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Abstract

The study aimed to develop agronomic interventions for improving cacao establishment and identifying cacao varieties which respond positively to the interventions. An experiment was conducted to study the water relations of three contrasting cacao clones (T 79/501, PA 150 and SCA 6) while using P 30 [POS] as a control genotype under greenhouse conditions. Another experiment was conducted (using the same genotypes) to study plant response under different shade intensities. Results from these two supplementary greenhouse and nursery experiments provided background information that was used to explain observations made in two field experiments [genotype * (shade * manure)] and [mulch * (shade * genotype)]. In all the experiments that considered seasonal influences, photosynthetic rates were lowest in the dry season. In the greenhouse watering experiment involving young, container-grown cacao plants optimal photosynthetic activity was observed when watering was done every 5^{th} day with the soil's field capacity of water. Results from the shade experiments showed that heavy shade is associated with low photosynthetic capacity and slower growth in cacao plants although greater dynamic photoinhibition may occur under higher light intensities. Under field conditions manure application and the use of mulches was associated with greater plant survival rates suggesting that in the present circumstance of unrelenting soil degradation the use of organic manure and mulching (or irrigation) at the establishment phase may have to be considered. Whereas no clear differences were observed in the photosynthetic efficiencies of the cacao genotypes, differences in growth rate were observed. The implications of the findings to field establishment of cacao have been discussed in this thesis.

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Plate 1 Young pod-bearing clonal cacao plant

A PHYSIOLOGICAL STUDY ON THE FIELD ESTABLISHMENT OF CACAO CLONES THROUGH THE IMPROVEMENT OF AGRO-ECOLOGICAL CONDITIONS

CHAPTER 1

Introduction and literature review

1.1 Introduction

Average cacao yields across the world are low. Hadley and Pearson (1996) estimated worldwide yields to be about 271-297 kg/ha. However, the potential yield of cacao is estimated to be much higher as higher yields have been produced under improved planting conditions. For example, under experimental conditions in Ghana, West Africa, Osei Bonsu et al. (2002) reported yields of over 2000 $kgha^{-1}$. Under optimum experimental conditions in Malaysia where rainfall distribution is good and soil nutrients are not a limiting factor Lim and Chan (1986) reported annual yields of between 3000 and 4000 $kgha^{-1}$

In West Africa, where the bulk of the world's cacao is produced, much of the difference between actual and potential yield is due to low tree population densities employed in many areas. A report by Ghana's Ministry of Finance (Ghana, 1999) for example, shows that of the 1.2 million hectares used for cacao production in Ghana, about 30% contributes very little to the yield of cacao because of lower tree density per hectare than recommended. Attempts to rehabilitate or replant such farms are fraught with difficult challenges of field establishment as the growing environment (both edaphic and aerial) is becoming increasingly hostile. Working on rehabilitation of cacao in Nigeria, Ayanlaja (1983) reported that the difficulty of seedling establishment, especially in soils carrying old and unproductive cacao trees, was the

major problem in cacao rehabilitation and blamed this as the main cause of a decline in the nation's cacao production. He compared some edaphic conditions (pH , % N and % organic carbon) of old cacao soils with counterpart forest soils and found the former to have values significantly lower than values considered to be adequate for cacao. It appears that a similar situation can be found in the rest of the cacao producing countries of West Africa. For example, Adu-Ampomah et al. (2006) reported that cacao production in Ghana is faced with many constraints which cause poor establishment of new plantations and low yields .

The deterioration of the aerial environment has mainly been blamed on deforestation. An investigation into the causes and consequences of deforestation in Cameroon between 1970 and 2000 by Gbetnkom (2005) cited erosion of agricultural lands, drying up of water bodies and modification of local and regional climatic conditions as some of the consequences. For example, Toxopeus (1968) noted that the traditional Amelonado cacao variety could no longer be established on a large scale in Western Nigeria. He cited the severe dry season and the progressive deforestation in the cacao belt as the two main factors which negatively affected young cacao establishment. He explained further that in earlier decades cacao plants survived better because the climate in the small farms was "dominated by the surrounding high forest which provided side shade during most of the day and a cool, humid, almost windless atmosphere with relatively small daily temperature fluctuations even during the dry season" (Toxopeus 1968). In spite of these known negative impacts on the environment and on crop production the rate of deforestation is still high. The rate of deforestation in Ghana, for example, was estimated by Ghana's Ministry of Environment and Science (2002) to be 1.5% per annum.

Since the earliest years of planting cacao as a crop it has tended to be planted following economic timber extraction from the primary forest. According to Lass (1999) this trend started

in Central America, moving to South America, to West Africa and now, to South-East Asia. Only in a few isolated cases was planting of cacao ever the prime reason for the clearing of primary forest.

As the growing environment for cacao deteriorates with more soils being degraded and with dwindling natural forests, the need for conducting studies that are aimed at identifying cacao varieties which are well adapted to the relevant environmental factors of establishment, growth and yield becomes greater.

Nearly 40 years ago, Toxopeus (1968) called for a study aimed at developing methods to enhance cacao establishment. However, unlike many temperate and a few tropical crops such as the oil palm, previous research to improve management practices for cacao has been based on pragmatic and empirical approaches without reference to a clear understanding of the physiological principles underlying what is observed. Moreover, most of the recent physiological work has been aimed at learning about yield development in young-bearing and mature cacao. As a complement to such recent work this thesis considers the factors that affect establishment with a view to gaining a better physiological understanding of how changes in the microenvironment and agronomic conditions affect establishment.

The study is expected to suggest answers to the following questions:

I. What simple agronomic practices could be designed to satisfy the water requirement of cacao during the establishment phase?
II. What is the best way to optimise cacao plant response to watering?
III. What is the shade requirement of young cacao plants?
IV. Can the application of organic soil amendments improve cacao establishment?
V. Is there any genetic variation among available cacao germplasm in the response to water stress and incident light intensity and
VI. what agronomic practices will help improve the field establishment of cacao ?

The adopted strategy was to conduct two field experiments together with two supplemen-

tary experiments involving the same factors but conducted under nursery and greenhouse environments. Results obtained from the supplementary work were utilised in the interpretation of field observations.

1.2 Literature review

History

Although cacao originated in South America, it was in Central America that its cultivation began in about 1000 B. C. According to Mossu (1992) the Maya Indians were the first to cultivate the tree. The Aztec Indians of the high Mexican plateaux extended their empire to the cacao-growing regions. After their conquest of the Maya Indians the Aztec people levied taxes on the Mayas in the form of seeds which they called 'cacahoatl' from which the word 'cacao' was derived.

Lass (1999) reports that in 1520 when Cortes discovered Mexico City (then the capital of the Aztec people) and met their leader (called Montezuma), he found that cacao beans were used in the preparation of a luxury drink – chocolatl – made by roasting whole cacao beans, grinding them and mixing with maize meal, vanilla and chilli. Those cacao beans had not been grown by the Aztec people but by the Mayas who gave them as a tribute to Montezuma. As they were valuable and easy to count, cacao beans were also used as currency.

Between 1520 and the middle of the 17th century, the main cacao areas were all in, or around, present-day Mexico, extending as far as Honduras. Columbus encountered cacao from Honduras in 1502 and introduced it to the "Old World". He carried some to Spain and introduced cacao drinks to the Spanish court. The drink became popular among the aristocracy in Spain and later in Italy, Flanders, France and England. Early in the 1800s, consumption of cacao increased but only as a chocolate drink. It was high in fat as it was made from

the whole cacao bean. In 1828, Van Houten designed a press to remove some of the fat and opened up a vast range of new products – including chocolate as we know it today (Wood, 1985).

As it gained popularity in Europe, cacao cultivation of Criollo types of cacao spread to Jamaica, Martinique and Trinidad in the West Indies, to the Philippines and from there to Sulawesi and Java, and perhaps to India and Sri Lanka. Brazil and Ecuador were the first countries to grow Forastero cacao in the 18th century.

Trading activities existed between Brazil and West Africa in the 19th century and from Brazil cacao was introduced into Principe, off the West African coast in 1822. As an agro-climatic analogue to the area of the Upper Amazon region of South America (the centre of origin and of diversity of *Theobroma cacao*) the equatorial forest region of West Africa provided a favourable environment for the crop (See Appendices 1 and 2) . However, large scale cultivation in West Africa only started in 1874 in Nigeria and in 1879 in Ghana.

According to Are and Jones (1973) cacao beans had been planted in Ghana (then the Gold Coast) earlier, but the first plantings were unsuccessful. The earliest planting of seeds was by the Dutch missionaries in 1815. Since the seeds were planted in the dry coastal belt of Ghana, it is not surprising that the venture was unsuccessful. Better success seems to have attended the efforts of the Basel missionaries in 1857 when cacao was planted at the mission station at Aburi and was recorded as flourishing in 1868. But the credit for starting up commercial growing of cacao in Ghana is usually given to Tetteh Quashie, who had been employed as a labourer on an estate in Fernando Po, but who returned to his home-town, Mampong, in the Akwapim area of Ghana in 1879, bringing with him a pod of cacao. The seeds were sown and the seedlings flourished and became the ancestors of many cacao trees in Ghana.

Economic importance of cacao

Significant proportions of the export earnings of many developing countries in the humid tropics are obtained from cacao. For example, revenue from cacao represents over 40% of Ghana's total export earnings. The six largest world producers of cacao are The Ivory Coast, Ghana, Indonesia, Nigeria, Cameroon and Brazil (see Table 1). Guyton (2006) forecast that West Africa will continue to be the leader in world cacao production into the foreseeable future with roughly 70% of the supply. Asia and the Americas are expected to produce 16% and 14%, respectively.

North America and Europe consume nearly two thirds of all cacao production but demand from emerging economies is growing (ICCO, 2008). Demand for confectionery products containing chocolate is rising by 4 - 5% per annum in any ten year period during the past 50 years and according to the World Cacao Foundation (www.worldcacao foundation.org) the demand for chocolate with a higher cacao solids content (43% minimum) is increasing whilst more small chocolate-manufacturing companies are being set up.

The profitability of cacao cultivation is significantly influenced by the suitability of the conditions in which it is planted and the quality of the planting material. It can be a rewarding crop for the smaller farmer as it appears to be one that is better suited to small holder cultivation. According to Lass (1999) a large number of Malaysian estates which planted cacao as a result of the high prices offered for cacao in the late 1970s have not found it an easy crop to manage on a large scale.

The first Latin name given for the cacao tree was *Amygdalae pecuniariae*- meaning 'money almond' in recognition of its status as currency. The Swedish, Linnaeus, himself a regular consumer of cacao, called the genus *Theobroma* which means 'food of the gods' (Lass 1999). This was prompted by the native Mayas' belief that the cacao tree was of divine origin.

When the Spanish were introduced to cacao, its usage was confined to the nobility and many claims were made about the new drink, one of which was its being aphrodisiac.

Table 1 World cacao production from the 2003/04 – 2007/08 crop year

COUNTRY	2003/04	2004/05	2005/06	2006/07	2007/08
Africa					
Côte d'Ivoire	1,550	1,410	1,516	1,387	1,510
Ghana	605	560	660	555	680
Nigeria	174	206	214	175	190
Cameroon	160	190	172	180	190
Other Africa	38	41	44	45	45
Total Africa	3,520	3,415	3,721	3,386	3,772
Asia & Oceania					
Indonesia	460	470	575	525	600
Malaysia	25	26	27	28	29
Other Asia	63	73	79	82	85
Total Asia & Oceania	548	569	681	635	714
Latin America					
Brazil	163	171	162	126	154
Ecuador	117	114	113	115	120
Others	165	152	160	167	169
Total Americas	446	437	434	409	443
World					
TOTAL	3,520	3,415	3,721	3,386	3,772

Units '000 tonnes
Source: (ICCO, 2008).

Nutritional benefits of cocoa

Lass (1999) quotes Johnston (1880) who suggested that there was a need to pay attention, not only to the nutritious effects of various foods, but equally to those that affect the 'nervous energy'. Johnston (1880) went on to compare a cacao drink with tea and milk - as having the advantages of both – "the exhilarating properties of tea and the strengthening and ordinary body-supporting qualities of milk."

The trend for people to reduce calorie intake and consumption of saturated fats that are linked to health risk factors has made chocolate a target for attack. Chocolate has been accused of causing headaches and migraines, promoting heart diseases, being addictive, causing dental decay, being a potent allergen, causing outbreaks of acne, and leading diabetics to, unreasonably, abandon dietary commonsense. Now, however, as research is demonstrating that not all saturated fats are equally harmful, many of the popularly held negative beliefs about cacao and chocolate have been largely disproved.

Reports on the nutritional benefits of cacao and chocolate are extensive. Work of Kris-Etherton et al. (1993) demonstrated that stearic acid (the main fatty acid in cacao butter) elicits a neutral cholesterolemic response which is likely to cause a decrease in plasma triglycerides. Cacao is known to contain more natural minerals than any other food item. Mossu (1992) mentions cacao as a rich source of calcium, phosphorus, iron, and vitamins A and D. Cacao and chocolate have been used as a major source of dietary copper for North Americans (Lass 1999).

According to Waterhouse et al. (1996) antioxidants are present in large quantities in cacao and it has been shown that their presence can protect consumers from many potential problems such as low-density lipoprotein (LDL), oxidation and stress. It is becoming increasingly accepted that cacao powder and chocolate are rich sources of high quality polyphenol anti-

oxidants (flavonoids), which are similar to those found in fruits, vegetables and red wine, that may have positive health benefits (ICCO, 2008). Pimentel et al. (2010) reported that “49 g of D71 dark chocolate has the same quantity of flavonoids as that of 196 ml of Tannat wine, which is the daily wine intake recommended to produce health benefits in an adult of 70 kg body weight”. In recent years numerous workers in medical practice have published information that highlight the potential of the antioxidants found in cocoa to protect consumers from free oxygen radicals that are linked to heart disease, certain cancers and physical degeneration sicknesses associated with ageing (Fisher and Hollenberg, 2005).

For example, Fisher et al. (2003) and Fisher et al. (2004) report that flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans and that "Cocoa that is rich in flavanols reverses endothelial dysfunction of human aging”. Also Addai (2010) hypothesises "Natural cocoa as a diet-mediated anti-malarial prophylaxis and Bayard et al. (2010) report that flavanol-rich chocolate may boost blood flow in the brain and reduce the risk of dementia.

Hollenberg (2007) studied the benefits of cocoa drinking on the Kuna people (who drink up to 40 cups of cocoa a week) in Panama and found that “the risk of four of the five most common killer diseases: stroke, heart failure, cancer and diabetes, is reduced to less than 10%”. This author attributed many of the health benefits to ‘epicatechin’- a flavonoid that natural cocoa contains in large quantity. Suggesting that epicatechin be classified as a vitamin Hollenberg (2007) reported that the Health Benefits of epicatechin could outshine penicillin."

Flavanols including epicatechin are removed for commercial cocoas because they tend to have a bitter taste. However, independent reports such as the above are prompting manufacturers to develop ways of maintaining flavonoid levels during processing. Chiva (1999)

points out that cacao also has the ability to provide an "overall feeling of well-being, which in itself is beneficial to the consumer."

Cacao botany

According to Toxopeus (1985) *Theobroma cacao* L. is the only member of the genus *Theobroma* which is widely cultivated due to its economic value. Previously, the genus *Theobroma* was regarded as belonging to the family Sterculiaceae (Cautrecasas, 1964) but more recently it has been reclassified as belonging to the Malvaceae family (Alverson et al., 1999). Wood (1985) considers that it is more correct to describe the area of the headwaters of the Amazon as the primary centre of diversity only but other people hold that the area was both the centre of origin and the primary centre of diversity of *Theobroma cacao* (Motamayor et al., 2002, Lass, 1999).

Theobroma species are lower storey trees of the evergreen rainforests of Central and South America (Toxopeus 1985). Traditionally, certain morphological and genetic characteristics of pods and beans as well as geographical origins of a germplasm have been used as the basis of classification into categories. Two main genetic groups, "Criollo" and "Forastero", have been defined. A third group, "Trinitario", has been recognized and consists of "Criollo" * "Forastero" hybrids (Bartley, 2005) as previously proposed by Cheeseman (1944). According to Motamayor et al. (2008a) botanists described, in parallel, two subspecies: *cacao* and *sphaeorocarpum*, corresponding to "Criollo" and "Forastero, respectively.

Criollo (*Theobroma cacao* L. spp. Cacao Caut) has a relatively soft pod husk, sometimes red in colour and contains an average of 20-30 white, ivory or very pale purple beans (Are and Jones 1973; Mossu 1992). Although Criollo cacao is regarded as having a higher quality, production is small due to its low yields and susceptibility to diseases and mirid infestation. Cri-

ollos are often not vigorous and if they form jorquettes, they will have three fan branches with small leaves. Forastero (*Theobroma cacao* L. spp. Sphaerocarpum Cuat.) populations are more widely grown, more vigorous and higher yielding. The group contains cultivated, semi-wild and wild populations among which are Amelonados (which are native to Brazil and cultivated in West Africa, Costa Rica, Mexico and Guiana), and the Middle Amazon populations. They are described by Pound (1938, , 1943) as having unpigmented but longer and more corrugated pods. Generally, the pods are hard and green in colour with 30 or more, pale to deep purple seeds.

Trinitario is generally considered as a variable hybrid of Criollo and Forastero. In the view of Cheeseman (1944) the Trinitarios are the most complex of the groups, yet in some respects, the best known because they happened to have been readily accessible for research in the countries which have the longest records of scientific study such as Trinidad and Ghana.

There are a few types of *T.* cacao which look different from all the populations described above. Examples are populations known as Catongo in Brazil which have more than 35 white beans in a hard pod; and Djati Roenggo (DR) in Indonesia. This one has a green, soft pod with 35 white beans (Toxopeus 1985).

Based on their recent work which aimed at improving the understanding of the origin, classification, and population differentiation within the species Motamayor et al. (2008b) have proposed a new classification of the cacao germplasm that is likely to enhance the management of the crop. In that work 1241 accessions covering a large geographic sampling were genotyped with 106 microsatellite markers and 10 genetic clusters, as opposed to the two genetic groups traditionally recognized within *T. cacao*, were found by applying Bayesian statistics. The 10 genetic clusters were named according to the geographical location or traditional cultivar most represented in each particular cluster. They are: Marañon, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Purús, Nacional and Guiana.

Plant growth

The fruit of cacao, commonly called a pod, contains seeds embedded in a mass of white, pinkish or brownish, acid to sweet, aromatic pulp developed from the outer layers of the testa. The pod husk may be thick and woody or thin and easy to cut through, depending on the variety.

Germination starts with the rootlet growing out first after which the hypocotyl raises the closed cotyledons about 3 cm above soil surface. This first phase of development, according to Toxopeus (1985), is referred to as the 'soldier stage'.

A mature tree has a tap root 120-200 cm long with lateral feeder roots often found in the top 20 cm of the soil but which may extend to 40 – 50 cm where the humic layer is deep. The lateral roots extend far beyond the limit of the tree's canopy forming a complex mat (Toxopeus 1985). Toxopeus (1985) reports that the tap root grows from 1 cm in length after 1 week to 16–18 cm after 1 month and 25 cm after 3 months. Thereafter, it takes more than 2 years for the tap root to reach 50 cm.

There are no buds on the stem of the hypocotyl, a situation that is exploited in grafting, as successful budding below the point of attachment of the cotyledons will prevent shoots arising from the rootstock. The second phase of development starts when the cotyledons open to expose the plumule. This phase ends with the appearance and hardening of the first four leaves. Subsequent growth continues at about six weekly intervals with the appearance of flushes of 4-6 leaves. When the plant reaches between 70 cm and 2 meters, it experiences its third growth phase when vertical growth ceases and 5 branches grow out sideways from the apex of the stem. The point from which the branches grow is called the jorquette (See Fig 1). Thus growth is dimorphic as the main stem is orthotropic and the fan branches are plagiotropic. Subsequently, the main stem continues vertical growth with the growth of a

stem (usually referred to as a chupon) from below a jorquette. This ultimately forms a jorquette of its own at a height above older jorquette. This process may be repeated several times and by such means the canopy of the tree becomes higher.

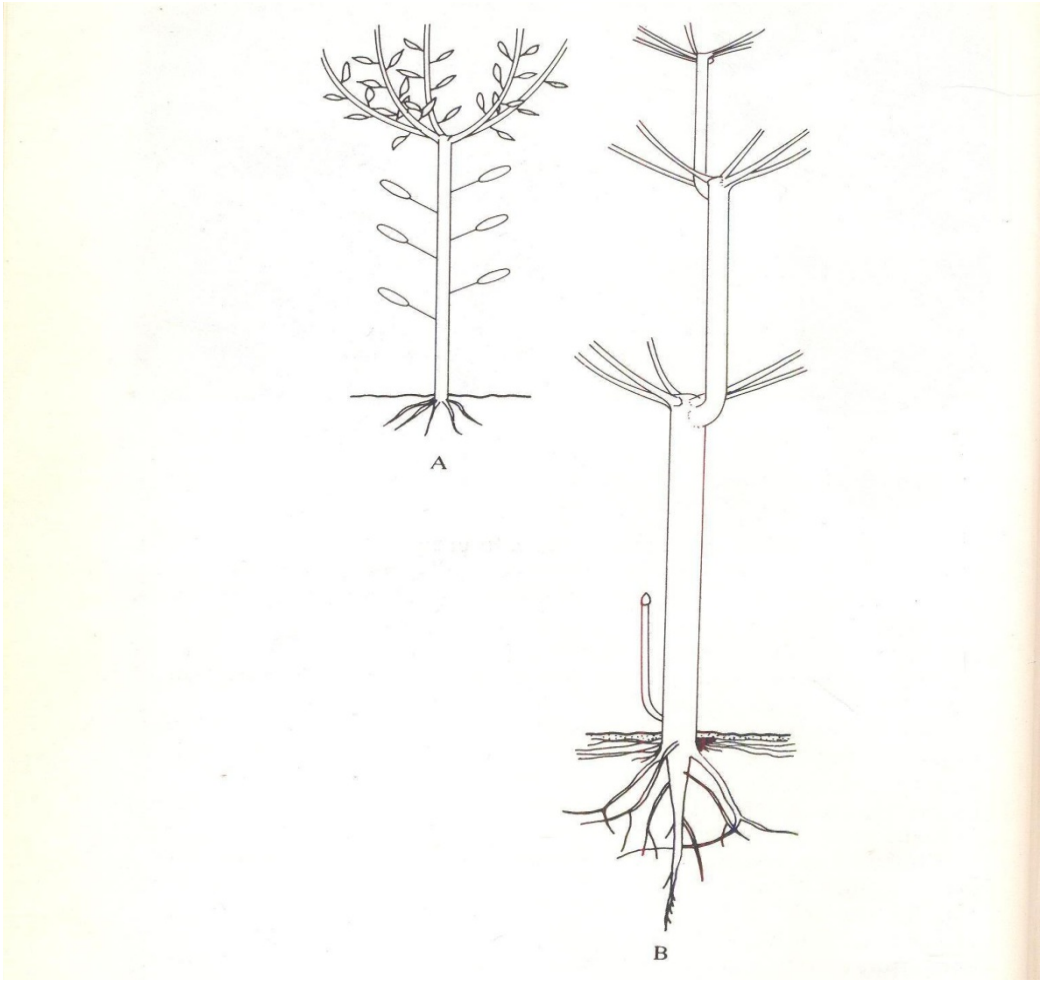

Figure 1 Stem growth of cacao tree. (a) Adult seedling with jorquette and five fan branches (b) older tree of three storeys with basal chupon.

Source: Toxopeus (1985) p16

The leaves also have dimorphic characters that are linked to the different types of stem on which they grow. On chupons, the leaves are arranged in a spiral phylotaxy and have long petioles which have a conspicuous pulvinus or swelling at the end. The leaves on the fan branches are arranged alternately down the branch, have shorter petioles and are compara-

tively asymmetrical. The leaves of cacao have stomata on the abaxial side only. Leaf production takes place as a series of flushes during which the terminal bud grows out, producing approximately three pairs of leaves. Heavy leaf flushing of individual trees may occur twice a year during the onset of the rainy seasons, each lasting about 8 weeks, but there is some leaf production at most times of the year. Initially leaves are soft but gradually harden following leaf expansion. After the leaves have expanded, the terminal bud remains apparently dormant for a variable length of time and then another flush of leaf growth occurs. According to Toxopeus (1985) the development of a new flush causes an increase in the need for plant nutrients which is partly met by the export of nutrients from the older leaves. Flushing in mature trees is stimulated by temperature and the alleviation of moisture stress and is mediated by changes in endogenous hormones as reported by Taylor and Hadley (1988). Exposed cacao trees flush more intensely than shaded ones. In addition, leaves that develop under shade are larger and greener than exposed leaves (Galyuon et al., 1996, Poorter et al., 2010).

Cacao reaches physiological maturity about 2 years after germination when the jorquette is formed and initial flowering begins (Toxopeus, 1985). The flowers are borne on the main trunk and branches and thus exhibit a cauliflorous flowering habit.

The inflorescence primordia are produced from old leaf axils in areas called flower cushions. Flowers take about one month from initiation to emergence of buds through the bark. Flower bud differentiation proceeds even during periods of water stress but flower bud growth only goes on when the plant is not under severe water stress. The flowers are small and have 5 sepals, 5 petals, 5 stamens, 5 staminoids and an ovary that is made up of 5 fused carpels. At bud maturity, the sepals split during the afternoon and opening continues during the night. Early the next morning, flowers are fully open and pollen grains are released (Billes

1941). In its natural setting flowers must be pollinated at this time to achieve pod set. Natural pollination is carried out by different kinds of small insects, including midges belonging to the family Ceratopogonidae. Species of the genus *Forcipomya* are the commonest pollinators (Billes, 1941). If pollination and fertilisation do not take place on that day the flower abscises.

Whereas a cacao tree can produce hundreds of flowers per year only 1 – 5 % of them are successfully pollinated to produce a pod. Thus in Trinidad a self-compatible tree was observed by Cope (1940) to have produced 4000 flowers a year and set 220 young fruits, which are referred to as cherelles. Only 8 of the fruits reached maturity, the loss of the majority being attributed to cherelle wilt.

Pod growth and cherelle wilt

After pollination, the pod takes about 75 days to reach maximum growth rate. Between 40 and 50 days after pollination, the zygote makes its first division to become an embryo. It takes between 5 and 6 months from pollination to pod ripening although there is considerable variation in the length of time depending on mean temperature and genotype (End, 1990). Alvim et al.(1972) showed that pod maturity was determined by temperature and the time taken to reach maturity can be predicted by thermal time using the following formula:

$N = 2500 / T – 9$, where N = number of days to maturity and T = the daily mean temperature (^{o} C)

Usually less than 5% of flowers are successfully pollinated and up to 80% of cherelles (young fruits) can be lost through cherelle wilt, a physiological mechanism that causes the fruit to cease growing, turn yellow, then blacken and shrivel, but do not abscise and instead remain mummified on the tree (McKelvie, 1956). A high proportion of wilting takes place within the

first 75 days of pod growth and does not completely stop before 95 days (End, 1990, McKelvie, 1956) after pollination. Thereafter, pods normally continue to mature without further cherelle wilt. Other cherelles may be lost to diseases such as black pod caused by the fungus *Phytophthora palmivora* or *Phytophtora megakarya* or to insect pests (Asare-Nyako and Amponsah, 1973).

The mature pod is botanically an indehiscent drupe which remains attached to the tree until harvested or long enough to decay away. Natural seed dispersal is done by monkeys, rats or squirrels which feed on the mucilaginous material inside the pod (Cheeseman 1944; Toxopeus 1985).

Senescence

Not much is known about the economic life of the tree but according to (Entwistle, 1972) it is commonly estimated to be from 40 - 60 years. It is not clear whether this broad limit represents the lifetime of the tree itself or a nutritionally depleted habitat especially when yields have been high but fertilizers have not been used. When the environment is favourable the economic life of the tree may be longer than 60 years if it is considered desirable to regenerate the trunk from a basal chupon (Lockwood, 1976, Entwistle, 1972).

1.2.1 Field establishment of cacao

Relationship between available plant resources and biomass production

Being autotrophs, plants obtain their energy from solar radiation which is used to fix other resources (CO_2, water and nutrients obtained from the soil) into carbohydrates. By accumulating carbohydrates in different forms the plant increases in size.

Blackman (1919) introduced the concept of relative growth rate (RGR) and presented an analysis showing the relation between resource capture and dry matter production. Shortly

thereafter many contributions to the subject laid emphasis on the importance of availability of plant resources to plant growth.

Today the relationships are better understood. For example, Hunt et al. (1990) that dry matter produced through photosynthesis can be expressed as a product of three terms:

I. The resource available per unit ground area
II. The amount of resource captured per unit of available resource, that is, efficiency of resource capture (or the rate of uptake of resource relative to the amount of resource available), and
III. The rate of dry matter production per unit of captured resource (efficiency of resource utilisation).

This suggested that the ability of plants to capture required resources and utilize them is as important to dry matter production as the availability of the resources. To capture resources effectively plants invest varying proportions of their assimilate into the root and shoot systems.

Relationship between leaf area and biomass production

The extent to which plants intercept radiation and absorb CO_2 depends to a large extent on the leaf area developed which, in turn, depends on the rate of leaf flushing. It has been shown that leaf flushing in many plant species is linearly related with thermal time for a given genotype and environment (Chapotin et al., 2006, Pirani et al., 2009, Ramirez et al., 2006). Besides the rate of leaf appearance, other factors which influence the rate of leaf area development include the production of branches, leaf size and disposition. Many workers have found a positive relationship between the developed leaf area and biomass production (Briggs et al., 1920, Gregory, 1926, Lambers, 1989, Whatley, 1992, Watson, 1947, Goudriaan and Monteith, 1990, Hunt and W., 1990)

Photosynthesis in cacao

Net photosynthesis is limited by the rate at which a leaf can assimilate CO_2 from the atmosphere and reduce it to carbohydrate and does not depend on gross photosynthesis alone. It is determined by gross photosynthesis, photorespiration and 'dark' respiration. These processes determine the efficiency with which intercepted photosynthetically active radiation (PAR) effects the conversion of CO_2 into plant dry matter. Thus, as explained by Fitter and Hay (2002), it is a balance between the process of taking up CO_2 (gross photosynthesis) and two processes that release CO_2 (photorespiration and 'dark' respiration).

A few workers have attempted to determine the efficiency with which the cacao plant uses light (as a resource) to produce dry matter. Raja Harun and Hardwick (1988) observed photosynthesis in cacao to have characteristics that are common in under-storey species; it has a low light saturation point, low rates of light saturated photosynthesis, and a low light compensation point.

Using leaves of a similar developmental stage Yapp (1992) obtained statistically different photosynthetic rates among mature clones planted on fertile soils in no-shade field plots in Malaysia. Although the magnitude and direction of the significant differences observed by Daymond (2000) were different at particular sampling times, he noted a number of definite trends in the cacao clones he worked on. For example, one of the clones, ICS-9 always had low photosynthetic rates whereas CP-519 had consistently high rates, again, suggesting genetic differences.

At the canopy level, differences in photosynthesis observed among genotypes are not caused by the efficiency of the photosynthetic apparatus alone. They may be caused by other plant factors such as plant architecture, leaf structure or the amount of photosynthetic material, such as the leaf area.

In this current work a few measurable leaf characteristics, including the specific leaf weight, leaf nitrogen, chlorophyll content and leaf water content were measured to estimate the influence that the different conditions (created as a result of the imposed treatments) had on them. Whether genetic differences exist in photosynthetic characters and biomass production among the germplasm to be studied were also determined, as far as possible. The interest was to add to the present understanding of the response of the photosynthetic mechanism of cacao genotypes to environmental factors which could provide a means by which their sensitivity to the environment would be assessed.

Transpiration in cacao

In order to allow CO_2 to diffuse through the stomata, plants need to keep the stomata open. However, as the stomatal pore also serves as the outlet for the efflux of water, plants lose water simultaneously with the uptake of CO_2. In times of water stress, in particular, plants may be said to be in a 'dilemma as they need to close their stomata to conserve as much internal water as possible but in doing so will hamper CO_2 uptake' (Sinclair et al., 1984). The ability of genotypes of a plant species to get a good balance is a desirable character that helps field establishment and growth.

In a situation such as in Ghana where a shortage of water (for example, during the 3-month 'harmattan' period between the middle of November and early March) restricts crop growth, it may be more valuable to consider the relation between crop growth and transpiration than the potential conversion efficiency of radiation. Hunt et al. (1990) provided a theoretical framework for using transpiration as a basis for calculating crop productivity. As reported by Squire (1990) there is evidence that for a particular crop and season, the relation between total dry weight and transpiration is linear with a slope , ϵ_w (g kg^{-1}), known as

the ‘dry matter : transpired water ratio’. Tanner and Sinclair (1983) observed that the value of ϵ_w does not seem to be significantly affected by plant nutrition. However, if the same crop is grown at different locations or in different seasons, the value of ϵ_w often varies from experiment to experiment. This suggests that plant water use is influenced by environmental conditions and it would be interesting to know if this feature could be exploited to aid field establishment in cacao.

1.1 Modulating environmental factors that affect photosynthesis, growth and establishment of cacao

Physiological processes which determine the growth of plants are influenced in various ways by environmental factors. Apart from sunlight, notable ones among the factors are soil and air temperature, soil fertility and moisture, and relative humidity. As observed by many workers these factors can, in turn, be modulated by agronomic practices such as the provision of shade, (Wood 1985), irrigation (de Almeida et al., 2002), the application of mulches (Oppong et al., 1998) and manures. Some of the effects of these factors are discussed below.

Shade

Murray (1953, , 1957) attributed the use of shade in cacao plantations to the early cultivators imitating environmental conditions of wild cacao trees in the forest of the Upper Amazon and Orinico River basin. Thus a primary question has been to find out whether shade is an intrinsic requirement of the cacao plant itself or whether it plays a secondary role such as maintaining appropriate soil conditions and insect population for the cacao plant. Recent publications seem to suggest that mature cacao requires moderate shade for sustainable cropping. Using simulated photosynthetic light response curves Ng (1982) provided guide-

lines as to how the potential yield of cacao could be predicted and showed how it can be affected by shade, canopy area and tree vigour. The outline indicated a big effect of shade on cacao productivity. A shade tree leaf area index of 0.5 to 1.0 gave as much as 25% to 50% reduction in cacao productivity. Unshaded cacao canopy photosynthesis (CAP) reached an asymptotic maximum at a leaf area index of 4.0, but under a shade tree (with leaf area index of 2.0) cacao canopy photosynthesis saturated at a leaf area index of 2.5. Similarly Zuidema et al. (2005) applied a physiological growth and production model for cacao (SUCROS-cacao) based on the SUCROS - family of physiological crop growth models. It calculates light interception, evapo-transpiration, biomass production and bean yield for cacao trees grown under shade trees. Simulations were carried out using climatic information of 30 locations in 10 cocoa-producing countries, three different soil types and varying shade levels. That study showed that more than 70% of the variation in simulated bean yield could be explained by a combination of annual radiation and rainfall during the two driest months. Concerning shade, moderate levels did not affect bean yield whereas more than 60% shade reduced yields. Such information indicates that yields of cacao are likely to be greater under no-shade situations.

In the view of Entwistle (1972), however, several adverse effects can offset the positive aspect of a no-shade situation; the most obvious being increases in insect pest attack. Higher weed growth is also observed under no-shade situations as are, greater nutritive demands of the cacao plants (Ahenkorah et al., 1974), as well as the production of a high percentage of small beans (Adu-Ampomah et al., 2006, Adu-Ampomah et al., 1998). Therefore, the ideal situation appears to be obtained through the provision of moderate shade since cacao has a low light saturation point (400 µmol $m^{-2}s^{-1}$) and a low maximum photosynthetic rate (4.0 µmol $m^{-2}s^{-1}$) at light saturation as observed by Hutcheon (1981). Raja Harun and Hardwick

(1987) reported that the photosynthetic rate of the crop decreases if the photosynthetic apparatus is exposed to light intensities exceeding 60% of full sunlight while prolonged exposure to high light intensities damages the photosynthetic apparatus of the leaves.

Beer et al. (1998) placed the major physiological benefits that cacao receives from shade trees in two groups both of which are associated with reduced plant stress:

I. Amelioration of climatic and site conditions through:
   (i) Reduction of air and soil temperature extremes
   (ii) Reduction of wind speeds
   (iii) Buffering of humidity and soil moisture retention
   (iv) Improvement or maintenance of soil fertility, and
II. Reduction in the quantity and changes in quality of transmitted light and hence avoidance of excessive vegetative growth.

According to Oppong et al. (1998) weed management in a newly established cacao plantation can take up to 70% of all costs during the first two to three years. In the view of Tomkins (2003) proper selection and management of shade tree species can reduce labour input and weeding costs considerably.

In spite of the benefits that can be obtained from the use of shade trees, a controversy rages on due to a number of physiological drawbacks including the observation made by Ahenkora et al. (1974) that shade trees compete with cacao for soil water. Despite this controversy, shade is invariably recommended for the establishment of cacao although very little quantitative data for shade in the establishment phase of cacao is available (Alvim, 1977, Evans and Murray, 1953, Wood, 1985). Obviously the need for shade is greater for young cacao plants because they have little self shading within their canopies. Without overhead shade almost all the leaves get exposed to high irradiance, resulting in high transpiration rates which according to Alvim (1977), is inimical to young cacao as they are very susceptible

to water stress. Young cacao needs shade of an appropriate density to produce the desired "shape and geometry". Too little light induces long internodes and few branches, while exposure to excessive light induces too much branching and short internodes which cause the production of bushy plants (Lee, 1978).

Although shade from other plant species is generally recommended for cacao, attention must be given to compatibility between the used plant species and cacao. The ideal shade tree must not be an alternate host species for the pests and diseases of cacao. A shade tree must give heavy enough and an even shade throughout the dry season. It must also be possible to thin the trees or remove them at a later stage without causing much damage to the cacao. *Gliricidia sepium* is a legume that is frequently used as shade. It establishes easily from planting long stems. Its light foliage falls in the dry season, but if the trees are lopped earlier the new stems which follow will retain their leaves. Other temporary shade plants include plantains (*Musa paradisiacal* Linn.), pawpaw (*Carica papaya* L), and cassava (Manihot *utilisima* Pohl.).

Mulching

Mulching cacao seedlings is beneficial during the first year of planting in the field, if applied before the onset of the first dry season. It is also useful to repeat its application during the second year and perhaps the third year.

Mulching has the potential of reducing seedling losses which usually occur during the first and second years. Jordan and Opoku (1966) observed that, if properly applied, it is possible to improve the soil moisture status by reducing soil moisture evaporation. Its greatest benefit may, therefore, be realised in the dry season and in marginal areas. It has been noted that mulching helps to create a porous, better aerated soil layer with an improved water holding

capacity making the soil a more suitable medium for the development of plant roots (Araya and Stroosnijder, 2010, dos Santos et al., 2010, Liu et al., 2010). In addition, organic mulches eventually break down to release variable but often significant amounts of plant nutrients usually including nitrogen, phosphate and potassium as well as trace elements. Unlike inorganic fertilizers, organic mulches release their nutrients slowly but constantly. Oppong et al. (1998) found weed control, protection of the soil against erosion, conservation of the crumb-soil layer as well as improved plant nutrition to be among the benefits of applying organic mulches in cacao plantations.

Despite the numerous benefits of mulching, the practice is not widely used in West Africa and only limited information on its effect on cacao establishment is available. An attempt was made in Ghana by Frimpong and Akuoko (1994) to study the combined effect of plantain pseudo-stems (used as a mulching material) and a water-absorbing polymer ('Grow Soak 400') on cacao seedling survival and growth under field conditions. Although the experiment did not run its full course first year results indicated a 19.7% greater seedling survival under the 'mulch-only' treatment than under the control treatment where there was no application of mulch or water-absorbing polymer. There is, therefore, a need to evaluate the application of mulches in cacao establishment but taking into consideration, the cost implications.

Manures

The capacity of plants to photosynthesize and translocate photosynthate is directly affected by the availability of mineral nutrients. Mineral nutrients play important roles in the synthesis, partitioning and utilization of assimilates. It is well known that mineral nutrient deficiencies reduce dry matter production. Partitioning of dry matter between plant organs (for ex-

ample, shoots and roots) is differentially influenced by mineral nutrient deficiencies. In a study on *Phaseolus sp*. Cakmak et al. (1994) observed that , under P deficiency, a greater proportion of dry matter was partitioned to roots, while under K and Mg deficiencies root growth was markedly decreased compared with shoot growth. These effects are likely to have impacts on the establishment of cacao in West Africa where the growing period is usually disrupted by a dry season.

Adams and Mckelvie (1955) indicated that the suitability of the soil for cacao cultivation depends on the soil type and soil phase. Soil type is largely determined by geology and topography, and soil phase, by the previous history of land use of the site. The history of land use is of particular importance to cacao establishment. Charter (1947) noted that tropical forests accumulate plant nutrients in the top few centimetres of soil. When such forests are cleared, the nutrients are released rapidly and they give the soil enough fertility for only a few years. The peasant farmer establishes cacao in cleared forest at this time to exploit the fertility. With appropriate vegetation cover in Ghana, Ahenkora et al. (1974) observed that cacao did not significantly respond to fertilizer until after two years of bearing. With the dwindling forest in most of West Africa, the need to completely replant cacao in the old cacao-growing areas has been recognised. In these areas, the soil type is suitable for cacao cultivation. There is also a pre-existing cacao infrastructure in the form of roads, warehouses and buying depots to support the cacao industry. However, re-establishing cacao on land that previously carried cacao has been difficult due, partly to poor soil phase, as observed by Adams (1962) and Ayanlaja (1983).

