of the stem excluding the leaves (Najihah et al., 2018), and stem diameter was assessed at 0.5 cm from the base of the stem.

5.2.12 Leaf Area

Areas of the first three fully developed leaves were recorded using a Portable Leaf Area Meter (Li-3000C, Licor, USA) after the end of the treatments. Averages were estimated and multiplied with total number of leaves to estimate total leaf area per plant assuming that all leaves had similar sizes.

5.2.13 Leaf Damage

Number of leaves were counted at the start and the end of the experiments, and number of leaves browning (Necrotic) or pale (Chlorotic) 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% damage; and 4 - full damage (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.

5.2.14 Plant Biomass

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

5.2.15 Data Analyses

The R statistical package version 4.1.1 was used for the data analyses using the nlme and lme4 packages. The model included the fixed effects of shade (S), heat (H), and time (T) as well as their interactions (SHT), while random effects included repetitions (rep), blocks (bl), plants (pl) and other error terms (ε).

$$Y_{(repeated\ measurments)} = \alpha(S) + \beta(H) + \Upsilon(T) + \mu(SHT) + A(rep) + B(bl) + C(pl) + \varepsilon$$

For measurements taken at the last day of heat imposition, shade (S), heat (H), and their interactions (SH) were used as fixed factors, while random factors included repetitions (rep), blocks (bl), plants (pl) and other error terms (ε).

$$Y_{(last\ mesurements)} = \alpha(S) + \beta(H) + \mu(SH) + A(rep) + B(bl) + C(pl) + \varepsilon$$

Assumptions of homoscedasticity and normality of residuals were investigated by residual and normal quantile plots followed by data transformation. Log transformation was done for stomatal conductance, transpiration, and water use efficiency while data on photosynthesis and chlorophyll fluorescence were transformed with square root and arcsine, respectively. Back transformation was then taken for mean separation.

Levels of significance of the treatments and treatment combinations were assessed with the F-test-based backward selection method (Pope and Webster, 1972) at $P < 0.05$. Multiple comparison test was done to separate treatment means that showed significance using Tukey Honest Significant Difference test (Tukey HSD).

5.3 Results

5.3.1 Environmental Conditions within the Treated Plots

Results of the average maximum and minimum air temperatures within the plots indicated a significant difference among the heat levels, shade levels and heat by shade level interactions (Table 5.3.1). The heat-treated plots on average recorded 2.0 – 4.0 ^{o}C more temperatures than the control plots. Full sun heat treated plots gave the highest average maximum temperature of around 39.2 ± 2.4 ^{o}C while the shade heat treated plots showed the highest average minimum temperature of 28.3 ± 2.1 ^{o}C

Table 5.3.1 Environmental Conditions within the Treated Plots

	Full Sun		Shade		Significant Levels (p < 0.05)		
Conditions	**Control**	**Heat**	**Control**	**Heat**	**SH**	**HL**	**SH*HL**
Microclimate							
¥Rel. Hum. (%)	76.6±4.4a	74.0±4.8b	78.4±4.7a	68.7±5.3c	0.051	0.015	0.025
¥Max. Temp (^{o}C)	38.4±2.4b	39.2±2.4a	37.0±2.1c	38.2±1.9b	0.005	0.006	0.032
¥Min. Temp (^{o}C)	25.6±1.8b	26.0±1.4b	25.9±1.4b	28.3±2.1a	0.024	0.022	0.032
¥Ave. Temp (^{o}C)	30.2±1.3bc	30.7±1.4b	29.9±1.2c	32.1±1.4a	0.036	0.013	0.022
PAR & Leaf Temp							
Leaf Temp (^{o}C)	23.8±1.5c	30.7±3.0b	24.6±1.5c	33.1±3.3a	0.001	<0.001	0.049
PAR (µmol m^{-2} s^{-1})	1485.0±32.0a	1514.0±19.8a	520.0±7.5b	446.0±7.2b	<0.001	0.516	0.117

¥Parameters were measured with ibuttons; Means in a row with different letters are significantly different at p < 0.05 according to Tukey HSD. SH – shade levels; HL – heat levels; Leaf temperature was measured with Infra-red thermometer; PAR was measured with light sensors of CIRAS 3. Means are ± standard error

Relative humidity of the treated plots was highly variable between 68% - 78%. Maximum percentages were recorded during the rainy days. Shade and full sun conditions showed interactive effects on relative humidity. Heat treated plots under the two shade levels had lower relative humidity compared with the control plots.

The levels of photons (PAR) reaching the surfaces of the leaves indicated significant differences among the shade levels with the full sun treatments producing more photons between 1400 – 1500 µmol m^{-2} s^{-1} (Table 5.3.1). Shade net used intercepted between 60 - 70% of the photons directed to the plants. No significant differences, however, were noted among the heat levels.

Predawn leaf temperature showed significant differences among shade by heat interactions and within the heat levels. Plants under heat showed increased leaf temperature of about 7.0 – 9.0 ^{o}C above the control plants. Shade heat-treated treatments had the highest predawn leaf temperature among all the treatments though lower than full sun treatments during the day (Figure 5.2.3).

5.3.2 Chlorophyll Fluorescence and Pigments

Figures 5.3.1 indicates predawn measurements of leaf chlorophyll fluorescence during the experimental time. Shade and heat levels were detected significant ($p < 0.003$; $p < 0.007$) during the two seasons. Shade grown plants showed increased response of fluorescence than full sun plants. Plants grown under heat conditions responded low to fluorescence as duration of heat imposition increased ($p < 0.001$). Shade and heat showed no interactive effects. Full sun grown plants gave the least response of less than 0.75 ± 0.12 during the two trials. Results from 2019 and 2020 trials, showed similar levels of fluorescence.

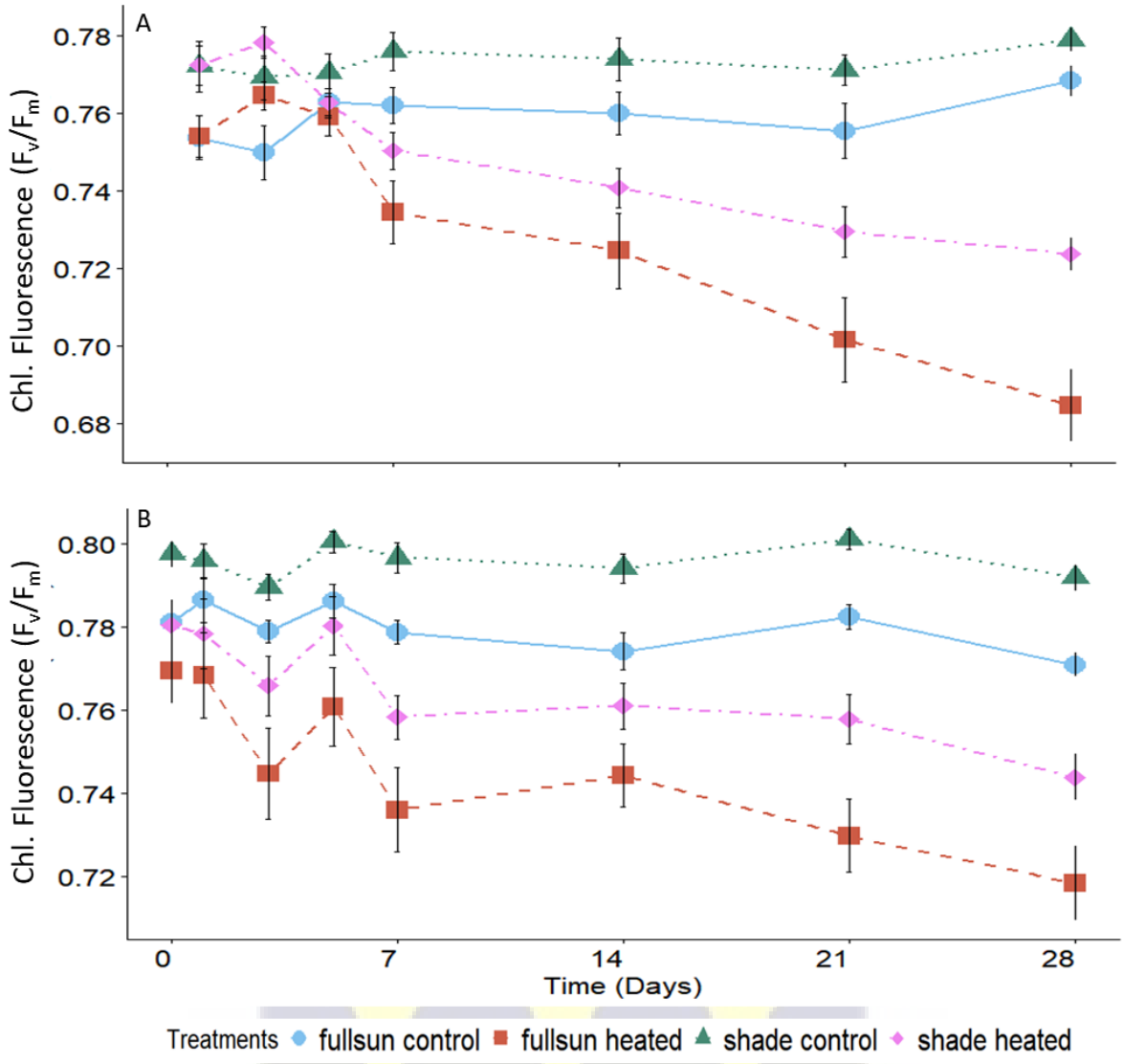

Figure 5.3.1 Chlorophyll Fluorescence of Cocoa Seedlings as Affected by Shade and Heat Levels, (A- 2019 Season, B – 2020 Season). Bars indicate standard error.