The soil fertility problem can be addressed through the use of organic and inorganic fertilizers. Afrifa et al. (2002) observed an increase in total yield over the control with the combined use of poultry manure at 1800 kg/ha and cacao pod husk ash (CPHA) at 135 kg/ha on

mature cacao in Ghana. It seems reasonable, therefore, to investigate the possibility of including organic manures in our effort to find ways to facilitate the re-establishment of cacao in lands with a long history of usage.

Water relations of cacao

Cacao is characteristically grown in areas of high annual rainfall (1500 – 3000 mm) and does not tolerate periods of prolonged drought (Bae et al., 2007, Bae et al., 2009). Compared with other crops such as coffee or kola, cacao is less efficient in the control of water loss because, as explained by Raja Harun and Hardwick (1988) stomata in the leaves of cacao are not controlled well enough under water stress and low relative humidity.

However, according to Balasimha and Daniel (1988) advantage could be taken of a few cacao genotypes which are capable of regulating their stomata more effectively and de Almeida et al. (2002) identified cacao clones which showed a degree of drought tolerance via osmotic adjustment. However, very little research effort has been made towards the improvement of drought tolerance in cacao (Balasimha and Rajagopal, 1988) or to develop agronomic interventions to ameliorate the effect of drought on cacao establishment.

Importance of biomass production to establishment of cacao

In West Africa the ability of young cacao plants to establish in the field is crucial to developing new plantings. This is because planting is best done in the major rainy season and gap filling due to failure to establish can effectively be done annually. Faster growth (or vigour) enhances field establishment as it leads to exponential increases in the ability to capture more resources from the environment.

Plant vigour is often measured by changes in above-ground structures (seedling stem diameter and seedling height) as well as time to jorquetting and branching intensity. In temperate

crops and a few tropical crops whose modes of partitioning have been investigated it has been noted that there can be large variations in plant vigour mostly because of variation in the proportions of photosynthate re-invested in the photosynthetic machinery. Thus fast growing plants direct most of their photosynthate successively into above-ground leaves and such species or varieties survive better (Dingkuhn, 1998, Tekalign and Hammes, 2005, Asch et al., 1999). In the case of cacao in West Africa where the growing period is disrupted, not long after transplanting, by a dry season (see Appendixes 1 and 2) the ability to capture resources and efficiently convert them into assimilates and translocate them into organs of establishment is essential. Since, in the tropics, soil water is more of a limiting factor than sunlight it may be reasonable to expect root growth to have a strong effect on the ability to establish in the field.

Assimilate partitioning in young cacao

The partitioning of photosynthate into the root rather than the shoot system has sometimes been considered a 'cost' to the plant (Gregory, 1994). It is regarded as a drain on assimilates since, as mentioned earlier, under field conditions crop growth is measured as increments in above-ground structures. However, in most crops roots are the organs adapted to water and mineral uptake and provide anchorage to the plant so that the shoot can expand without lodging. Also the idea of considering the root system as being too large in some species and therefore 'wasteful' is dispelled if the requirement to take up less mobile resources such as phosphorus is considered. According to Gregory (1994) the relative growth of root and shoot systems is an "integrated process" in which "homeostasis" is upheld as a result of both 'size and activity' of the two systems. Earlier, Brouwer (1962) suggested that plants achieve optimal growth in an environment if they are able to achieve a 'functional balance' between

the relative increases in root and shoot biomass. Sharing a similar opinion Evans (1972) noted that since one or more of required plant resources may be inadequate in supply in a particular environment, plants need strategies that ensure a balanced uptake of resources to minimise the limitation to growth that will otherwise be caused by the resource(s) in short supply. This, according to Evans (1972), usually requires more allocation of assimilate to the structures that capture the most limiting resource(s). Slightly modifying his own view later Brouwer (1983) explained that 'functional equilibriums' cannot exist for long periods of time due to uncontrollable 'disturbances' in existing relationships. For that reason this author further suggested the idea of a 'functional control' (for example, 'nutritional control') as a better description of what is observed. However, the fundamental concept of photosynthate being partitioned to roots and shoots in "inverse proportion to the rates at which they capture resources" still remains (Li et al., 2006).

Küppers et al. (1988) refer to this adaptation as a "compensating response" in plants in order to sustain total carbon uptake. This response has been demonstrated in radish (*Raphanus sativas* L.) by Mooney et al. (1984) who showed that when, for some reason, the photosynthetic capacity per unit leaf area declines the plant partitions more dry matter into leaf material and thus partly compensates for the shortfall in assimilate production. Compensation responses are also observed in forest tree species as noted by some workers (Küppers et al., 1996, Paliwal et al., 1994, Kueppers, 1984, Riddoch et al., 1991) who explain that photosynthetic capacity declines from pioneer to successional species although later species are stronger competitors and replace the early ones.

Very little information has been published about the relationship between root and shoot growth in cacao. From a greenhouse experiment Taylor and Hadley (1988) showed that cacao seedlings have alternating phases of root and shoot growth. Muños and Beer (2001)

found in cacao under field conditions, that the growth of fine roots was positively correlated with the frequency of rainfall and that there was an internal competition between leaf flushing and fine root renewal. It would be interesting to find out if, like the frequency of rainfall, light intensity had any effect on root growth relative to shoot growth in cacao.

Based on the idea of compensation responses that are under the influence of the plant, a representation (Figure 2) of factors which determine a plant's carbon supply may be adapted for cacao seedlings [from Küppers et al.(1988)]. It depicts a compensation effect that takes into account the four important processes involving plant resources three of which were mentioned earlier:

I. The resource available per unit ground area
II. The amount of resource captured per unit of available resource (that is, the efficiency of resource capture)
III. The rate of dry matter production per unit of captured resource (efficiency of resource utilisation) and
IV. The amounts of dry matter partitioned into the root or shoot over a period of growth.

It will be interesting to pick up differences in biomass allocation as an indicator of genetic variation between the cacao clones being tested under this study.

Aims and objectives

It is already known that the establishment of cacao is (as with all plants) influenced by sunlight, soil moisture and soil fertility – environmental factors the levels of which were optimal for cacao in West Africa. For example rainfall, relative humidity, aerial temperature and sunlight were all ameliorated in small cacao farms by the surrounding forests. With the changing environment, primarily as a result of deforestation, and possibly global warming, there is an increasing need for improved establishment methods and cacao varieties which can tolerate suboptimal environments. Unlike many temperate and a few tropical crops such as the oil

palm previous research to improve management practices of cacao has been based on practical approaches rather than on unambiguous comprehension of pertinent physiological principles. Moreover, most recent physiological work has focused on understanding yield development in cacao. Together with such recent work, this study considers the factors that affect establishment with a view to gaining a better physiological understanding of how changes in the microenvironment and agronomic condition affect establishment and how they may be improved.

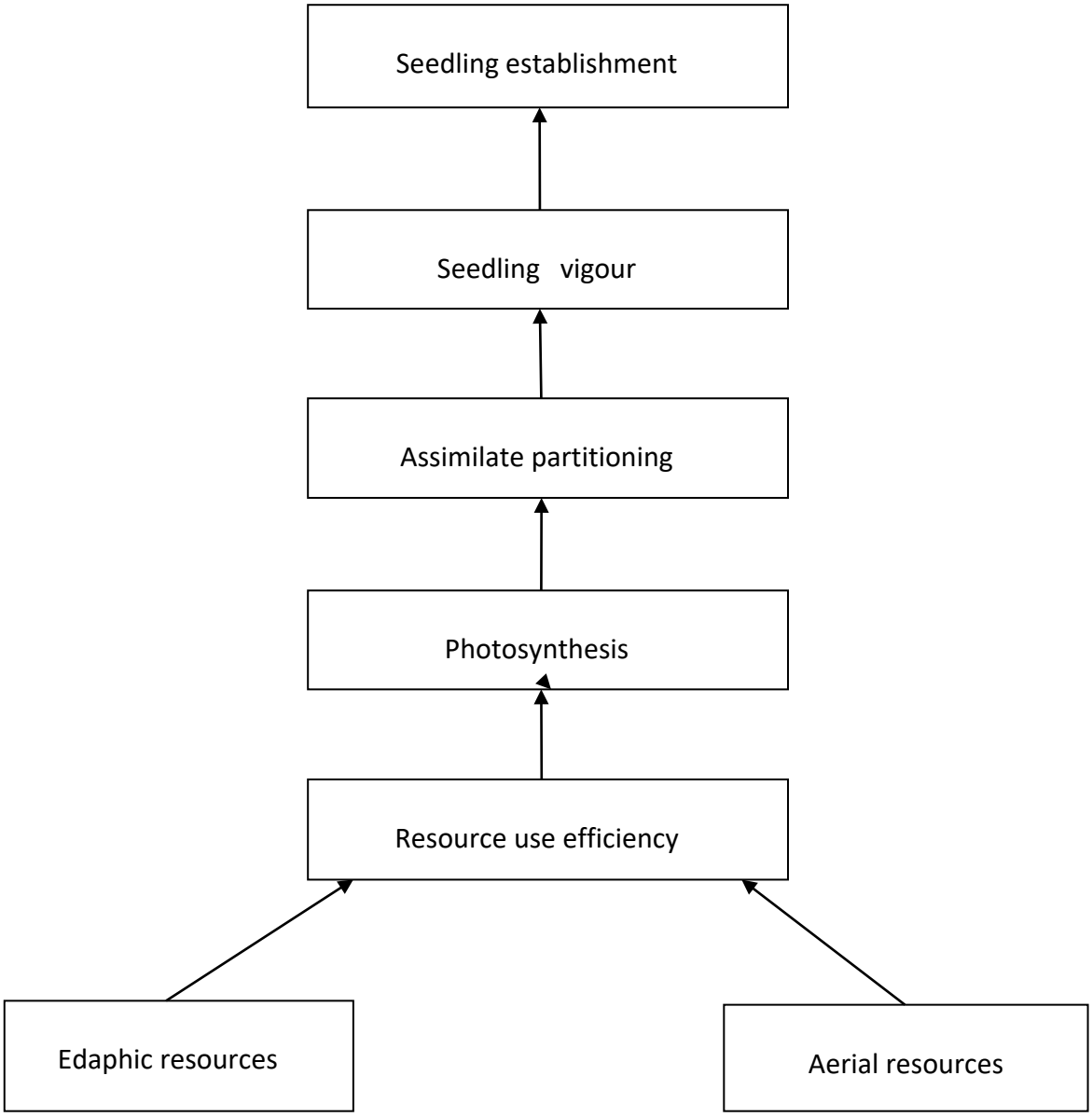

Figure 2 Schematic diagram showing factors affecting establishment ability (Adapted from Küppers et al. 1988)

CHAPTER 2
General materials and methods:

2.1 Plant material

Three cacao clones T 79/501, PA 150, SCA 6, representing, respectively, a vigorous, an intermediate, and a low vigour genotype, and P 30 [POS] were propagated by side grafting using a common seedling rootstock (T 60/887 x Amelonado) between July and November 2006 and used in all the experiments. Care was taken to insert the scion below the point of attachment of the cotyledons of the rootstock to prevent chupons growing from below the graft union. The rootstock had been raised in 25 cm x 32.5 cm polythene bags containing topsoil.

SCA 6 (or Scavina 6) was among the collections made by Pound in 1938 (Pound, 1943). It produces relatively small leaves with pink/red flush colour and has a dry bean productivity of about 1076 kg ha^{-1} and a pod index of 52.3 (pods per kg dried beans) (Sounigo et al., 1993). T 79/501 [POS] was selected in Ghana by Posnette and is grown commercially in many countries. Its flush colour is green and produces pods that are similar in shape to Amelonado. It has a productivity of between 1093 kg/ha and 4708 kg/ha. PA 150 (or Parinari 150) is grown in many countries. Reports on its flush colour vary from country to country (Sounigo et al., 1993). Its dry bean yield is about 1456 kg/hectare and has a pod index of 38.5. P 30 [POS] is a derivative of Amelonado and is grown only in Ghana and Togo. Amelonado cacao has been a traditionally cultivated genotype in Ghana and most of the early work in West Africa involved this cultivar. With SCA 6 and T 79/501 included the growth rate of P 30 [POS]was expected to be average in this experiment.

According to Bartley (1998) there may be some confusion between the P 30 [POS] selection

made by Posnette in Ghana (used in this study) and Pound 30 [POU] which was collected in Peru by Pound. The immature fruit is green and has a shape similar to Amelonado

2.2 Meteorological data

A weather station was located approximately 300 metres from the greenhouse and nearby vegetative propagation shade house where the two supplementary experiments were carried out. Records of greenhouse temperature were collected daily in the greenhouse itself using a screened maximum – minimum thermometer.

2.3 Photosynthesis measurements

An infra red gas analyzer (LC pro+, ADC Bioscience, UK) was used to measure stomatal conductance and the rates of photosynthesis and transpiration on single attached leaves approximately once every month in all the experiments with or without an artificial light source depending on the experiment. To reduce differences caused by diurnal fluctuations in photosynthesis, measurements were taken between 9:00 am and 11:00 am. For each replication three out of five plants (in the case of the greenhouse and nursery experiments) and three out of six plants (in the case of the field experiments) were tagged and the youngest expanded and hardened leaf of a tagged plant was selected for gas exchange measurement.

2.3.1 Measurement of leaf chlorophyll fluorescence:

Leaf Chlorophyll Fluorescence was measured by means of “Fluorpen” Leaf Chlorophyll Fluorescence Meter (FP100; Photon Systems Instruments, Brno, the Czech Republic). Data were collected between the mid morning and early afternoon (11:00 am and 12:15 pm) on leaves selected for photosynthetic measurements. Leaves were dark adapted for 30 minutes (using

strips of aluminium foil) before measurements were taken.

2.3.2 Leaf traits

2.3.2.1 *Determination of specific leaf weight (SLW)*

A cork borer (No. 5: diameter = 9.43mm) was used to punch 20 leaf discs from leaves that had previously been used for photosynthesis measurements. They were dried in a ventilated oven at 80 °C for 48 hours, cooled in a set of desiccators and their weights (W) measured. The Specific Leaf Weight (SLW) was calculated according to equation (1):

$SLW = [\{W * 1000\} / \{20\pi * (4.715)^2\}]$ mg/cm²..... (1)

2.3.3 Determination of leaf water content:

Three leaves of approximately uniform size and physiological age were selected from plants that had been tagged for other physiological data. The leaves were cleaned and, avoiding the midrib and lateral veins, a cork borer was used to obtain leaf discs of equal area. A set of ten leaf discs (from all three leaves) of a selected plant were weighed rapidly and then floated in 20 ml of distilled water in a Petri-dish which was then covered and left to stand for 24 hours to allow the discs to become 'saturated' with water. The discs were then removed and quickly surface- dried between layers of filter paper, pressed by a 1 kg metallic weight for one minute (to dry off externally held water) and then weighed. The discs were then dried in a hot-air oven at 80 °C for 24 hours. After cooling the dry leaf discs in a set of desiccators their dry weights were measured. The weight at 'saturation' was considered as showing the weight when the leaf discs were fully turgid and was used as an index to determine the relative water content at the time of destructive harvesting.

The relative leaf water content was calculated according to equation 2:

Leaf Water Content (%) = [(fresh weight – dry weight) / (saturated fresh weight – dry weight)] * 100%..... (2)

2.3.4 Leaf Chlorophyll content determination:

Leaf chlorophyll concentration was measured by means of an automatic- calibrated and temperature - compensated Leaf Chlorophyll Meter (CL-01 chlorophyll content meter, Hansatech, UK) with a capability range of 0 – 2000 chlorophyll units. For each replication three out of five plants in the case of the greenhouse and nursery experiments and three out of six plants in the case of the field experiments were tagged and the leaves that had previously been used for photosynthesis measurements were selected for leaf chlorophyll content determination.

2.3.5 Determination of leaf nitrogen:

Leaf samples (0.5 g) of approximately uniform size and physiological age that had, previously, been used for photosynthesis measurement were dried overnight at 80˚C. With assistance from the Soil Science Division of the Cocoa Research Institute of Ghana samples from different treatments and replications were ground separately using a coffee grinder and then, using copper as a catalyst, were digested for two hours with 12 ml of nitrogen-free concentrated H_2SO_4 to digest the leaf tissue.

The digestate was transferred into a distillation tube and distilled by the Kjeldahl method for 3 minutes using an automated Kjeldahl Unit according to the methods of Stuart (Stuart, 1936) using methyl red as an indicator. 120 ml of the distillate was collected with which a back titration was done to determine the end point. % Nitrogen (N) was calculated as:

(%N) = [Titre value * N (= 0.02) * 1.401) / 0.5] %..... (3)

Where,

Normality = 0.02N; weight of sample = 0.5g; Constant = 1.401

2.3.6 Determination of leaf total phosphorus (P)

Samples of approximately 1 g of leaves that had previously been used for photosynthesis measurement were weighed and placed in digestion tubes. 15 ml of Nitric acid (HNO_3) was added to each sample and then digested for 30 minutes at 150°C in a digester. 10 ml of per-chloric acid ($HCLO_4$) was then added to each digest. Each digest was re-digested at 200 °C for one hour. The digested sample was allowed to cool down, filtered into 250 ml volumetric flasks, topped up with distilled water to the 250 ml mark and shaken to mix well. Total phosphorus (P) in each sample solution was then determined via the ascorbic acid method (Twine and Williams, 1971) using spectrophotometry.

2.3.7 Determination of leaf K

1.0 g of oven-dried leaves of each of two selected plants per treatment which had been finely ground in a mortar was weighed into digestion tubes. 15 ml of nitric acid (HNO_3) was added to each sample and digested for 30 minutes at 150 ^{o}C in a digester in a fume cupboard. 10 ml of 1 molar perchloric acid ($HClO_4$) was then added to each of the digested samples and re-digested for another one hour at 200 ^{o} C. The digests were allowed to cool in a fume cupboard and were then filtered through Whatman No. 42 filter papers into 250 ml volumetric flasks. The filtrates were topped up with distilled water to the 250 ml mark and the flask was shaken by hand to mix well. The sample filtrates in the 250 ml volumetric flasks were used to determine the leaf potassium content on an atomic absorption spectrometer (de Almeida et al., 2009).

2.4 Determination of water holding parameters of topsoil used for the [genotype x (quantity of water x frequency of watering)] greenhouse and the [shade * genotype] nursery experiments

2.4.1 Field capacity of topsoil:

Five samples of the topsoil were watered until they were completely saturated and then allowed to drain freely until the water ceased to drip out. At that stage the water that remained in the soil was assumed to be at field capacity. Measurement of the field capacity was carried out by first weighing, then oven-drying the moist soil at 100 ^{o} C until a constant weight was obtained and then by subtraction of weights.

2.4.2 ii) Wilting coefficient of topsoil:

The point at which the force with which the soil holds its water is equal in magnitude to the suction force exerted by the roots of the cacao seedlings was determined by potting 5 seedlings (girth = 1.2 cm; height = 47 cm) into 5 plastic containers of equal volume that had no drains and that contained 14 kg loosely-packed soil at field capacity. The surface of the soil was sealed with a layer of wax in order to prevent evaporation. When the seedlings had extracted as much water as they could and had wilted, the residual moisture content of the soil was determined as before (Section 2.4.1). The values obtained from the two pilot experiments were used as baseline information for the clone x (quantity of water x frequency of watering) experiment (described in section 3.1).

Data analysis

Results were analyzed (using Genstat) by analyses of variance, and significance among mean values was determined by least significant difference (LSD) values where P = 0.05.

2.5 General materials and methods for the field experiments

2.5.1 Study sites

Two field experiments, [(1) genotype * { shade * manure} and (2) mulch * {shade * genotype} experiments] were conducted at the Cocoa Research Institute of Ghana, located in the cacao growing region of Ghana, West Africa (latitude 6 º 13' N, Longitude 0 º 22' W).

2.5.1.1 *Selection of sites*

A 1.6 - and a 2.4 - hectare site were selected for the two field experiments, respectively. Both sites carried moribund cacao trees, *Gliricidia sepium* and forest trees used, in previous trials, as shade for the cacao (Plate 2). Land preparation (carried out between December 2006 and May 2007) involved stumping out the old cacao and felling the other trees (Plate 3). Although both sites fell under the ideal soil type classification (based on geology and topography) the soil phase which depends on the previous history of land use, was rated as 'low' for cacao establishment.

Plate 2 Vegetative cover of the site for the genotype * (shade * manure) trial

Plate 3 Clear-felled site for the genotype * (shade * manure) trial

2.6 Meteorological data

A weather station was located within 1.5 km from the field trials. In addition, 'Tiny- tag' data loggers (Gemini Data Loggers, Chichester, UK) placed inside Stevenson screens were used to measure temperature and relative humidity on days when physiological data were measured.

2.6.1 Soil sampling and chemical analysis

In each of the field experiments four spots were randomly selected per block for soil sampling. The soil samples were taken after scraping away the surface litter and then using a hand trowel the top soil was collected from the surface to a depth of 15cm and kept in a plastic container. Thereafter, a sample was taken from 15-30 cm deep from the same hole and kept separate in a different container. Collection of soil samples from the two depths was repeated for the blocks. Samples in each of the buckets were, homogenized and 100 g of each sampled through quartering and kept in a black polythene bag and labelled. The 100 g samples were air-dried, crushed and screened (to remove coarse materials), passed through a 2 mm sieve and stored in polythene bags at room temperature for laboratory analysis (which was done on my behalf by the Soil Science Division of the Cocoa Research Institute of Ghana) as described below.

2.6.1.1 *Methods of chemical analysis*

Soil pH

10 g of the air-dried and sieved soil was weighed into a 100 ml beaker. 25 ml of distilled water was added to obtain a soil: water suspension ratio of 1: 2.5 W/V. The suspension was allowed to stand for 30 minutes and then stirred occasionally with a glass rod. The pH was

then measured with a Jenway 3020 pH meter.

Soil organic carbon and organic matter

The organic carbon in the soil was determined by the Walkley method (Walkley, 1947) The conversion of % organic carbon to % organic matter was done with the van Bemmelem's empirical factor 1.724 (Read and Ridgell, 1922).

Total soil nitrogen

2.5 g of air-dried soil was weighed into a digestion tube and digested with 12 ml concentrated sulphuric acid for about two hours, using a Foss Digester 2006. When digestion was complete the digest was allowed to cool. 70 ml of de-ionized water was carefully added to the tube, and distillation was then carried out in a Tecator Kjeltec distiller (Model Kjeltec 2100).

20ml of Boric acid solution was added to a conical flask and placed into the distillation unit. The platform was raised so that the distillate outlet was submerged in the receiver solution. The digestion tube was also placed in the distillation unit and the safety door was closed. 20 ml of a 40% sodium hydroxide (NaOH) solution was dispensed into the tube. The steam valve was opened and the digested material was distilled for three minutes. The receiver solution (boric acid solution) in the distillate flask turned green, indicating the presence of ammonia. A 'blank' was also determined in the same manner. The distillate was titrated with 0.02N sulphuric acid using two drops of 0.1 g methyl blue dissolved in 50 ml Ethanol. The nitrogen content of the soil was determined using the formula:

$$\% \text{ Nitrogen} = [(a - b)/s \times M \times 14 \times Mc \times (V/t)] \quad (4)$$

where

1. a = volume of H_2S0_4 required for sample titration
2. b = volume of H_2S0_4 required for blank solution
3. s = weight of air-dried soil sample in grams
4. M = molarity of H_2S0_4
5. 14 = atomic weight of Nitrogen
6. V = total volume of digest
7. t = volume of aliquot taken for distillation and
8. Mc = moisture correction factor.

Soil phosphorus

Available phosphorus was extracted from the soil with 0.002N sulphuric acid. This was followed by the ascorbic acid method for developing the blue colour for measurement of the phosphorus. 5 g of air-dried soil was weighed into a shaking bottle. 100 ml of 0.002N H_2S0_4 was added and shaken on a shaking machine (Model K5 501 digital) for two hours. It was then filtered with Whatman No.42 filter paper. 5ml aliquot was used for the phosphorus determination with ascorbic acid as a reductant.

In the ascorbic acid method, 1.056 g of ascorbic acid was dissolved in 200 ml of a reagent obtained by dissolving 12 g of ammonium molybdate in 250ml of distilled water, and 0.2908g of antimony potassium tartrate separately dissolved in 100ml of distilled water. These solutions were added to 1 litre of 5N H_2S04, mixed thoroughly and made up to 2 litres with distilled water.

For the colour development, 10 ml aliquot was pipetted into 25ml volumetric flask. Distilled water was added to make it up to about 20ml. 4ml of the ascorbic acid reagent was added and made to volume with distilled water. The blue colour developed was then read on Cecil Spectrometer (Model Aquarius) at a wavelength of 882 nm.

2.7 Field planting of cacao and shade plants

Field planting of cacao clones in both trials was carried out in June (2007) which is within the ideal period for transplanting cacao in Ghana.

2.7.1 Imposition of temporary shade treatments:

After lining and pegging the fields, three temporary shade regimes were created using plantains (*Musa sapientum*) and *Gliricidia sepium.* They were:

1) **The 'control' shade regime**:

   1.5 m tall *Gliricidia sepium* stakes and plantain suckers planted in April 2007 for the mulch * (shade * clone) experiment and in May 2007 for the clone * (shade * manure) experiment at 3m x 3m each as shown in the sample spot plan (Fig 3). Plates 4 and 7 show the shade situation four and ten months after planting the plantain suckers, respectively.

Cacao (x) + *Gliricidia* (G) + Plantain (P)														
x	p	x	p	x	p	x	p	x	p	x	p	x	p
	G		G		G		G		G		G		G
x	p	x	p	x	p	x	p	x	p	x	p	x	p
	G		G		G		G		G		G		G
x	p	x	p	x	p	x	p	x	p	x	p	x	p
	G		G		G		G		G		G		G
x	p	x	p	x	p	x	p	x	p	x	p	x	p
	G		G		G		G		G		G		G

Figure 3 Sample spot plan for the 'control' shade treatment

2) **The 'triangular *Gliricidia*' shade regime**:

Consisted of the 'control' shade plus three stands of 0.5 m tall *Gliricidia sepium* stakes planted at 0.6m away from the cacao in a triangle to enclose the cacao as shown in the sample spot plan (Fig 4, not drawn to scale). The added *Gliricidia sepium* stakes were planted later, in August 2007. Plates 5 and 8 show the shade situation soon after planting the *Gliricidia* stakes and seven months thereafter, respectively.

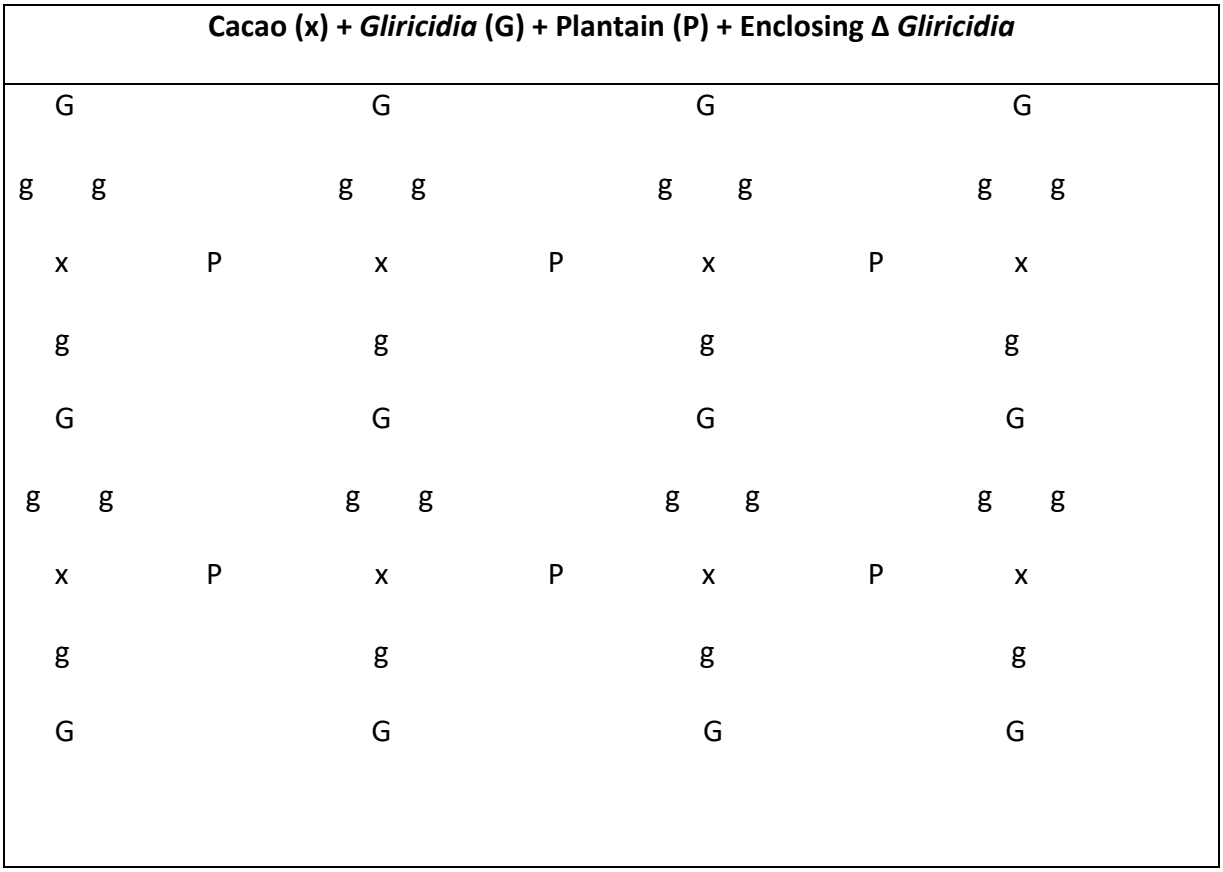

Figure 4 Sample spot plan for the "triangular *Gliricidia*" shade regime

3) **'Close plantain' shade regime**:

The same in all respects as the control shade except that the plantain suckers were planted 0.6 m away from the cacao plants (instead of 1.5 m) and, in relation to the position of the cacao, towards the west (Fig 5). Plates 6 and 9 show the shade situation four and ten months after planting the plantain suckers, respectively.

Plate 10 shows the general shade situation 12 months after planting the plantain suckers and the taller *Gliricidia* stakes.

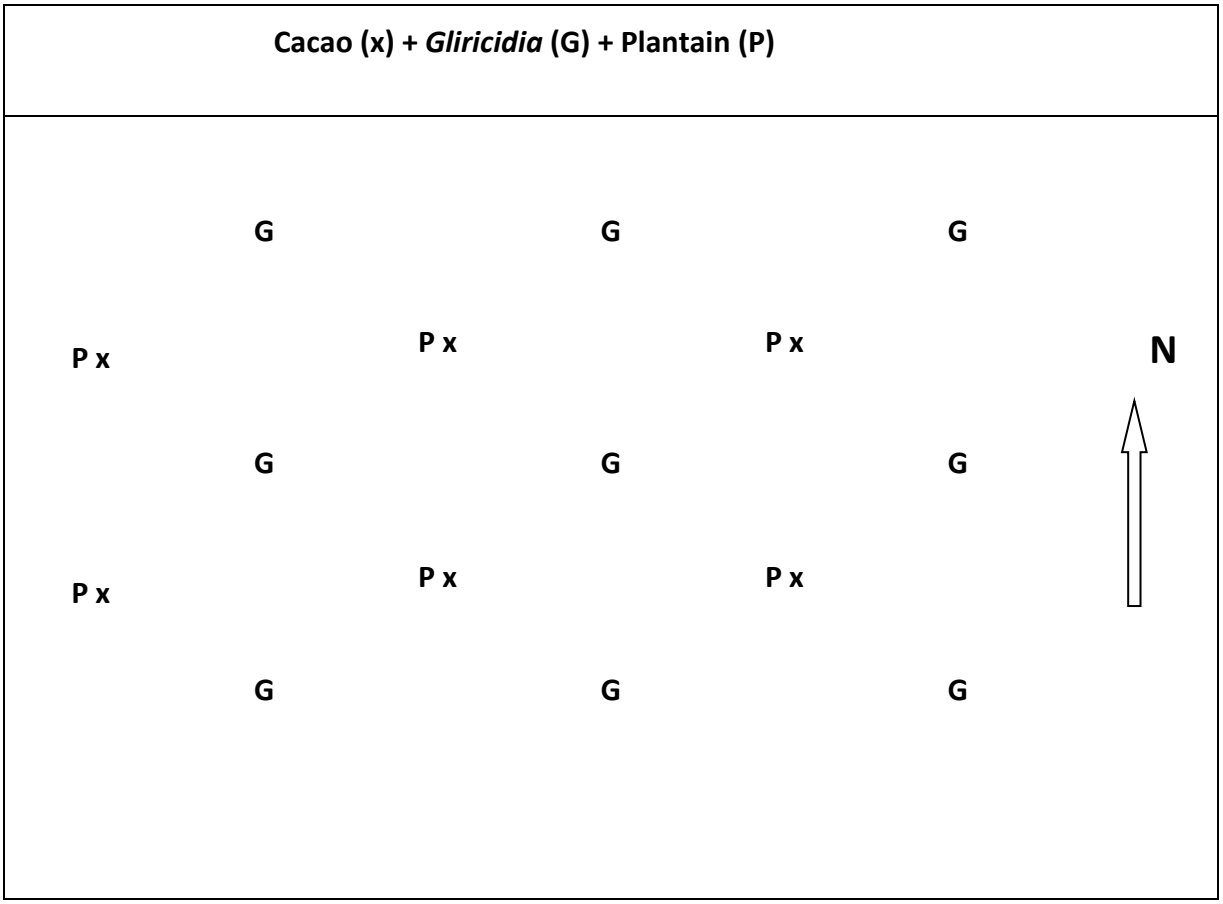

Figure 5 Sample spot plan for the 'close plantain' shade regime

2.8 Attained shade levels

Shade levels were measured twice in each of the three seasons by means of a 'SunScan' System SS1 (Manufacturers: Delta-t, UK). Measurements were taken around midday with the light sensor held below the canopy of the shade plant and just above the young cacao plants. With reference to the position of the test plants [in the mulch * (shade * clone) experiment], the shade provided by the 'close plantain' shade regime (the heaviest shade regime) ranged from 57% to 63% shade (860 µmol $m^{-2}s^{-1}$ light - 740 µmol $m^{-2}s^{-1}$ incident light) after eight months of planting the plantains. The 'triangular *Gliricidia*' treatment (the medium shade regime) attained 54.0% to 57.5% (920 µmol $m^{-2}s^{-1}$ incident light- 850 µmol $m^{-2}s^{-1}$ incident light) and the 'control' shade treatment (the 'light' shade regime) attained 50% to 54% shade

(1000 µmol $m^{-2}s^{-1}$ incident light- 920 µmol $m^{-2}s^{-1}$ incident light). In the clone * (shade * manure) experiment the close plantain shade treatment (the heaviest shade regime) attained 58-61% shade (840 µmol $m^{-2}s^{-1}$ incident light - 780 µmol $m^{-2}s^{-1}$ incident light), the 'triangular *Gliricidia*' shade treatment (the medium shade regime) attained 52-57% shade (960 µmol $m^{-2}s^{-1}$ incident light - 1140 µmol $m^{-2}s^{-1}$ incident light), and the 'control' shade treatment attained 50 - 52.5% shade (1000 µmol $m^{-2}s^{-1}$ incident light – 950 µmol $m^{-2}s^{-1}$ incident light) eight months after planting the plantains. In both experiments the lower levels of shade for each shade treatment were obtained in the dry season.

Plate 4 The control shade treatment 4 months after planting the plantains

Plate 5 The triangular *Gliricidia* stakes at planting

Plate 6 The close plantain shade treatment 4 months after planting the plantains

Plate 7 The control shade treatment 10 months after planting the plantains

Plate 8 The "triangular *Gliricidia*" shade treatment 7 months after planting the stakes

Plate 9 The close plantain shade treatment 10 months after planting the plantains

Plate 10 The general shade situation 12 months after planting the plantains

CHAPTER 3

Photosynthesis, survival and growth of cacao genotypes grown under different watering regimes in a greenhouse

3.1 Introduction

Water stress is known to cause negative effects on the growth and development of plants (Kaiser, 1987). However, unlike temperate crops, information on the water relations of tropical crops is inadequate and narrowly focused. Earlier work on the water relations of cacao, for example, focused on the effect of soil water on leaf flushing and cacao yield (Alvim, 1977, Alvim and Alvim, 1978, de Almeida et al., 1987). Atanda (1972) found rainfall distribution to be more important to cacao crop yield than its magnitude.

Because changes in environmental conditions are making it more difficult to achieve successful cacao establishment in the field, more recent work has been directed toward improving our understanding of plant response to water stress in young cacao plants. For example, Balasimha and Daniel (1988) showed that genotypes of *T. cacao* that effectively regulate their stomata are capable of reducing transpirational water loss and survive better under water stress. However, Raja Harun and Hardwick (1988) reported that leaves of cacao do not show high stomatal resistance under water stress and low relative humidity.

Although some information about the water relations of cacao is available, it is far from complete particularly for young cacao. As very little work has been carried out on genotypic variation in water stress response, this experiment was conducted to determine the sensitivity of the four cacao clones (SCA 6, T 79/501, P 30 [POS] and PA 150) to water stress and to increase our understanding of the basic physiological mechanisms of drought tolerance in cacao. As both the quantity and distribution of precipitation are known to be important en-

vironmental factors that affect cacao production under field conditions, artificial water stress conditions were created by varying both the quantity of water supplied and the frequency of watering, and the relative importance of these factors on photosynthetic activity of young cacao were studied. Any observations that could be applicable to the aim of finding ways to improve methods of raising seedlings under nursery conditions as well as to improve field establishment were noted.

3.2 Materials and methods:

The experiment was set out as a split-plot experiment designed to examine the supply of pot-grown cacao plants with different quantities of water in three different treatments:

(a) 100% 'field capacity' (169g of water/ kg soil),
(b) 50% 'field capacity' (84.5g of water/ kg soil), and
(c) 25% 'field capacity' (42.2g of water/ kg soil).

Changes that occurred in soil water content with time were erratic (due to constant fluctuation of relative humidity and temperature and the fact that the rate of plant water uptake changes with plant growth). Owing to logistic constraints (such as the lack of suitable equipment to measure soil water of potted plants) and the desire to keep the growing medium and plant root disturbance at the minimum, the same volumes of water that brought the soil to the respective moisture levels at the initial stage were applied to appropriate subplots each time watering was done.

These respective quantities of water were combined with three 'frequency of watering' treatments:

(a) Watering every other day (3rd day) (b) Watering every fifth day (5th day) and (c) Watering every 9th day (9th day).

The experiment was set up on 16 benches each measuring 2.9 m * 1.8 m in a greenhouse compartment (15 m * 10 m) at the Cocoa Research Institute, Tafo, Ghana under approximately 36.4 ^{0}C daytime maximum and 22.5 ^{0}C night-time minimum temperatures. CO_2 concentration was ambient.

Experimental design and layout:

The layout is shown in Figure 6. Five hundred and forty grafted cacao plants (135 for each of the four clones) of the same age (6 months) and approximately the same size (girth = 0.85 cm; height = 36 cm) were potted into bigger (32 cm x 37.5 cm) polythene bags. Taking into consideration the fact that cacao is intolerant to prolonged waterlogged situations (Sena Gomes and Kozlowski, 1986), the growing bags were provided with a 3.5 cm diameter drainage hole at the bottom. Each bag contained 12 kg of the top soil of known water relations (see sections 2.4.1 and 2.4.2).

There were three replications each comprising thirty-six (36) clone x (quantity of water x frequency of watering) plots laid out in a split-plot design with clones being arranged in the split plots. The clones therefore constituted the main plot factor and each sub plot contained 5 grafted plants (Plate 11).

Gas exchange measurement

Starting from April 2007 the infra red gas analyzer was used to measure stomatal conductance and the rates of transpiration and photosynthesis once every month sampling experimental plants as described in section 2.3.

Leaf traits

In March 2008, specific leaf weight, relative water content, Leaf chlorophyll concentration and leaf nitrogen content were measured once on a leaf on each of the three plants which were used for photosynthetic data collection in each treatment (see section 2.3.2 for details)

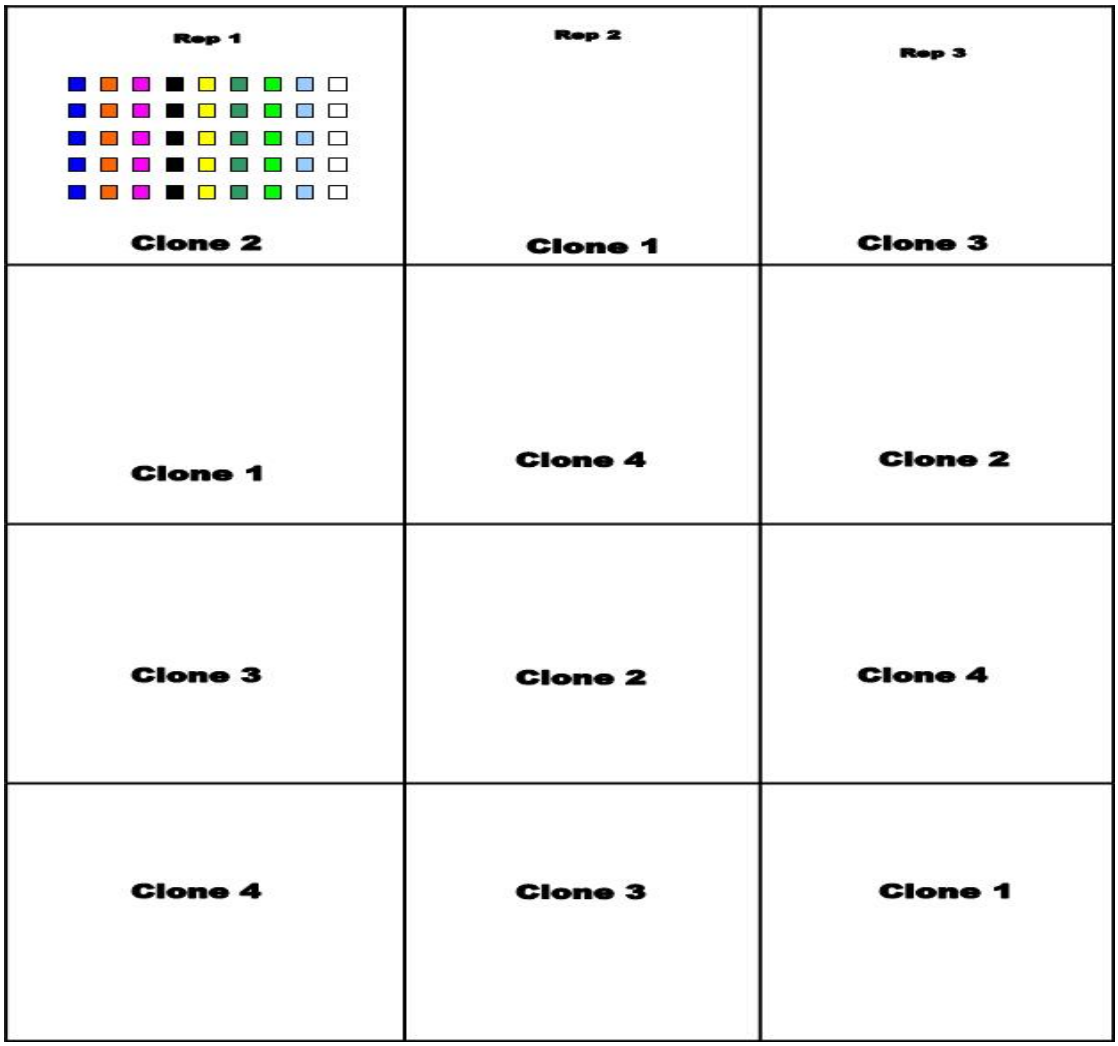

Figure 6 Layout of the clone * (quantity of water supplied * frequency of watering) experiment.

The columns of differently coloured square blocks (as shown in the first box of Rep 1) represented the different watering treatment combinations.

Plate 11 Setup of experiment on greenhouse benches

3.3 Results

3.3.1 Soil moisture levels

Table 2 shows the mean soil moisture levels for the various “quantity of water x frequency of watering” treatments a day before re-watering.

Table 2 Percent soil moisture before re-watering

Watering treatment	Full 'field capacity'	50% 'field capacity'	25% 'field capacity'
3rd Day	88.04	62.22	34.62
5th Day	46.70	36.80	27.91
9th Day	31.76	29.90	24.50

Stomatal conductance

A significant (p = 0.04) quantity * frequency interaction term was observed between watering treatments in the stomatal conductance of the cacao plants. The highest stomatal conductance rates (mean = 0.1181 mol $m^{-2}s^{-1}$) were attained by plants which were watered with full 'field capacity' every 5th day followed by those which were watered with 50% 'field capacity' every 3rd day. The stomatal conductance rates of cacao plants which were watered with the lowest quantity of water over time (25% 'field capacity' every 9th day) were the lowest (mean = 0.0306 mol $m^{-2}s^{-1}$) and were the main cause of the significant interaction. Plants which were watered with the largest amount of water over time (full 'field capacity' every 3rd day) had slightly lower stomatal conductance rates (mean = 0.1097 mol $m^{-2}s^{-1}$) than plants which were watered with the full 'field capacity' of water every 5th day and those which were watered with 0.5 'field capacity' every 3rd day (mean = 0.1118 mol $m^{-2}s^{-1}$).

Significant ($p = 0.033$) differences in stomatal conductance were observed among the cacao genotypes. T 79/501, P 30 [POS], SCA 6 and PA 150 had stomatal conductance rates of 0.0784, 0.0737, 0.0711, and 0.0710 mol $m^{-2}s^{-1}$, respectively (Fig 7).

The clone * quantity and clone * frequency interaction terms were not significant. There were also no significant 2nd order interactions.

Transpiration

In parallel to the observations made on stomatal conductance a significantly ($p = 0.035$) different quantity * frequency interaction term was observed in the transpiration rates of cacao plants. The highest transpiration rate (2.28 mmol $m^{-2}s^{-1}$) was observed under the 50% 'field capacity' treatment when watering was done every 3rd day. The lowest rate (0.361 mmol $m^{-2}s^{-1}$) was, however, obtained when watering was done with the smallest quantity every 9th day.

There were significant ($p < 0.001$) differences among the mean transpiration rates of the cacao genotypes (Fig 8). PA 150 showed a mean transpiration rate of 1.511 mmol $m^{-2}s^{-1}$ which was 9.5 %, 20.8% and 28.1% higher than the mean rates at which P 30 [POS], T 79/501 and SCA 6 transpired, respectively. The clone * quantity and the clone * frequency interaction components were not significantly different.

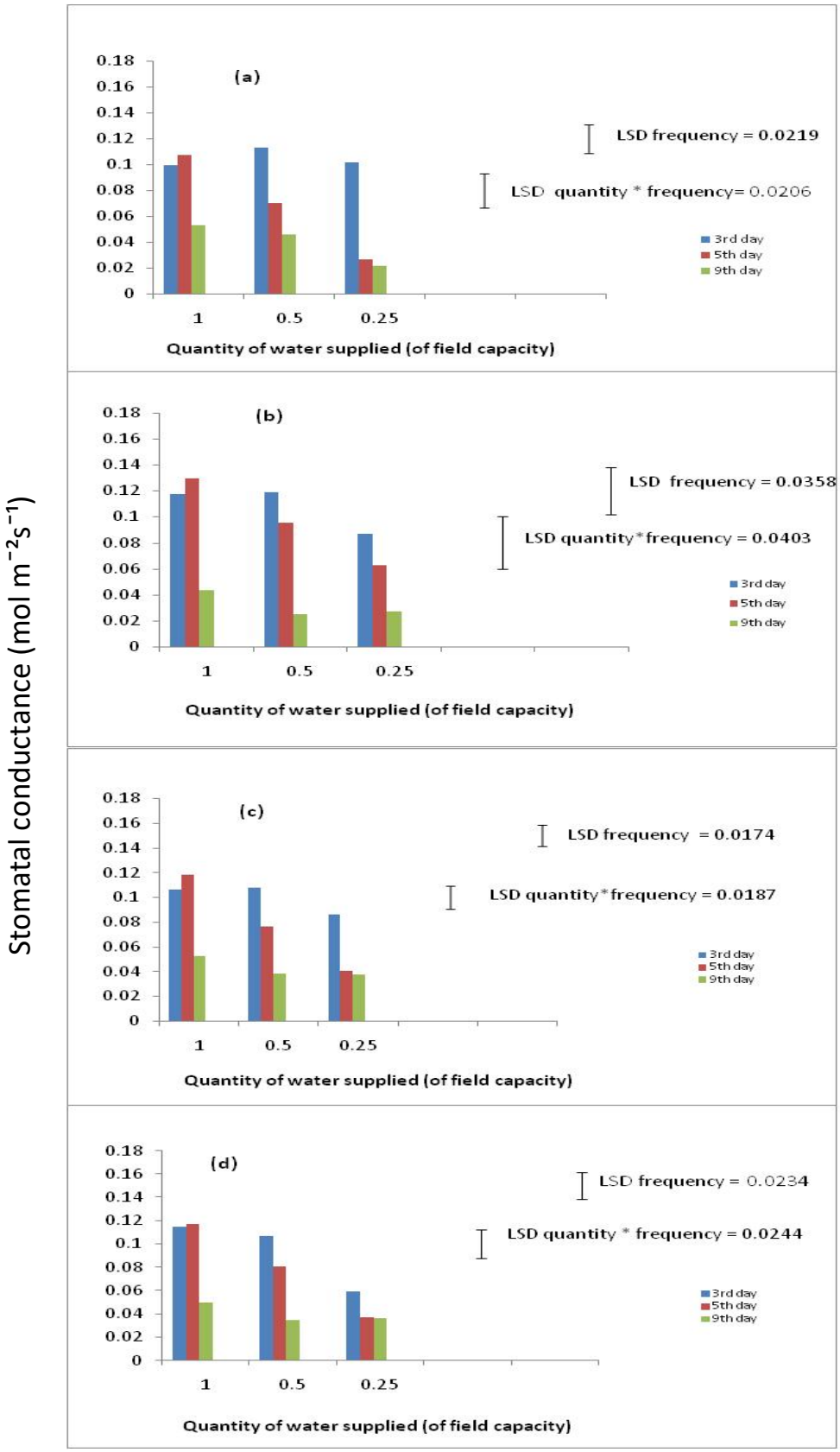

Figure 7 Stomatal conductance of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean of five measurements on 9 plants per treatment.

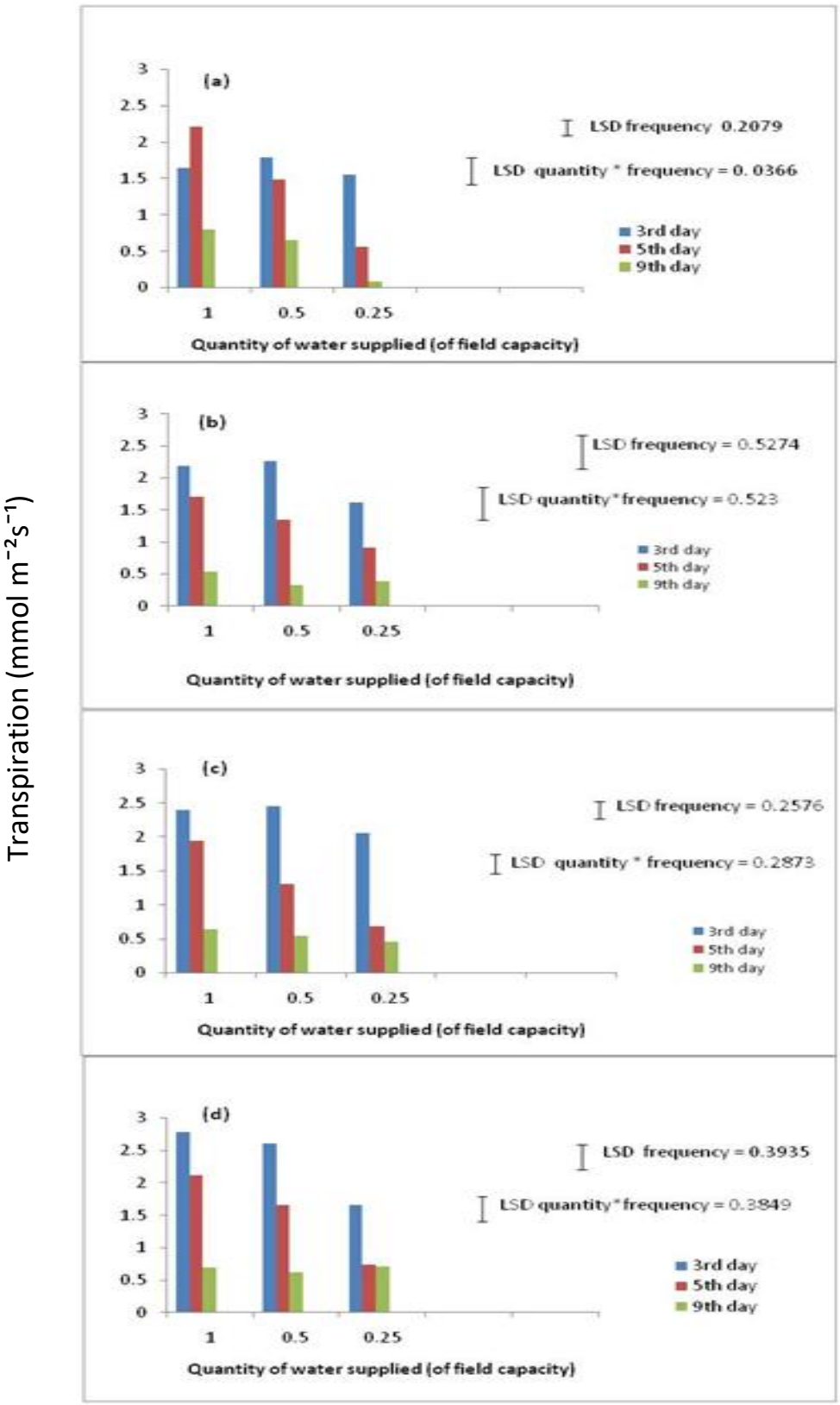

Figure 8 Transpiration of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean of five measurements on 9 plants per treatment.

Photosynthesis

A significant (p = 0.001) quantity * frequency interaction term was recorded in the rates of photosynthesis of the cacao plant. Similar to the observation in stomatal conductance and transpiration, plants supplied with the smallest quantity of water over time (watering with 25% 'field capacity' every 9th day) had the least photosynthetic rates (1.611 µmol $m^{-2}s^{-1}$). The highest mean photosynthetic rate (3.944 µmol $m^{-2}s^{-1}$) was recorded in plants supplied with the soil's 'field capacity' of water every 5th day. Their mean rate was 10.62% higher than that of plants which were watered with the highest volume of water over time (with full 'field capacity' every 3rd day) (Fig 9).

There were no significant differences in photosynthetic rates between clones. The clone * quantity and the clone * frequency interaction components were not significant and there were no significant 2nd order interactions. For most of the clones a high photosynthetic rate was maintained at a high watering frequency (Fig 9).The frequency of watering appeared to have had a higher impact on photosynthesis than the quantities of water that were applied at each irrigation (Fig 10).

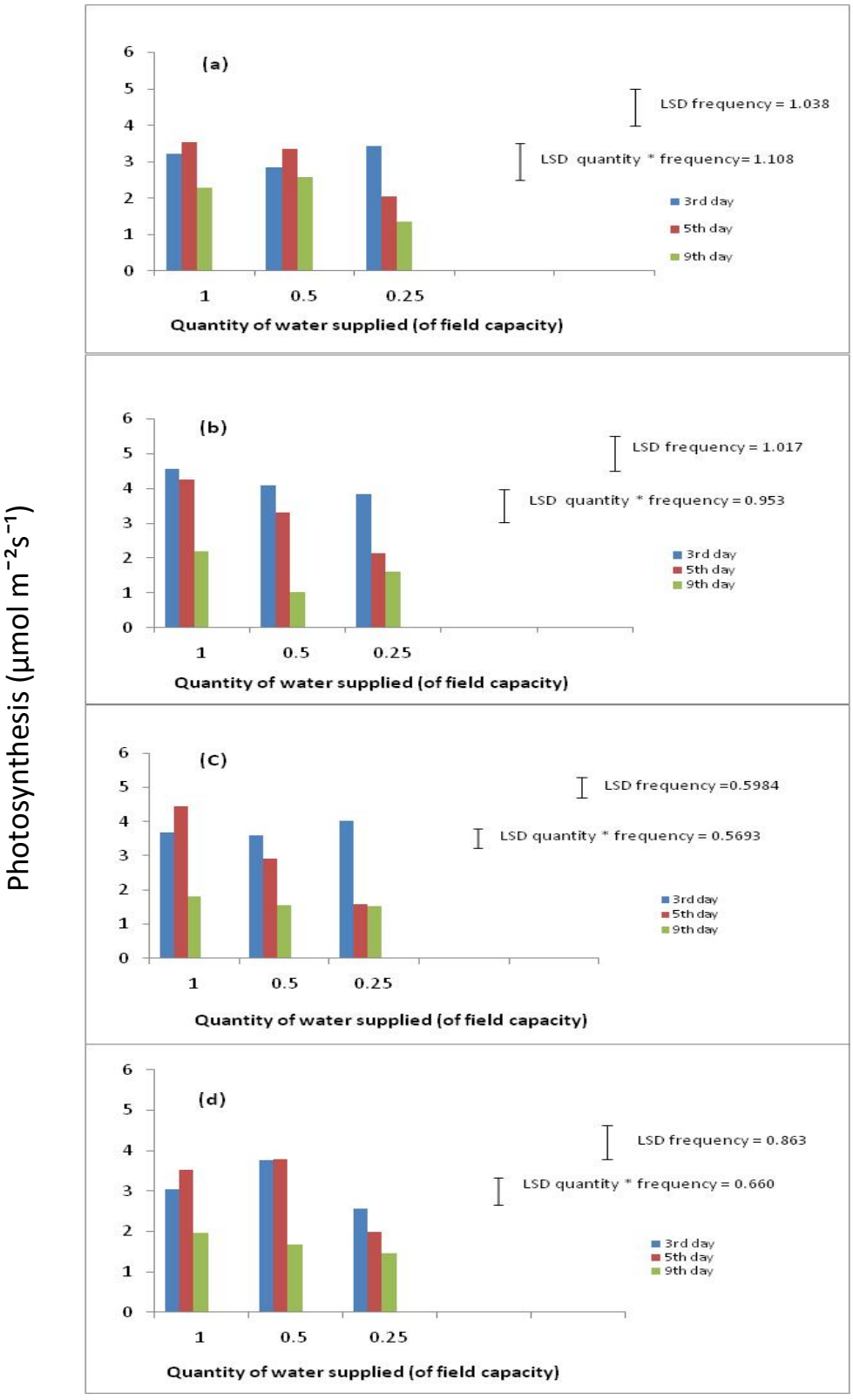

Figure 9 Photosynthesis of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and PA 150 under different watering treatments.

Each bar represents the mean of five measurements on 9 plants per treatment.

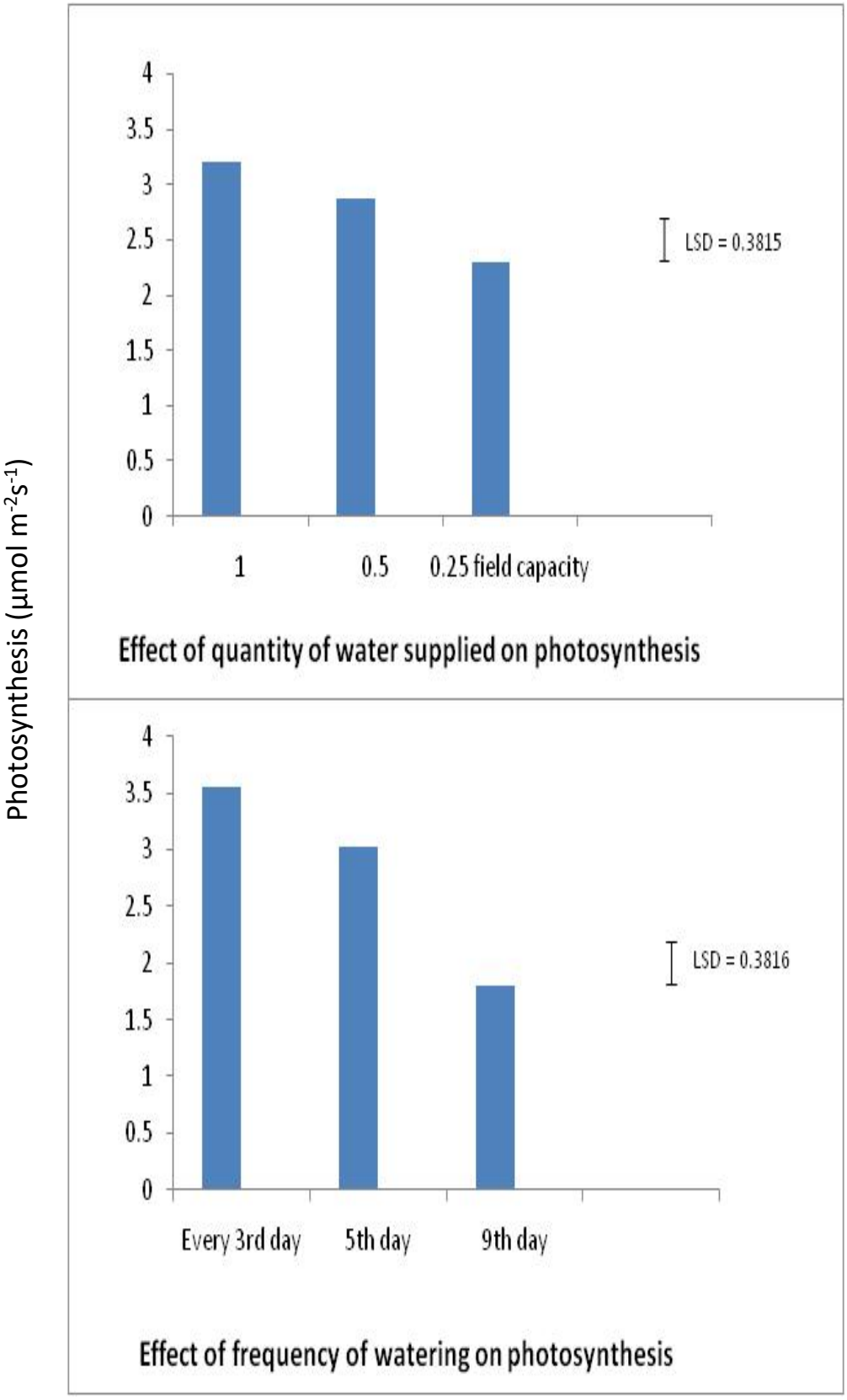

Figure 10 Photosynthesis of cacao under different quantity of water supplied and frequency of watering.

Each bar represents the mean of five measurements on 108 plants per watering regime.

Water use efficiency

There were significant ($p = 0.029$) differences among the clones in their water use efficiencies over the entire period (Fig 11). Their water use efficiencies ranged from 2.36 [µmol (CO_2) $mmol^{-1}$ (H_2O)] for PA 150 to 3.28 [µmol (CO_2) $mmol^{-1}$ (H_2O)] for T 79/501. Intermediate water use efficiency were recorded for P 30 [POS] and SCA 6 (2.59 [µmol (CO_2) $mmol^{-1}$ (H_2O)] and 2.63 [µmol (CO_2) $mmol^{-1}$ (H_2O)], respectively).

The quantity of water supplied had no significant effect on water use efficiency. However, plants which were watered less frequently had a more efficient water use ($p = 0.001$).

None of the interactions was significant.

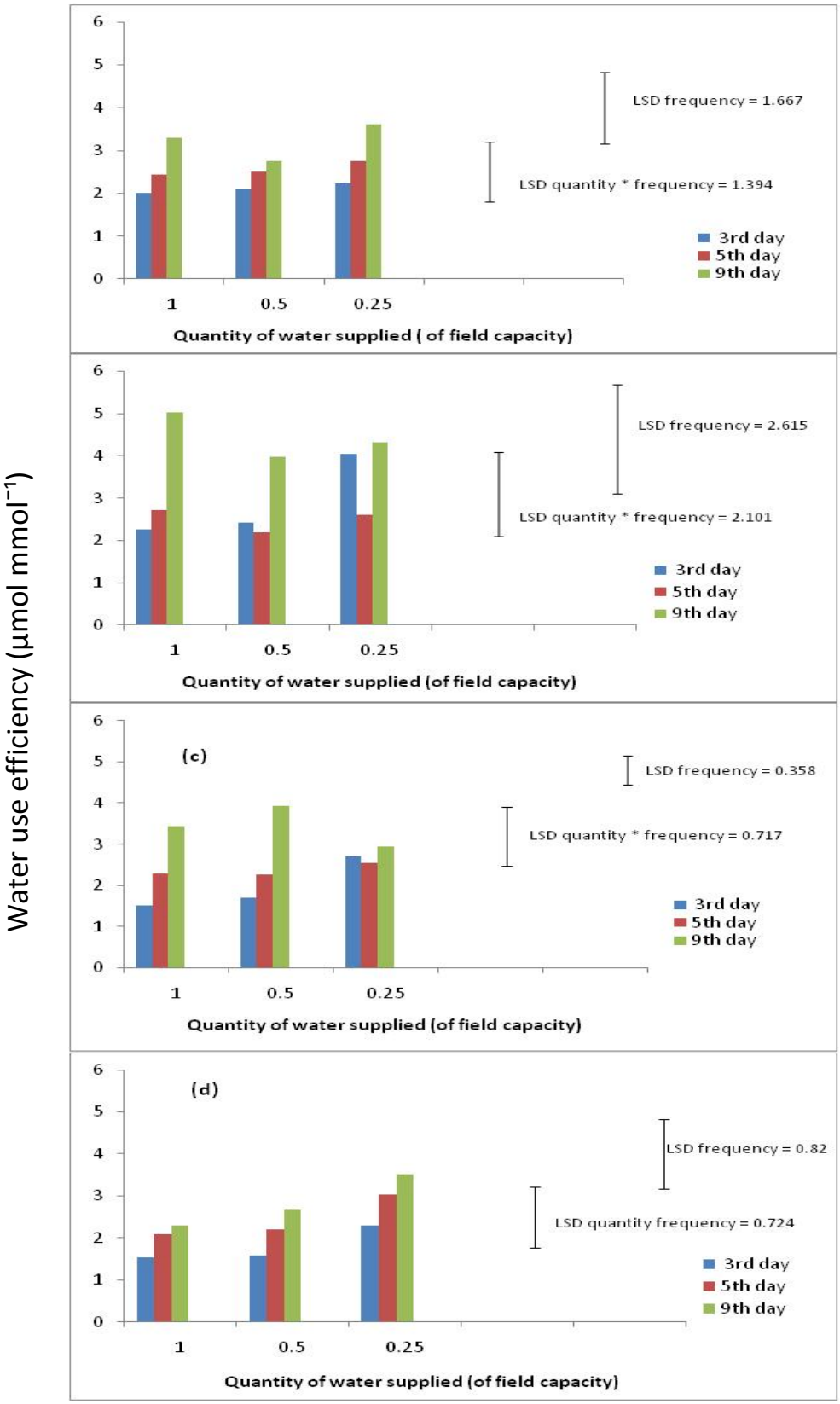

Figure 11 Water use efficiency of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean of five measurements on nine plants per treatment.

Leaf chlorophyll fluorescence

The quantity of water supplied was not associated with significant differences in the Fv/Fm ratio. The highest value (0.565) was obtained by plants supplied with 0.5 of 'field capacity'.

The frequency of watering was associated with significantly different ($p = 0.01$) Fv/Fm ratios (Fig 12). Plants that were watered every 5th day had the highest mean Fv/Fm ratio (0.584) which was 6.29% higher than the value obtained by plants watered every 3rd day and 6.76% higher than the value obtained by plants that were watered every 9th day.

The quantity * frequency interaction term was not significant. Consistently, plants that were watered with 0.5 of the soil's 'field capacity' of water had the highest values [ranging from 0.570 (for those which were watered every 3rd day) to 0.598 (for those which were watered every 5th day). The group of plants which was watered every 3rd day had the highest mean Fv/Fm ratio (0.560) under the smallest instantaneous quantity of water (0.25 'field capacity') treatment. Conversely plants which were watered every 9th day had the lowest Fv/Fm ratio values (0.534) under the smallest instantaneous quantity of water (0.25 'field capacity') treatment.

There were no significant differences between clones in the ratio of Fv/Fm. The clone * quantity and clone * frequency interaction terms were not significantly different. All the clones had their highest Fv/Fm ratio when they were watered every 5th day and all the clones (except PA 150) had their highest Fv/Fm ratio when supplied with 50% 'field capacity' (Fig 12). The 2nd order interaction term was not significant.

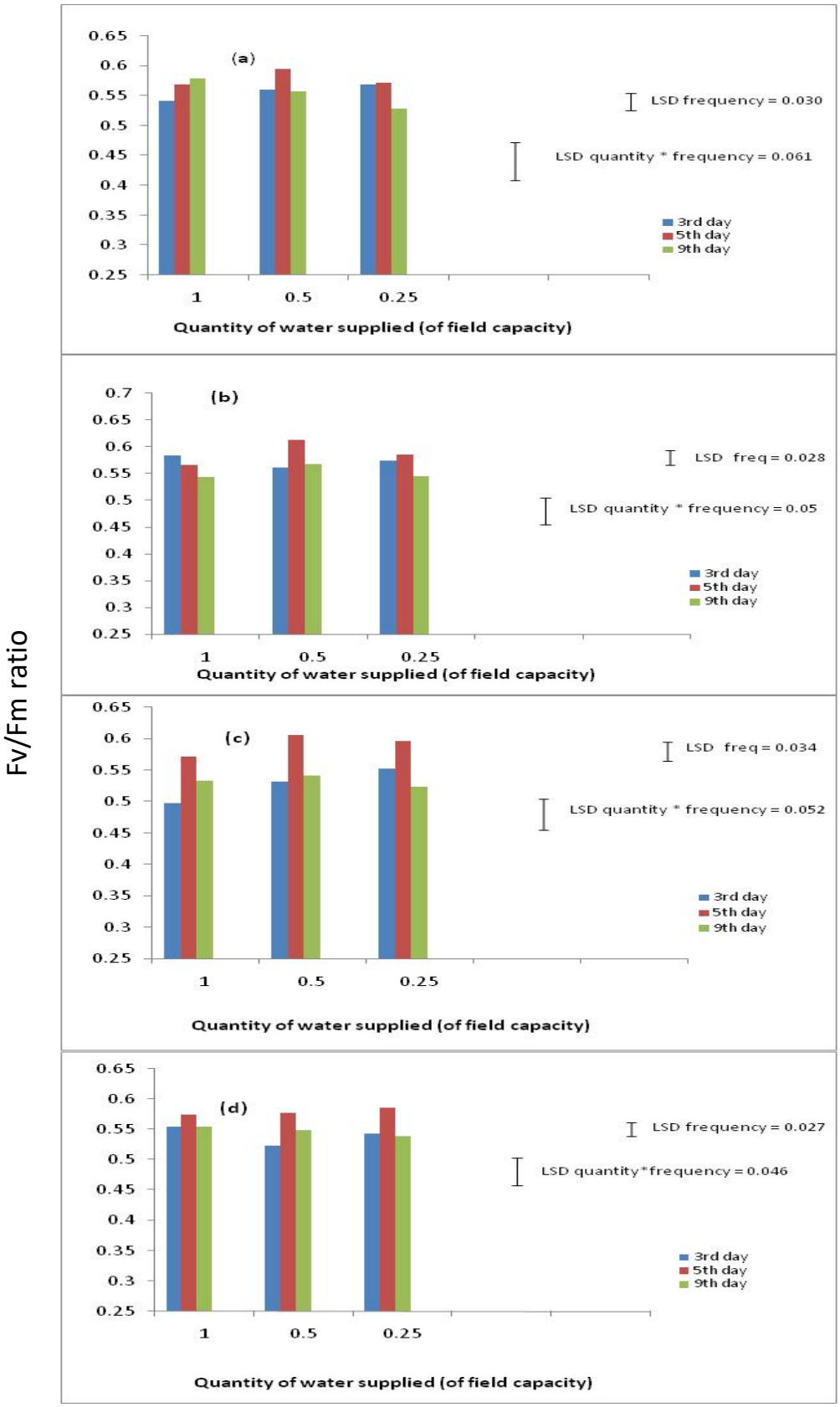

Figure 12 Leaf chlorophyll fluorescence of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean of five measurements on nine plants per treatment.

3.3.2 Plant growth

In this present work no data were collected on root growth. Plate 12 gives an impression of differences in root development in the growing pots under the 50% 'field capacity' treatment.

Plate 12 A sample of roots developed in plants watered with 50% field capacity every (a) 3rd (b) 5th (c) 9th day (from left to right)

Stem girth

In harmony with the observations on stomatal conductance, transpiration and the rate of photosynthesis, a significant ($p = 0.032$) quantity * frequency interaction was observed in the stem diameter of plants. The thinnest stems (0.78 cm) were recorded for plants watered every 9th day with the smallest quantity (0.25 of 'field capacity') of water at each watering. Plants which were watered every 3rd day with 50% 'field capacity' had the thickest stems (1.14 cm). Their stems were 0.04% and 8.75% thicker than those of plants which were watered every 3rd day with the soil's full 'field capacity' and 0.25 'field capacity', respectively.

There were no significant differences between cacao genotypes in their stem diameters. T 79/501 had the thickest mean stem (1.017cm) which was 3.84%, 4.73% and 9.6% thicker than the mean stems of PA 150, P 30 [POS], and SCA 6, respectively.

The clone * quantity interaction term was not significant but the clone * frequency interaction term was significant ($p = 0.038$). Whereas all the clones had smaller diameters in response to reducing frequency of watering, the magnitude of the observed reductions associated with less frequent watering was different from clone to clone (Fig 13). Also whereas SCA 6 and P 30 [POS]) suffered a greater reduction when the frequency of watering was reduced from every 3rd day to every 5th day PA 150 and T 79/501 were more affected when the frequency of watering was reduced from every 5th day to every 9th day . T 79/501 and PA 150 experienced greater reductions with diminishing frequency of watering when the quantity of water supplied at a time was the minimum than SCA 6 and P 30 [POS].

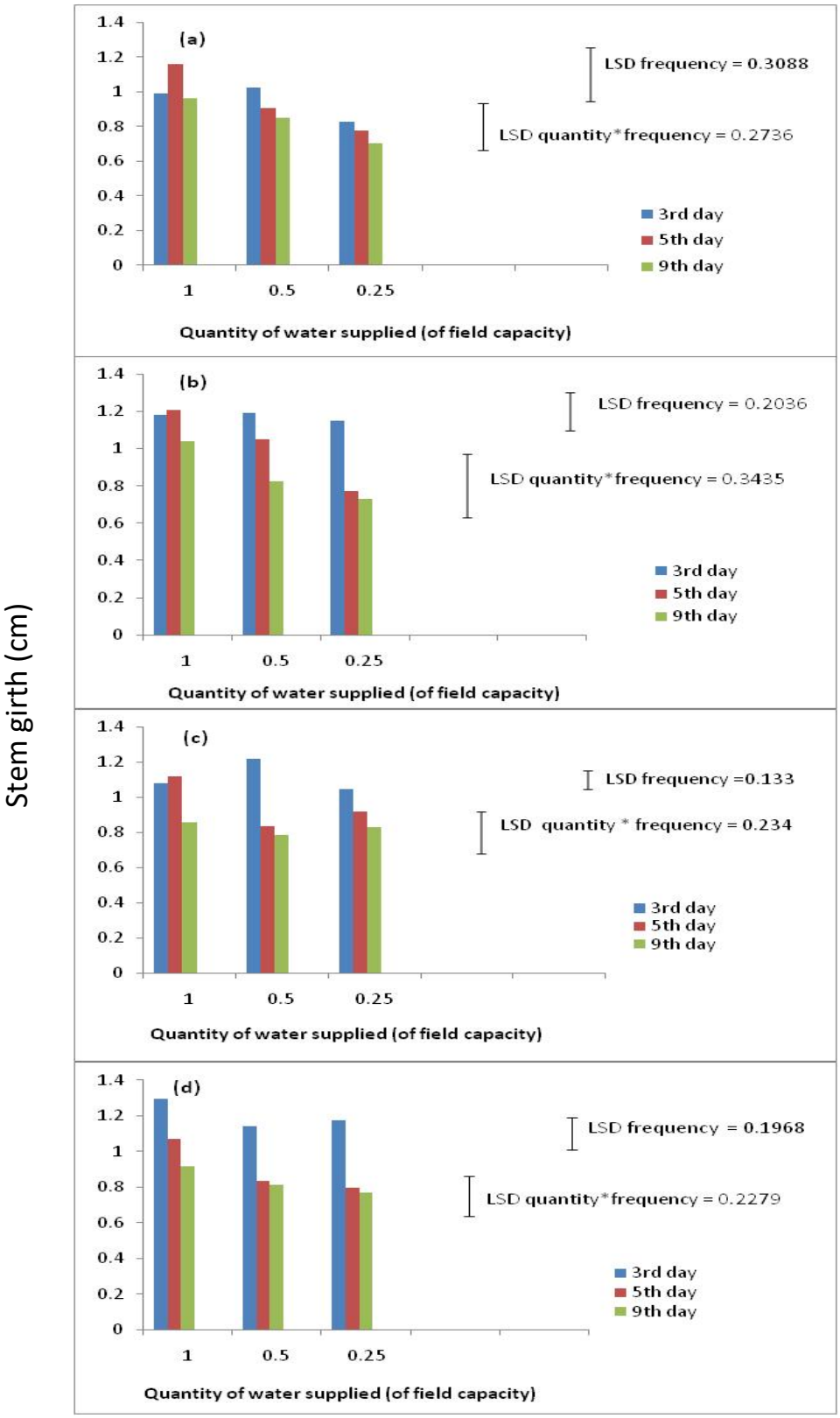

Figure 13 Stem girth of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and PA 150 under different watering treatments.

Each bar represents the mean of five measurements on nine plants per treatment.

Plant height

A significant (p = 0.004) quantity * frequency interaction was also seen in plant height. With the supply of the soil's 'field capacity' of water the tallest (59.35 cm) plants were obtained, not under the most frequent watering schedule but when watering was done every 5th day. With the 50% 'field capacity' treatment, increasing frequency of watering was associated with the production of taller plants with the tallest (mean height = 54.94 cm) plants being produced under watering every 3rd day. When 25% 'field capacity' of water was supplied, the tallest plants (49.94 cm) were produced under the most frequent watering schedule (Fig 14). There were no significant differences between the cacao genotypes in their heights. With the exception of P 30 [POS] all the clones produced their tallest plants when full 'field capacity' of water was applied every 5th day and, without an exception, all the clones produced their shortest plants when watering was done every 9th day (Fig 14). The clone * quantity and clone * frequency interaction terms were also not significant.

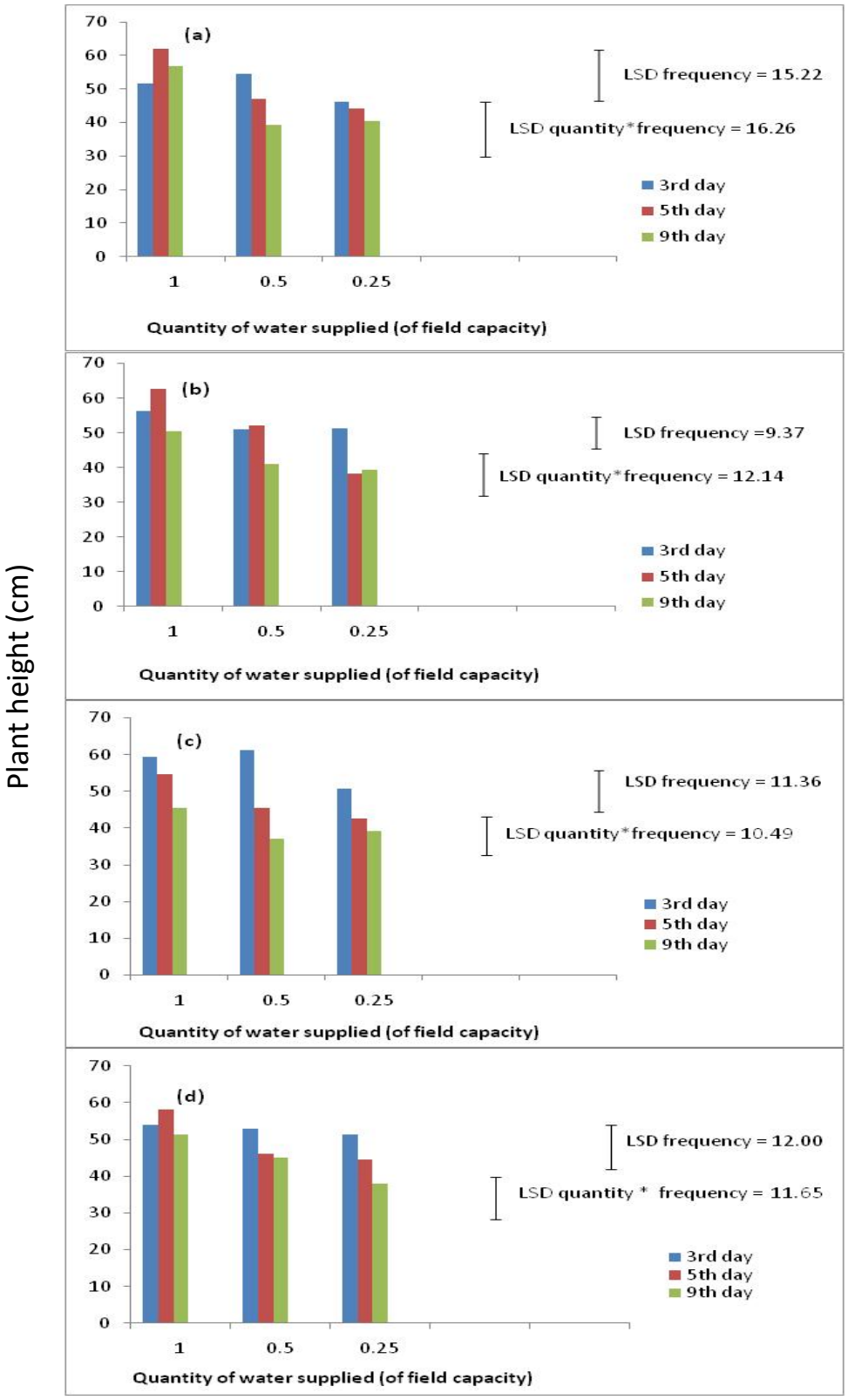

Figure 14 Height of (a) SCA 6, (b) T 79/501, (c) P 30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean of five measurements on nine plants per treatment.