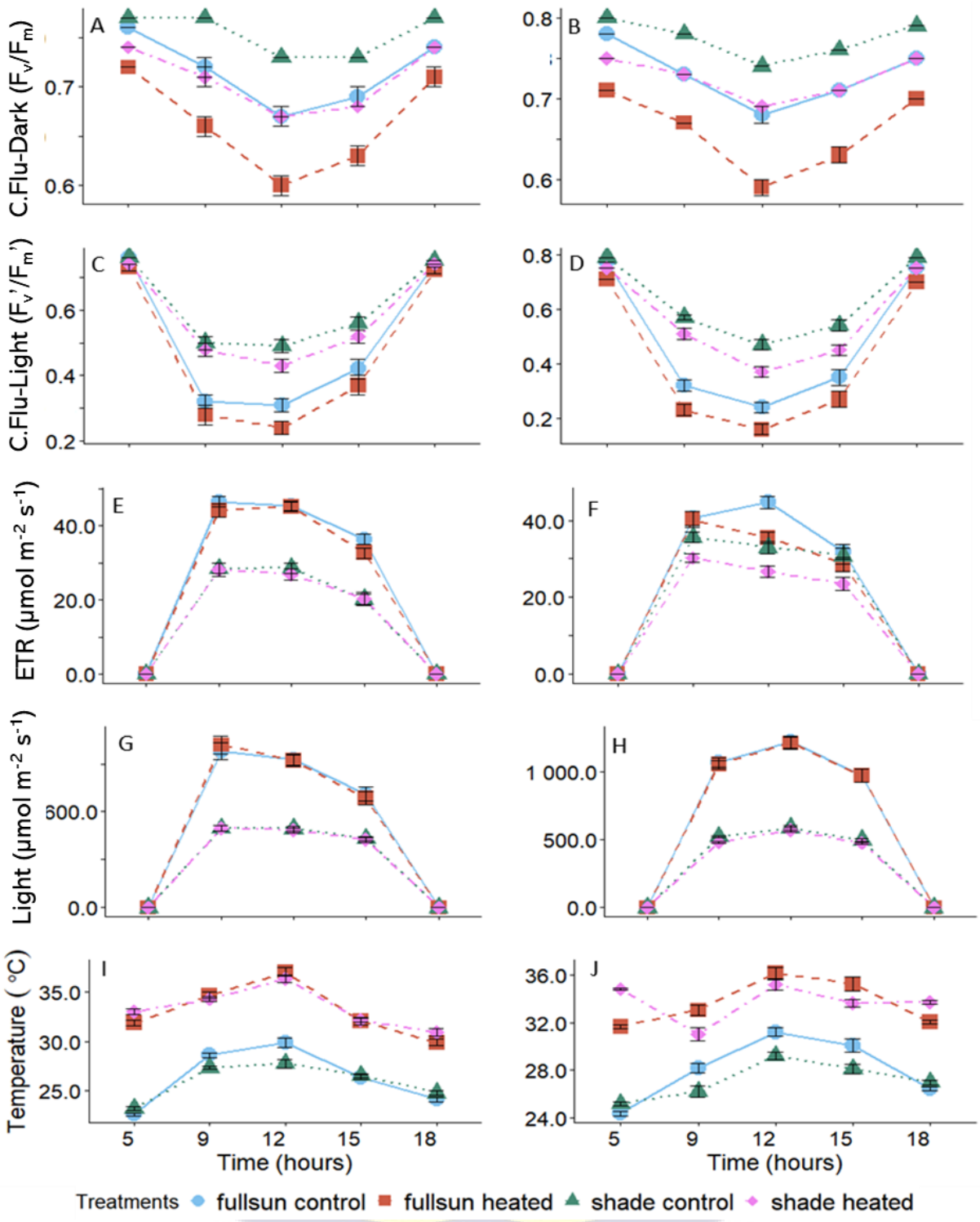

Figure 5.3.2 Diurnal Variations of Chlorophyll Fluorescence, Leaf Temperature and Electron Transfer Rate under Natural Light Conditions During the Two Seasons; (A, C, E, G, I -2019 Season; B, D, F, H, J – 2020 Season); A and B – Chlorophyll Fluorescence at Dark Adaption; C and D – Chlorophyll Fluorescence at Light Adaption, E and F - Electron Transfer Rate (ETR); I and J – Leaf Temperature; Measurement done at the hours of 5, 9, 12, 15 and 18 on weekly intervals for 4 weeks. Bars indicate standard error

Leaf activity in terms of fluorescence, electron transfer and leaf temperature were studied based on diurnal variations of light conditions (Figure 5.3.2). Response to fluorescence for all treatments was high during early and later part of the day and low at middays (Figures 5.2.4 A and B). Shade grown plants comparatively gave higher response on both light and dark acclimated leaves (Figures 5.3.2 A and B). Electron transfer, on the other hand, was as high as 40 µmol m^{-2} s^{-1} on full sun grown plants but below 35 µmol m^{-2} s^{-1} for shade grown plants during the midday. Transport of electrons were recognized to have a negative (-0.8, $p < 0.001$) relationship with fluorescence responses and positively correlated with leaf temperature (0.23, $P<0.001$) and light (0.77, $P<0.001$). Predawn and night temperatures increased in shade, the pattern, however, changed during the midday when light intensity was high. Leaf surface temperature was raised to almost 7 – 10 ^{o}C higher on the heat grown plants than the control plants at midday. Also, shade reduced leaf surface temperature to about 2 – 5 ^{o}C during the midday. The two trials showed the same diurnal patterns of fluorescence, electron transfer, light, and leaf temperature.

Table 5.3.2 Probability Values of Predawn Fluorescence, Leaf Temperature, Diurnal Measurements of Chlorophyll Fluorescence, Temperature, Weekly Measurements of Stomatal Conductance, Photosynthesis, Transpiration and Water Use Efficiency

P Values p < 0.05	Heat	Shade	Time	Heat*Shade	Shade* Time	Heat* Time	Heat*Shade* Time
2019 Season							
Pred. C.Flu	0.001	0.005	0.001	0.330	0.058	<0.001	0.251
Pred.Temp	<0.001	0.002	<0.001	0.425	0.141	<0.001	0.554
Diur. C.Flu-Light	<0.001	<0.001	<0.001	0.454	<0.001	0.003	0.639
Diur. C.Flu-Dark	<0.001	<0.001	<0.001	0.384	<0.001	0.028	0.151
Diur. ETR	<0.001	<0.001	<0.001	0.241	<0.001	<0.001	0.076
Diur Light	0.314	<0.001	<0.001	0.791	<0.001	0.907	0.992
Diur. Leaf Temp	0.001	<0.001	0.055	0.016	<0.001	<0.001	<0.003
2020 Season							
Pre.C.Flu	0.001	<0.028	<0.001	0.817	0.112	<0.001	0.736
Pre. Temp	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.002
Diur.C.Flu-Light	0.001	<0.001	<0.001	0.881	<0.001	0.045	0.742
Diur. C.Flu-Dark	<0.001	<0.001	<0.001	0.468	<0.001	0.001	0.323
Diur.ETR	<0.001	<0.001	<0.001	0.343	<0.001	<0.001	0.045
Diur Light	0.817	<0.001	<0.001	0.881	<0.001	0.955	0.977
Diur.Leaf Temp	0.005	<0.001	<0.001	0.002	<0.001	<0.001	0.004
g_s	0.833	0.001	<0.001	0.838	0.001	<0.001	0.034
P_n	<0.001	<0.058	<0.001	0.818	<0.001	<0.001	<0.001
E	0.727	<0.402	<0.001	0.883	<0.001	<0.001	<0.001
WUE	0.007	0.295	<0.001	0.677	<0.001	<0.001	<0.001

Pred. C.Flu – Predawn measurement of chlorophyll fluorescence on weekly intervals; Pred.Temp – Predawn measurements of leaf temperature on weekly basis; Diur. C.Flu. Light – Diurnal measurement of chlorophyll fluorescence under light conditions; C.Flu.Dark – Diurnal measurement of chlorophyll fluorescence when leaves were dark adapted for 30 minutes with light exclusion clips; Diur. Leaf Temp - Diurnal measurements of leaf temperature; Diur. ETR – Diurnal Measurement of Electron Transfer Rate; g_s – Stomatal conductance; P_n – Rate of photosynthesis; E – Transpiration; and WUE – Water use efficiency.

3.3.3 Effects of Light on Photosynthesis

Figure 5.3.3 shows response of photosynthesis to different levels of photosynthetically active radiation (PAR). Increasing PAR from 0 µmol m^{-2} s^{-1} to 2000 µmol m^{-2} s^{-1} had differential effects on the treatments. At lower light levels, rate of photosynthesis (P_n) increased linearly with increasing light intensity. Then P_n decreased briefly to saturation. Getting to the saturation point, shade plants had slightly sharper arch than full sun grown plants, but significant effects were not detected.