Plant volume

Similar observations to what was found on photosynthetic activity were made on plant stem growth where the quantity * frequency interaction term was significant (p = 0.006). Generally, smaller stem volumes were obtained when smaller quantities of water were supplied over a period of time. However, as also observed for stem diameter and plant height, with the supply of the soil's 'field capacity' of water, the biggest mean stem volume (22.07 cm^3) was obtained when watering was done every 5^{th} day rather than every 3^{rd} day. With the supply of 50% 'field capacity' of water, the biggest stem volumes (mean = 20.47 cm^3) were obtained under the most frequent (every 3^{rd} day) watering schedule. There were no significant differences between the cacao genotypes in their stem volumes. SCA, P 30 POS], PA 150 and T 79/501 had mean stem volumes of 13.16 cm^3, 13.77 cm^3, 14.16 cm^3 and 16.18 cm^3, respectively.

Whereas the clone * quantity interaction term was not significant, the clone * frequency interaction term was significant (p = 0.032). In all the genotypes, plants which were watered less frequently produced smaller stem volumes. However, the magnitude of the response to decreasing frequency of watering differed from clone to clone. A 25.99% reduction in stem volumes was recorded in SCA 6 plants when the frequency of watering was reduced from every 3rd day to every 5th day whereas under the same change in the frequency of watering T 79/501 became 96.47% smaller and PA 150 and P 30 [POS] became 2.41 and 2.57 times smaller, respectively.

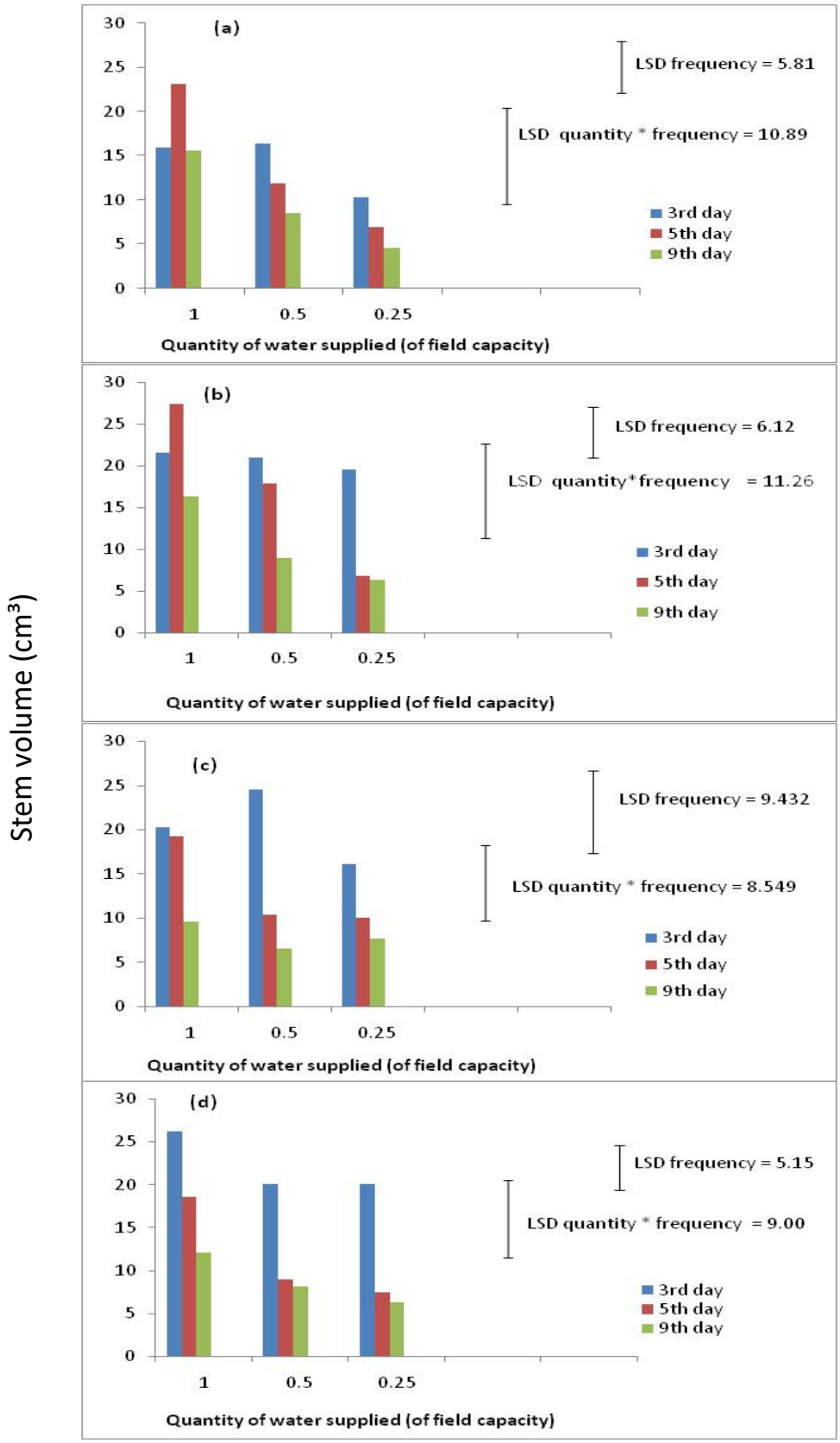

Figure 15 Stem volume of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean of five measurements on nine plants per treatment.

Fig 16 shows positive linear relationships between the rates of photosynthesis and plant stem volumes in all the cacao genotypes given by the regression equations 1 to 4:

$V = 8.13 + 2.02A$; $p = 0.005$, $r^2 = 0.70$ (for SCA 6)……..(1)

$V = 6.94 + 1.85A$; $p = 0.007$, $r^2 = 0.67$ (for T 79/501)…….. (2)

$V = 5.12 + 1.87A$; $p = 0.029$, $r^2 = 0.51$(for P 30 [POS]) …….. (3)

$V = 6.98 + 3.64A$; $p = 0.09$, $r^2 = 0.34$ (for PA 150)…….. (4)

where V = stem volume and A = instantaneous photosynthetic rate.

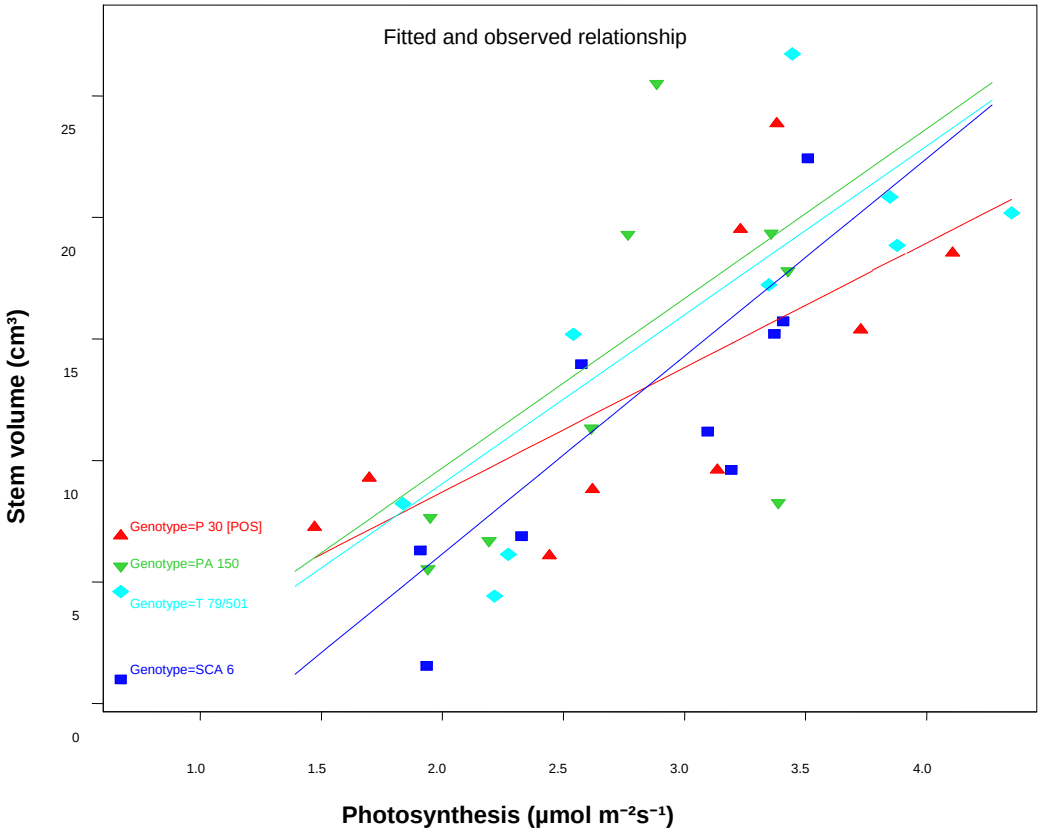

Figure 16 Relationships between mean instantaneous photosynthesis and final stem volume of the cacao genotypes

Figure 17 shows a generic graph that disregards genotypic differences and shows the positive linear relationship between instantaneous photosynthetic rates and final stem volume

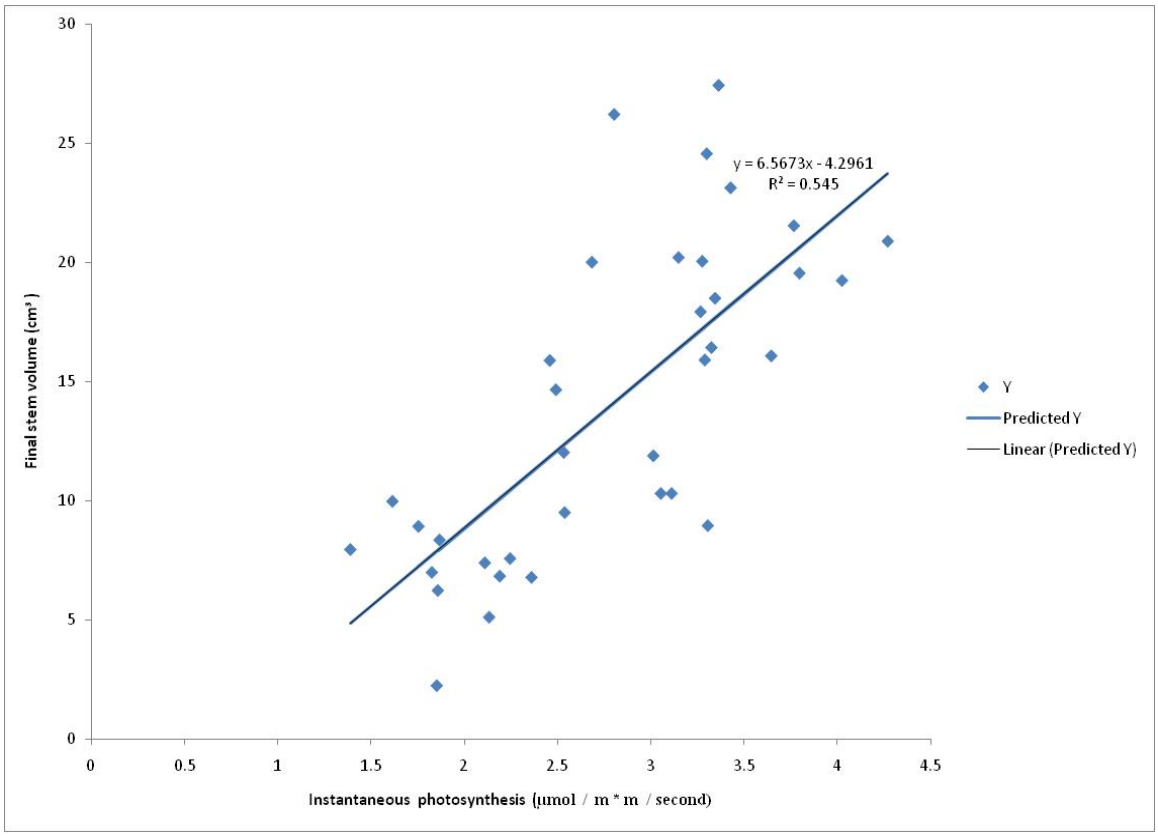

Figure 17 Generic relationship between instantaneous photosynthetic rate and final stem volume of young cacao clones

3.3.3 Leaf traits

Leaf nitrogen content

Differences among the clones were on the borderline of significance (p =0.054) with respect to the leaf nitrogen content. PA 150, SCA 6, T 79/501 and P 30 [POS] had 1.776%, 1.669%, 1.585% and 1.573% leaf nitrogen contents, respectively (Fig 18). The quantity * frequency interaction term was significant ($p < 0.001$). The highest leaf nitrogen concentrations (1.853% and 1.779%) were recorded in plants which were watered with 50% 'field capacity' of water every 5th day and every 9th day, respectively. Plants which were watered every 9th

day with 25% ‘field capacity’ of water had the lowest leaf nitrogen content (1.339 %N).

Leaf chlorophyll concentration

The quantity of water supplied did not have a significant effect on leaf chlorophyll concentration. However, a trend was observed in which plants that were supplied with larger quantities had lower chlorophyll concentration (Fig 19). Plants supplied with full, 50% and 25% ‘field capacity’ of water had 6.97 chlorophyll metre units, 7.68 chlorophyll metre units and 8.06 chlorophyll metre units leaf chlorophyll concentration, respectively.

The quantity * frequency interaction term was significant ($p = <0.001$). Under the full ‘field capacity’ of water treatment plants which were watered more frequently had lower leaf chlorophyll concentrations. Plants which were supplied with 50% and 25% of the soil’s ‘field capacity’ of water had higher leaf chlorophyll concentrations when watered every 5^{th} day and the lowest concentrations when watered every 3^{rd} day.

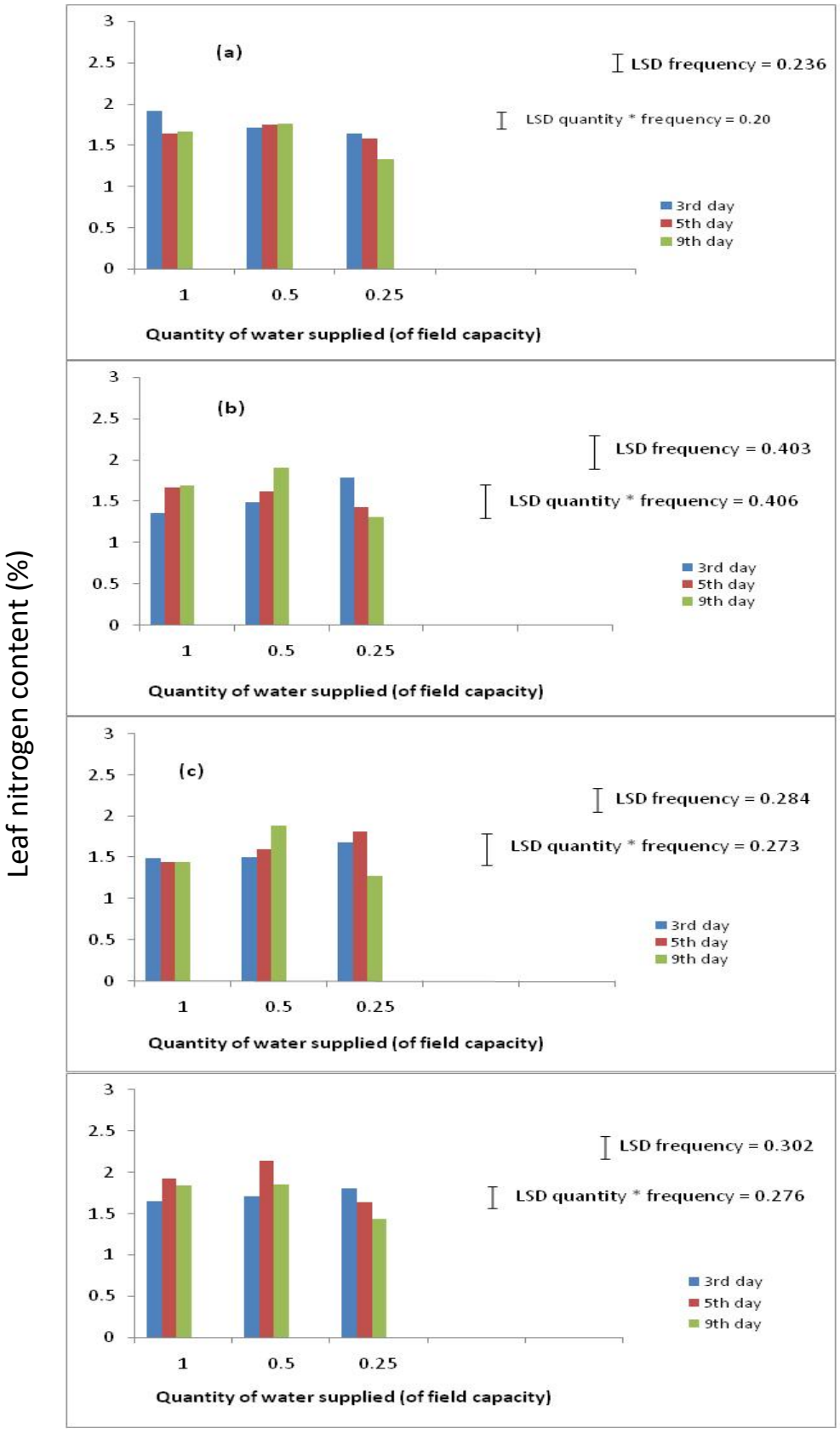

Figure 18 Leaf nitrogen content of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean value for 6 plants per treatment.

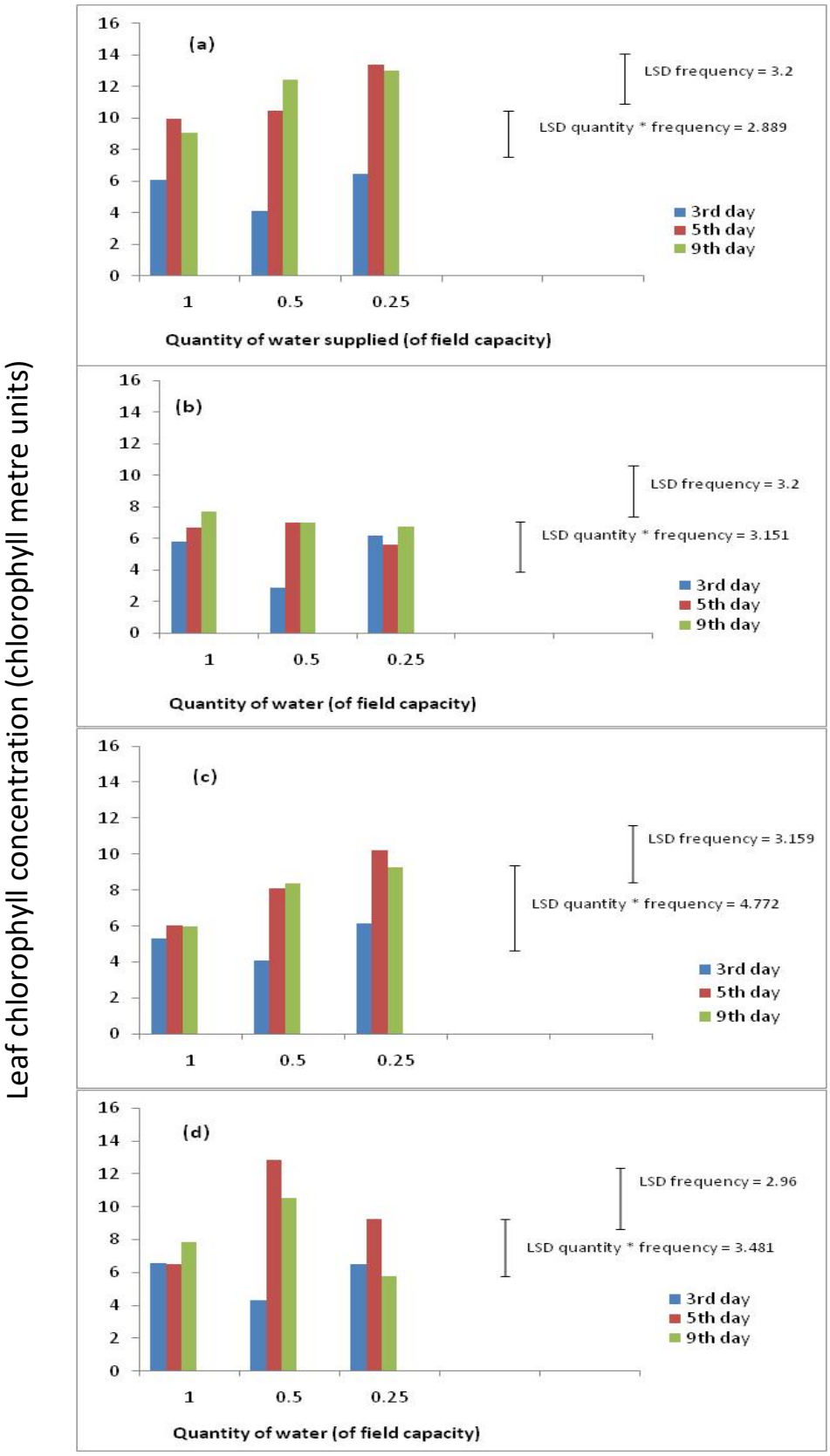

Figure 19 Leaf chlorophyll concentration of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean of nine plants per treatment.

The genotype * quantity interaction component was significant (p = 0.016). SCA 6 and P 30 [POS] had increasing leaf chlorophyll concentrations with reducing instantaneous water quantity. Consistent results were not obtained for T 79/501. PA 150 had its highest value (9.26 chlorophyll metre units) under the 50% 'field capacity' treatment and the lowest under the full 'field capacity' treatment.

The genotype * frequency interaction component was also significant (p = 0.031). All the clones had their lowest leaf chlorophyll concentrations with the most frequent watering schedule (watering every 3rd day). Also all the clones (except T 79/501) had their highest values when watered every 5th day. There were no significant 2nd order interactions.

Relative water content

The cacao genotypes did not have significantly different relative water contents of leaves (Fig 20).

However, a significant (p = 0.016) quantity * frequency interaction term was detected. Although under all three 'quantity of water' treatments, plants that were watered less frequently had lower relative water contents, more pronounced reductions in leaf water content were observed under the 25% 'field capacity' treatment when the frequency of watering was reduced. Whereas with the full 'field capacity' and 50% 'field capacity' of water plants that had their watering frequency reduced from every 3rd day to every 9th day had 62.83% and 62.19% as relative water content, respectively, plants which experienced the same reduction in frequency of watering under the 25% 'field capacity' of water treatment had a relative water content of 52.15%, reducing their relative water contents by a quarter.

The genotype * frequency interaction term was also significant (p = 0.012). With the exception of T 79/501 which lacked consistency in its response, all the clones experienced lowered relative water contents when the frequency of watering was reduced. The magnitude of the

response to decreasing frequency of watering differed from clone to clone, however. When watered every 3rd day PA 150 had a relative water content of 69.11% but when watering was done every 9th day the value reduced to 63.58%.For the same reduction in frequency of watering, SCA 6, T 79/501 and P 30 [POS] had their relative water contents reduced from 65.86% to 54.92%, 62.68% to 61.72% and from 70.83% to 56.02%, respectively. The genotype * quantity interaction element was not significant.

Specific leaf weight

Plants that received increasing quantities of water supplied at a time had significantly ($p = 0.011$) lower specific leaf weight. Plants watered with 25% 'field capacity' of water had 5.60% and 7.29% higher specific leaf weights than plants watered with 50% 'field capacity' and the full 'field capacity' of water, respectively (Fig 21). No significant differences were established among the frequency of watering treatments. Only a trend was observed in which plants that were watered more frequently had lower specific leaf weights. Plants which were watered every 9th day had specific leaf weights which were 1.49% and 3.46% higher than plants which were watered every 5th day and every 3rd day, respectively. The quantity * frequency interaction term was not significant. Differences among the specific leaf weights of the cacao genotypes were not significant. The genotype * quantity, the genotype * frequency and the 2nd order interaction terms were also not significant.

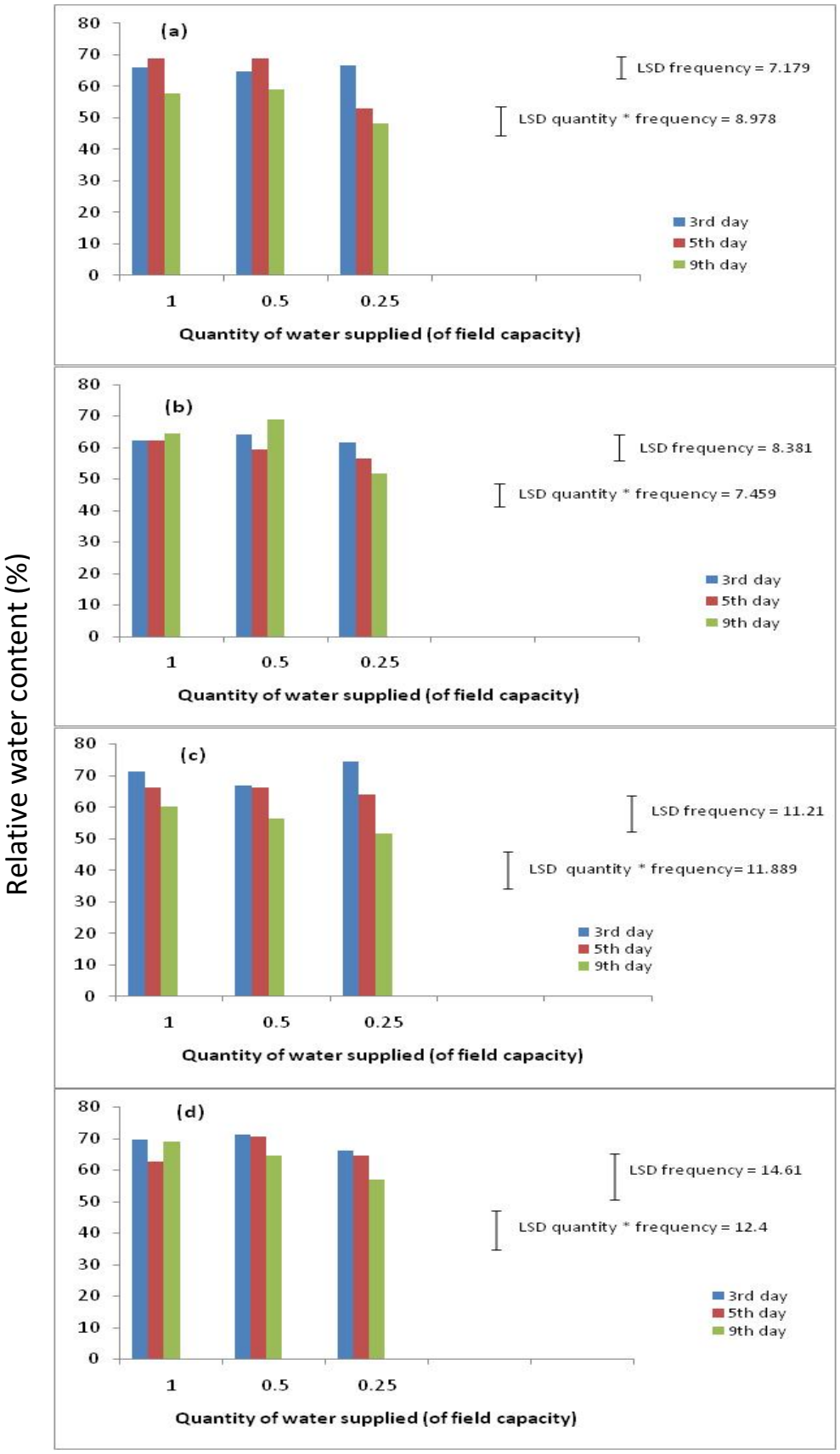

Figure 20 Relative water content of (a) SCA 6 (b) T 79/501 (c) P30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean of six plants per treatment.

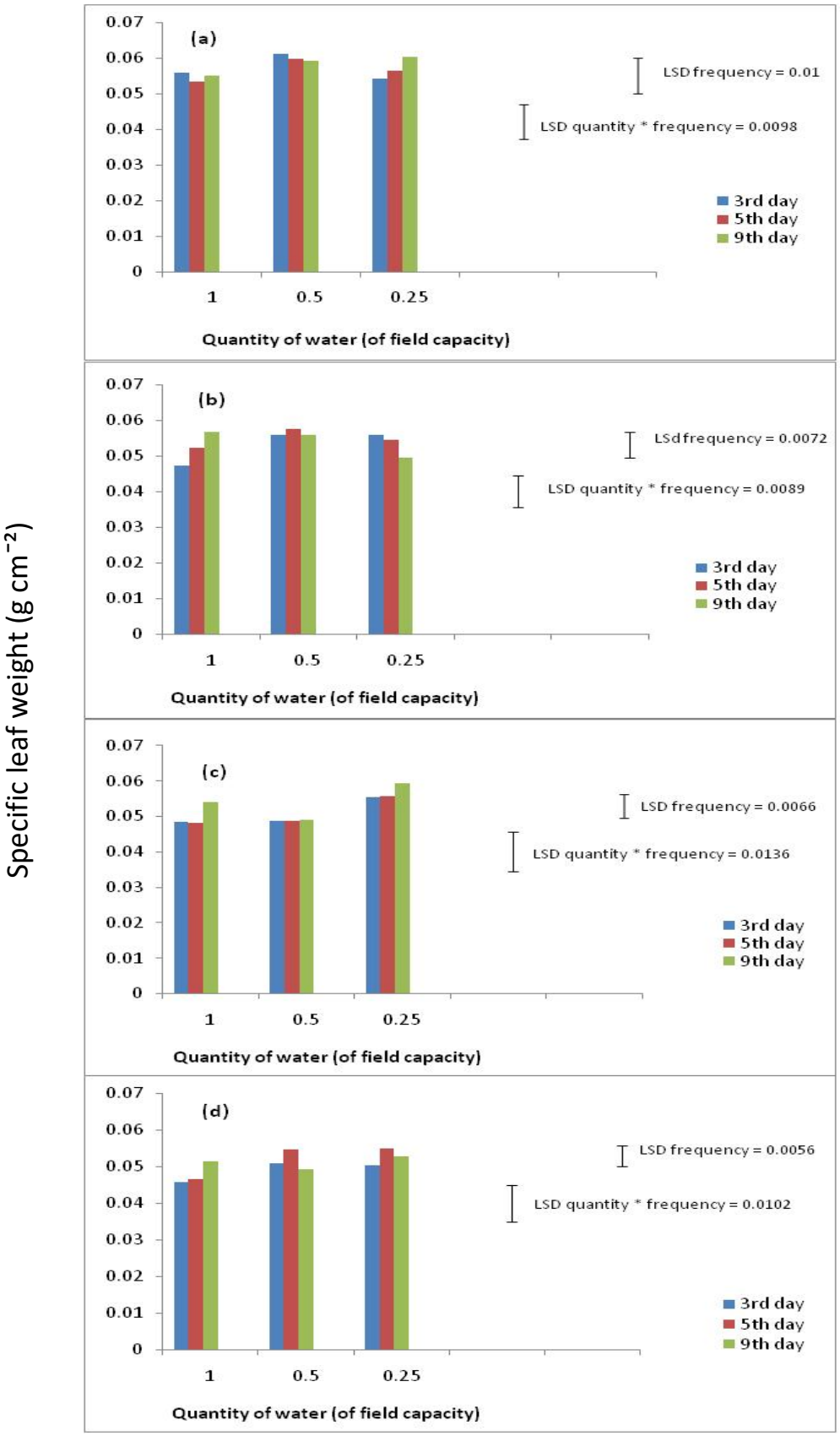

Figure 21 Specific leaf weight of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different watering treatments.

Each bar represents the mean of six plants per treatment.

3.3.4 Plant survival

Ranging from 2.5% to 7.2% plant mortality rates were not very different among the clones.

The quantity * frequency interaction term was significant at ($p < 0.001$). Whereas all the plants watered every 5th day with 0.5 of the soil's 'field capacity' of water survived 1% of plants watered at the same frequency but with the soil's full capacity of water and 2% of plants watered at the same frequency but with the 0.25 of the soil's 'field capacity' of water died (Fig 22).

The clone * frequency interaction term was on the borderline of significance ($p = 0.08$). Under watering every 5th day all SCA 6 and P 30 [POS] plants survived whereas 1% and 2% of T 79/501 and PA 150 plants died, respectively. But when watering was done every 9th day 2.4% of SCA 6 and 0.9% of P 30 [POS] died as against 0.8% of T 79/501 and 0.3% of PA 150, showing that more of SCA 6 and P 30 [POS] died under less frequent watering than plants died of T 79/501 and PA 150.

The 2nd order interaction term was not significant.

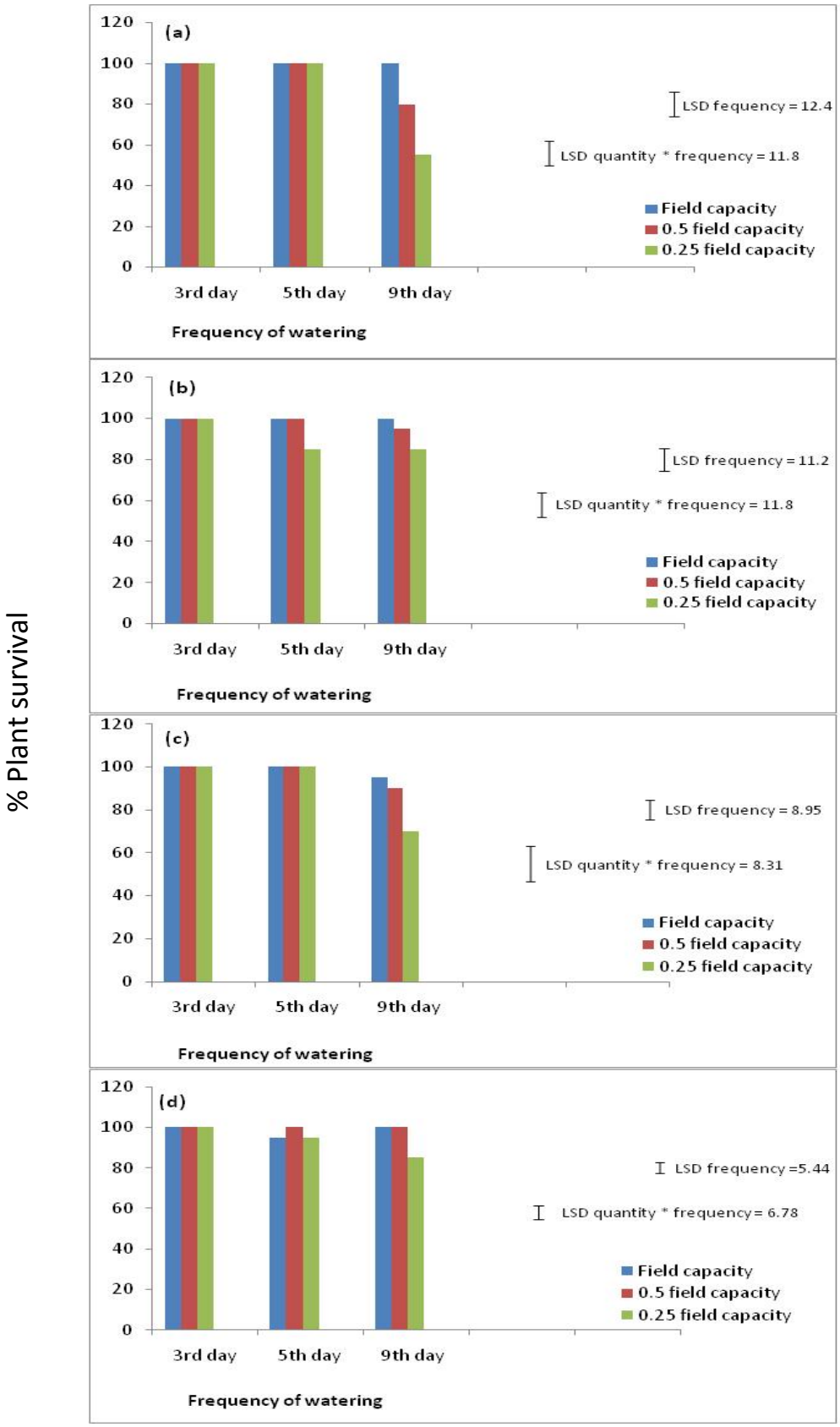

Figure 22 Survival of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different watering regimes.

Each bar represents the value for 20 plants.

3.4 Discussion

Photosynthesis of plants under different soil water levels

Cacao plants which received the lowest amount of water over time had the lowest stomatal conductance rates. This confirms previous observations by Rada et al. (2005) which indicate that when watering frequency was reduced from 3 to 12 and 25 days, stomatal conductance in cacao plants declined with increasing water stress. The significantly different stomatal conductance rates that were found among the clones may be an indication that genetic variability exists among the genotypes with respect to stomatal regulation and or stomatal density in response to soil water stress. A wider range of stomatal conductance values was observed in this work than that observed by Rada et al. (2005) on four-year-old, field planted cacao under lower temperatures and that may be a reflection of differences in environmental conditions.

Similar responses to intensifying water stress have been noted in other plant species including soybean (Guan-yu et al., 1991), the woody species *Vitis vinifera* L. (Perez-Martin et al., 2009), and the oil palm (Smith, 1989). Hutcheon (1977) showed, in cacao, that changes in stomatal opening during the day are influenced mainly by the water status of the plant. This author also observed that whereas in well watered cacao stomatal closure is not very complete even during the night, there is a virtual closure during the day on occasions when both soil and air were dry. Joly and Hahn (1989a) explained that the leaves of cacao are especially responsive to plant water deficit, the stomata reacting quickly to moderate levels of stress such as is caused by a slight decrease in root water status in drying soil. These authors cited the separate works of Cornish and Zeevart (1985), Lachno and Baker (1986) and Zhang et al. (2008) which suggest that ABA, synthesized in roots (in response to water deficit) acts to induce stomatal closure.

The observation that plants which were watered with full 'field capacity' of water every 3rd day (the largest amount of water over time) had a slightly lower mean stomatal conductance rate than those which received about 20% less water would suggest mild symptoms of asphyxiation in the former group of plants (Adams, 1962). Possibly the opening provided at the base of the growing pots for drainage was not big enough to eliminate the creation of occasional hypoxic soil conditions under the highest watering regime. Other possible reasons could be increased soil density as a result of soil particle compaction under the highest watering treatment (Kim and Kim, 2010, Sun et al., 2010) as well as a higher level of soil nutrient leaching in that treatment (Kannan, 2010, Nzokou and Cregg, 2010).

The observation that generally plants supplied with smaller amounts of water (by supplying smaller quantities at a time or by reducing the frequency of watering) had smaller transpiration rates is consistent with observations in previous work which show that water-stressed plants had a lower stomatal conductance and transpiration (Cameron et al., 1999). Balasimha and Rajagopal (1988) and Rada et al. (2005) observed in separate experiments under water stress conditions that transpiration rates in young cacao generally decreased as water stress increased. In a potted-plant experiment in which soil moisture was varied between 13% and 41%, Nunes (1967) observed in cacao seedlings (varieties Amelonado and Laranja) that when soil moisture dropped to below 30% transpiration rates showed significantly lower values. The observed differences in that experiment were ascribed to decreases in stomatal opening.

According to Öpik et al. (2005), when a soil is at field capacity plant water uptake can go on without restraint. Field capacity is the ideal soil state for plants since it has a high water potential but also has air-filled spaces. These authors explain that as the soil begins to dry, the 'lowered soil water potential leads to a correspondingly lower plant water potential, but wa-

ter uptake continues until ultimately, the plant can no longer get adequate water to compensate for transpiration losses'. In this current work, however, the highest rate of transpiration was observed in plants supplied with 50% 'field capacity' of water every 3rd day rather than in plants that received the largest amount of water over time. This was a reflection of the response found in stomatal conductance and is in line with Joly and Hahn's (1989a) remark that transpirational water loss from leaves is controlled by the stomata.

Results of this current experiment appear to follow a trend observed by Nunes (1967) who presented data on young cacao which showed increasing transpiration rates between 13% and 32% but decreasing transpiration rates between 32% and 41% soil moisture (the maximum soil moisture treatment in that experiment).

The fact that plants which received either larger quantities of water or increasing frequency of watering had significantly higher stomatal conductance is consistent with the higher photosynthetic rates that were observed here. Bae et al. (2007, , 2009) also observed a 29% and 96% reduction in photosynthesis when the frequency of watering of cacao seedlings was reduced from a 3- day cycle to a 13- day cycle. Rada et al. (2005) reported from their diurnal study of young cacao that although osmotic adjustment increased as water stress intensified, CO_2 assimilation declined. In addition, Deng et al. (1990) found average photosynthetic rates of about 2.2 µmol CO_2 $m^{-2}s^{-1}$ when the leaf water potential of cacao was between -1.4 MPa and -1.5 MPa, and close to zero when the leaf water potential fell below -1.6MPa. Working on two Amazonian types (EET – 399 and EET – 400) and one Trinitario type (United Fruit cultivar UF – 613) of cacao, Joly and Hahn (1989a) observed a rapid reduction in stomatal conductance soon after the imposition of drying cycles on the plants.

The reduction in stomatal opening lowers transpiration and leads to the conservation of water, delaying the start of low tissue water potential. Since the stomatal cavity concurrently

provides a conduit for CO_2 from the atmosphere to leaf mesophyll cells, soil water deficits restrict net CO_2 assimilation. In another experiment Joly (1987) noted in cacao that the extent to which net photosynthesis was reduced by leaf water deficits depended on both the intensity and duration of the moisture stress regime.

Unlike the observation made by Yapp (1992) and Daymond (2000) the cacao genotypes used in this work had similar photosynthetic rates suggesting that there was little genetic variability among them respecting their rate of CO_2 assimilation per unit leaf area although the clones did differ in relation to their water relations.

The mean Fv/Fm ratio values were smaller than those reported elsewhere and that could partly be attributed to the high day-time temperatures (mean 36.4 ^{0}C) under which the plants were grown. The observation that 50% 'field capacity' of water (rather than full 'field capacity') supplied to the plants every 5th day (and not every 3rd day) gave the highest Fv/Fm ratio values [not significant] suggests that this was the best soil moisture content for photosystem II activity in this experiment and that soil moisture contents outside what was obtained by using this watering regime were sub-optimal for the young cacao plants. Relevant results obtained earlier by other workers show that outside the optimal soil water range photosynthetic activity of cacao is adversely affected. For example Sena Gomes and Kozlowski (1986) noted that excessive soil water is a barrier to the establishment of cacao. Working on the seedlings derived from the cacao cultivar 'catongo', these authors observed significant reductions in stomatal conductance and transpiration rates within two hours of imposing a flooding treatment. There were also reductions in leaf formation and growth, as well as stem and root growth. In contrast, Balasimha and Daniel (1995) found lower leaf chlorophyll fluorescence in cacao accessions during drier months of the year. These reports show that both soil moisture deficits and excessive soil water are stressful to cacao plants. In

the current work, whereas only a trend was observed among the quantities of water supplied at a time, a significant difference was established among the frequencies of watering suggesting that the frequency of watering has a greater impact on leaf chlorophyll fluorescence than the quantity of water supplied at a time. This suggests that to optimize photosynthesis, growth and survival rate more attention may have to be given to the frequency of irrigation than the volumes of water supplied at a time and under rain-fed conditions cacao plants benefit from more even rainfall distribution than higher rainfall intensity.

Balasimha and Namboothiri (1996) compared the leaf chlorophyll fluorescence of excised leaves of two accessions (NC 42/94 and PA 7 * Na 32) of cacao representing drought-tolerant and susceptible cacao cultivars, respectively. These authors found similar Fv/Fm values (0.774 and 0.764, respectively) for the two cultivars before a heat stress treatment (dipping of excised leaves in a water bath at 47^{o}C for 5 minutes) was imposed. Significantly different Fv/Fm values (0.623 and 0.539, respectively) were recorded for the cultivars 30 minutes after the heat stress treatment had been stopped "indicating a smaller reduction (19.9%) of quantum yield in the drought-tolerant cacao than in the susceptible cultivar (30.1%)". Under the different soil water treatments of the current experiment, no significant differences were observed among the cacao genotypes and probably this explains why no significant differences were observed in the rates of photosynthesis between the clones.

Soil water and Leaf traits

The observation that plants supplied with the smallest quantities of water over time had the lowest leaf nitrogen content may be explained by the fact that soil nutrients are taken into plant roots in the form of charged ions through the root hairs, along with water (Fitter and Hay, 2002). Hence the lack of adequate soil moisture would result in low leaf nitrogen contents. The observation that plants supplied with 50% 'field capacity' of water every 5th day

had the highest nitrogen seems to suggests a narrow range of soil moisture contents within which nitrogen absorption can be optimized for young cacao. As optimal photosynthetic activity was observed when the plants were watered with 50% 'field capacity' but at a higher frequency (every 3rd day), this observation suggests that a slightly lower soil water content than the optimum for photosynthetic activity was required to maximize leaf nitrogen concentration. In this respect young cacao may be regarded as responding differently to soil water content from some other plant species. For example a wider range of soil moisture contents may be tolerated (without changes in leaf nitrogen content) by maize (Bahrun et al., 2003) and rice (Otoo et al., 1989). The marginal differences observed among the cacao clones with regard to their leaf nitrogen content are probably an indication of some genetic variability in nitrogen uptake.

Similar to its effect on leaf nitrogen, cacao plants which experienced water stress had lower leaf chlorophyll concentrations. Joly and Hahn (1989b) also noted that chlorophyll synthesis in expanding leaves of cacao was supported by the availability of substrates (principally from older leaves) and is delayed in water-stressed plants; under soil moisture stress either synthesis of the total quantity of substrates is delayed or the rate of their import into younger leaves is delayed. Disparate results have been reported, in different plant species, on the effect of soil water on leaf chlorophyll content. For example, De-Souza et al. (1997) reported that soybean plants subjected to drought stress in a greenhouse lost their leaf chlorophyll concentration much faster than control plants but Schelmer et al. (2005) reported that drought stress had no significant effect on leaf chlorophyll content of maize. Mensah et al. (2006) reported that severe drought stress resulted in an increase in leaf chlorophyll content of maize and Beeflink et al. (1985) observed an increase in chlorophyll content in onion plants under drought stress.

On the other hand, the results obtained here suggest that maintaining soil water close to the 'field capacity' was associated with reduced leaf chlorophyll concentration so that maximum leaf chlorophyll concentration is obtained when cacao plants are grown within a short range (about 50 - 60 %) of soil moisture levels. Probably, as mentioned earlier, there was a higher level of soil nutrient leaching where larger quantities of water were applied over time. The significant genotype * frequency of watering interaction suggests that cacao genotypes differ in their optimal soil water requirement for leaf chlorophyll synthesis. It is probable that in SCA 6 and P 30 [POS] (in which consistent trends were observed) plant nutrient uptake - which is important in chlorophyll development- was adversely affected by supplying larger volumes of water at a time.

The observation that plants which were supplied with smaller quantities of water over time had lower leaf relative water contents is consistent with previous work and shows the importance of adequate soil moisture on plant water status. Jiang Xiong Liao et al. (2008), for example, report that soil water depletion caused decreases in the relative water content of *Adiantum reniforme*, an endangered fern. A similar response was found by Joly and Hahn (1989a) who observed, in cacao seedlings, that irrigated plants had higher relative water contents than others that did not have as much soil water. Hassanzadeh et al. (2009) states that relative water content is preferred to plant water potential as a criterion for determining plant water status because it more accurately indicates the balance between absorbed water and consumed water through transpiration. These authors contend that genotypes that have higher drought tolerance qualities should also have higher relative water contents under water stress and cite Liu et al. (2002) as stating that the relative water content of a plant is an indication of its vigour under conditions of water stress. The cacao genotypes used in this work did not have significant differences in their relative water contents, and

that probably is an indication of limited differences among them as regards tolerance to soil moisture stress and explains why the transpiration rates were not significantly different among the genotypes.

The observed association of lower specific leaf weight with increasing soil water was not as striking as those that have been found in other plant species such as peach (Johnson et al., 1992), cucumber (Yuan et al., 2006) and *Eucalyptus globules* (Steinbauer, 2000). The observed effects in the present work may be an indication that increasing soil moisture stress leads to increased leaf toughness (Lansberg, 1990, Steinbauer, 2000) which, according to many authors (Choong, 1996, Choong et al., 1992, Lansberg, 1990), is a high index of schlerophyll development and low leaf water content in many plant species.

Balasimha (1999) found a significant difference among cacao accessions that he worked on and associated higher specific leaf weight with drought tolerance. In this present work only non-significant differences were observed among the clones and that was consistent with the observation that no significant clonal differences were observed in photosynthetic rates.

Plant growth and survival

The fact that, separately, plants which were supplied with greater volumes of water at a time and those which were watered more frequently produced bigger plant stem volumes explains the higher survival rates observed as water stress reduced (Cameron et al., 2006). Cunningham and Burridge (1960) submitted cacao seedlings to full sunlight and showed that soil water partially ameliorates the stressful effects of full sun exposure.

It was observed in this present work that, separately, plants which were supplied with greater volumes of water at a time and those which were watered more frequently had a higher plant water status (as indicated by the higher relative water content), stomatal conductance, transpiration and net photosynthesis all of which are known to promote plant growth. It has

been reported that reductions in plant water status cause restrictions in both plant cell division and cell expansion which, as reported by Balasimha (1999), is one of the earliest symptoms of plant water deficit (Meyer and Boyer, 1972).

In this present work no data were collected on root growth. However, based on the experience of Silva and Kummerow (1998) it can be supposed that increased water supply was associated with greater root development (See plate 2).

Joly and Hahn (1989b) and Bradford and Hsiao (1982) reported that for many plant species leaf expansion growth is the physiological process most sensitive to water stress. Zuidema et al. (2005) mentions soil water as one of the important factors that influence leaf dynamics. Although leaf area development was not assessed in this work the bigger stem volumes associated with increasing soil water could be a reflection of the relative leaf areas developed in response to the various watering treatments.

According to Joly and Hahn (1989b) who developed an empirical model for leaf expansion in cacao, the production of photosynthetically active leaves is an important requirement for the productivity of a crop. These authors observed that as water stress increases, reductions take place in individual leaf size, total leaf area and net photosynthesis per unit leaf area. It also accelerates the rate of senescence in older leaves. Balasimha (1988) stated that a decrease in plant leaf area coupled with stomatal regulation helps to reduce transpiration and these are a major adaptation for water conservation. It is, therefore, not surprising that, in this work, increasing quantity of water supplied at a time and also a higher frequency of watering separately led to increased plant growth.

The significant quantity and frequency interaction which showed that plants which were watered with the 'field capacity' of water every 5th day (rather than every 3rd day) produced the biggest plants does not necessarily imply that maintaining soil water close to the 'field ca-

pacity' hampers the growth of young cacao under all conditions. As explained earlier, reasons such as the creation of periodic hypoxic conditions as a result of possible insufficient drainage could have occurred in this experiment under the highest water supply treatment. Such conditions (of excessive soil water) have been known to reduce total leaf area and general vegetative growth in cacao (Sena Gomes and Kozlowski 1986). Hence the present data was interpreted as showing that it is important to supply the right quantity of water (considering important factors such as drainage) over time to ensure plant growth and survival. The significant quantity of water supplied * frequency of watering interaction term showed that this could be achieved, to some extent, by adjusting either the quantity of water or the frequency of watering. However, the data showed that, just as was observed in photosynthetic processes, the frequency of watering has a greater impact on plant survival than instantaneous quantity of water supplied.

The observations made in this experiment may be usefully applied in cacao nurseries in Ghana, where, owing to inadequate information, not much attention is paid, especially to the quantity of water supplied at each time of watering. The result is that sometimes young cacao plants have been under-watered or over-watered and plant survival and growth have been adversely affected. The fact that reasonably similar results can be obtained in the plants by slightly adjusting either the quantity of water supplied or the frequency of watering gives room for flexibility. In this experiment it was clearly observed that maintaining the soil water at around 60 to 62% 'field capacity' would give optimal results.

CHAPTER 4

Photosynthetic activity and growth of four cacao genotypes grown under different shade regimes in a nursery condition

4.1 Introduction:

Although conventionally cacao has been grown under shade, it is not known whether shade is an intrinsic requirement of young cacao plants or whether shade only plays a secondary role such as by causing a reduction in insect pest attack and contributing to the recycling and sustainability of soil nutrients for cacao. Nevertheless, cacao is often considered to have an obligatory shade requirement as it has many of the attributes of a shade species such as low photosynthetic rates (Raja Harun and Hardwick, 1988), low light saturation levels of about 400 µmol m^{-2} s^{-1} that correspond to about 20% of full sunlight (Raja Harun and Hardwick, 1988) and a high photoinhibitory stress (Serrano and Biehl, 1996). Young cacao is considered to require shade to aid establishment. However, the physiological basis of this shade requirement still needs to be more clearly defined. Moreover, there is considerable inconsistency in what is considered to be the optimal light levels for cacao. For example, studies conducted by Da Matta et al. (2001) suggest that cacao seedlings may show increasing photosynthetic rates as the photosynthetically active radiation (PAR) increases to 750 µmol m^{-2} s^{-1} corresponding to 30% of PAR at full sunlight. Working on four-year old 'Guasare' (a cacao cultivar), Rada et al. (2005) observed higher photosynthetic rates between PAR 800 and 1000 µmol $m^{-2}s^{-1}$. Thus the shade requirement for the different stages of the growth of cacao is still not clearly defined. Moreover, under field conditions where other living plants are usually used as shade for cacao plants, it is difficult to determine a clear effect of shade on cacao plants since other factors such as inter-specific competition for soil water and nutri-

ents come into play. Furthermore, whilst a short-term study on the interaction between shade and cacao genotypes in Trinidad has been reported (Galyuon et al., 1996a) no such studies have been conducted incorporating both the wet and dry seasons of West Africa. The experiment described here was therefore conducted to assess the shade requirement of four cacao clones (SCA 6, T 79/501, P 30 [POS] and PA 150 all grafted on a T 60/887 root-stock) in the different seasons of Ghana (in West Africa) and to determine the physiological basis of their respective shade requirements. To circumvent complications that could have emanated from inter-species competition the experiment was conducted in a nursery environment using shade netting to vary the intensities of incident light. Results from this experiment were used later to provide useful further insights into similar experiments conducted under field conditions.

4.2 Materials and methods:

The experiment was carried out inside a 35 m x 30 m nursery shade house that was covered artificially on all sides with a single-layer of shade netting, supported by galvanized pipes and rafters. Inside the shade house, pits 4.5 m long * 1.35 m wide * 1 m deep were constructed of concrete blocks. To provide drainage, large stones were put in the bottom of the pit and then gravel was added to bring the level up to half way (about 50 cm) below the top of the pit. A polythene sheet, perforated with five 1 cm - diameter holes across its length at 90 cm intervals was spread on top of the gravel. An 8 cm deep layer of sand was put on top of the polythene sheet. Plants of the four cacao clones were potted into large (32 cm x 37.5 cm) black polythene bags (growing pots) perforated with about 28 evenly distributed 0.7 cm - diameter holes and filled with top-soil. On 8th March 2007 the growing pots were placed on

top of the layer of river sand and more sand was added between the pots until the river sand layer was approximately 27 cm deep, leaving the top 15 cm of the pit empty (see Fig 23). Thus a greater part of the stems of the cacao plants in the growing pots projected vertically above the side walls of the pit (Plate 13). The holes in the growing pots provided contact between the topsoil in the pots and the river sand in the pits whiles the holes in the spread polythene sheet ensured drainage down the pit. Watering was not direct to the pots, but was applied to the river sand when its water content declined from 'field capacity' (12.5%) to about 10%. In the absence of any rainfall the river sand was able to supply the plants with water for 14 – 18 days depending on the season, the shade regime and the age of the cacao plants.

The experiment consisted of three shade regimes: light shade (32.5% shade, Plate 13), medium shade (55% shade, Plate 14) and heavy shade (76% shade, Plate 15) created by means of (a) the outermost single net (b) the erection of an additional single-netted or (c) double-netted cage, respectively, over the cacao growing in the pits.

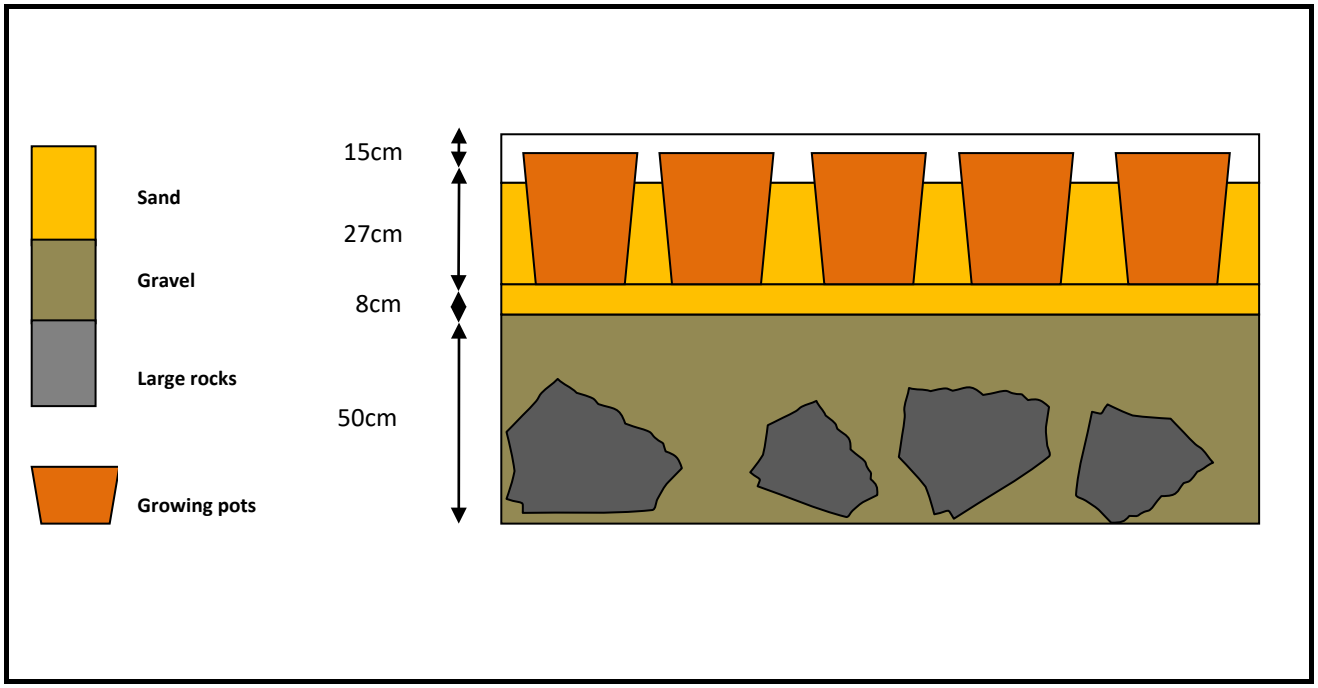

Figure 23 Diagram showing the placement of growing pots in a propagation pit

The experiment was therefore set out as a split-plot with shade as the main plot factor and the four clones as sub-plots. There were five plants per sub plot and each was replicated three times. The layout was as shown in Figure 23.

Plate 13 Cacao growing under the light (32.5%) shade

Plate 14 Cacao growing under the medium shade

Plate 15 The heavy shade situation under which the cacao was grown

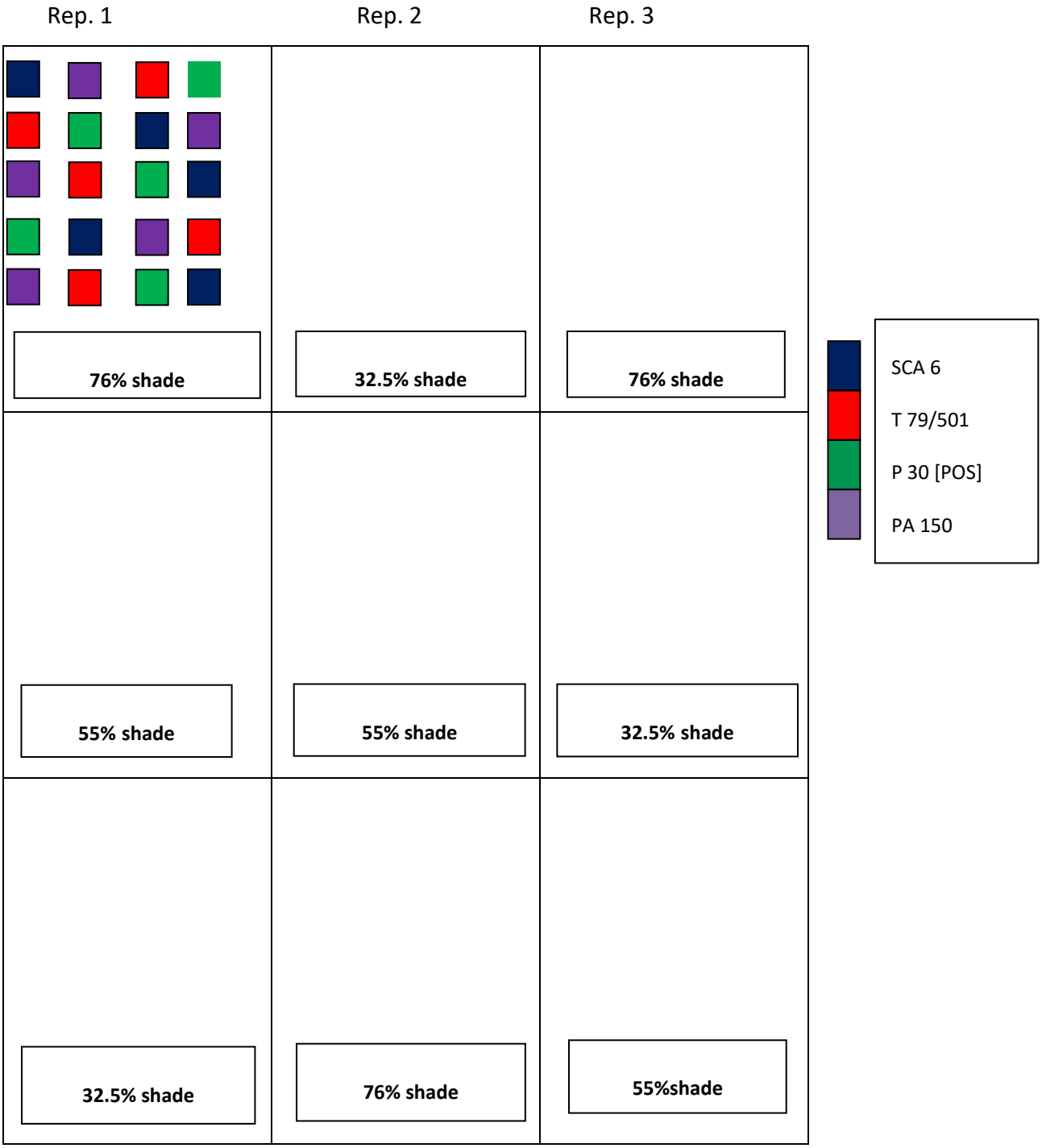

Figure 24 Layout of the shade * genotype nursery experiment

Photosynthesis measurement:

The infra red gas analyzer (LC pro+, ADC Bioscience, UK) was used to measure stomatal conductance and the rates of photosynthesis and transpiration once every month for 12 months

beginning in April 2007 (See section 2.3 for details). Measurements were taken under ambient light.

To reduce effects caused by diurnal fluctuations in photosynthesis, measurements were taken between 9:00 am and 11:00 am. For each replication three out of five plants were tagged and the youngest expanded and hardened leaf of a tagged plant was selected for data collection.

A diurnal study was also made on 28 April 2008 in which an artificial light source provided a constant saturated light intensity (1200 µmol photons $m^{-2}s^{-1}$) to assess diurnal photosynthesis under constant light conditions.

Data on Relative Humidity and Temperature:

Screened Tiny-Tag data loggers (Gemini Data Loggers, Chichester, UK) were used to record the temperature and relative humidity at 15 minute intervals in the three micro-environments of the trial during days of photosynthesis data collection.

Diurnal relative humidity and temperature values for 28 April 2008 are shown in (Figures 24 and 25)

Diurnal relative humidity

The diurnal relative humidity values under the three shade treatments were between 93 and 96 % around 08:30 hours, and declined progressively during the day. Diurnal relative humidity values were generally lower under increasing light (Fig 25).

Diurnal Temperature

Diurnal temperatures ranged between 25 and 26 °C by 08:30 hours under all the shade treatments to 29°C, 30°C and 32°C under the heavy, medium and light shade treatments, respectively at 12:45 hours and then declined to about 24 °C by 16:30 hours (Fig 25).

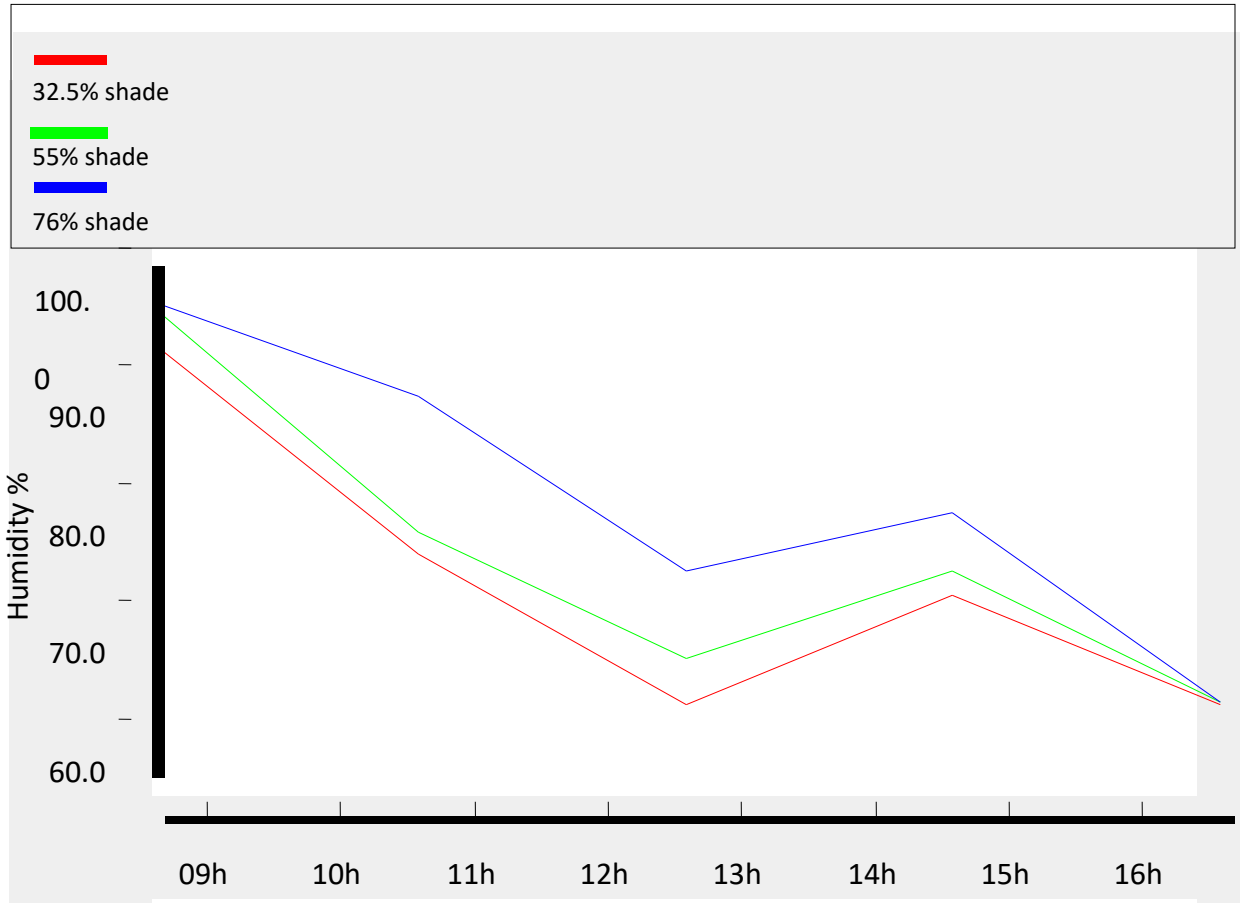

Figure 25 Relative humidity values under the different shade treatments

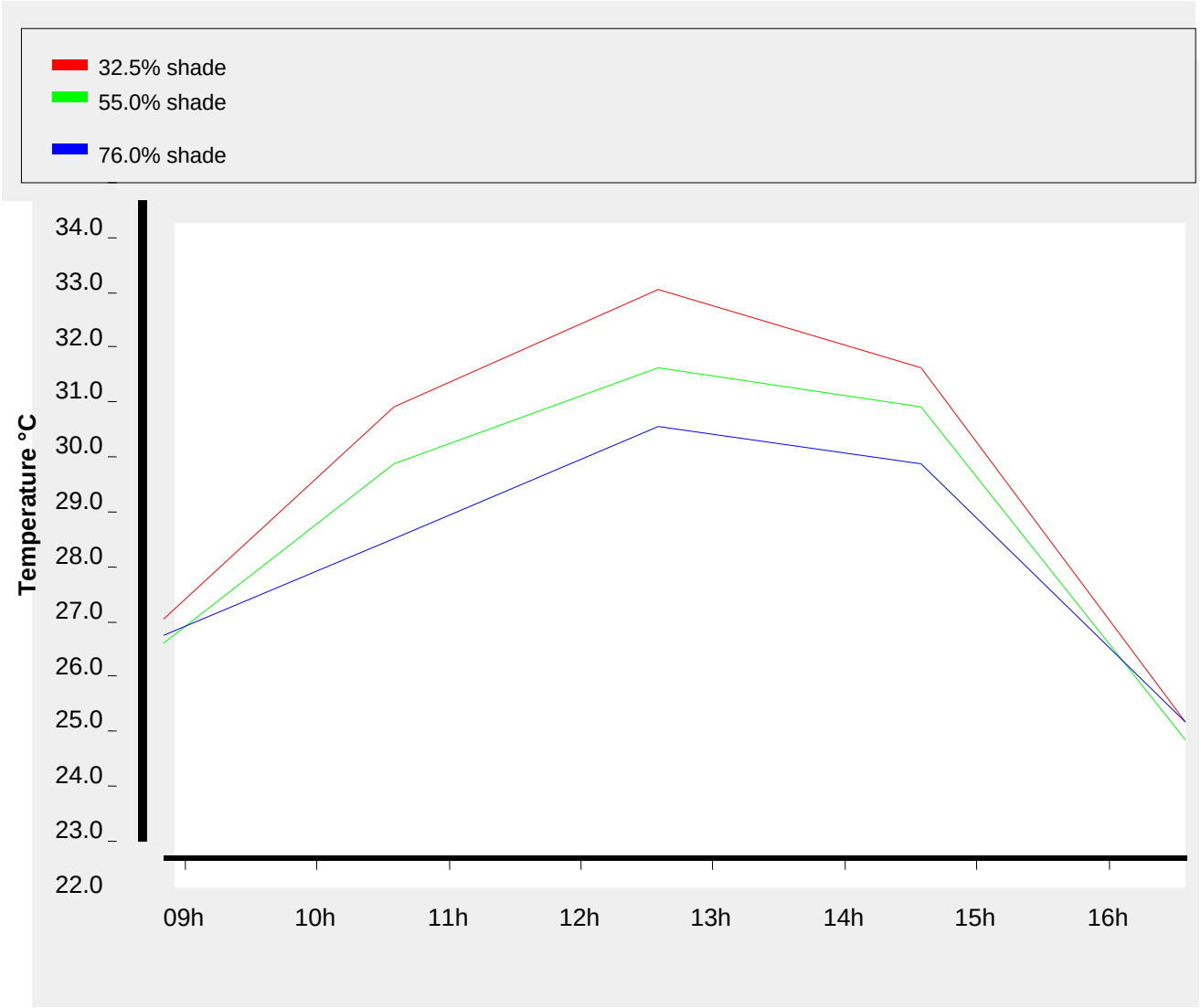

Figure 26 Diurnal aerial temperature under the different shade treatments

Measurement of Leaf Chlorophyll Fluorescence:

Leaf chlorophyll fluorescence was measured using the leaf chlorophyll fluorescence meter (FP100; Photon Systems Instruments, Brno, the Czech Republic) between 11:00 am and 12:15 pm on the same days that photosynthesis measurements were carried out (see section 2.3.1 for details). Leaves that had been selected for photosynthesis measurement were also dark adapted for 30 minutes by means of strips of aluminium foil before leaf chlorophyll fluorescence measurements were taken.

Determination of fresh and dry weight of experimental plants:

At the end of the experiment (in May 2008) two out of the three plants on which growth and physiological measurements had been made per treatment per block during the course of the experiment were randomly selected for destructive harvesting. Selected plants were bulked and their organs were separated for fresh and dry weight determination. Drying was done in a ventilated oven at 80 °C for 60 hours after which no changes in weight were observed.

Determination of leaf area

Before determining dry weights of the organs of experimental plants selected for destructive harvesting fresh leaves of the selected plants were taken for leaf area determination using an automatic leaf area meter (Delta- T Devices Ltd., Burwell in Cambridge, U.K.). The leaf areas of all the leaves of selected individual plants were measured.

Leaf traits

The leaf traits including the leaf chlorophyll concentration, leaf nitrogen and phosphorus contents, relative water content and specific leaf weight were determined as described in the 'General Materials and Methods' chapter (See section 2.3.2 for details).

4.3 Results

In the cacao-growing belt of West Africa three seasons are generally recognized: the major rainy season (March to Mid-July), the minor rainy season (Mid-July to October) and the dry season (November to February) and these are accompanied by changes in climatic factors such as rainfall, relative humidity and temperature (Appendices 1 and 2) that affect plant physiological activity.

Table 3 Mean seasonal relative humidity (% RH) and temperature (T °C) under different shade regimes over the experimental period.

	32.5% shade		55.0% shade		76.0% shade		Mean	
	RH	T	RH	T	RH	T	RH	T
Major rainy season	70.0	36.9	72.8	34.8	75.0	32.2	72.6	34.6
Minor rainy season	64.9	31.9	73.1	31.2	76.8	31.0	71.6	31.3
Dry season	50.2	36.8	51.1	35.2	58.5	33.3	53.3	35.1
Mean	61.7	35.2	65.7	33.7	70.1	32.2	65.83	33.67

Mean aerial temperatures were approximately 3.5^{o}C lower during the minor rainy season compared with the major rainy season and the dry season as a result of overcast conditions that characterise much of the minor rainy season. Also the temperature under the heaviest shade was approximately 3^{o}C lower than under the lightest shade (Table 3).

Over the experimental period the mean relative humidity for the two rainy seasons was 72.1% compared with a mean value of 53.3% for the dry season. The mean relative humidity under the heaviest shade was 70.1% compared with 61.7% under the lightest shade (Table 3).

Stomatal conductance

Stomatal conductance rates varied significantly ($p = 0.001$) between seasons. The highest rate (0.162 mol $m^{-2}s^{-1}$) was observed in the minor rainy season which was 13.6% higher than the mean rate in the major rainy season and 50.2% higher than that of the dry season of the 2007/2008 crop year (Fig 27).

Over the entire experimental period the stomatal conductance of the cacao clones under the medium shade (55% shade) had the highest rate (0.1462 mol $m^{-2}s^{-1}$) and the lightest shade (32.5% shade) having the lowest rate (0.1372 mol $m^{-2}s^{-1}$) ($p = 0.002$). However, stomatal

conductance under the different shade treatments seemed to have been influenced by the seasons. During the dry season, plants under increasing shade had higher stomatal conductance; whereas in the rainy seasons plants that were growing under the heaviest shade (76% shade) had 34% lower mean stomatal conductance rates compared with those under the lightest shade (32.5% shade) during the dry season plants that were growing under the heaviest shade had 50% higher stomatal conductance rates than those under the lightest shade. Thus there was a significant ($p < 0.001$) season * shade interaction on stomatal conductance. Over the entire experimental period, differences in stomatal conductance between cacao clones were not significant. However, a significant ($p < 0.001$) season * clone interaction was observed. With the exception of SCA 6, all the clones had lower stomatal conductance rates in the dry season compared with highest rates obtained in the minor rainy season. P 30 [POS] had an 18.7% reduction in its stomatal conductance during the dry season and T 79/501 and PA 150 had 37.9% and 59.4% reductions, respectively. Thus the reductions (in the dry season as compared with the rates in the minor rainy season) experienced by the clones varied considerably (Fig 27).

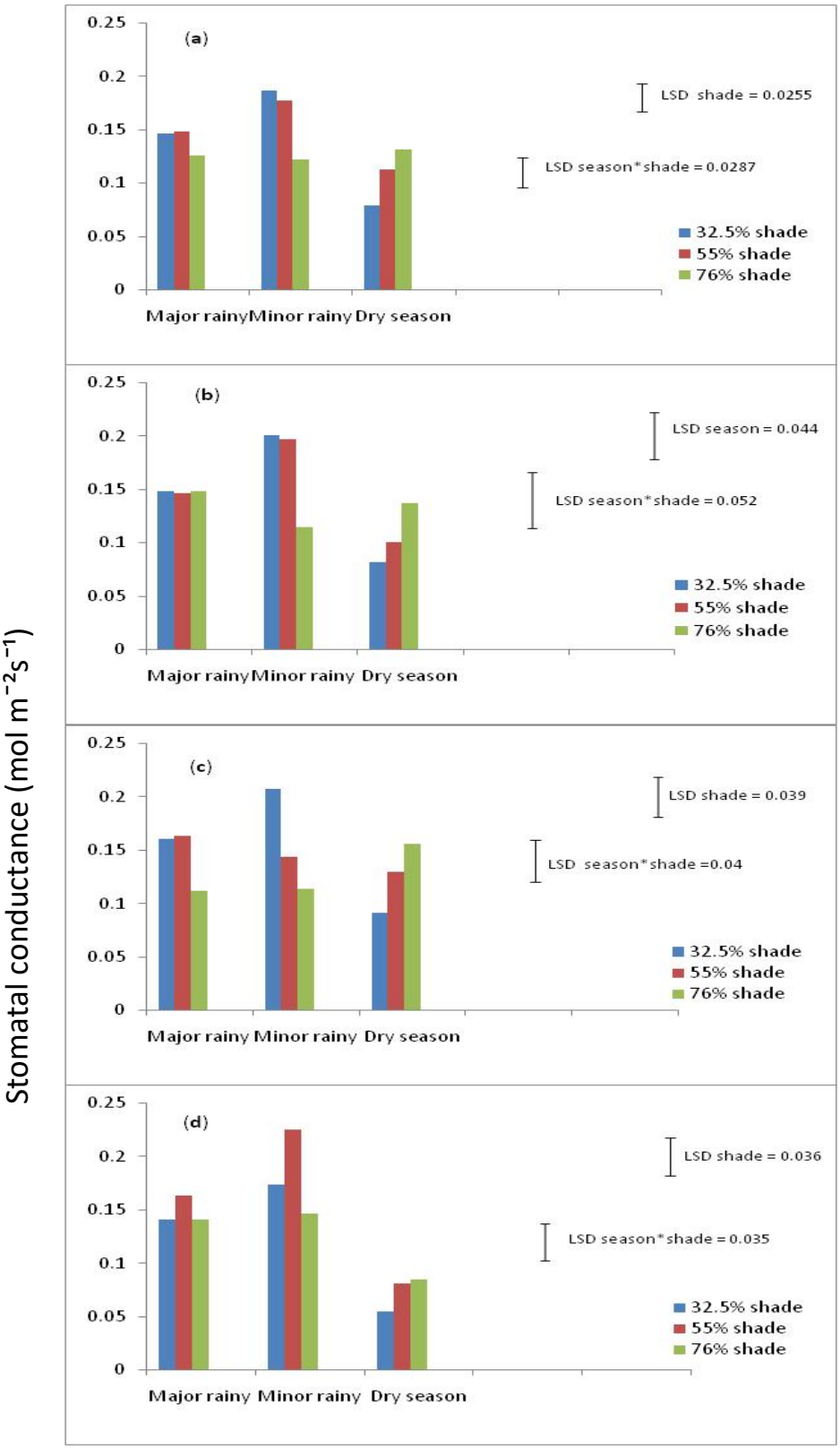

Figure 27 Mean stomatal conductance of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under varying shade levels.

Each bar represents the mean of 12 measurements on three plants per treatment per block.

In the two rainy seasons PA 150 had the highest rates (mean 0.1655 mol $m^{-2}s^{-1}$) whilst SCA 6 had the lowest (with 21.9% lower values than PA 150). T 79/501 and P 30 [POS] had intermediate values (0.1589 mol $m^{-2}s^{-1}$ and 0.1504 mol $m^{-2}s^{-1}$, respectively). In the dry season, however, P 30 [POS] and SCA 6 had the highest rates (above 0.12 mol $m^{-2}s^{-1}$) whilst T 79/501 and PA 150 had 16.2% and 41.4% lower rates than that obtained by P 30 [POS]). The differences between clones were generally consistent under the shade treatments. Therefore, the shade * clone interaction term was not significant.