Shade grown plants saturated between 325 – 453 µmol m^{-2} s^{-1} of PAR (Table 5.2.5). On the other hand, full sun grown plants showed higher saturation point of 427 – 523 µmol m^{-2} s^{-1} of PAR. In general, heat treatments gave lower rates of photosynthesis at saturation points with no significant differences between shade and full sun treatments. Light compensation point (LCP) differed among the treatments. Heat grown plants produced higher LCP than control plants while at the shade levels, plants under shade gave lower rates between 0.8 - 14.3 µmol m^{-2} s^{-1}. Full sun plants compensated at light levels between 11.2 – 17.5 µmol m^{-2} s^{-1}. Shade and heat levels had no effects on dark respiration (R_d) and Convexity (θ).

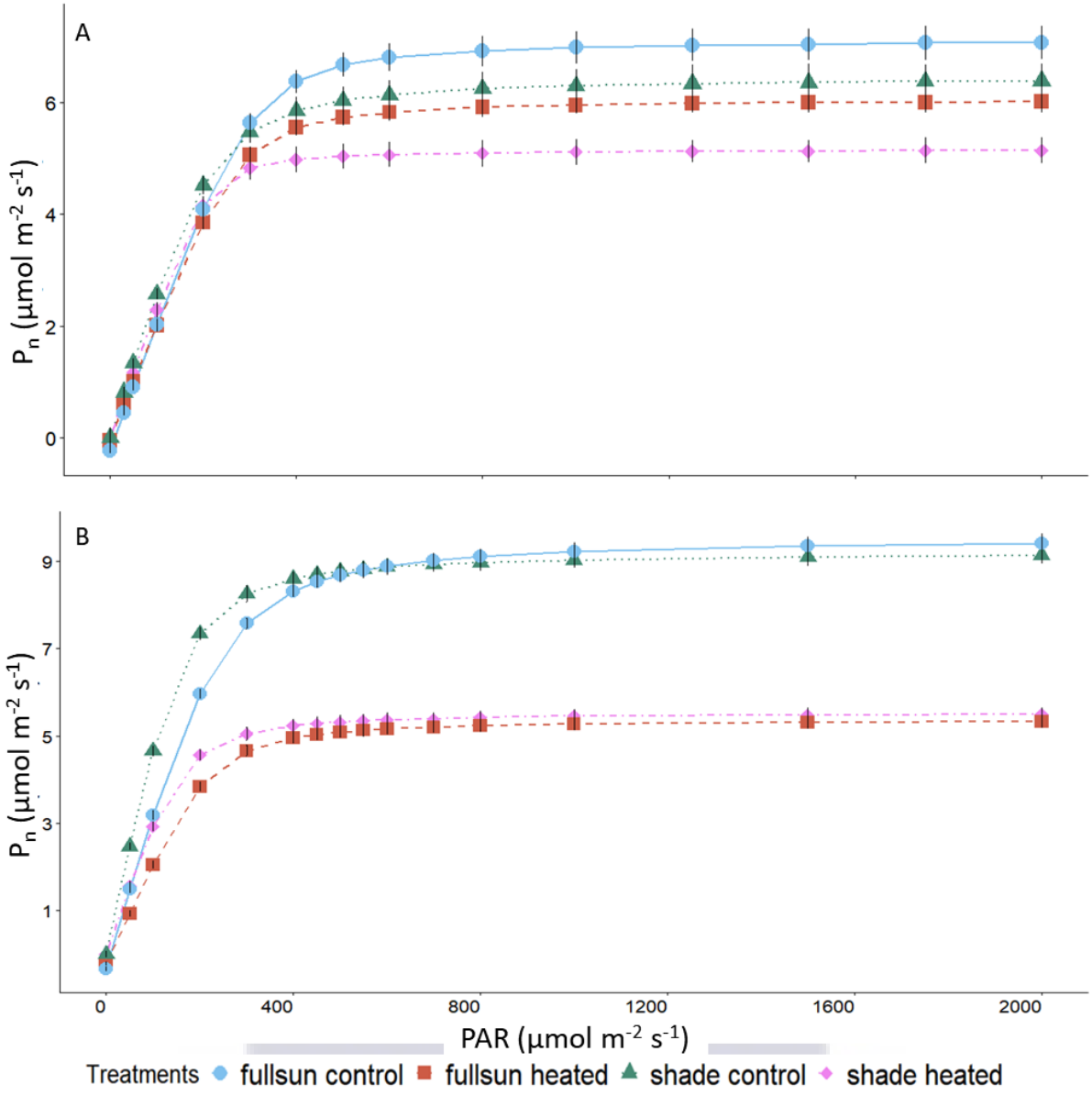

Figure 5.3.3 Rate of Photosynthesis at Different Levels of Light During the 2019 Season (A) and 2020 Season (B); PAR – Photosynthetically Active Radiation; P_n – Rate of Photosynthesis; 2019 season was conducted in September to October 2019 while the 2020 season was conducted in March to April 2020. Bars indicate standard error.

5.3.4 Effect of Temperature on Photosynthesis

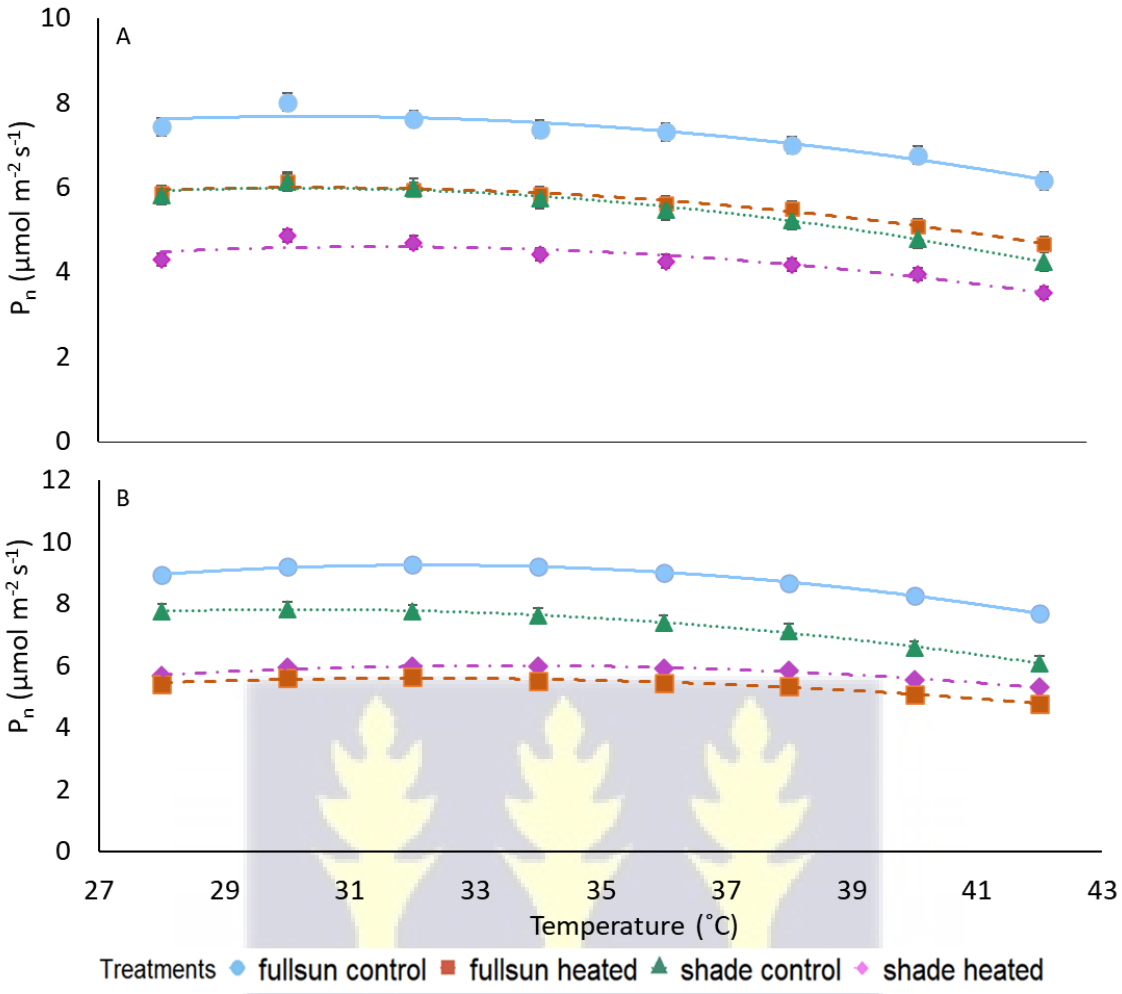

Figure 5.3.4 Effects of Different Levels of Temperature on Rate of Photosynthesis; A – 2019 Season, B – 2020 Season

Photosynthetic rate (P_n) responded significantly to different levels of temperature and was noted to increase from 28 ºC to 33 ºC (Figure 5.3.4). All treatments produced the same pattern and had maximum rates at 30 – 33 ºC to reveal optimum temperature for cocoa plants in that range (Table 5.3.3). Outside this range, photosynthesis was noted to decline as temperature increased. However, rate of decline of photosynthesis was faster in the control than the heat treatments. Full sun grown plants showed the highest rates of photosynthesis with the peak at

8.0 – 9.0 mmol m^{-2} s^{-1} at 32 ^{o}C while the shade heated plants gave the least rates at the optimum temperature. Optimum temperature during the 2019 cool and wet season was 2 – 3 ^{o}C lower than the 2020 hot and dry season which might indicate a shift of optimum temperature due to weather variations between the two seasons.

Table 5.3.3 Rate of Photosynthesis at Different Levels of Light and Temperature

	Full Sun		Shade		Significant Levels p < 0.05		
	Control	Heated	Control	Heated	SL	HL	SL*HL
Light Response							
2019 Season							
n =96							
$P_{g(max)}$	7.6±0.5a	6.4±0.3ab	6.7±0.4ab	5.8±0.3b	0.041	0.011	0.742
$P_{n(max)}$	6.8±0.5a	5.6±0.3ab	5.8±0.5ab	4.9±0.4b	0.056	0.018	0.740
LSP	523.1±42.9a	432.6±26.3ab	453.4±36.5ab	348.2±17.8b	0.021	0.004	0.822
LCP	13.1±3.7	14.8±2.8	8.6±3.2	14.3±3.3	0.526	0.570	0.172
R_d	0.4±0.7	0.3±0.1	0.4±0.2	0.5±0.2	0.399	0.861	0.231
Convexity	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.505	0.983	0.250
2020 Season							
n=96							
$P_{g(max)}$	10.0±0.4a	6.0±0.2b	9.2±0.3a	5.9±0.2b	0.123	<0.001	0.183
$P_{n(max)}$	8.9±0.3a	5.0±0.3b	8.4±0.4a	5.2±0.3b	0.564	<0.001	0.283
LSP	521.0±29.6a	427.8±24.1b	378.9±29.5bc	325.3±13.8c	<0.001	<0.001	0.117
LCP	11.2±2.3b	17.5±2.8a	0.8±0.5d	5.7±1.7c	<0.001	0.004	0.432
R_d	0.4±0.1	0.6±0.1	0.1±0.1	0.3±0.1	0.550	0.700	0.440
Convexity	0.9±0.1	0.9±0.1	0.8±0.4	0.8±0.1	0.360	0.220	0.253
Temperature Response							
2019 Season							
n = 60							
T_{opt}	30.5±0.7	30.8±0.7	29.5±1.4	30.6±1.7	0.594	0.571	0.738
$P_{n(max)}@T_{opt}$	7.9±0.6a	5.9±0.4bc	6.1±0.6bc	4.7±0.4c	0.006	0.003	0.620
2020 Season							
T_{opt}	32.0±0.3	32.1±0.6	31.00±0.8	33.1±0.7	0.959	0.118	0.043
$P_{n(max)}@T_{opt}$	9.3±0.6a	5.8±0.3c	7.8±0.3b	6.0±0.3c	0.146	0.040	0.040

Where $P_{g(max)}$ - maximum gross photosynthetic rate (µmol (CO_2) m^{-2} s^{-1}) ; $P_{n(max)}$ - net photosynthetic rate (µmol (CO_2) m^{-2} s^{-1}); LSP - light saturation point (µmol (photons) m^{-2} s^{-1}); LCP - light compensation point (µmol (photons) m^{-2} s^{-1}; R_d – dark respiration(µmol m^{-2} s^{-1}) (respiration that occurs during photosynthesis); θ – convexity, measuring the sharpness of the arch of the graphs as affected by treatments; T_{opt} – optimum temperature for maximum photosynthetic rate (^{o}C).); $P_{n(max)}@T_{opt}$ – maximum photosynthetic rate (µmol (CO_2) m^{-2} s^{-1}) at optimum temperature; SL – shade levels; and HL – heat levels. Means in a row with different letters are significantly different at p < 0.05 according to Tukey HSD. Means are ± Standard Error

5.3.5 Presence of HSPs

Plate 5.3.1 indicates the production of heat shock proteins (HSPs) as plants were affected by treatment regimes. Results indicated that all the treatments showed the presence of HSP 22 and HSP 70 proteins at different band sizes though band sizes were not quantified. It is, therefore, suspected that the treatments might have influenced the concentration of the HSPs in the plants as indicated by the sizes of the bands. The two HSPs were detected at very low base pairs with HSP 22 around 120 bp while HSP 70 bands were detected close to 150 bp.

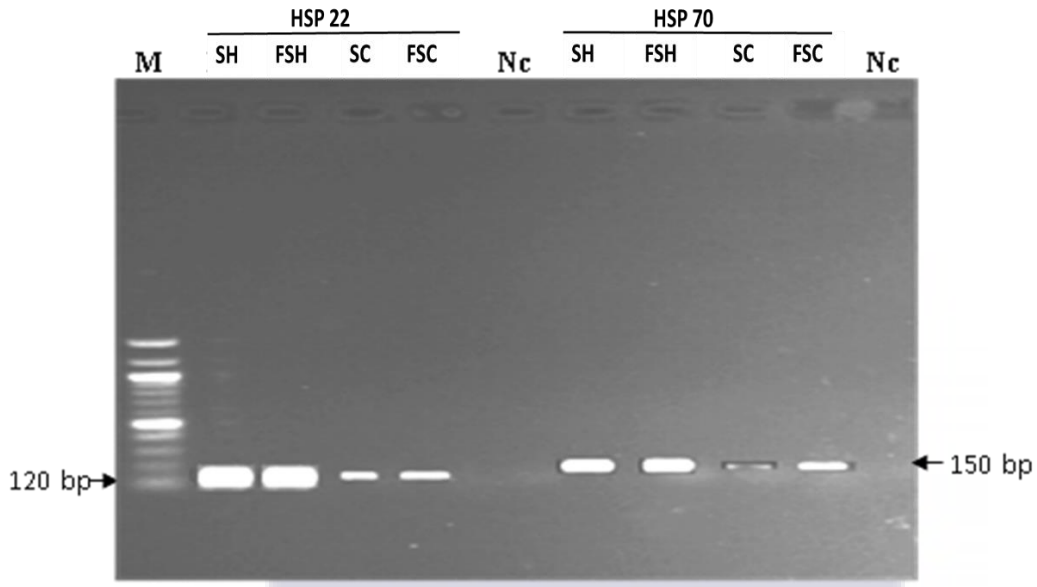

Plate 5.3.1 Amplified cDNA Fragments of Heat Shock Proteins (HSPs) Using Heat Shock Specific Primers (HSP 22 and HSP 70) in Six-Month Old Cocoa Seedlings Subjected to Heat and Shade over a Period of 28 Days. M = Molecular size marker, quick-load purple 1 kb plus DNA ladder (0.1-70.0 kb); Nc = Negative controls (Sterile nuclease-free PCR water); SH = Shade heated; FSH = Full sun heated; SC = Shade control; FSC = Full sun control.

5.3.6 Photosynthesis: Weekly Responses

Treatments effect on heat levels was high ($p < 0.001$) though shade gave no effect ($p < 0.057$) (Figure 5.3.5). Control plants showed improved rates of photosynthesis during the periods measurements were taken as compared with heat-treated plants.

Control of gases in and out of the stomata were affected by shade levels ($p < 0.00I$) and time of the day ($p < 0.001$) but not heat levels. Plants under shade showed improved opening of the stomata compared with plants under full sun. All the treatments had the same effects on leaf transpiration though plants from the control showed slightly increased water use efficiency.