Transpiration

Similar to the observation on stomatal conductance, transpiration tended to be higher under lighter shade such that under the heaviest shade (76% shade) transpiration was on average, 25.7% less than under the lightest shade (32.5% shade).

The rates of transpiration were significantly ($p < 0.001$) different between the seasons (Fig 28). A mean rate of 2.066 mmol $m^{-2}s^{-1}$ was observed in the major rainy season which was 50.2% higher than the rates observed in the dry season. Inverse responses on transpiration were observed under the shade treatments as the seasons changed. In the rainy seasons the lowest transpiration rates were observed under the heaviest shade; in the dry season, however, the heaviest shade had plants with the highest rate (1.2 mmol $m^{-2}s^{-1}$) which was 56.5% higher than rates observed under the lightest shade. Thus the season * shade interaction term was significant ($p = 0.001$).

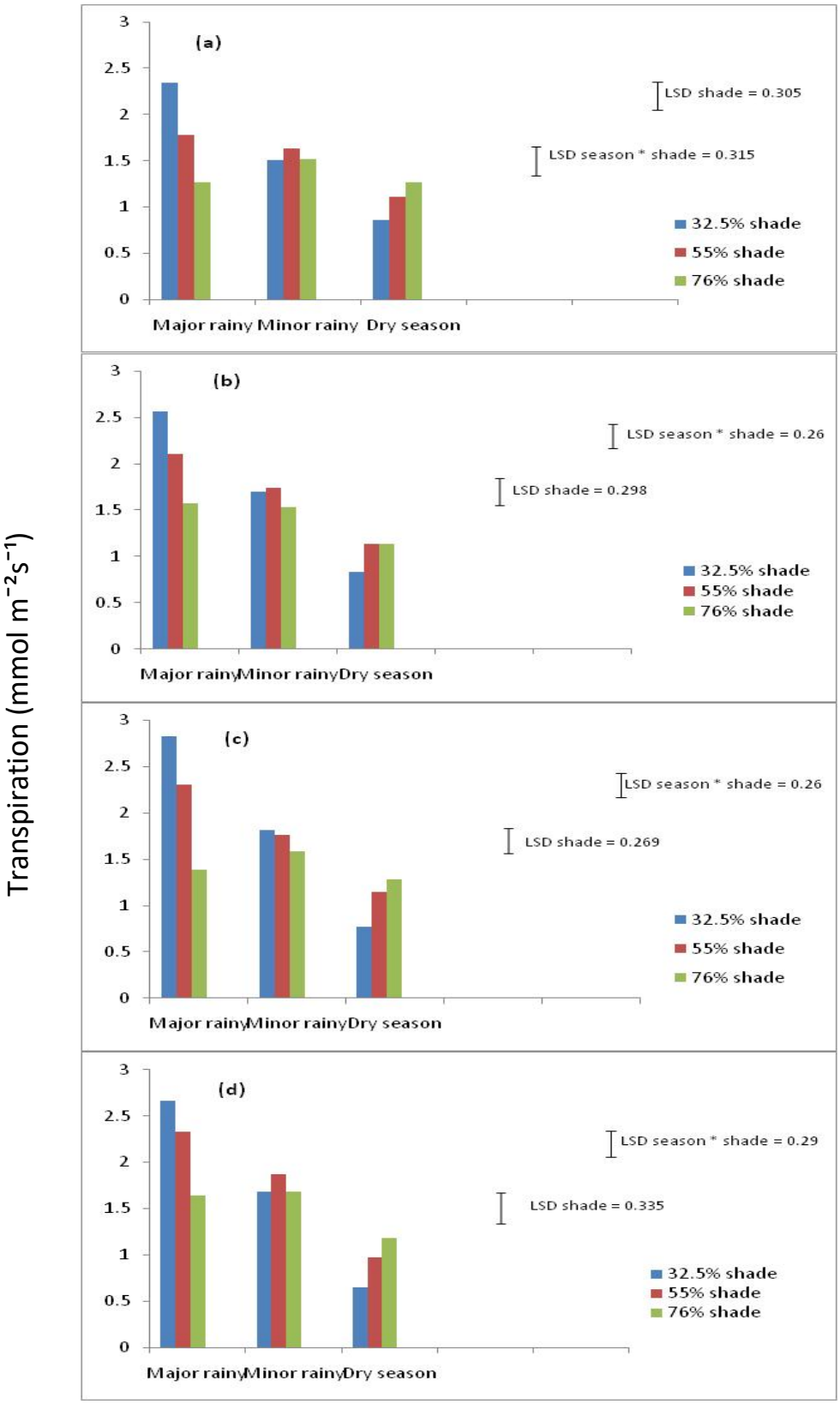

Figure 28 Transpiration of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and in different seasons.

Each bar represents the mean of 12 measurements on nine plants per treatment.

There were significant ($p < 0.001$) differences in mean transpiration rates between the clones over the experimental period (Fig 28). P 30 [POS], PA 150, T 79/501 and SCA 6 had mean transpiration rates of 1.654 mmol $m^{-2}s^{-1}$, 1.632mmol $m^{-2}s^{-1}$, 1.589 mmol $m^{-2}s^{-1}$ and 1.476 mmol $m^{-2}s^{-1}$, respectively. This order was maintained under all the shade treatments. Hence the shade * clone interaction term was not significant. However, changes occurred in their respective ranking positions of the clones in each season, so that SCA 6 which had the lowest transpiration rate during the rainy seasons (mean = 1.674 mmol $m^{-2}s^{-1}$) had the highest rate (mean = 1.081 mmol $m^{-2}s^{-1}$) during the dry season. In the dry season, its transpiration rate was 1.2% higher than that of P 30 [POS], 4.4% higher than that of T 79/501 and 13.6% higher than the rate for PA 150. Hence the season * clone interaction term was significant ($p < 0.001$) (Fig 27). The season * shade * clone interaction term was not significant.

Photosynthesis

The highest photosynthetic rates (mean = 3.37 µmol $m^{-2}s^{-1}$) were observed in the major rainy season ($p < 0.001$). That was 34.6% higher than the rate for the minor rainy season and 2.9 times higher than the rate during the dry season. Photosynthetic rates were significantly ($p < 0.001$) different under the shade treatments so that plants under the lightest shade had, on average, a rate (3.26 µmol $m^{-2}s^{-1}$) which was 34.6% higher than that of the medium shade and 3.16 times as high as that of the heaviest shade. However, season seemed to have influenced photosynthesis under the different shade treatments. During the dry season (unlike the rainy seasons), plants under the medium shade had the highest rate of photosynthesis (0.823 µmol $m^{-2}s^{-1}$) which was 4.7% higher than the rate for the heaviest shade and 62.8% higher than that of the lightest shade. The season * shade interaction term was, therefore, significant ($p < 0.001$).

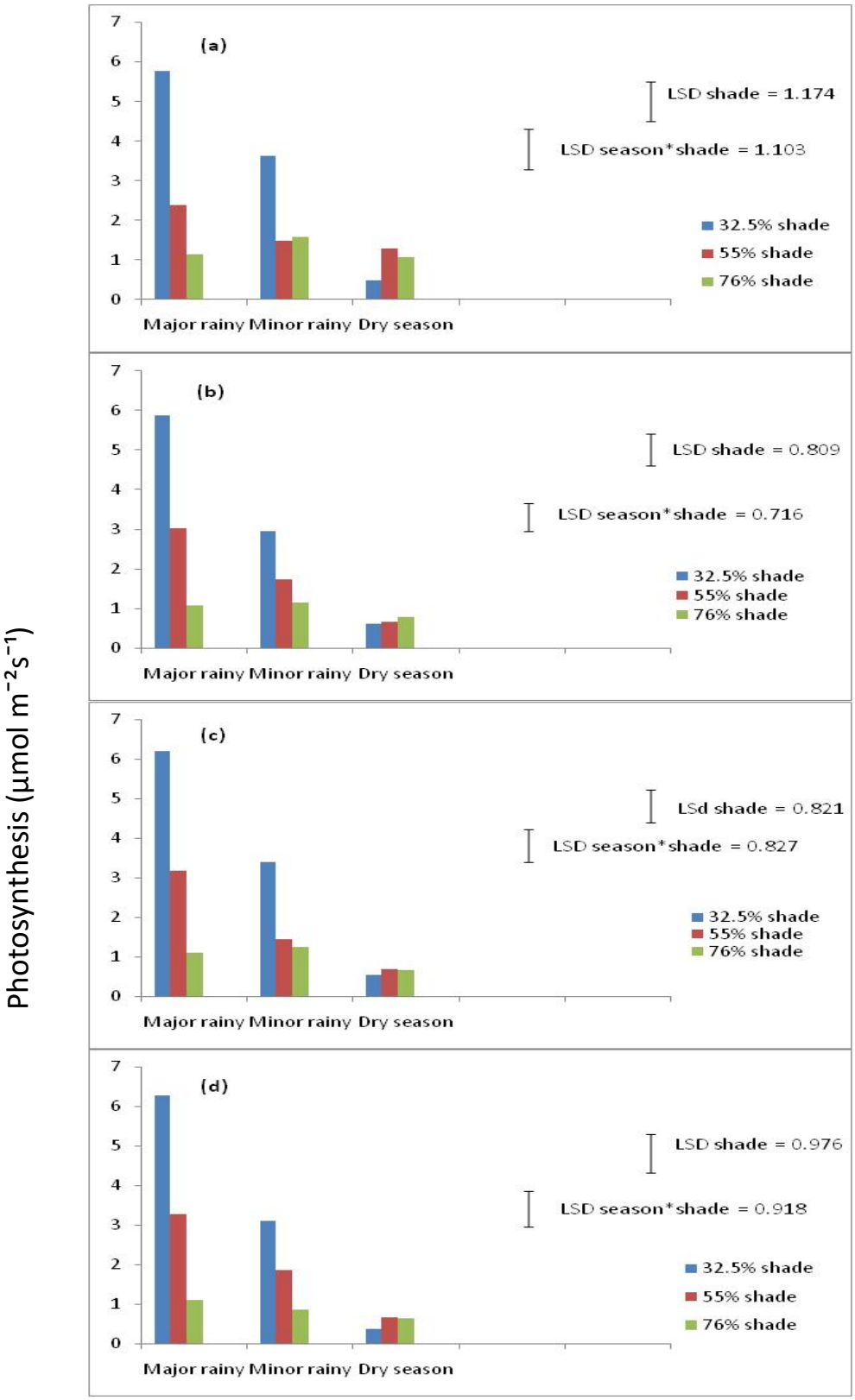

Figure 29 Photosynthesis of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and in different seasons.

Each bar represents the mean of 12 measurements on nine plants per treatment.

Stomatal conductance had a positive relationship with the rates of photosynthesis in all the cacao genotypes. Although when averaged over the shade and season treatments, clones were not significantly different in different seasons, some changes were observed in the order of the clones such that PA 150 which had the highest rate (3.54 μmol $m^{-2}s^{-1}$) during the major rainy season had the lowest rate (0.551 μmol $m^{-2}s^{-1}$) in the dry season whereas SCA 6 which had the lowest rate (3.11 μmol $m^{-2}s^{-1}$) during the major rainy season had the highest rate (0.957 μmol $m^{-2}s^{-1}$) in the dry season. All the clones had their lowest photosynthetic rates in the dry season (Fig 29). Similarly, all the clones had their highest mean photosynthetic rates under the lightest shade and their lowest rates under the heaviest shade. Thus the shade * clone and season * clone interaction terms were not significant and there were no significant second order interactions.

Leaf Chlorophyll Fluorescence

Chlorophyll fluorescence values did not vary between the seasons.

Fv/Fm of the cacao plants increased significantly ($p < 0.001$) with increasing shade (Fig 29). The heaviest shade (76% shade) had plants with an Fv/Fm value of 0.7184 which was 23.6% higher than the ratio for plants under the lightest shade. Although plants under all the shade treatments had lower Fv/Fm values in the dry season (compared with their respective Fv/Fm values in the major rainy season) the magnitudes of the decrease were not equal. For plants under the heaviest shade, their mean Fv/Fm was 3.8% lower during the dry season than during the major rainy season whereas under the lightest shade plants experienced a 7% decrease during the dry season compared their values during the major rainy season. Thus the season * shade interaction element was significant ($p = 0.013$) (Fig 30).

There were significant ($p = 0.006$) differences between the cacao genotypes with their Fv/Fm values ranging from 0.6434 (for T 79/501) to 0.6689 (for SCA 6). These differences were con-

sistent through the seasons and the season * clone interaction term was not significant. The shade * clone interaction component was also not significant. All the clones had higher Fv/Fm values under increasing shade.

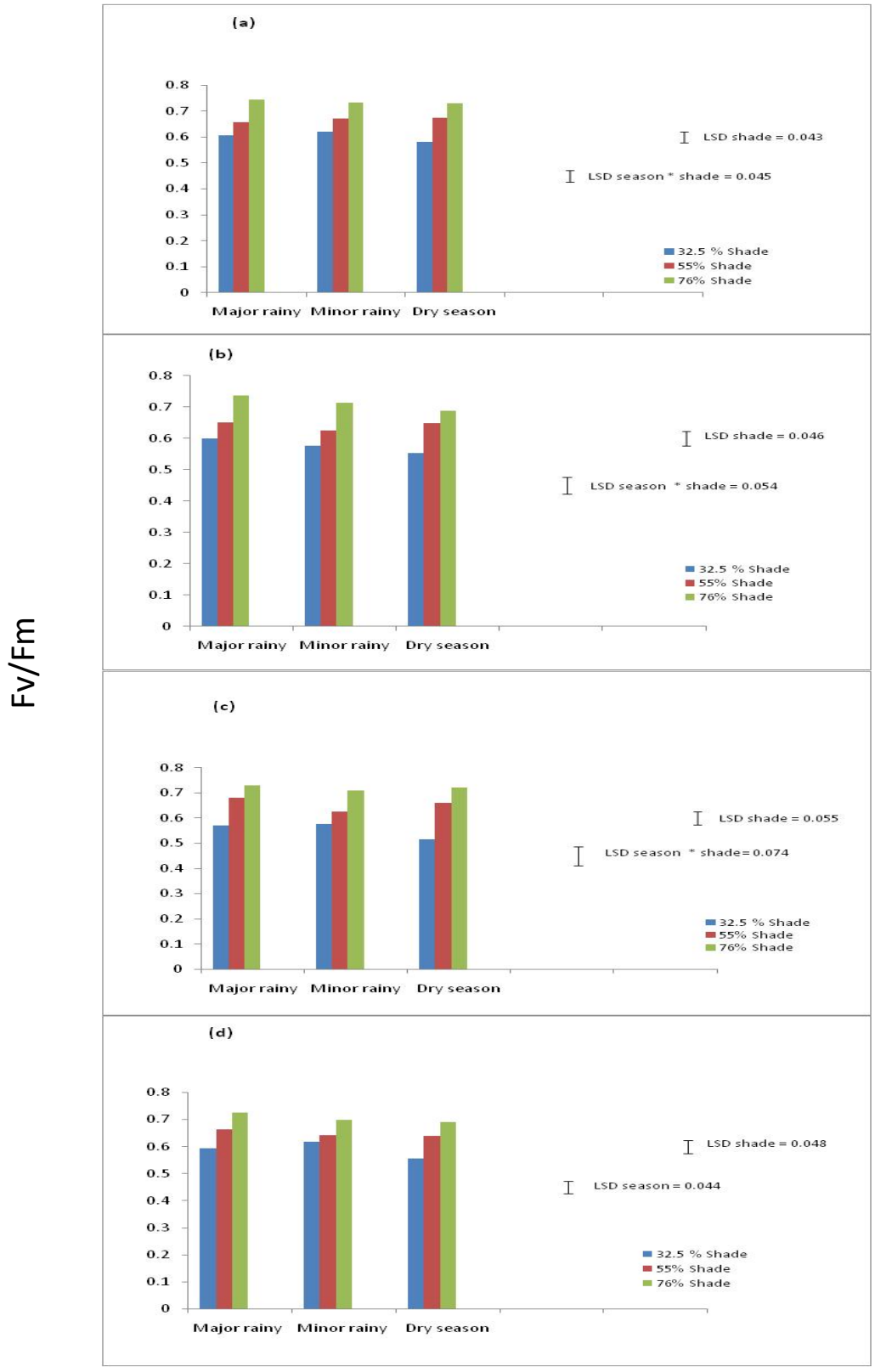

Figure 30 Leaf chlorophyll fluorescence of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade levels and in different seasons.

Each bar represents the mean of 12 measurements on three plants per treatment per block.

4.3.1 Diurnal stomatal conductance

Stomatal conductance decreased as the day progressed. For all the cacao genotypes there was a striking drop in stomatal conductance between 8:00 and 10:00. In addition, stomatal conductance declined with increasing shade level ($p < 0.001$) so that the highest mean stomatal conductance (0.2124 mol $m^{-2}s^{-1}$) occurred under the lightest shade and was 30.7% and 77.2% greater than the values under the medium and heaviest shades, respectively (Fig 31).

A significant ($p = 0.018$) time * shade interaction was observed. Although there were consistent differences in stomatal conductance between the shade levels, the magnitude of the differences became smaller as the day progressed.

The shade * clone interaction term was not significant and there were no significant second order interactions.

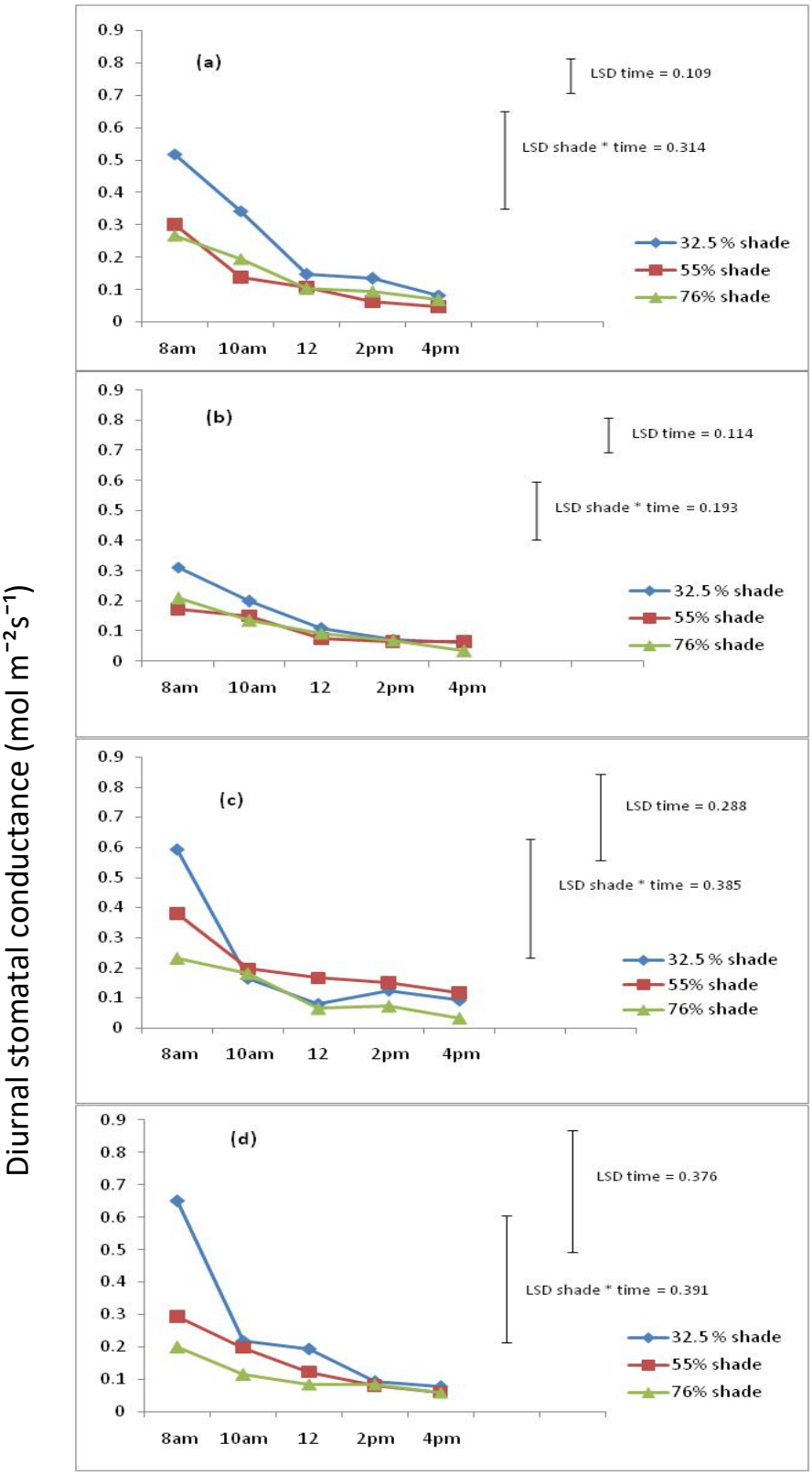

Figure 31 Diurnal stomatal conductance of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade levels.

Each line describes the response of 3 plants per shade treatment per block.

Diurnal Transpiration

Diurnal transpiration in the plants did not change significantly with time or shade level. Also the time * shade interaction component was not significant (Fig 32). Significant ($p = 0.033$) differences in transpiration rates were observed between clones (Fig 32). T 79/501 had a transpiration rate of 1.69 mmol $m^{-2}s^{-1}$ which was over 16% lower than those of the other clones.

No significant time * clone interactions were observed. However, the shade * clone interaction term was significant ($p = 0.012$). Whereas T 79/501 maintained the same rate (1.6 -1.7 mmol $m^{-2}s^{-1}$) PA 150 had a 26.1% reduction in transpiration rate under the heaviest shade (Fig 31).

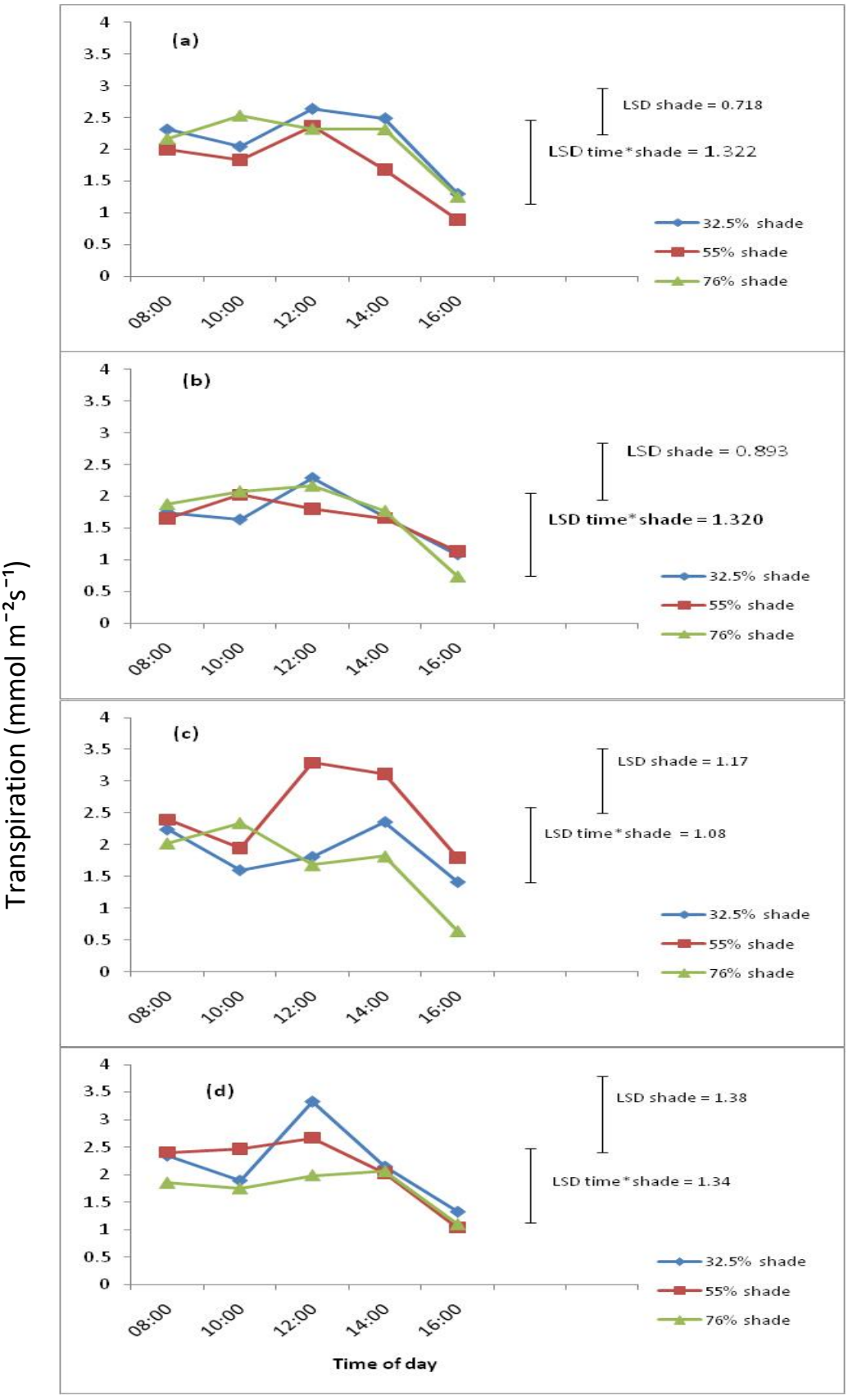

Figure 32 Diurnal transpiration of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade intensities.
Each line describes the response of three plants per shade treatment per block

Diurnal Photosynthesis

Significant ($p = 0.004$) differences were observed in photosynthesis with time, higher rates occurring during the first half of the day (between 8:00 and 12:00 hours). The peak rates of light-saturated photosynthesis (4.51 µmol $m^{-2}s^{-1}$) were recorded around 10:00 hours after which time the rates declined steadily till the end of the day ($p = 0.004$) (Fig 33).

Photosynthetic rate generally increased with reducing shade level ($p < 0.001$). The time * shade interaction component was not significant (Fig 33).

There were no significant differences between the cacao genotypes.

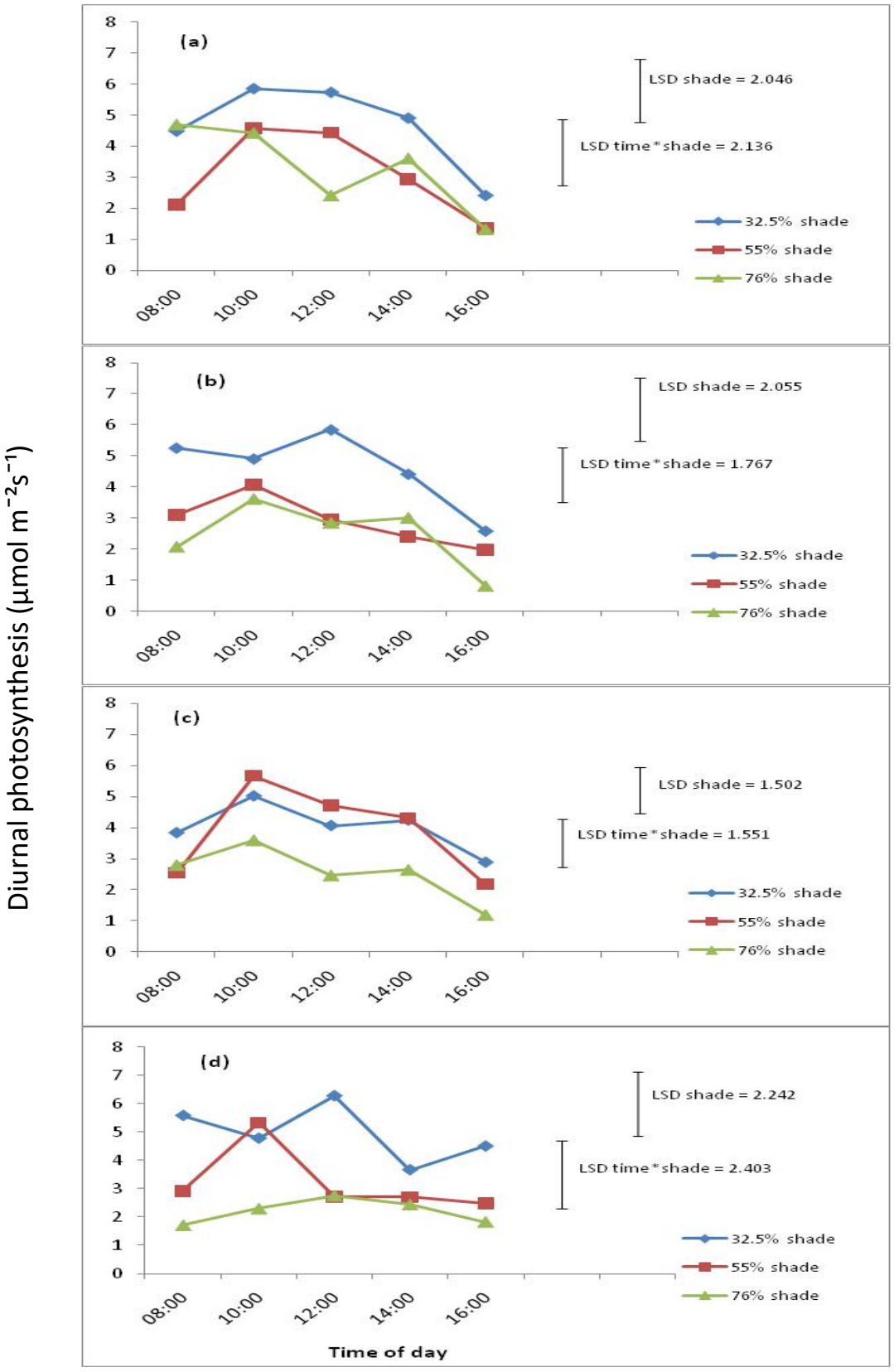

Figure 33 Diurnal photosynthesis of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under varying shade.

Each line describes the response of three plants per shade treatment per block

The shade * clone interaction component was significant (p = 0.02). SCA 6 experienced a smaller reduction in photosynthesis under increasing shade than the other clones. The photosynthetic rate of SCA 6 under the lightest shade was 29.6% higher than under the heaviest shade. On the other hand photosynthetic rate of the other clones declined between 37% and 55% (compared with their respective rates under the lightest shade). The time * clone interaction term and the second order interactions were not significant.

Diurnal leaf chlorophyll fluorescence

Overall mean Fv/Fm declined from 0.6774 at 8:00 hours to 0.6250 at 10:00 (when photosynthesis was highest) and then it began to rise steadily (until 16:00) to a maximum of 0.7373 (p=0.001) (Fig 34).

The mean Fv/Fm value under the heaviest shade was (0.7237) being 20% higher than under the lightest shade (Fig 34) (p=0.001). The time * shade interaction element was not significant. Differences between the leaf chlorophyll fluorescence of the clones were significant (p = 0.001) (Fig 34). T 79/501 had the highest mean chlorophyll fluorescence value (0.6864) and PA 150 had the lowest value. A significant (p = 0.001) shade * clone interaction was observed. Although for all the cacao clones, plants under the heaviest shade had the highest Fv/Fm value, the magnitudes of the difference that occurred as light levels increased were different for the clones. Under the lightest shade (32.5% shade) SCA 6 and PA 150 had lower Fv/Fm values than under the other two shade treatments. For T 79/501 and P 30 [POS] Fv/Fm values declined significantly under both 32.5% and 55% shade levels.

The time * clone interaction term was not significant. The highest leaf chlorophyll fluorescence values were observed at 16:00 hours when photosynthesis was lowest (Fig 34).

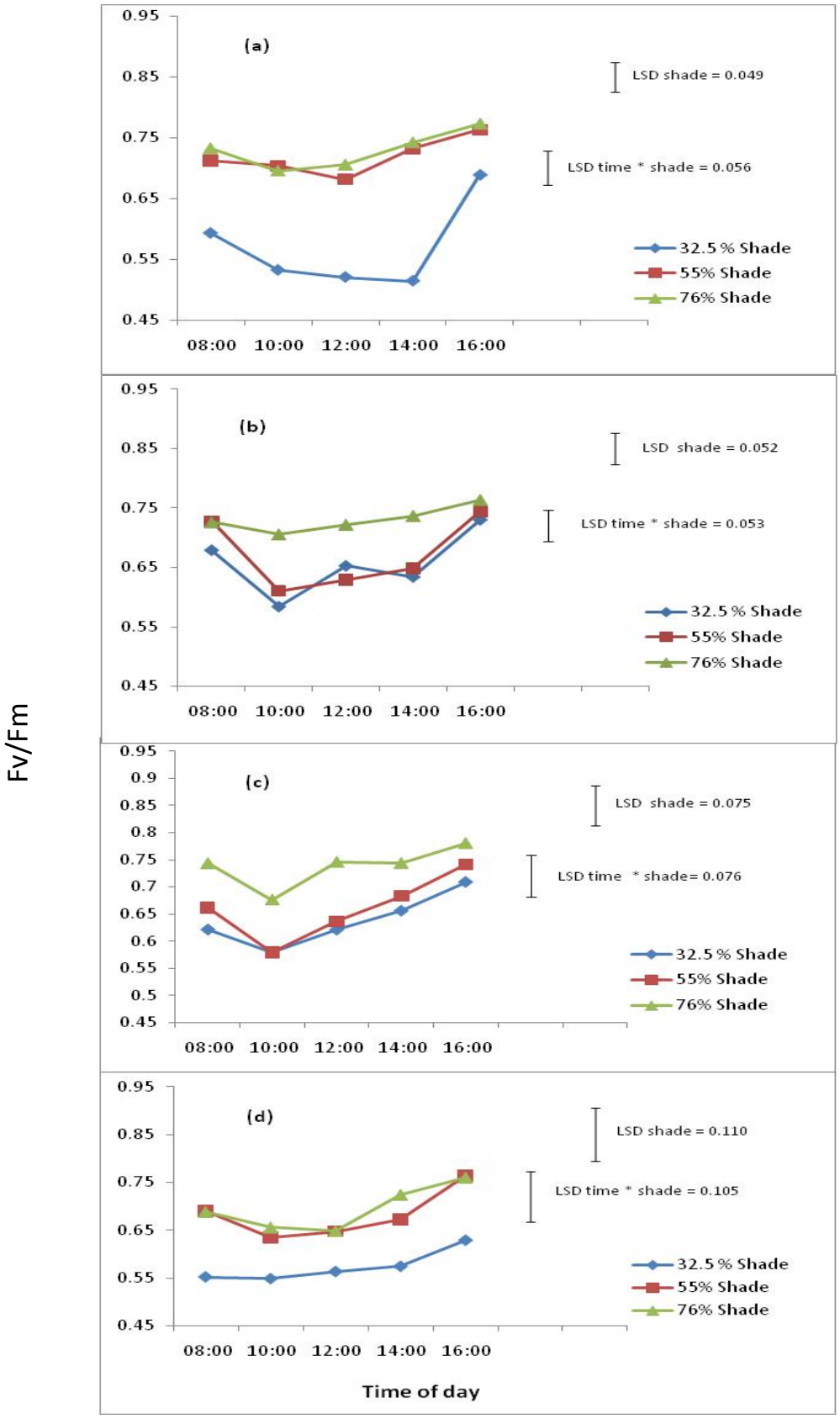

Figure 34 Diurnal leaf chlorophyll fluorescence of (a) SCA6 (b) T 79/501 (c) P 30 [POS] and PA 150 under varying shade.

Each line describes the response of three plants per shade treatment per block

4.3.2 Leaf traits

Leaf Nitrogen

Leaf nitrogen concentrations ranged from 1.91% to 2.01% and were not significantly different under the shade treatments.

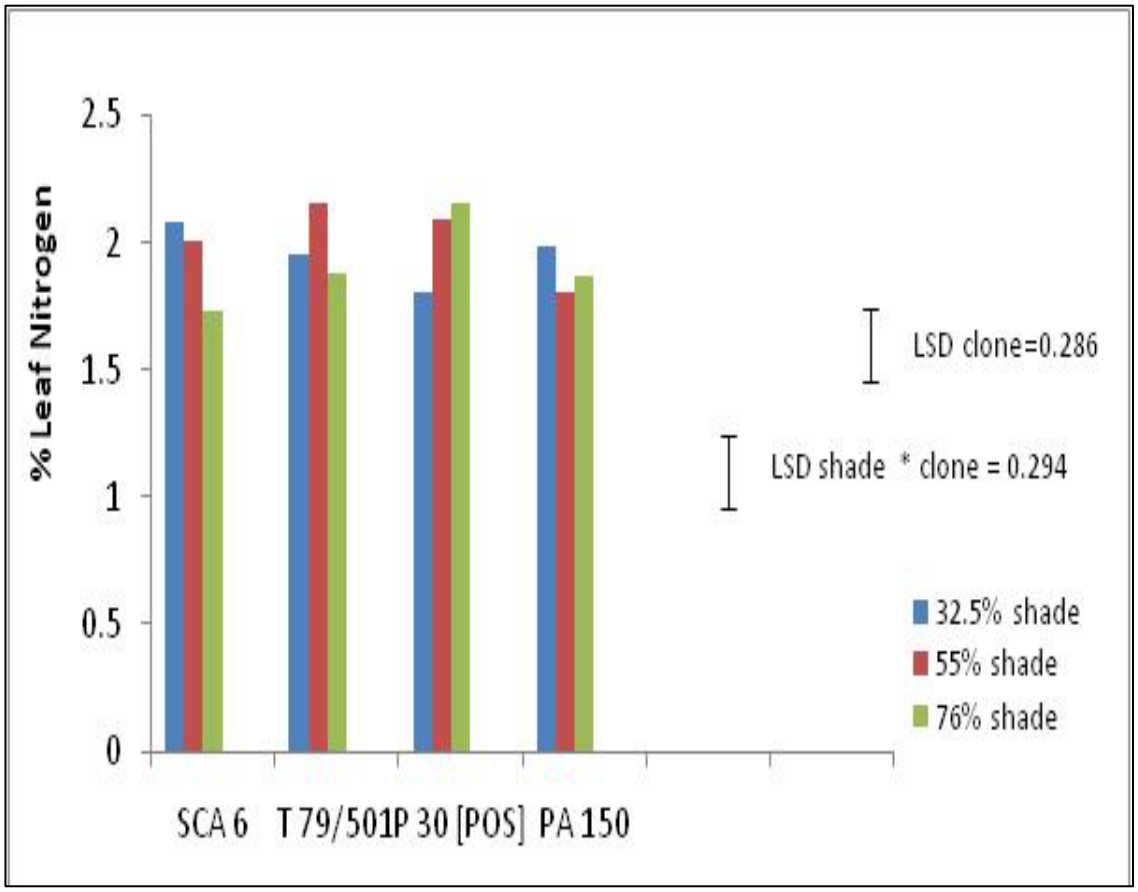

Figure 35 Leaf nitrogen concentration of cacao clones under different shade treatments.

There were also no significant differences between cacao genotypes. However, there was a significant shade * clone interaction ($p = 0.035$) so that SCA 6 plants that were under 76% shade had a 20% reduction in leaf nitrogen concentration whilst P 30 [POS] plants experienced a 19% increase in leaf N concentration. In contrast, N content was not much affected by shade in T 79/501 and PA 150 (Fig 35).

Leaf chlorophyll concentration

Although there were no significant differences in leaf chlorophyll concentrations between clones (Fig 35), chlorophyll content increased significantly with increased shade level($p = 0.032$). Chlorophyll content was 53.5% higher under the heaviest shade than the value for plants under the lightest (Fig 36).The shade * clone interaction term was not significant.