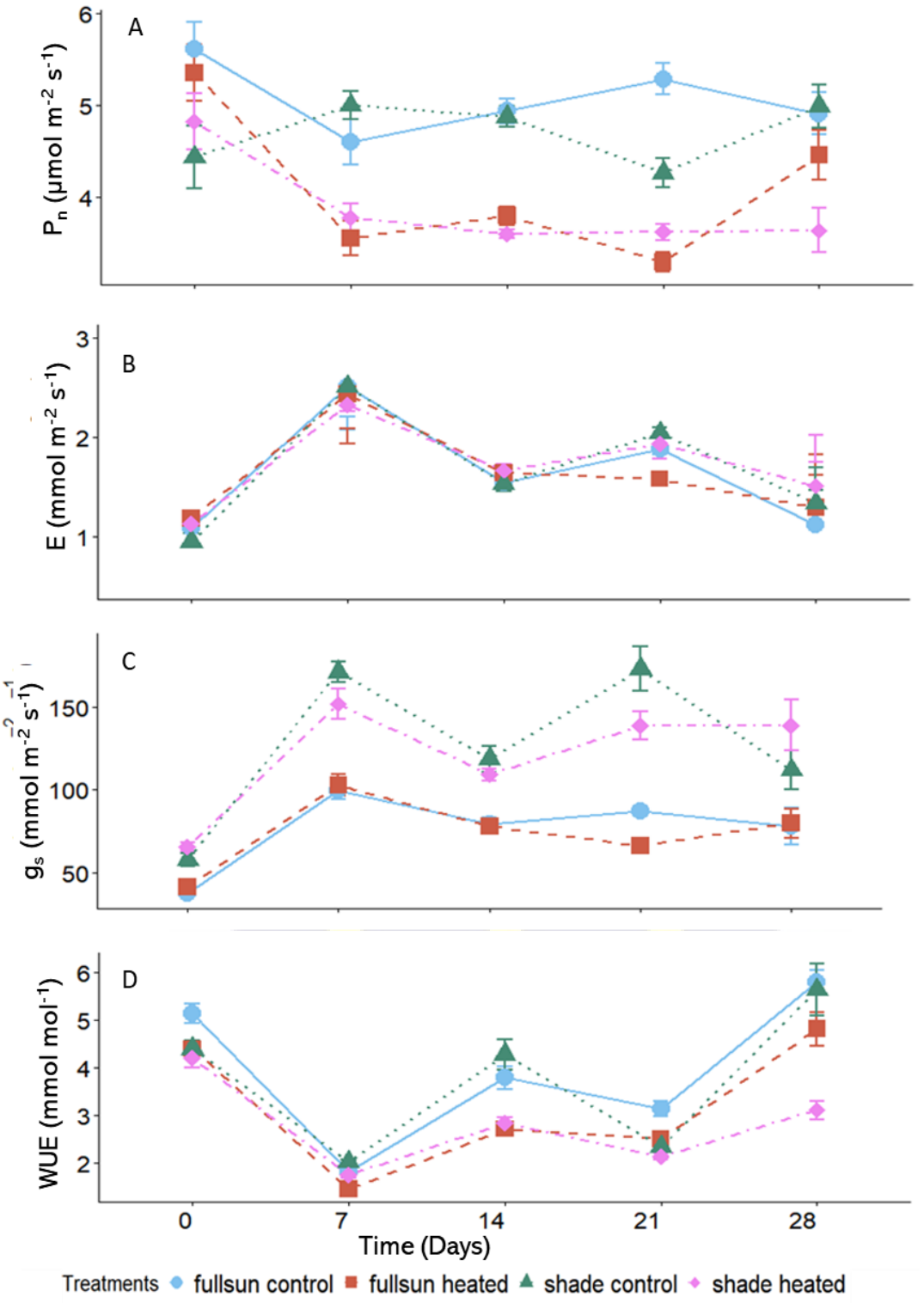

Figure 5.3.5 Gas Exchange as Affected by Duration of Treatments and Time of the Day; A) Rate of photosynthesis (P_n); B) Transpiration (E); C) Stomatal conductance (g_s); and D) Water use efficiency (WUE). Bars indicate standard error

5.3.7 Chlorophyll Pigments

Tables 5.3.4 shows relative chlorophyll contents of leaves measured before and after the treatments with SPAD meter during the second trial, and chlorophyll a and b components studied by UV spectrophotometry. Full sun grown plants significantly showed lower values of total chlorophyll contents before the imposition of the treatments. Loss of chlorophyll could be due to acclimatization period where the plants were kept under full sun for some weeks. Greater loss ($p < 0.001$) of chlorophyll was observed after the treatments compared with the shade grown plants. Heat levels affected the contents of leave relative chlorophyll ($p < 0.001$) with seedlings under heat giving the highest average loss of 2.4 ± 0.3 nmol/cm^2. Heat treated plants under full sun gave the highest loss of 10.1 ± 0.5 nmol/cm^2 while the shade control plants gave the least loss of 1.0 ± 0.1 nmol/cm^2. No significant effect was, however, detected among the shade by heat interactions.

Table 5.3.4 Assessment of Leaf Chlorophyll Contents as affected by Heat and Shade

	Full Sun		Shade		P values ($p < 0.05$)		
Chlorophyll Content	Control	Heat	Control	Heat	SL	HL	SL*HL
2019 Season							
Chl a (ug/g)	18.1±1.3ab	12.8±1.0bc	22.0±1.7a	14.2±1.8b	0.009	0.001	0.131
Chl b (ug/g)	11.1±1.6	9.8±1.7	12.7±2.7	10.1±1.8	0.370	0.074	0.547
Chl a/Chl b	1.7±0.2ab	1.4±0.1b	1.8±0.2a	1.4±0.1b	0.344	0.010	0.743
2020 Season							
¥Chl Before (nmol/cm^2)	39.4±0.7b	39.0±0.7b	41.9±1.3a	40.6±0.9ab	0.001	0.137	0.491
¥Chl After (nmol/cm^2)	34.4±0.5b	28.9±1.0c	40.9±1.3a	35.2±0.7b	<0.001	<0.001	0.676
¥ΔChl (nmol/cm^2)	5.0±0.5b	10.1±0.5a	1.0±0.1c	5.4±0.4b	<0.001	<0.001	0.085
ChlA (ug/g)	14.8±1.6b	8.6±1.6c	20.8±2.7a	13.2±0.8b	0.012	0.002	0.712
ChlB (ug/g)	8.7±0.8c	6.6±0.6d	12.6±1.6a	10.5±1.0b	0.003	0.022	0.984
ChlA/ChlB	1.8±0.3a	1.3±0.2b	1.7±0.2a	1.3±0.2b	0.821	0.038	0.830

Chl - Chlorophyll; ChlA – Chlorophyll a; ChlB - Chlorophyll b; ΔChl – change of chlorophyll content after treatments. Means in a row with different letters are significantly different at $p < 0.05$ according to Tukey HSD. Means are ± Standard Error; ¥ - measured with SPAD meter and others measured with UV Spectrophotometry; SL – shade levels; HL – heat levels; $P_{0.05}$ – probability at 0.05

Both shade and heat levels were observed to have significant effects on chlorophyll a (Chl a) and chlorophyll b (Chl b) pigments during the 2020 trial while Chl b was not affected by the treatments in 2019 trial (Table 5.2.7). Shade grown plants showed increased contents of Chl a and Chl b. Heat reduced both Chl a and Chl b contents to about 2.0 – 7.0 ug ml^{-1} of plants grown under the two shade levels. Heat also affected chl a/b ratio ($p < 0.038$) with control plants producing higher ratios than heat treatments. Shade had no observable effects on Chl a/b ratios.

5.3.8 Plant Growth

Shade and heat levels interacted to affect growth in height (ΔHeight) during the 2020 season however this was not observed during the 2019 season (Table 5.3.5). On the other hand, heat significantly affected growth in height during the two seasons.

Table 5.3.5 Mean Growth of Plant Height, Diameter and Number of Leaves as Affected by Shade and Heat Levels

	Full Sun		Shade		P Values (p < 0.05)		
	Control	Heat	Control	Heat	SL	HL	SL*HL
2019 Season							
Height (cm)	58.0±1.7	58.5±1.1	59.3±2.1	55.5±1.7	0.599	0.321	0.203
Diameter (cm)	8.9±0.3	9.4±0.4	8.8±0.5	7.8±0.4	0.065	0.600	0.104
Leaves	14.0±1.0	13.0±1.0	15.0±1.0	11.0±1.0	0.800	0.097	0.471
ΔHeight (cm)	3.4±0.6ab	2.1±0.5b	3.6±0.6a	2.6±0.5ab	0.443	0.020	0.774
ΔDiameter (cm)	1.5±0.2	1.2±0.3	1.5±0.2	1.1±0.2	0.858	0.092	0.743
ΔLeaves	1.0±1.0	0.0±1.0	1.0±1.0	0.0±1.0	0.928	0.590	0.964
2020 Season							
Height (cm)	62.6±0.6ab	60.5±0.7b	65.3±1.1a	60.8±0.8b	0.166	0.008	0.225
Diameter (cm)	9.8±0.2	9.4±0.2	9.8±0.3	9.5±0.2	0.803	0.098	0.803
Leaves	9.0±1.0	9.0±1.0	10.0±1.0	8.0±1.0	0.618	0.196	0.618
ΔHeight (cm)	4.2±0.3b	2.7±0.2c	6.4±0.5a	3.1±0.2b	0.002	<0.001	0.010
ΔDiameter (cm)	1.1±0.1	0.8±0.1	1.1±0.2	1.0±0.1	0.395	0.218	0.444
ΔLeaves	3.0±1.0	2.0±1.0	3.0±1.0	2.0±1.0	0.876	0.184	0.364

Means in a row with different letters are significantly different at p < 0.05 according to Tukey HSD. Observations in a row without letters are not statistically different. Means are ± Standard Error

In both seasons, seedlings had growth reduction of about 35 - 50% under heat conditions. Stem expansion and leaf number showed no significant differences among the treatments.

5.3.9 Leaf Functions

Leaf browning (necrosis) was studied before and after the treatments (Figure 5.3.6). Necrosis was more severe under heat conditions than under control treatments and increased in full sun grown seedlings than in shade grown seedlings. As twice as many leaf damages were recorded in the heat treatments from the two shade levels than the control during the two trials.

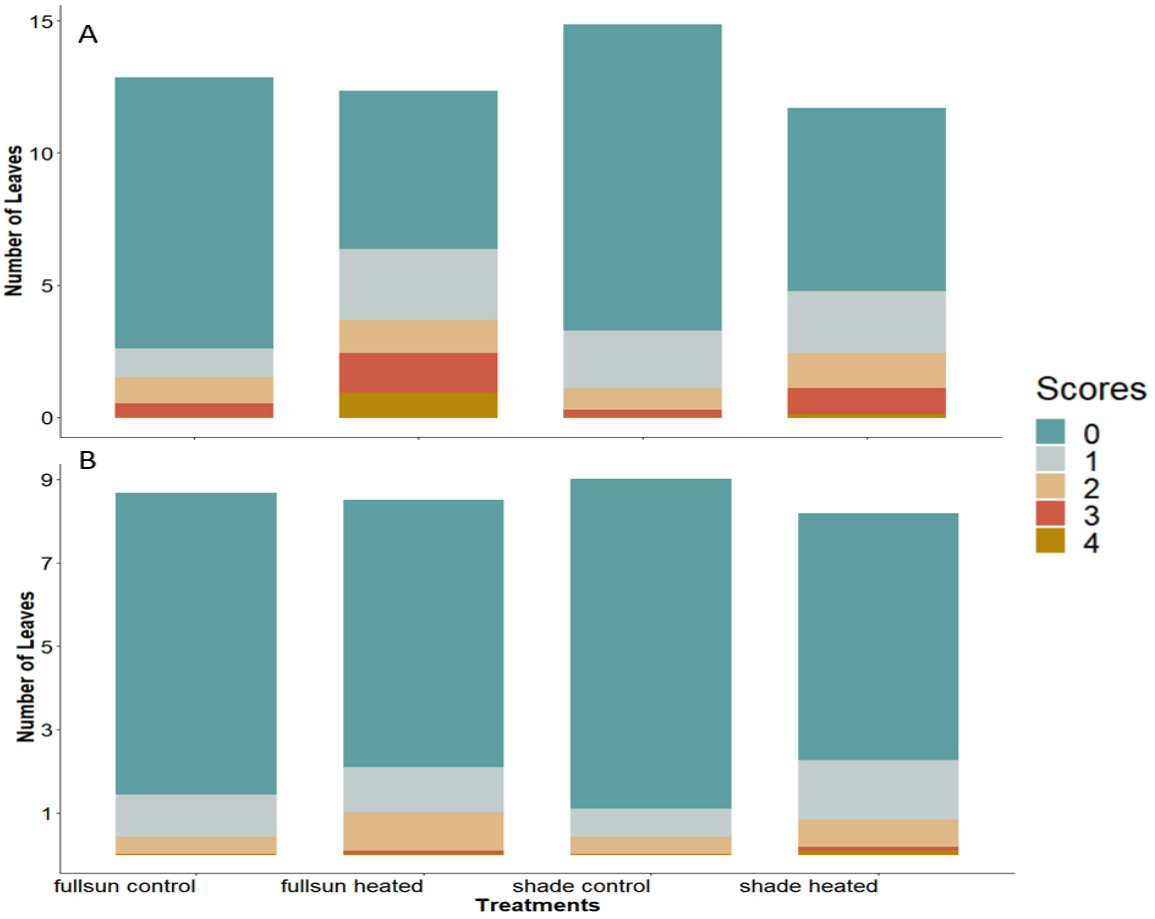

Figure 5.3.6 Number and Severity of Leaves Damage (Necrosis); A – 2019, B – 2020

Most of the brown leaves identified were more speckled (score 1) and less than 50% damaged (score 2) for all the treatments. Score-two was more frequent in the full sun heat-treated seedlings than shade heat-treated seedlings though no significant differences existed between the two treatments. Few leaves were noted to be more than 50% and fully damaged among all the treatments.

5.3.10 Leaf Area, Stomata Density and Specific Leaf Area

Shade grown plants significantly produced leaves with broad surface areas (Table 5.3.5) than full sun grown plants. Heat, however, affected the leaf surface area in the two conditions to as high as 56% reduction on shade grown plants and 31% on full sun treatments. Plants under heat showed lower values of specific leaf area (sla), especially during the second season, to indicate denser leaves than control plants. Also, shade affected sla in full sun grown plants giving lower values than shade grown plants. Specific leaf area ranged between 20.1 ± 0.7 to 26.1 ± 0.5 for all the treatments.

The number of stomata produced per unit area were significant among heat levels, shade levels and heat by shade interactions. Leaves from heat-treated plants under full sun produced more stomata per unit area comparing with the same treatment under shade. The same pattern was also noted among the control plants under the two shade levels.

Table 5.3.5 Effects of Shade and Heat on Leaf Area, Stomata Density and Dry Weight of Leaves, Stems and Roots

	Full Sun		Shade		Significant Levels ($p < 0.05$)		
	Control	Heat	Control	Heat	SL	HL	SL*HL
2019 Season							
Leaf Area (cm^2)	90.8±7.6b	86.0±7.2b	148.1±9.7a	128.3±7.6a	<0.001	0.121	0.345
Tot. Leaf Area (cm^2)	1396.5±180.8b	1354.7±195.9b	2473±224.6a	1671.6±168.7ab	0.013	0.101	0.134
SLA (m^2kg^{-1})	21.4±0.3bc	20.1±0.3bc	26.9±1.4a	25.4±1.2ab	<0.001	0.185	0.931
St. Density (mm^2)	904.5±10.5b	1007.2±14.5a	670.8±11.2d	801.0±11.1c	<0.001	<0.001	0.403
2020 Season							
Leaf Area (cm^2)	77.8±5.0b	43.9±2.6c	130.04±8.6c	85.29±6.4b	<0.001	<0.001	0.178
Tot. Leaf Area (cm^2)	717.8±89.1b	408.1±40.0c	1264.6±114.4a	700.3±72.3b	0.001	0.001	0.355
SLA (m^2kg^{-1})	23.4±0.5b	20.1±0.7c	26.1±0.5a	24.0±0.6bc	<0.001	<0.001	0.495
St. Density (mm^2)	921.9±18.9b	1077.2±22.8a	614.3±19.8d	833.9±20.6c	<0.001	<0.001	0.040

SLA – Specific leaf area; RWC% - Leaf relative water content. Means in a row with different letters are significantly different at $p < 0.05$ according to Tukey HSD. SH – shade levels; HL – heat levels; Means are ± Standard Error

5.3.11 Biomass

Stem dry weight was not affected by shade though affected by heat during the first season (Table 5.3.6). Leaf dry weight differed between heat-treated plants and control plants during the two seasons with the former giving lower yield. Shade control plants gave the maximum yield. Total dry weight and root dry weight were affected by heat during the second season. The control treatments showed improved total dry weight under shade and under full sun.

Table 5.3.6 Effects of Shade and Heat on Leaf Dry Weight, Stem Dry Weight, Roots Dry Weight, Total Dry Weight, Leaf to Stem Ratio, Leaf to Root Ratio and Stem to Root Ratio for 2019 and 2020 Seasons

	Full Sun		Shade		Significant Levels ($p < 0.05$)		
	Control	Heat	Control	Heat	SL	HL	SL*HL
2019 Season							
Leaves DW (g)	6.4±0.5a	5.7±0.4ab	6.3±0.5a	4.6±0.4b	0.068	0.008	0.128
Stem DW (g)	8.6±0.5ab	8.1±0.4ab	8.7±0.5a	7.1±0.4b	0.292	0.028	0.180
Roots DW (g)	4.9±0.6	4.9±0.5	7.5±0.6	5.6±0.6	0.060	0.225	0.195
Total DW (g)	19.9±1.4ab	18.6±1.1ab	22.5±1.4a	17.3±1.3b	0.806	0.061	0.152
Leaf/stem	0.8±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.101	0.098	0.542
Leaf/root	1.6±0.2a	1.4±0.2a	0.9±0.1b	0.9±0.1ab	0.014	0.889	0.619
Stem/root	2.1±0.3a	2.1±0.3a	1.2±0.2b	1.6±0.3ab	0.009	0.728	0.316
2020 Season							
Leaves DW (g)	3.6±0.4ab	2.6±0.2b	4.9±0.4a	2.7±0.4b	0.124	0.004	0.131
Stem DW (g)	6.3±0.4	5.2±0.2	6.7±0.4	5.7±0.2	0.427	0.102	0.956
Roots DW (g)	3.0±0.2a	2.3±0.1b	3.5±0.3a	2.6±0.1b	0.163	0.024	0.980
Total DW (g)	12.9±0.9ab	10.1±0.4b	15.1±0.9a	10.9±0.4b	0.226	0.016	0.552
Leaf/stem	0.6±0.1bc	0.5±0.1c	0.7±0.1ab	0.5±0.1c	0.057	0.001	0.011
Leaf/root	1.2±0.1ab	1.1±0.1ab	1.5±0.2a	1.0±0.1b	0.519	0.028	0.041
Stem/root	2.1±0.1b	2.4±0.1a	2.0±0.1b	2.2±0.1ab	0.045	0.007	0.925

Means in a row with different letters are significantly different at $p < 0.05$ according to Tukey HSD. SH – shade levels; HL – heat levels; $p_{0.05}$ – probability at 0.05. Means are ± Standard Error

In terms of biomass partitioning, shade affected stem/root ratio during the two seasons and in addition leaf/root ratio during the first season. Heat rather, affected leaf /stem ratio, leaf/root ratio and stem/root ratio of plants during the second season. Plants under heat showed lower ratios of leaf/stem and leaf/root but higher ratios of stem/root.