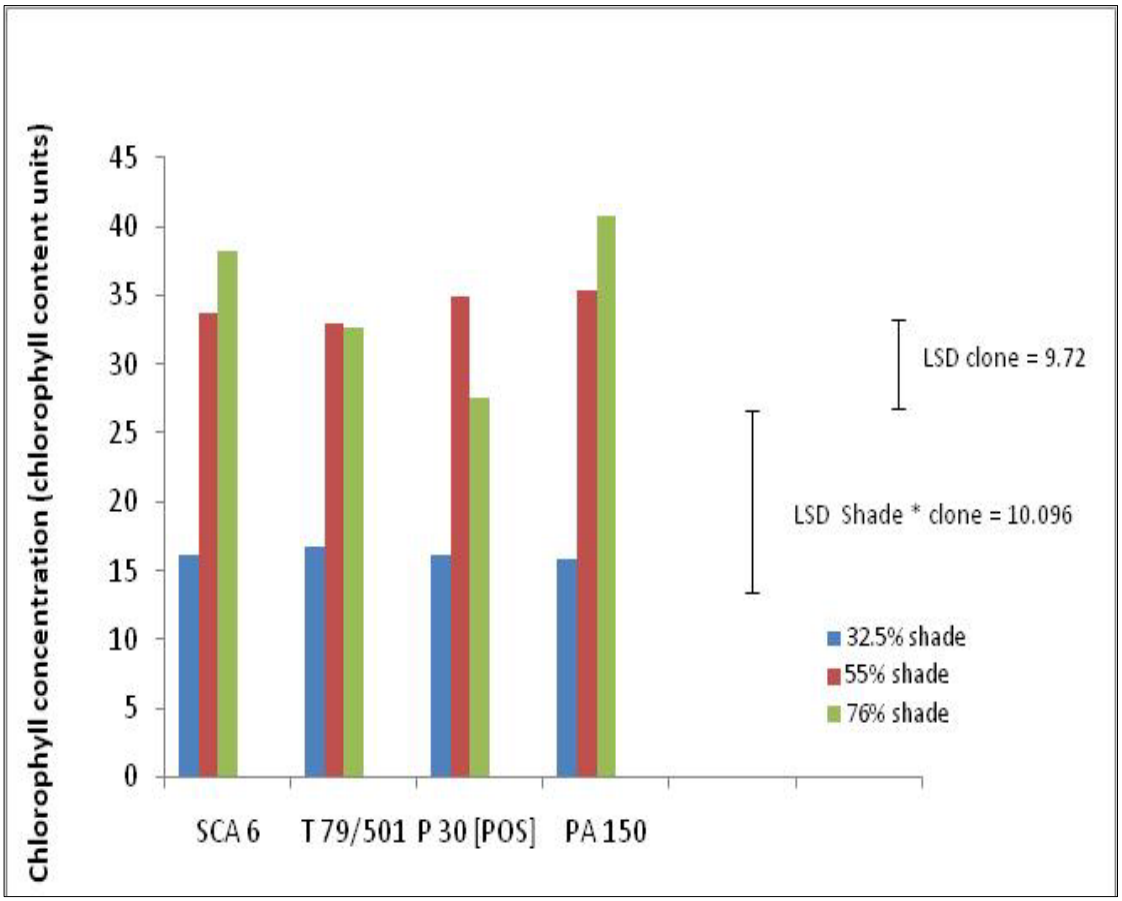

Figure 36 Leaf chlorophyll concentration of cacao genotypes under different shade levels

Leaf Phosphorus Concentration

No significant differences in cacao leaf phosphorus concentration were observed under the shade treatments. However, there were significant ($p < 0.001$) differences in leaf phosphorus concentrations between clones so that T 79/501 and PA 150 had a significantly higher phosphorous content than P 30[POS] and SCA 6 (Fig 37). The shade * clone interaction term was

not significant.

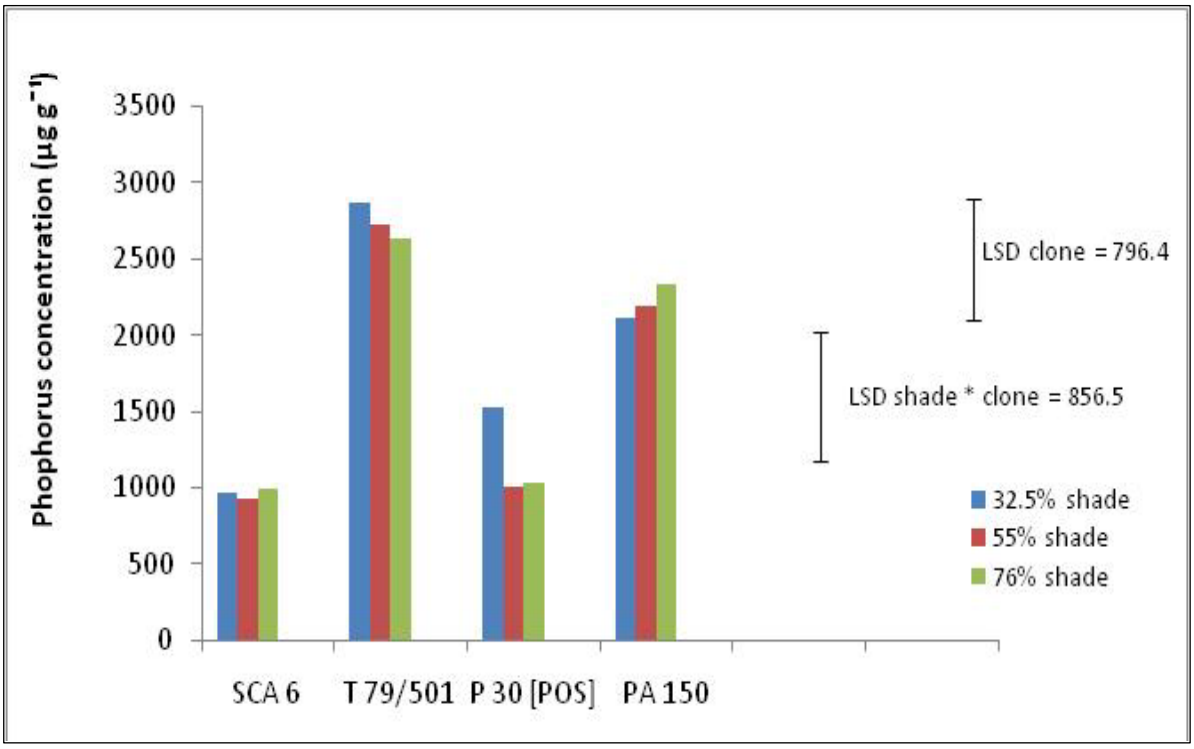

Figure 37 Leaf phosphorus concentration of cacao genotypes under different shade levels

Relative water content

There were no significant differences between the cacao genotypes or the shade treatments in the relative water content of plants. However there was a significant (p = 0.003) shade * clone interaction term so that whereas the relative water content of SCA 6 and PA 150 increased with increasing shade, the other clones showed no consistent trends under the shade treatments (Fig 38).

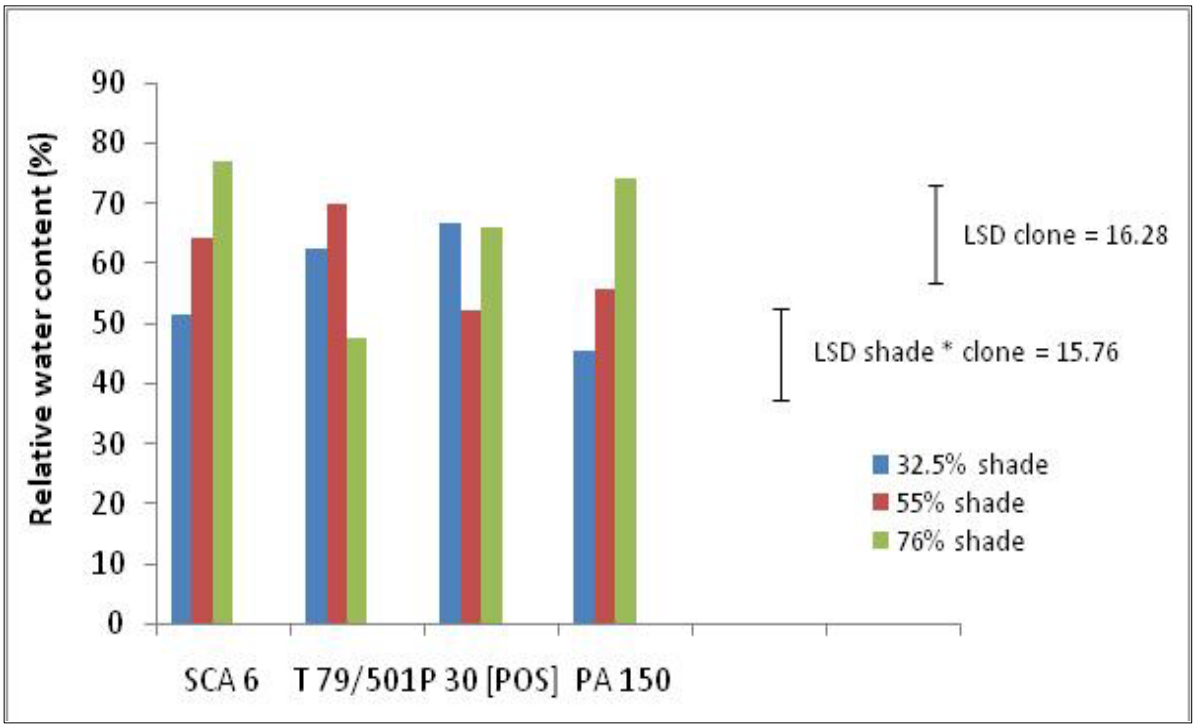

Figure 38 Relative water content of the leaves of cacao clones under varied shade levels

Specific leaf weight

Although the cacao clones did not differ significantly on specific leaf weight, plants that grew under increasing shade showed a reduction in the specific leaf weight so that the mean value was 44.1% higher under the lightest shade than that under the heaviest shade. The shade * clone interaction component was not significant (Fig 39).

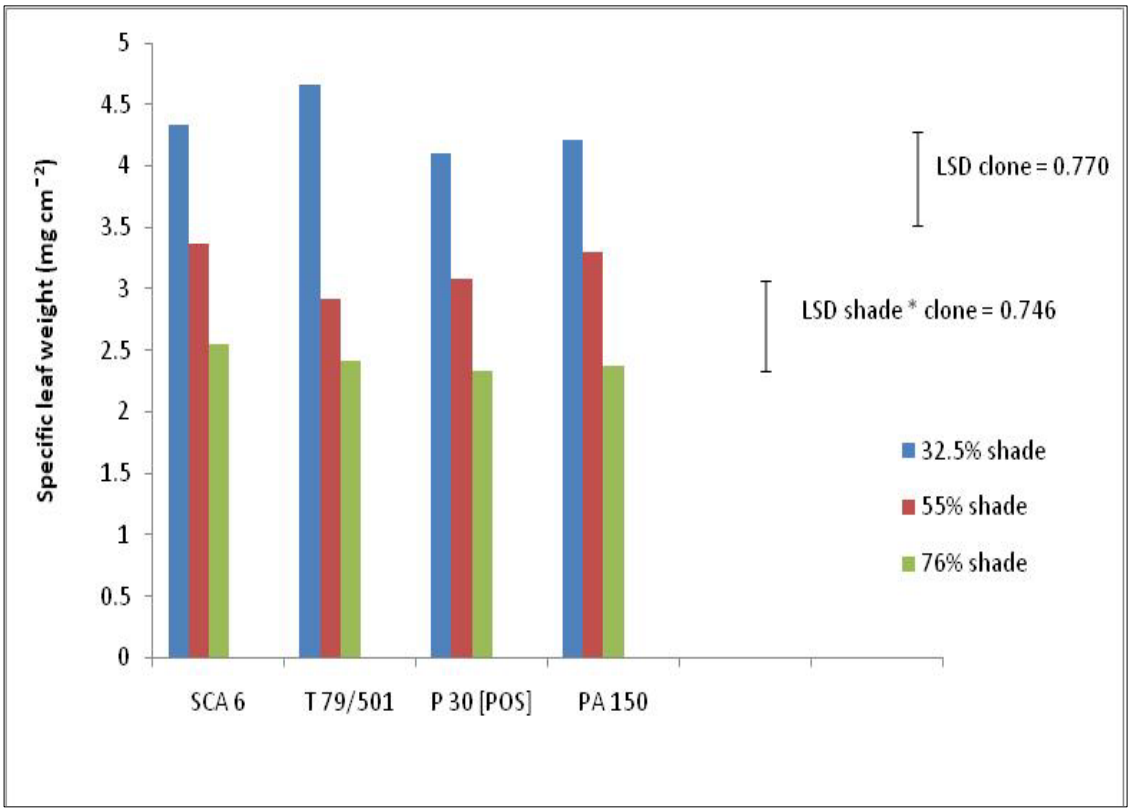

Figure 39 Specific leaf weight of 12 month old cacao under different shade levels

4.3.3 Growth parameters

Leaf number

The number of leaves produced per plant differed significantly (p = 0.002) under the shade treatments, plants under heavier shade having smaller numbers. At the time of destructive sampling, the lightest shade treatment had plants with a mean of 202 leaves which was 39.4% and 76.8% more than the number for plants under the medium and the heaviest shade treatments, respectively (Fig 40).

There were also differences (p = 0.009) between clones in leaf number. SCA 6 had the highest number of leaves (177) per plant. That was 23.9%, 40.0% and 41.2% more than P 30 [POS], PA 150, and T 79/501, respectively. The shade * clone interaction term was not significant (Fig 40).

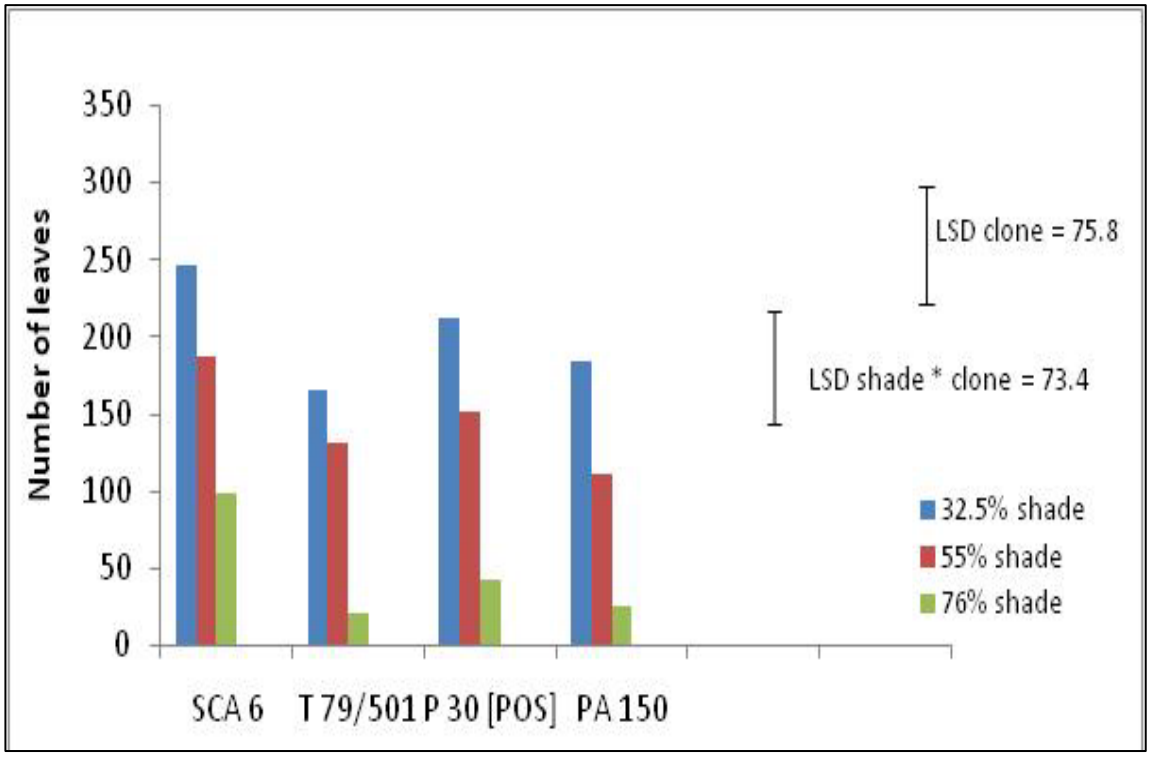

Figure 40 Number of leaves produced/plant by cacao under different shade levels Each bar represents the mean value for six plants

Individual leaf size

Plants under different shade treatments had significantly different (P = 0.013) mean leaf sizes, with those under heavier shade producing bigger individual leaves. The lightest shade had plants with a mean leaf size of 128.16 cm² which was 7.7% and 22.6% smaller than the leaf sizes of plants under the medium and heaviest shade treatments, respectively (Fig 41).

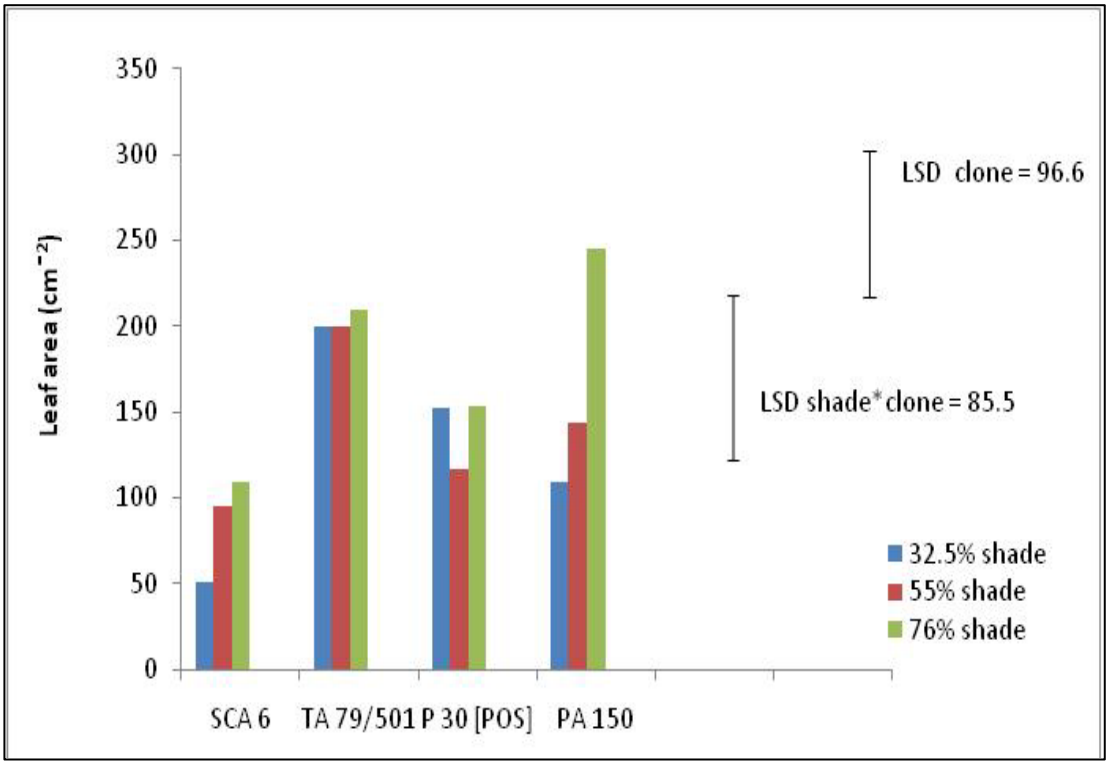

Figure 41 Individual leaf size of cacao plants under different shade intensities Each bar represents the mean value for six plants

There were also significant (p = 0.003) differences between clones. PA 150, T 79/501, P 30 [POS] and SCA 6 had mean sizes of 166.09 cm², 202.99 cm², 140.86 cm² and 85.05 cm², respectively (Fig 41). The mean leaf size of T 79/501 was not very different under varying shade and P 30 [POS] did not show consistent results under the shade treatments. However, both SCA 6 and PA 150 consistently had increasing leaf sizes with increasing shade. The shade * clone interaction element was marginally significant (p = 0.053).

Leaf area per plant

Differences between clones in leaf area per plant were on the borderline of significance (p = 0.05). The leaf areas were 1.31 m², 1.32 m², 1.91 m² and 2.34 m² for SCA 6, PA 150, P 30 [POS] and T 79/501, respectively.

The shade * clone interaction term was significant (p = 0.048) with SCA 6 and T 79/501 having their biggest leaf areas under the medium shade and P 30 [POS] and PA 150 having their

biggest values under the lightest shade (Fig 42).

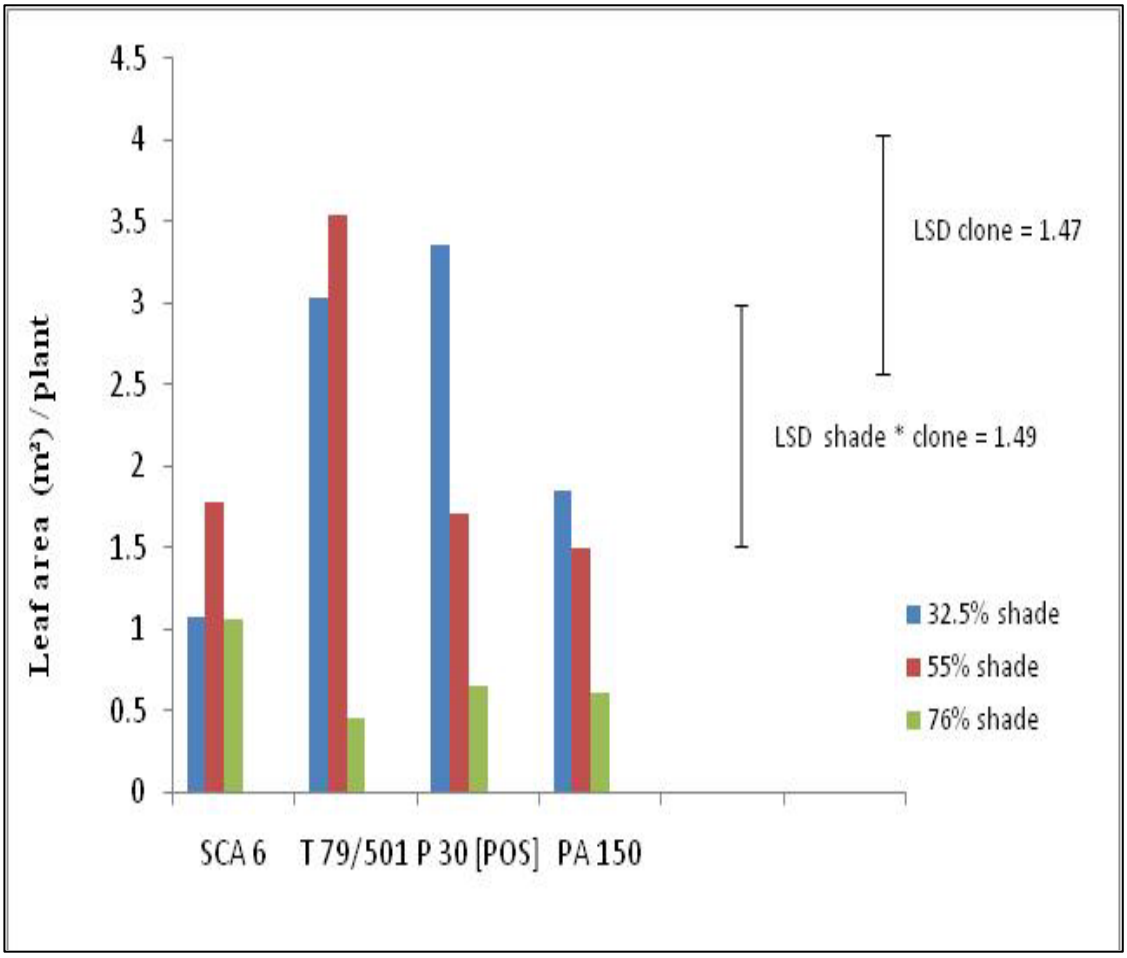

Figure 42 The leaf area per plant under different shade levels
Each bar represents the mean value for six plants

Leaf dry weight / plant

Similar to the observation on leaf area, plants under heavier shade had lower ($p = 0.05$) total leaf dry weight so that those under the lightest shade had a total leaf biomass that was 8.1 times heavier than that of those under the heaviest shade (Fig 43).

There were no significant differences between clones and the shade * clone interaction term was also not significant.

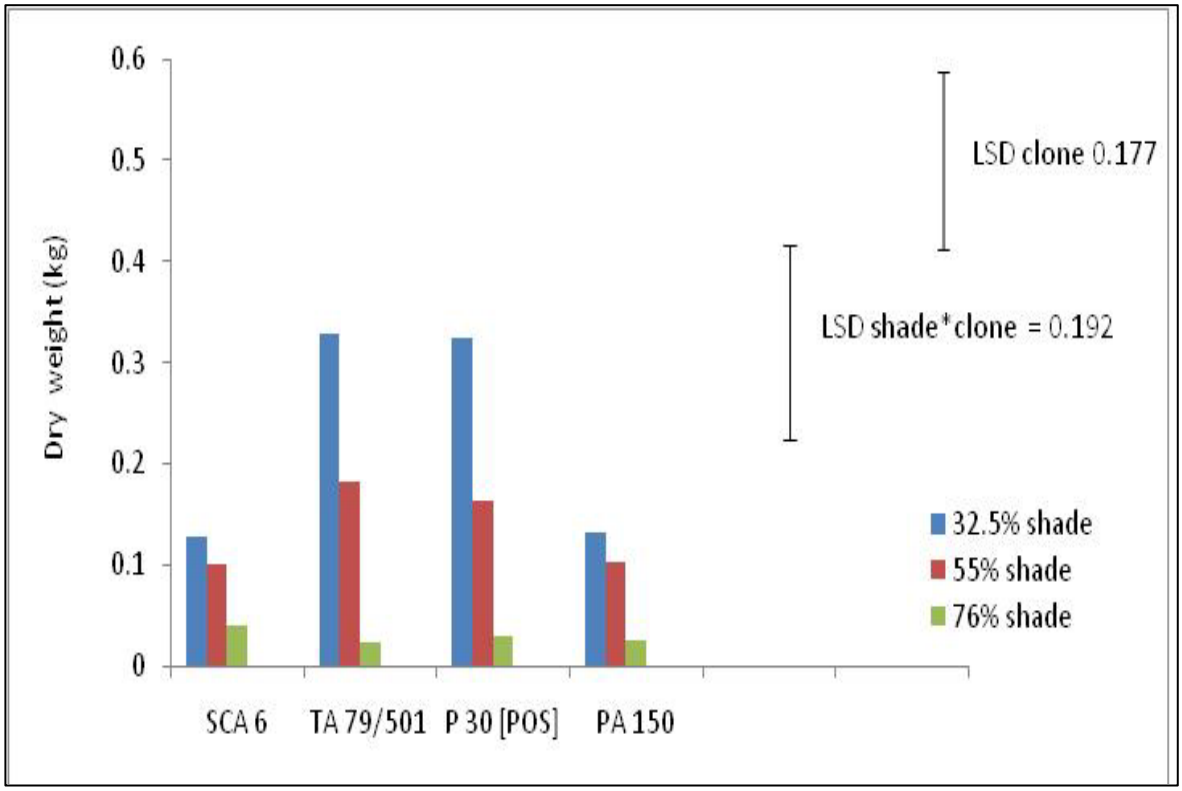

Figure 43 Leaf dry weight of cacao plants under different shade levels
Each bar represents the mean value for six plants

Stem girth

At the end of the experiment the stem diameter of the plants differed significantly (P = 0.001) under the shade treatments with plants under heavier shade having smaller stem diameters (Fig 44). The lightest shade regime had plants with a mean stem diameter of 23.51mm. That was 32.6% and 52.9% greater than those of plants growing under the medium and heaviest shade treatments, respectively.

The clones also had significant differences between them (p = 0.032). The biggest clone, T 79/501 had a mean stem diameter of 19.3 mm and was 8.3%, 10.2% and 20.8% greater than P 30 [POS], PA 150 and SCA 6, respectively (Fig 44). The shade * clone interaction term was not significant.

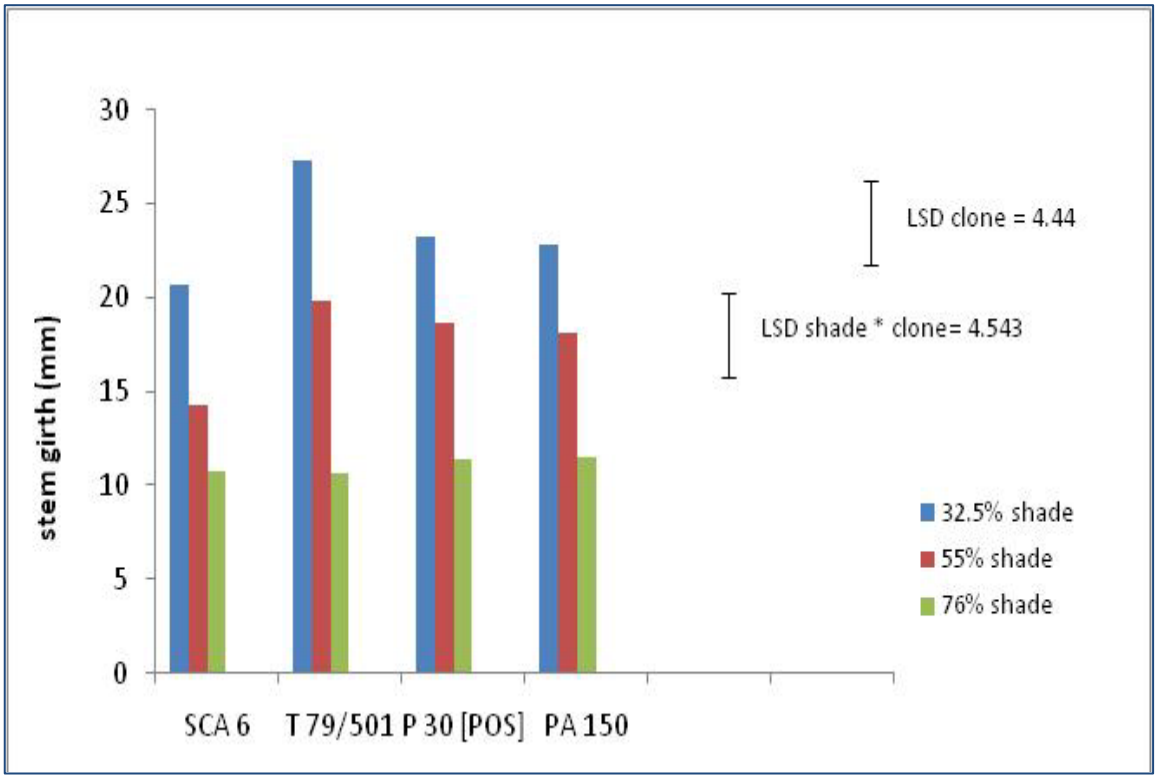

Figure 44 Stem girth of cacao clones under different shade 12 months into the experiment

Each bar represents the mean value for six plants

Plant height

At the end of the experiment, the clones were not significantly different in height. Their mean heights ranged from 117 cm (for SCA 6) to 131 cm (for T 79/501) (Fig 45).

Plant height differed significantly ($p = 0.012$) under the shade treatments. The lightest shade treatment had plants that were 154 cm tall on average and 9.7% taller than those under the medium shade but 49.9% taller than those under the heaviest shade (Fig 45).

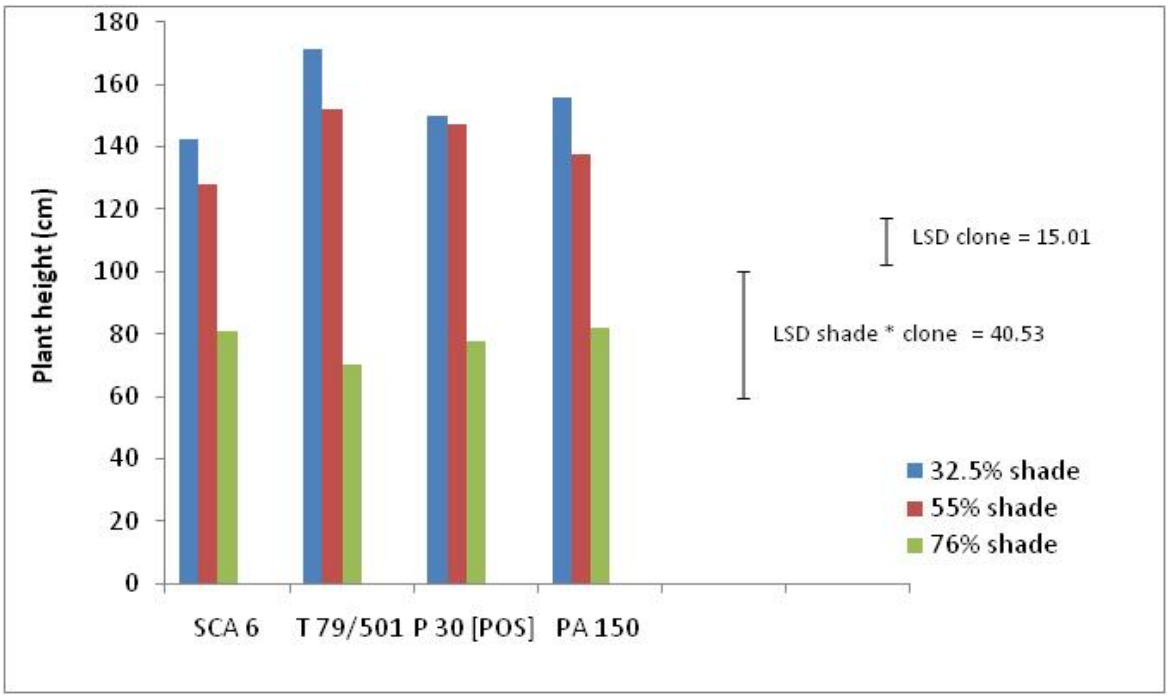

Figure 45 Plant height of cacao clones 12 months into the experiment
Each bar represents the mean value for six plants

4.4 Cacao stem volume

Plant stem volumes were significantly (p = 0.007) different under the different shade levels (Fig 46). The lightest shade regime had plants with a mean stem volume of 248.3 cm^3. That was 88.96% bigger than that of plants under the medium shade and 9.4 times bigger than those under the heaviest shade. There were also significant (p = 0.015) differences between clones. The biggest was T 79/501 (193 cm^3). P 30 [POS], PA 150 and SCA 6 had 131.3 cm^3, 125.3 cm^3 and 91.4 cm^3 stem volumes, respectively.

The shade * clone interaction term was not significant (Fig 46).

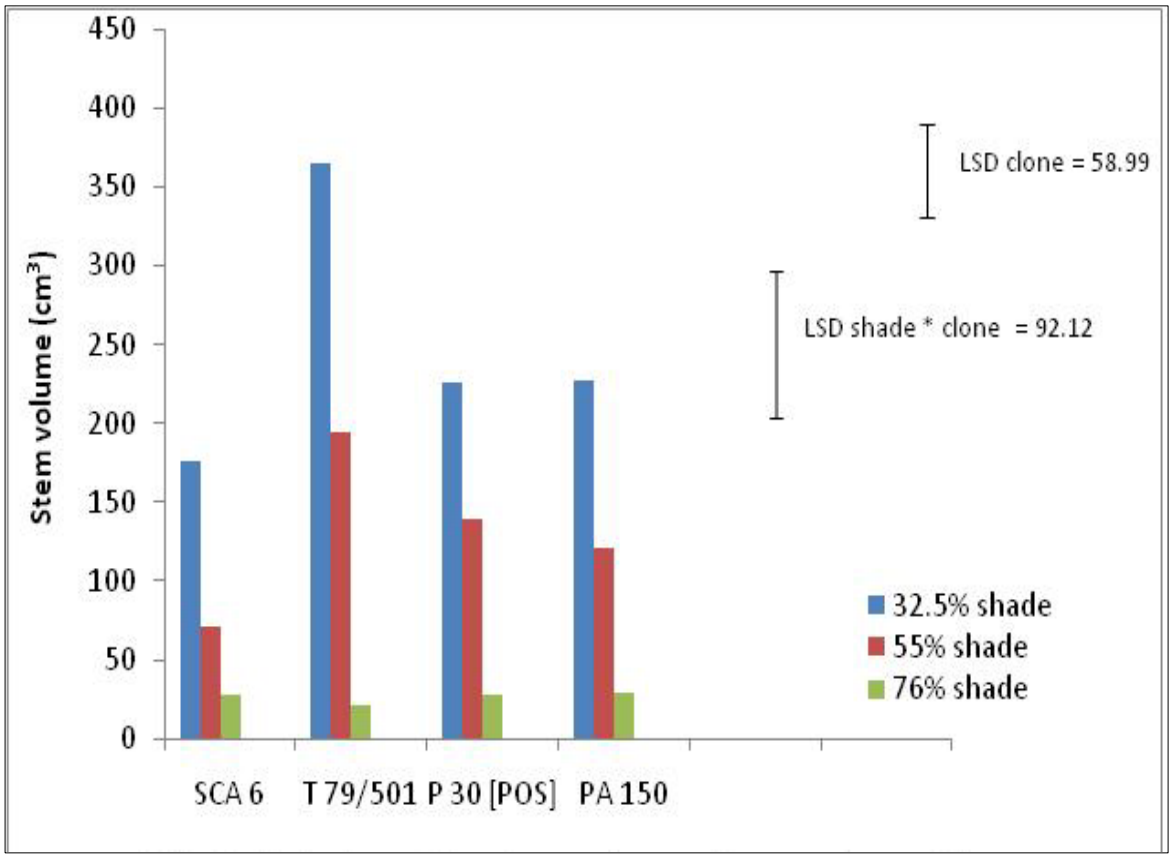

Figure 46 The stem volumes of cacao clones under varying shade
Each bar represents the mean value for six plants

Stem dry weight

Stem dry matter accumulation was significantly (p = 0.003) lower under increasing shade so that the lightest shade had plants with a mean stem dry weight of 0.3015 kg/plant which was 67.1% higher than that of plants under the medium shade and 9.48 times greater than that of the plants that grew under the heaviest shade (Fig 47).

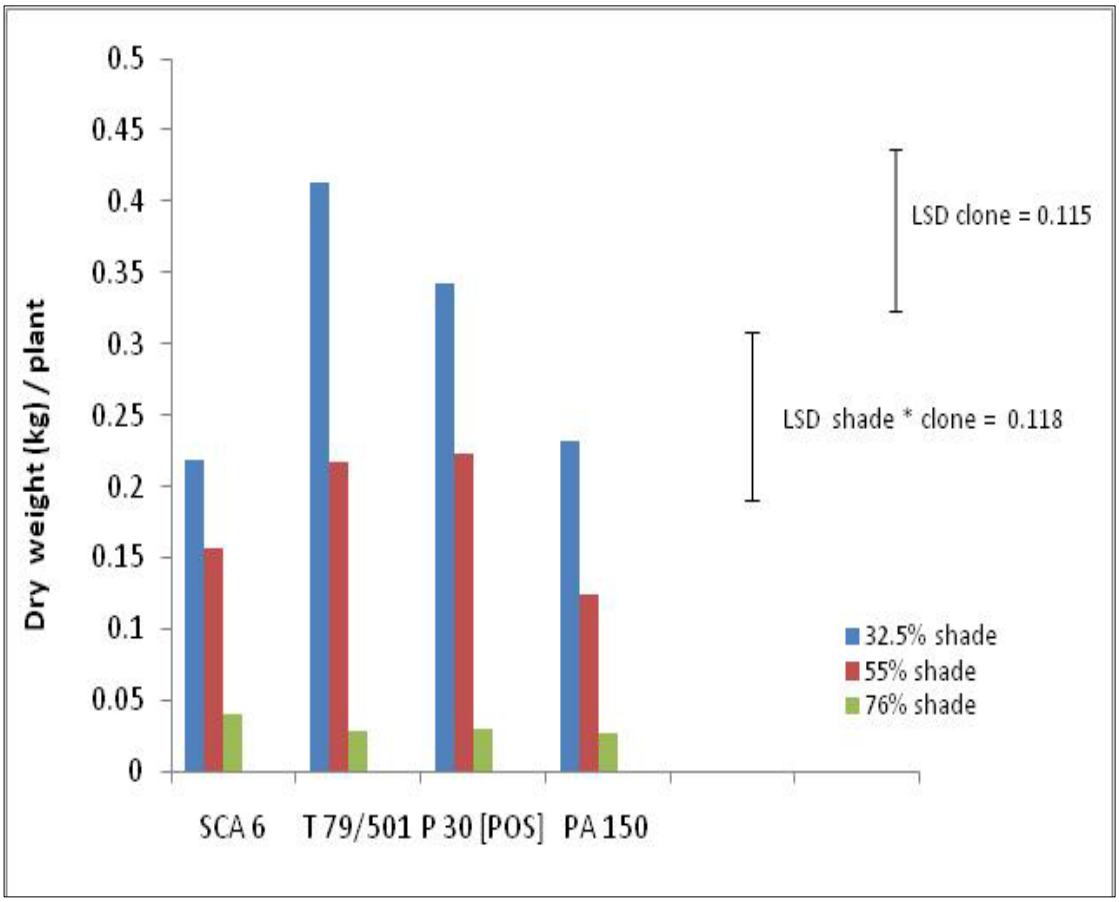

Figure 47 Mean stem dry weight of cacao under different shade levels. Each bar represents the mean value for six plants

Significant (p = 0.026) differences were also found between clones in stem dry weight per plant, with T 79/501 having the highest value (0.2195kg) which was 10.7% greater than that of P 30 [POS], 57.9% greater than that of SCA 6 and 71.2 % greater than that of PA 150. The shade * clone interaction term was not significant.

Stem dry weight: fresh weight ratio

The stem dry weight: fresh weight ratio was not significantly different between clones or shade treatments (Fig 48). The shade * clone interaction term was not significant.