5.4 Discussion

Results of chlorophyll fluorescence and photosynthesis indicate individual effects of heat and shade on cocoa physiology. Shade was observed to modify the effects posed by heat. Seedlings from shade treatments and the two heat levels comparatively recorded higher fluorescence (Fv/Fm), an indication of an improved Photosystem II (PS II) efficiency under shade conditions

at predawn and during the day. Lower values observed in the full sun treatments especially, treatments under heat might be due to temporal heat injury associated with the PSII complexes. Photosystem II complexes with the associated cofactors are known to be the primary target for heat injury (Chen et al., 2012). Yamamoto (2016) explained this to be due to plants adjusting to the stress effects by damaging some proteins in the PS II complexes and replacing them with new ones. At very high light and heat, the protein (DI) is irreversibly aggregated making turnover impossible and removal by protease difficult. High photosynthetic photon flux density (PPFD) which is typical of full sun conditions is anticipated to cause intercepted light energy to exceed capacity of photosynthetic machinery (Jaimez et al., 2018) causing photoinhibition to decrease maximum quantum efficiency of PSII and photosynthesis. The problem is manifested in the rate of photosynthesis at different levels of light. Seedlings from heat treatments recorded lower rates of P_n at increasing light conditions. Carboxylation capacity and RuBP regeneration capacity directed by electron transport are known to depend on temperature (Warren, 2008; Greer and Weedom, 2012). At high or low temperature, Farguhar et al. (1998) noted that electron transfer rate (ETR) declines to diminish the slope of photosynthesis at increasing light conditions. The situation could increase oxygenation instead of carboxylation at Rubisco site to reduce demand of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) (Weber and Bar-Even, 2019) and then affects maximum rate of carboxylation. Optimum temperature for photosynthesis was observed to be between 31 – 33 ^{o}C. The values are in line with Balasimha et al. (1991) that photosynthesis in cocoa is more efficient between temperature range of 31 – 33 ^{o}C, but slightly lower than Yapp et al. (1992) at 33 – 35 ^{o}C also on cocoa. Although much has not been done in cocoa on temperature and photosynthesis response curves, works on other plants indicate that high temperature inhibits the repair of PSII (Allakhverdiev et al., 2008) thereby affecting rate of photosynthesis. However, the extent of the damage depends on the balance between damage and repair

processes during the stress (Allakhverdiev et al., 2008). The effects could be due to damages to the thylakoid membrane due to increased strength of hydrophobic bonds and decreased strength of hydrophilic bonds (Ribeiro et al., 2006). At high temperature, oxygenating reaction of rubisco increases more rapidly than carboxylation (Lambers et al., 2008). Photorespiration then occurs to reduce assimilates production. An adaptation of plants to increased higher temperature is a shift of optimum temperature to higher temperature (Berry and Bjorkman, 1980; Medlyn and Delzon, 2002) which was noted in the shade heated treatments. These shifts could be injuries to the photosynthetic apparatus. It increases transpiration that would put much demand on available soil water in already drier areas (Qaderi et al., 2019). However, rate of transpiration was not significant among treatments though stomata density was significant. The higher number of stomata per unit area recorded in the full sun and the heat treatments might be influenced by leaf specific area. Full sun produced denser leaves with lower leaf area which might cause stomata closely parked to each other. The mechanism could be a strategy to reduce light penetration to prevent photodamage. The tightly arranged stomata therefore did not significantly increase transpiration. It is anticipated that the size of the stomata as well as opening, and closure might be the major factors of water regulation between leaves and the environment instead of number per unit area. Cocoa plants are, however, reported to be poor regulators of water loss (De Almeida and Valle, 200). Although, precise functions of HSPs are not well defined, there is considerable evidence to indicate that HSPs are essential for survival at both normal and elevated temperature (Kregel, 2001). A transgenic *Arabidopsis thaliana* with HSP 70 was identified to confer tolerance to high heat stress by exhibiting higher chlorophyll fluorescence, more intact cell membranes, lower accumulation of hydrogen peroxide (H_2O_2) and lower superoxide anion free radicals (O_2^-) than the wild type (Zhao et al., 2019). Expression of HSP 70 and HSP 22 at different band sizes might indicate different levels of tolerances to heat under shade and full sun conditions. The small HSPs such as HSP 22, are

reported to be effective indicators of heat resistant (Elthon, et al., 2003) by conferring a protective function to prevent thermal aggregation of proteins through binding to non-native forms (Park and Seo, 2015). The HSPs do not repair the damage but rather function to prevent the damage (Allakhverdiev et al., 2008).

No observable differences were shown between shade and full sun grown plants on rate of photosynthesis though many publications such as Agele et al. (2016), Avila-Lovera et al. (2016), De Araujo et al. (2017), and Salazar et al. (2018) indicate higher rates under full sun. Results, however, confirm earlier reports by De almeida and Valle (2008); Tee et al. (2018) and Asare et al. (2018) that photosynthetic rate, growth, and yield of cocoa are improved with shading and could modify the effects of heat on photosynthesis. According to Pallardy (2008), plants that can grow under shade have ability to adapt their photosynthetic apparatus to low light intensity and such plants must have the capacity to efficiently trap available light and convert them into chemical energy, maintain a low rate of respiration and partition a large fraction of assimilates into leaf growth. Low light saturation points (LSP) of 400 µmol m^{-2} s^{-1} and low maximum photosynthetic rate of around 7µmol m^{-2} s^{-1} at light saturation are reported in cocoa (Anim-Kwapong and Frimpong, 2004; Hutcheon, 1981). Salazar et al. (2018) reported LSP between 200 - 750 µmol m^{-2} s^{-1} and LCP around 5 – 57 µmol m^{-2} s^{-1} for shade tolerant cocoa plants grown in Colombian Amazon while in Venezuela, Avila-Lovera et al. (2016) reported LSP between 400 – 500 µmol m^{-2} s^{-1}, and LCP of 11.1 µmol m^{-2} s^{-1}. These findings are in line with the results of this work and provide evidence of cocoa as a shade tolerant plant.

Higher chlorophyll content recorded under the shade treatments may depict low or no photoinhibition under shade conditions, similar results have been reported as a strategy to survive under shade conditions and for efficient utilization of limited light available to the plants (Sorrentino et al., 1997; Salazar et al., 2018; Lambers et al., 2008). It is anticipated that loss of chlorophyll from the heated plants might be a factor for increased browning of the leaves

as observed by Knudson et al. (1977) that there is a high correlation between chlorophyll reduction and percent visible necrosis or chlorosis. High temperature inhibits chlorophyll synthesis (Li et al., 2018). In effect plants tend to produce more of Chlorophyll b, a thermally stable pigment (Erge et al., 2008) to augment Chlorophyll a and protect leaves from heat injury (Lambers et al., 2008).

Shaded plants responded better to growth and biomass parameters over full sun plants. Heat retarded growth as well as biomass production. Vascular cell damage or death can occur at either extreme temperatures or exposure to prolonged heat stress (Qaderi et al., 2019). Extreme temperature is also noted to disrupt hydraulic conductance and create unsuitable levels of xylem tension which reduce water movement because of cavitation (Qaderi et al., 2019). It is anticipated that reduced growth of plants under heated conditions could be due to increasing tension on xylem water, reducing water and solutes transport to the shoots, and causing reduction in photosynthesis, growth, and biomass production while optimum shading has been identified to increase plant growth and yield. With broader leaves of low specific density and higher volumes of air spaces, conductivity of materials between leaves and environment is enhanced to facilitate photosynthesis under shade conditions. Semchenko et al. (2011) identified 50% increased biomass yield under shade of all the 46 species of plants they studied. Shading was observed to modify light levels, wind speed, air temperature and humidity (Semchemko, et al., 2011)

The results from this study confirm the hypothesis that heat can alter physiological functions of cocoa, but the effects can be modified by shade. Heat caused leaf's damage and reduced chlorophyll fluorescence to affect photosynthesis; growth and yield of biomass were thus affected. Plants responded to heat with narrower leaves, higher number of stomata per unit area and lower leaf specific area. There seems to be a shift of optimum temperature to higher levels to adapt or tolerate the heat stress. Shade on the other hand increased yield of fluorescence, leaf

area, and chlorophyll pigments of leaves. Low light saturation points between 325 – 380 µmol m^{-2} s^{-1}, comparing with 427 – 529 µmol m^{-2} s^{-1} in full sun conditions, reveals efficient utilization of light under shade. This protects leaves from injury and enhance leaf physiology. Shading can be a means of modifying the bad effects of climate change on cocoa growth to increase longevity of cocoa plants and improve yield. Agroforestry may be a possible solution to safeguard cocoa against heat.

CHAPTER VI

CONCLUSION AND RECOMMENDATION

6.1 General Conclusion

Elevated temperature and rainfall variabilities are currently the key factors expected to affect the cocoa industry. This study has shown that performances of cocoa including physiology, growth and yield are affected by the two climatic conditions.

Water suppression affected soil moisture reducing moisture content to as low as 0.2 m^3/m^3 among the severe stressed plots. The effects of low soil moisture among the water suppressed treatments were shown in the water potential levels of the stem where control plants recorded higher water potential than the water suppressed plants. Leaf chlorophyll fluorescence indicated weakened photosystem II efficiency among water suppressed treatments in both predawn and diurnal measurements. Leaf stomatal conductance was not affected by water suppression levels indicating poor stomatal control of loss of water in cocoa leaves. Water suppression decreased transpiration rate affecting photosynthesis and assimilates production. Litter fall was therefore higher among the severe water suppressed plants indicating reduced leaf surface area for photosynthesis and thus affecting assimilates production. Yield of cocoa in terms of pods density and dry weight decreased proportionally with water suppression levels.

Elevated temperature increased leaf surface temperature and reduced leaf chlorophyll fluorescence as duration of imposition of heat increased. This affected maximum rate of photosynthesis as lower values were recorded on plants under heat. Leaf chlorophyll pigments degraded under heat to increase leaf damage and then reduced growth in terms of height. Leaf area was reduced under heat but leaf density (SLA) and number of stomata per unit area increased showing thick leaves under heat stress, a means of reducing easy penetration of solar

energy to ensure energy balance in the leaves. Yield of biomass was high under control plots correlating with rate of photosynthesis.

Shade provided favourable environmental conditions reducing leaf surface temperature and air temperature and then increasing relative humidity around the plants to reduce soil moisture evaporation. Shade reduced the effects of water suppression on leaf chlorophyll fluorescence, litter fall and then increased photosystem II efficiency and promoted growth of vegetative and reproductive parts. Though rate of photosynthesis was high under full sun conditions, flower development to matured pods was low perhaps due to higher air temperature that might have affected floral development. Canopy and Pods density were high under shade conditions and dry bean yield was shown to be higher under shade conditions in contrast to earlier reports.

Shade modified the effects of heat on cocoa plants through higher leaf chlorophyll fluorescence, lower day leaf temperature, broader and lighter leaves for wider area of light catch and penetration. Light saturation points (LCP) of shaded plants were low to indicate efficient use of limited light available to the plants and hence the postulation that cocoa does well under shade.

Shade can be a mitigative strategy to protect cocoa plants against drought and heat. The experiment has shown that shade improves plants microclimates to increase plant's water status enhancing photosystem II efficiency to protect photosynthetic machinery. It is assumed that survival of cocoa plants will likely be improved under shade conditions compared with full sun conditions. Nonetheless, higher humidity under shade could serve as a medium for spread of black pod diseases causing loss of cocoa pods.

Cocoa agroforestry could be a tool to sustain cocoa industry and protect the environment from degradation. Trees that are used for cocoa agroforestry could help sequester carbon and thereby contribute to the mitigation of climate change. Trees could also serve as long term investment

and farmers could fall upon them as timber to buffer against unpredictable events. Provision of wood for fuel, medicine, fruits, wind belt for other crops and materials for building are other added benefits with cocoa agroforestry.

6.2 Recommendations

Farmers are encouraged to practice cocoa agroforestry to help protect the plants from bad weather.

The uniformity of shade and type of tree used for the shade is very important in the quest to go into cocoa agroforestry. There is the need of further research to select shade trees that will give uniform shade and at the same time will provide less competition for soil moisture and nutrients.

Further research is needed to provide more evidence on the stomatal regulation of water loss in cocoa plants – whether the species is an isohydric or anisohydric plant.

Also, the levels of expression of heat shock proteins in the cocoa under stress could not be quantified. Further research is needed to understand the mechanisms behind the production of HSPs under stress conditions.

Finally, a trial should be conducted in a dry and hot environment to provide more information on effects of heat on cocoa production.

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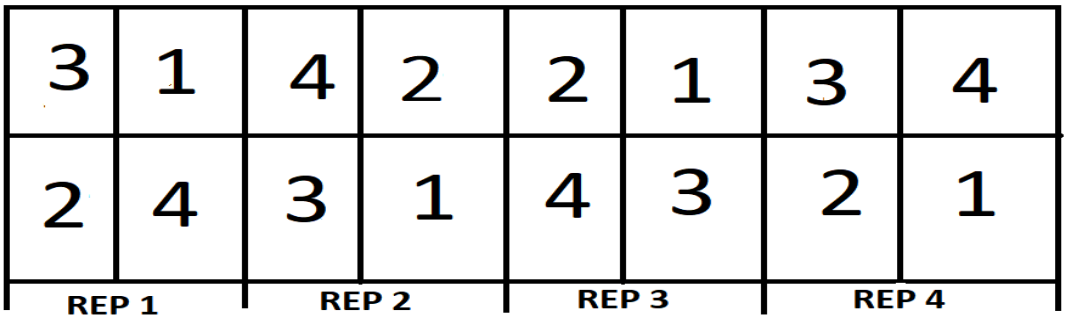
APPENDICES

Appendix 1. Field Lay-out of the Randomized Complete Block Design of the Heat Stress Experiment



Treatments were kept 2 meters apart from each other.

Appendix 2. Chemical Constituents of Soil used for the Repotting of Cocoa Seedlings. Analysis was Conducted at the Ecological Laboratory of University of Ghana

Parameter	Value	Recommended	Reference	Status
pH	5.00	5.10 – 7.00	Snoeck et al. (2016)	Acidic
Ec ds/cm	50.70	70.00– 90.00	Brito-Vega et al. (2018)	Low
Available P	50.44	>20.00	Horneck et al. (2011)	Adequate
%C	2.07	1.70 – 3.20 %	Snoeck et al. (2016)	Adequate
% Total Nitrogen	0.09	>0.20	Singh et el. (2019)	Low
Ca cmol+/kg	2.34	4.00 – 18.00	Snoeck et al. (2016)	Low
Mg cmol+/kg	1.32	0.90 – 4.00	Snoeck et al. (2016)	Adequate
K cmol+/kg	0.09	0.20 – 1.20	Snoeck et al. (2016)	Adequate
Na cmol+/kg	0.04	<3.00	Botta (2015)	Adequate
Cu mg/kg	<0.01	0.40 – 1.80	Snoeck et al. (2016)	low
Mn mg/kg	9.00	3.00 – 12.00	Snoeck et al. (2016)	Adequate
Soil Texture		Texture		
% Sand	71%			
% Silt	19%	Sandy Loam		
% Clay	10%			

Appendix 3. P Values of Effects of Shade, Water Suppression and Time on Canopy Cover, Flower, Pod, and Cherelle Productions

	Soil Moisture	Canopy	Flower	Cherelles	Aborted Cherelles	Pods	Damaged Pods
Sources of Variation *(P<0.05)*							
Shade	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.014
Suppression	<0.001	<0.001	<0.001	<0.001	0.462	<0.001	0.723
Month	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Shade*Suppression	<0.001	<0.001	<0.001	0.129	0.233	0.439	0.301
Shade*Month	<0.001	0.287	0.054	0.024	0.011	<0.001	0.024
Suppression*Month	0.196	0.025	0.743	0.544	0.766	<0.001	0.004
Shade*Suppression*Month	0.004	NA	0.974	0.810	0.363	0.337	0.043
Observations							
No. of Observations	7020	3599	3555	3570	3569	3518	3526

Appendix 4. P Values for Effects of Shade and Water Suppression on Pods and Beans Physical Characteristics

P values for effects of shade and water suppression on pods and beans physical characteristics (n = 9)

	Shade Level	**Suppression Level**	**Season**	**Shade * Suppression**	**Shade*season**	**Suppression *season**	**Shade*season *suppression**
Sources of Variation							
Pods Physical Appearance							
Pod Weight [g/pod]	<0.001	<0.001		0.313			
Pod Length(cm/pd)	0.018	0.010		0.122			
Pod Diameter[cm/pod]	<0.001	0.002		0.027			
Length/Diameter	0.731	0.053		0.011			
Beans Quantity and Weight							
Beans per Pod(n/pod)	0.002	0.691		0.860			
Beans weight pod[g/pod]	<0.001	<0.001		0.927			
Beans(g)/Pods(g)	0.116	0.120		0.027			
% Beans Content per pod	0.116	0.120		0.027			
Fresh Weight per Bean [g/ bean]	<0.001	<0.001		0.457			
Dry Weight per Bean[g/bean]	0.212	0.003		0.813			
Moisture of Bean(g/bean)	<0.001	<0.001		0.359			
Dry bean yield (kg/ha/season)	0.035	0.008	<0.001	0.945	0.483	0.006	0.872

Appendix 5. Main and Mid Seasons of Cocoa Production in the World

Country	Main crop	Mid-crop
Brazil	Oct-Mar	Jun-Sep
Cameroon	Sep-Feb	May-Aug
Colombia	Apr-Jun	Oct-Dec
Congo, Democratic Republic	Sep-Mar	Apr-Sep
Costa Rica	Jul-Feb	Mar-Jun
Côte d'Ivoire	Oct-Mar	May-Aug
Dominican Republic	Apr-Jul	Oct-Jan
Ecuador	Mar-Jun	Dec-Jan
Ghana	Sep-Mar	May-Aug
Grenada	Apr-Nov	Dec-Mar
Haiti	Mar-Jun	Jul-Feb
Indonesia	Sep-Dec	Mar-Jul
Jamaica	Dec-Mar	Apr-Nov
Liberia	Oct-Mar	Apr-Sep
Malaysia	Oct-Dec	Apr-May
Mexico	Oct-Feb	Mar-Aug
Nigeria	Sep-Mar	Jun-Aug
Panama	Mar-Jun	Jul-Feb
Papua New Guinea	Apr-Jul	Oct-Dec
Sri Lanka	Nov-Feb	Mar-Oct
Togo	Oct-Mar	Apr-Sep
Trinidad	Dec-Mar	Apr-Nov
Venezuela		Mar-Sep

The table gives an idea of the main and the mid cocoa seasons in the major cocoa producing countries in the world. Source: Wood, G.A.R. and Lass, R. A. (1985). Cocoa. Longman, 4th Edition.

Appendix 6. Plastic Sheet Platforms and Aluminium Trenches

Appendix 7. Aluminium Trenches Extended 10 m to Move Water from the Treated Plots

Appendix 8. Shade Nets Covering the Cocoa Plants

Appendix 9. Heat Stress Experimental Set-Ups

Seedlings under Shade

Seedlings Acclimated to Full Sun

Full Sun + Heat

Full Sun – Heat (+ Mock Heater)

Shade + Heat

Shade – Heat (+ Mock Heater)