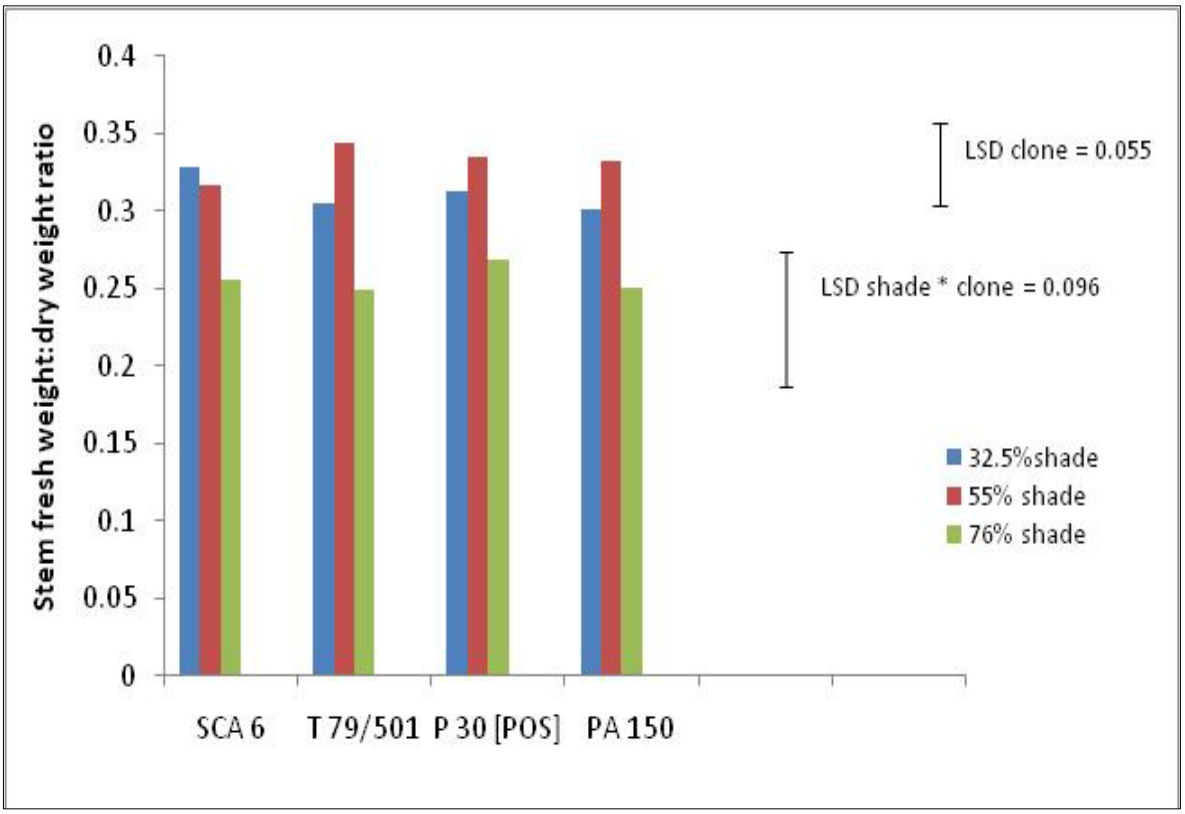

Figure 48 Stem dry weight: fresh weight ratio of cacao under different shade levels. Each bar represents the mean value for six plants

Root dry weight

There were significant (p = 0.018) differences in root dry weight for plants that grew under different shade treatments with those under increasing shade having lower values. The lightest shade had plants with a mean of 0.141 kg/plant root dry matter and that was 20.5% more than that of plants under the medium shade and 5.9 times greater than that of plants under the heaviest shade. There were no significant differences between clones and the shade * clone interaction term, was also not significant (Fig 49).

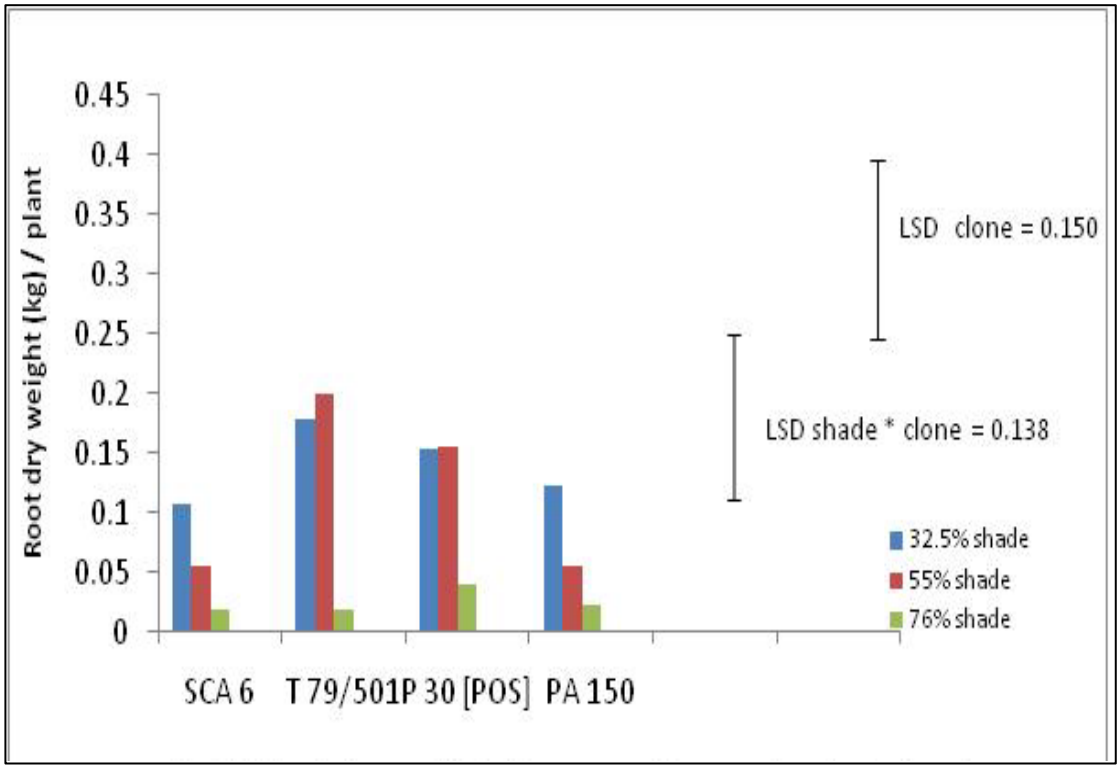

Figure 49 Root dry weight of cacao under different shade levels 12 months into the experiment.

Each bar represents the mean value for six plants

4.5 Root dry weight: fresh weight ratio

The root dry weight: fresh weight ratio was not significant among the clones. They ranged from 27.60% (for SCA 6) to 30.55% (for T 79/501). Plants under the different shade intensities had significant ($p = 0.034$) differences between them, the heaviest shade having plants with 23% less dry matter compared with those under the lightest shade which had the highest (33.65%) dry matter content (Fig 50) . The shade * clone interaction term was not significant.

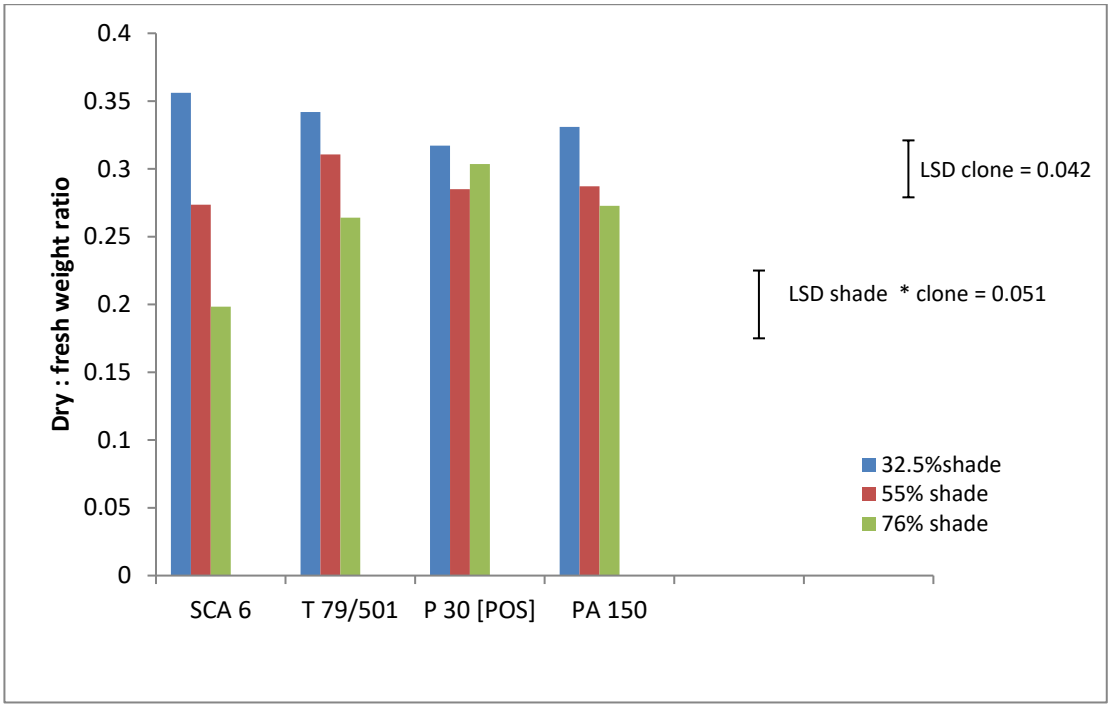

Figure 50 Root dry weight: fresh weight ratio of cacao grown under different shade intensities.

Each bar represents the mean value for six plants

Shoot: root ratio

SCA 6, P 30 [POS], T 79/501 and PA 150 had shoot: root ratios of 4.09, 3.69, 3.42 and 3.21, respectively (Fig 51), the differences being significant at ($p = 0.045$). The shade treatments had plants with significant ($p = 0.031$) differences. The medium shade had plants with the highest shoot: root ratio (4.26) followed by the lightest shade treatment (3.65) and then by the heaviest shade treatment (2.9). The shade * clone interaction term was not significant (Fig 51).

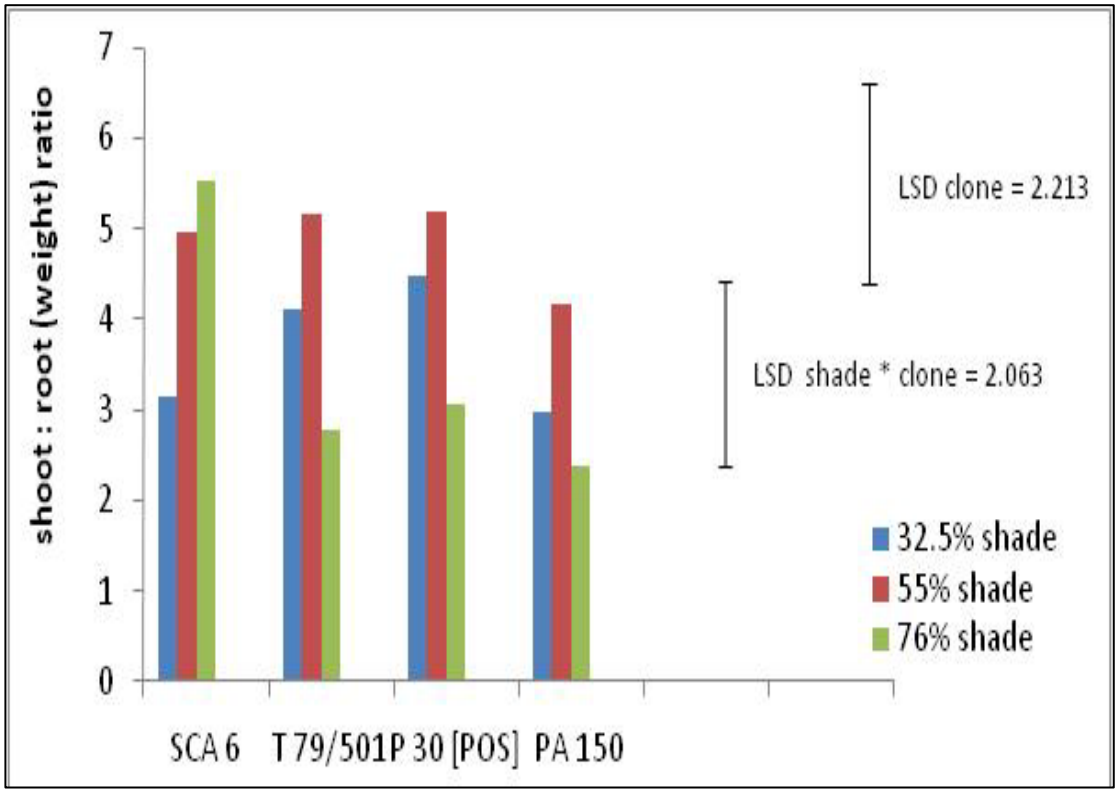

Figure 51 The shoot: root ratio of cacao grown under different shade intensities Each bar represents the mean value for six plants

Leaf weight ratio

There were no significant differences in leaf weight ratios between clones (Fig 52). The shade treatments were also not significantly different although a trend was observed in which plants under increasing shade tended to have increased leaf weight ratios. The shade * clone interaction term was not significant (Fig 52).

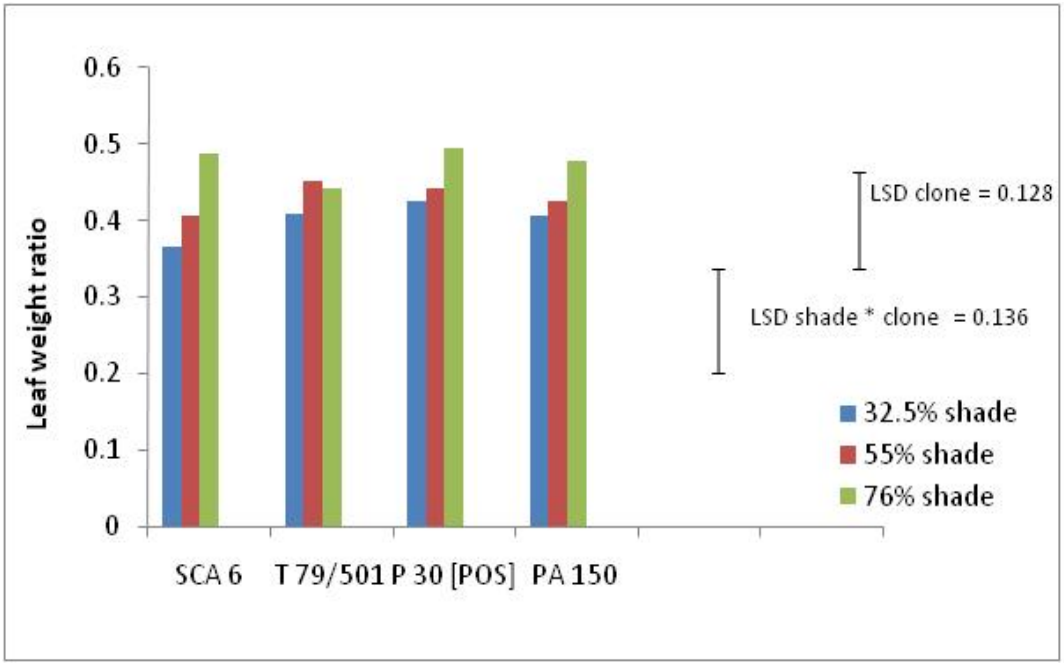

Figure 52 The leaf weight ratio of cacao grown under different shade levels Each bar represents the mean value for six plants

4.6 Discussion

Stomatal conductance and transpiration

The significant seasonal differences in stomatal conductance rates observed in the present work, with the lowest stomatal conductance rates occurring in the dry season may be explained by changes in environmental variables notably the relative humidity and air temperature (Table 2). The low relative humidity and high ambient temperature during the dry season must have contributed to the creation of a high water vapour pressure deficit (WVPD), causing the plants to restrict stomatal opening in order to limit plant water loss and conserve plant tissue water. With restricted stomatal opening, transpiration too was lowest during the dry season.

In contrast with the dry season the minor rainy season, in which the plants had the highest stomatal conductance rates, was characterized by high relative humidity and low tempera-

ture. Probably the high relative humidity in the minor rainy season took precedence over the lower ambient temperature and thus supported the creation of a condition of high water vapour pressure in that season.

Data from the diurnal study gave further proof of the influence of increased water vapour pressure deficit on CO_2 uptake and transpiration in cacao. In that study higher aerial temperature and reducing relative humidity values were recorded as the day progressed towards the early afternoon presumably causing a higher water vapour pressure deficit around midday. In a 'trade- off' fashion the plants apparently responded by restricting stomatal opening in order to reduce transpirational water loss but by so doing had their CO_2 uptake reduced (Hall et al., 1976). Sheriff and Kaye (1977) explained that stomatal closure due to an increase in WVPD is a "hydroactive response to water stress in the epidermis". Raja Harun and Hardwick (1988) cited Slatyer (1966) as suggesting that stomatal closure in response to high WVPD is due to increased drying of the mesophyll cell surfaces.

The significant season * clone interaction in stomatal conductance and transpiration rates is, probably, indicative of genetic differences expressed under varying conditions. The observation that SCA 6 had the lowest stomatal conductance and transpiration rates in the major rainy season but the highest rates during the dry season suggests that SCA 6 was not under as much stress (during the dry season) as the other clones, indicating a higher tolerance to increased water vapour pressure deficit than the other genotypes. On the other hand, it could simply mean that it has a poorer ability to regulate stomata and conserve water even under atmospheric stress. The greater stomatal closure in PA 150 (59.5%) and T 79/501 (37.9%) than in the other genotypes during the dry season (as compared with the rainy seasons) suggests a more effective stomatal control under low humidity conditions in PA 150 and T 79/501 than in the other genotypes. Balasimha (1988) and (1999) noted that an effec-

tive stomatal regulation in cacao genotypes is an important “acclimation strategy” against drought as such genotypes tend to maintain leaf turgidity and a high water potential under dry conditions. Thus reduced transpiration, especially under dry conditions, is regarded as a mechanism of drought tolerance. As mentioned in the General Introduction Raja Harun and Hardwick (1988) also noted that the few genotypes of cacao which have an efficient mechanism of stomatal regulation show a decrease in transpiration under water stress. Therefore, based on the latter assumption, PA 150 (followed by T 79/501) would be regarded as the most suitable (of the genotypes studied here) for conditions of high water vapour pressure deficit as it seemed to possess greater stomatal regulation ability than the other clones.

Joly and Hahn (1989a) explain that stomata of cacao leaves are particularly sensitive to some signal induced by plant water deficit, with stomatal conductance being affected by very mild levels of stress. These authors cited separate works by Cornish and Zeevart (1985) and Lachno and Baker (1986) which suggest that ABA, synthesized in roots in response to water deficit, acts to induce stomatal closure. Responses triggered by such internally controlled mechanisms are likely to differ between genotypes.

Plant reaction under the varied shade levels may be interpreted in terms of the direct effect of varied available light itself and/or indirect effects such as changes that occur in other environmental factors including relative humidity, air and soil temperature and soil moisture as shade intensity changes. Thus the observation that plants which grew under increasing light intensities tended to have higher stomatal conductance and transpiration rates during the two rainy seasons could have been caused by the direct effect of increased light in those seasons. It appears that under the higher light intensities the cacao plants increased their CO_2 uptake through wider stomatal opening so as to utilize the increased light energy that became available. That observation confirmed an earlier observation of Balasimha (1999)

which showed that stomata of intact cacao leaves generally open in response to increases in light intensity. The response could also have been influenced by the higher aerial temperature observed under lighter shade. The result was similar also to an observation by Okali and Owusu (1975) who reported that when there was adequate soil moisture, leaf temperatures of unshaded cacao seedlings were higher than shaded ones, and transpiration was higher under the no shade situation. Due to the high ambient relative humidity in those seasons the water vapour pressure deficit was not expected to be unduly high although temperatures were higher and the evaporative demand on plants would be moderate enough to be met by soil water uptake by the plants. Although similar observations have been made by other workers, many of them recommend a degree of shading for young cacao as the benefits it confers are often considered enough to outweigh its disadvantages.

Showing that some shade is required in young cacao Ahenkorah et al. (1974) and Alvim (1977) reported excessive leaf transpiration under no shade and explained that this condition is inimical to young cacao plants as they are susceptible to water stress. As mentioned in the 'Introduction' chapter the two groups of major physiological benefits that cacao receives from shade trees are associated with reduced stress (Anim-Kwapong, 2003, Beer et al., 1998). Estimating the reducing effect of shade on transpiration, Valle et al. (1987) referred to data modelled for transpiration and showed that on a sunny day a shaded cacao tree could transpire 40 litres of water assuming that all the leaves transpired at the same rate. On the other hand cacao planted without shade could transpire 90 litres of water (2.4 litres $m^{-2}d^{-1}$) if all of its leaves were exposed to incident light. Results from the current experiment seem to support the idea of shade especially during the dry season.

During the dry season stomatal conductance and transpiration rates were lower under increasing light. This indicated that the shade treatments modulated the effect of the seasons

in different ways. It is not known with certainty how the change in direction of plant response under shade was brought about as the seasons changed. However, the observation was consistent with Balasimha's (1999) earlier observation that a condition of low relative humidity reverses the increasing effect of light on stomatal opening. Probably, the greater stomatal opening under increasing shade during the dry season was caused by the observed higher relative humidity values under increasing shade in that season. Increasing shade would then result in a lower water vapour pressure deficit which would reduce atmospheric stress on the plants.

As stomatal conductance is a primary event for photosynthesis, the observed changes in it in the different seasons and under the shade levels were similar to the changes observed in photosynthesis.

Leaf chlorophyll fluorescence and photosynthesis

The fact that photosynthetic data were collected under different growing environments and across the different seasons of the year explains the wide range of photosynthetic rates (0.373 µmol $m^{-2}s^{-1}$ to 6.26 µmol $m^{-2}s^{-1}$) observed in this work.

As explained above the plants experienced restricted CO_2 uptake in the dry season. Also lower chlorophyll fluorescence values were recorded in the dry season than in the other seasons reflecting lower PS II efficiency. Therefore, reduced CO_2 uptake (due to stomatal closure) and reduced efficiency of PS II activity (also due to environmental stress) may have been the main reason for the lower photosynthetic rates observed in the dry season.

According to Daymond and Hadley (2006) "high temperature stress may be experienced during the dry season" in a number of cacao growing areas including Ghana" where, although the monthly maximum temperatures reach 31 - 32^{o}C (Wood, 1985), "absolute temperatures of up to 39 ^{o}C have been recorded more recently ".

The fact that (when assessed over the entire period) stomatal conductance was not very different among the clones may partially explain the reason why net photosynthesis was also not significantly different for the clones. This shows that at the leaf level, not only are their ability to capture CO_2 similar, but their carbon utilization may also be similar. A similar observation was made by Balasimha (1999) who found no significant differences in net photosynthesis between the cacao accessions he worked on. It was interesting, however, to find (in the current experiment) that when their photosynthetic rates were arranged on a simple magnitude, their order changed from season to season such that PA 150 which had the highest rate of photosynthesis in the major rainy season had the lowest rate in the dry season and SCA 6 which had the lowest rate in the major rainy had the highest rate of photosynthesis in the dry season, that being a reflection of the trends observed in stomatal conductance and transpiration. That observation suggested that SCA 6 is a more 'productive' clone than the others under high water vapour pressure deficit. The fact that SCA 6 had the highest mean Fv/Fm value (0.6689) during the dry season is probably an indication of its ability to withstand atmospheric stress. This explains why it had a higher photosynthetic rate during the dry season than the other clones. Similar to this observation, Daymond and Hadley (2006) found SCA 6 to be the least heat sensitive among four cacao clones that were subjected to a leaf chlorophyll fluorescence technique.

On the other hand, PA 150 (followed by T 79/501) was capable of having higher rates of assimilation when the atmospheric vapour pressure deficits were more conducive. This trend was, to a limited degree, a reflection of the significant season * clone interaction observed in stomatal conductance and transpiration.

The over three times faster rate of photosynthesis recorded (over the entire period) under 67.5% full sunlight than under 24% full sunlight gives an indication of the light requirement

of the genotypes used in this work. It was mentioned in section 4.1 how some workers, including Joly and Hahn (1989a), Da Matta et al. (2001) and Rada et al. (2005) have shown, in some cacao genotypes, that increased photosynthetic activity occurs with increasing incident light up to the light saturation point. Asomaning et al. (1971) observed that the improvement in the growth of cacao in response to reduced shade can be explained in terms of increased carbohydrate production and the observations made in this work confirm those findings.

However, there have been reports which indicate that even in the case of mature cacao, some shade is desirable. Galyuon et al. (1996b) reported that the photosynthetic rate of cacao decreases if the photosynthetic apparatus is exposed to light intensities above 1800 µmol $m^{-2}s^{-1}$. Raja Harun and Hardwick (1988) added that exposure of the cacao leaf to high light intensities for long periods, damages the leaf photosynthetic mechanism. The shade requirement, however, appears to be affected by other environmental conditions.

As observed with stomatal conductance and transpiration, the plants altered their relative photosynthetic activity under the imposed shade treatments with seasonal changes in water vapour pressure deficit, stomatal conductance and transpiration.

Thus, although when averaged over the entire experimental period absolute net photosynthesis was higher under increasing light, there was greater photoinhibition in the plants that grew under higher light intensities. The ameliorating effect of increasing shade is shown by the significant season * shade interaction in which heavier shade led to lower photosystem II activity in the rainy seasons but higher photosystem II activity in the dry season.

In the rainy seasons when the water vapour pressure deficit is low, the plants can utilise higher light for photosynthesis and the light requirement increases. Thus this seems to suggest that cacao exhibits some acclimation of photosynthesis to light as can also be seen in

the diurnal profile reported on in this work which suggests that the light requirement of cacao is dynamic. The fact that other environmental factors influence the light requirement of plants also seems to explain why there is considerable inconsistency in what is considered to be the optimal light levels for cacao (Da Matta et al., 2001, Joly and Hahn, 1989a, Rada et al., 2005, Raja Harun and Hardwick, 1988).

Results in this work indicate, therefore, that the ideal shade management practice for cacao should involve providing shade on the basis of the plant's "current light requirement". This implies the provision of lighter shade during the rainy seasons and heavier shade in the dry season. It appears that this will be needed to maintain optimal photosynthesis over the cropping year.

Diurnal chlorophyll fluorescence and photosynthesis

This positive relationship between stomatal conductance and photosynthesis (Sena Gomes et al., 1987) was not seen (in the diurnal experiment) during the early hours of the day. Thus, although stomatal conductance started to decrease between 8:00 and 10:00 hours GMT, photosynthesis was still high between those hours. In the early hours photosynthesis may have been boosted by substantially high light use efficiency.

The observation that the plants had their highest rate of photosynthesis during the first half of the day but declined towards the midday and afternoon may be explained in terms of the observed significant depression in leaf chlorophyll fluorescence (Fv/Fm) around midday. That was an indication that the photochemical efficiency of photosystem II was significantly impeded around midday as a result of a high photoinhibition of photosynthesis which, according to Galyuon et al. (1996b) is associated with increased quenching of Fv due to increased thermal dissipation of excitation energy (Raja Harun and Hardwick, 1988).

A similar observation was made by Balasimha (1999) in cacao seedlings that net photosyn-

thesis was highest around the mid-mornings and declined thereafter. It was explained by this author that at midday, increased light intensity decreased the rate of photosynthesis due to soil and atmospheric drought (that lead to high water vapour pressure deficit and low leaf water potential). This explanation focused on the indirect way by which increased light intensity affects photosynthetic activity. Commenting on a similar observation Raja Harun and Hardwick (1987) explained that under a situation of high water vapour deficit a greater proportion of captured light energy would not be used for photosynthesis, a situation which, can lead to chlorophyll damage.

According to Senevirathna et al. (2003) there is evidence that high irradiances induce depression of photosynthetic productivity in many crops including rubber (*Hevea brasiliensis*) even though they may be known to be light-adapted species. Citing earlier work by Powles (1984), Demmig-Adams and Adams (1992), Osmond (1994) and Senevirathna et al. (2003) described photoinhibition as commonly affecting plants growing in the tropics, and causing depressions in photosynthetic efficiency which depressions may be dynamic or chronic depending on relaxation times. Although the relaxation time was not determined in this present work, it was likely that the photoinhibition levels that were prevailing immediately before data collection did persist for a while. This is seen from the fact that although a constant saturating irradiance was applied artificially at the different times of the day, the recorded photosynthetic rates varied through time. The observation that the Fv/Fm values increased after the midday depression suggests that the depression resulted from dynamic photoinhibition and not from photoinhibitory damage to photosystem II (Osmond 1994). This means that the photosynthetic apparatus of cacao suffers midday depression, and provided its upper limit is not exceeded, it recovers in the course of the night and normal photosynthetic capabilities are restored.

As the work seems to point to the need for good shade management for field-planted young cacao, it would be interesting to know how long it takes for cacao to adapt to a changed shade level.

Assessing the influence of temperature on the initial growth of young cacao, Daymond and Hadley (2004) observed genetic differences in response to temperature stress. Therefore, the observation, in this work, of significantly different Fv/Fm values for the clones suggests genetic variability under photoinhibitory conditions. PA 150 had the lowest Fv/Fm and this might have contributed to its low photosynthetic rate during the more stressful conditions of the dry season. Using excised leaves, Balasimha (1999) observed higher Fv/Fm values in more tolerant cacao under heat stress which suggested that the efficiency of PS II activity was maintained better in those accessions. However, if, as some workers contend, photoinhibition is a protective mechanism used by the plant to protect the photosynthetic apparatus then the varieties that exhibit more of it under adverse conditions may be more suitable for planting in marginal areas. Orts and Baker (2002) regard processes such as photorespiration and cyclic electron flow around PS I (that take place under photoinhibition) as strategies to reduce the harmful effects of high photosynthetic photon flux density (PPFD).

The plants showed significantly different diurnal photosynthetic activity under the different shade treatments. Absolute net photosynthesis values were lower in the plants that grew under heavier shade. Yet, as explained earlier, photoinhibition very likely caused a greater reduction in photosynthetic efficiency in the plants that grew under higher light intensities than in the others that grew under heavier shade. This is corroborated by the lower Fv/Fm that was observed under increasing light. The observation that the diurnal Fv/Fm values were higher under increasing shade is comparable to the mentioned observation by Senevirathna et al. (2003) on rubber seedlings. These authors explained the observation as oc-

curring because unshaded plants (unlike others in that experiment that grew under 33%, 55% and 77% shade) received “saturating irradiances throughout the day”.

4.6.1 Leaf production

Sale (1968) found, under controlled environments, that increasing temperatures between 23.3°C and 30 °C accelerated flushing initiation. In a review publication De Almeida et al. (2007) reported that in Bahia (in Brazil), the main leaf flushing period was from September to February (the period of increasing solar radiation and temperature) while periods of decreasing energy coincided with months in which little leaf flushing occurred.

Although there is evidence that under field conditions, the leaf flushing cycle is triggered by changes in the environment, the cycle persists under constant conditions such as ones provided under controlled environments. Based on the observation, Orchard et al. (1978) and (1981) suggest the presence of an endogenous form of control, and that may be an explanation of the observed significant differences in the number of leaves produced by the different clones. Being an Amelonado accession, P 30 [POS] was expected to produce a small number of leaves. The results presented here, however, did not show this. This may be explained by Hutcheon’s (1977) observation that the loss of leaves by seedlings of Amelonado occurs only if they are subjected to light levels exceeding 70% daylight.

Orchard et al. (1981) found high levels of auxin and cytokinin in expanding cacao leaves. The leaves at that stage could be acting as a source for auxin and cytokinin production or as sinks that receive auxins and cytokinins from other parts of the plant. Machado and Hardwick (1987) showed that other photoassimilates including carbohydrates are transported into expanding leaves. The probable transportation of substances into expanding leaves of cacao

may explain the different leaf sizes observed in the clones in the current work since leaves of different plant materials could be expected to have different sink strengths. Generally, genotypes that have higher leaf numbers tend to have smaller leaf sizes. The concurrent development of more leaves can lead to a higher carbohydrate utilization incident that may use up available photosynthate and limit the potential of the leaves to grow big.

Okali and Owusu (1975) noted that high light intensity caused constraints to leaf expansion and Galyuon et al. (1996a) observed a leaf-size reduction of about 30% under full sunlight compared with 50% sunlight.

Leaf area per plant

The hypothesis by Miyaji et al. (1997) that heavily shaded cacao trees can be more efficient in photosynthesis and leaf dynamics was not supported by observations in this work as plants under the heaviest shade had significant reductions in leaf area. This does not contradict the observation by Galyuon et al. (1996a) when he noted a higher leaf area per plant under 50% sunlight as these authors compared two light intensities (50% sunlight with full sunlight) that were different from those compared in the present work. Although plants under the highest light intensity had smaller leaves, the greater leaf numbers under higher light conditions more than compensated for the smaller leaf sizes which would have reduced the total leaf area. It may also be an indication that many of the leaves that were produced as a result of the greater number of flushes under higher light intensities were also retained by the plants. What is shown here is that although increasing light intensities causes reductions in leaf size, if the intensities are not high enough to lead to substantial restrictions on leaf expansion and / or cause high leaf abscission, total leaf area can be increased due to increases in the number of leaves produced by the plant. The apparent genotypic differences in leaf area under the different light levels suggest that to maximise photosynthesis at the canopy level the shade requirement of individual clones must be taken into consideration.

4.7 Leaf traits

Leaf structure

The relative water content of the leaves was generally lower than those obtained by de Almeida et al. (2002) who assessed the effects of water stress on cacao leaves. The observed lower leaf relative water content under lighter shade was probably an expression of higher leaf water stress (Okali and Owusu 1975). The observation was consistent with those made in other studies. Daymond (2000) made a similar observation on cacao clones when he found that on the same tree, 'sun' leaves had lower leaf water contents than leaves in the middle of the canopy that experienced more self-shading. In their review on the ecophysiology of cacao, de Almeida and Valle (2007) noted that shaded leaves show higher relative water content than exposed ones. On the other hand the observed significant positive effect of increasing light on specific leaf weight (independent of plant material) agrees with earlier observations made by other workers including Costa et al. (1998), Galyuon et al. (1996), and Daymond (2000). Higher light intensities are known to contribute to the formation of a greater number of palisade cells, better developed mesophyll parenchyma and more starch deposition (Thompson et al., 1992) all of which increase specific leaf weight and are expected to enhance photosynthesis.

The photosynthetic machinery

The significant shade * clone interaction in leaf nitrogen content suggests genetic variability with respect to leaf nitrogen content. The high leaf nitrogen content of SCA 6 and PA 150 under the highest light regime is what came out to be consistent with earlier observations made by Daymond (2000) who found, in cacao, that exposed 'sun' leaves had a higher nitrogen content per unit leaf area than self-shaded leaves and that leaf nitrogen content was

positively linked to higher photosynthesis. The fact that leaf phosphorus content also differed significantly among the cacao clones showed that they possessed different strengths for phosphorus uptake. It was noticed that plants under the different shade treatments did not have significantly different leaf P levels. A similar observation was made by Raaimakers et al. (1995) who investigated the relationships between P availability and photosynthetic capacity of nine trees growing in a heavily weathered soil of a rainforest in Guyana. These authors observed that leaf P content was a more important determinant of light saturated photosynthesis (A sat) than leaf N. It was also observed, among all the species, that the light environment did not alter the leaf P but all the species had constant leaf P concentrations across different light climates. Thus leaf P was able to limit the photosynthetic rates of plants regardless of light level.

The observation was made in the present work that heavier shaded plants had higher leaf chlorophyll concentrations. Probably that is an adaptation of the cacao tree, being an understorey species of the tropical rainforest, to increase its photo-utilization capacity under lower light intensities and increase protection of the photosynthetic apparatus under high light intensities. A similar observation was explained by Galyuon et al. (1996b), who found lower total chlorophyll levels and a reduction in the total chlorophyll to carotenoid ratio under full sunlight (compared to 50% sunlight), that that response in the plants may help protect leaf chlorophyll from photo-oxidation. The observations are consistent also with earlier ones made by Guers (1974) and Daymond (2000) who observed higher leaf chlorophyll contents in self- shaded cacao. Similarly Costa et al. (1998) recorded substantial increases in leaf chlorophyll content of shade leaves of cacao seedlings especially under nitrogen application.

4.7.1 Biomass production

Under normal growing conditions, the stem girth is often used as an important index of vegetative growth in cacao. Thus the observed bigger stems under reducing shade and the significantly different stem girths between the clones were an indication of relative plant growth.

Galyuon et al. (1996a) explained the greater heights of plants that were grown under 50% sunlight than those grown under full sunlight as having been caused by observed longer internodes in the former. Raja Harun and Kamariah (1983) made a similar observation when cacao plants grown under 45% sunlight were compared with others under full sunlight. The dissimilar observation (in this work) in which plants under higher light levels had taller plants may be explained by the fact that 67.5% sunlight (instead of 100% sunlight) was used as the highest light intensity in this present work. Hence inter-node length (not measured) may not have been reduced by the highest light intensity.

As the plant heights were not very different among the clones, the significant difference in the stem girths of the clones contributed more towards the significant differences in mean clonal stem volumes. The stem volume of cacao plants has often been regarded as an index of plant growth and is a better approximation for total dry matter production than stem girth alone. Being significantly different in leaf area, the clones were expected to produce total dry matter at different rates although their rates of photosynthesis per unit leaf area were not very different. In the case of seedlings (in which self shading is minimal) total photosynthesis depends on the size and efficiency of the photosynthetic apparatus. Thus in this work (involving young plants) the basis of genetic differences in dry matter production, was the rate of leaf area development which determined the relative amount of sunlight available to each clone. As the growing period of cacao in West Africa is disrupted by the three -

month dry period only five months after transplanting, the ability of a genotype to avail itself of resources, capture resources efficiently convert them into assimilate for growth is essential. For that reason cacao genotypes in West Africa which exhibit early vigour are regarded as good candidates for field establishment (Balasimha 1999).

The fact that plants growing under increasing light intensity had greater biomass production is a reflection of the higher rates of photosynthesis. Considering the differences in net photosynthesis under the different shade treatments, it can be seen that the highest light intensity provided in this work fell within the range that can be considered 'appropriate' (at least for most of the experimental period). This was the case although under increasing light lower values of some desirable factors of photosynthesis including leaf chlorophyll fluorescence, leaf nitrogen content, leaf chlorophyll concentration and relative water content were recorded. The 'positive' influence of increased light on photosynthetic parameters including stomatal conductance and transpiration as well as morphological characters such as specific leaf weight, and total leaf area per plant must have outweighed the 'undesirable' influences of increased light. According to Galyuon et al. (1996a) increased sunlight leads to an increase in "leaf thickness, stomatal density and specific leaf weight" which enhance the "efficiency of light exploitation and reduce the stressful effects of high light intensity". According to Hunt et al. (1990) in areas where soil water and nutrients are not limiting, the growth of a crop is initially related to the development of leaf area, the amount of PAR it absorbs, and the photosynthetic conversion ratio (Singh et al., 1990, Singh et al., 1989). Since more leaf area was developed and PAR was not limiting under the highest light intensity, assimilate production would be higher provided photoinhibition was manageable.

4.7.2 Biomass partitioning

The observation that the heaviest shade had plants with the highest shoot: root and leaf weight ratios and vice versa is in agreement with that of Galyuon et al. (1996a) in which greater partitioning of photosynthate to root growth occurred at the expense of leaf growth under full sunlight than under 50% sunlight. It is also expected that a greater root development will improve plant water uptake and thereby enhance plant survival under high light intensity conditions which cause high transpirational water loss. The observation in the current work is also consistent with Gregory's (1994) supposition that the growth of root and shoot systems is an integrated course of action in which 'homeostasis is preserved 'as a result of both size and effectiveness of both systems. Furthermore the observation shows the validity of the observation by Li et al. (2006) that assimilates are partitioned into shoot and root in "inverse proportion to the rates at which they capture resources".

Thus heavier shaded plants translocated more assimilate into leaf production to aid light interception at the canopy level.

The experiment has shown that for most of the cropping year young cacao can be grown under less shade than what has generally been provided (50%) (Lee, 1978). It is only in the dry season that heavier shade (about 55% shade) may be required. However, the experiment was carried out with minimal edaphic stress as enough soil water was ensured. It would be interesting to study how the cacao genotypes respond to shade under field conditions where, in addition to atmospheric stress, soil water stress also comes into play. That aspect is considered in the field experiments involving the four cacao clones.

CHAPTER 5

Photosynthesis, survival and growth of cacao genotypes grown under different shade levels and organic manure treatments

5.1 Introduction:

Reviewing "the main stocks and flows of nutrients" in cacao ecosystems for a number of cacao-growing regions in the tropics Hartemink (2005) observed that most soils under cacao had a lower fertility when compared to primary forests due to the fact that "large amounts of nutrients in cacao ecosystems are transferred each year". For such reasons the difficulty in rehabilitating or replanting cacao farms is partly attributed to inadequate soil fertility especially where there is scanty overhead shade (Ayanlaja 1983). Thus there is a need to replenish 'mined' soil nutrients in order to maintain cacao production.

In mature cacao it seems possible to address the soil fertility problem through the use of fertilizers (Cunningham and Arnold, 1962). Because of health and environmental concerns there has been a steady growth in the demand for organically produced cacao in recent times. Therefore it seemed appropriate to investigate whether organic fertilisers aid in the re-establishment of young cacao on a piece of land with a previous history of cacao cultivation. Therefore, a field experiment was established involving the four cacao clones (SCA 6, T 79/501, P 30 [POS] and PA 150) under three different shade (called the 'close plantain', the 'triangular *Gliricidia*' and the 'control' shade) treatments. The hypotheses tested were:

I. Can the soil application of organic fertilisers improve cacao plant nutrition and help plant establishment?
II. Under the prevailing conditions will shade alter cacao plant response to organic fertiliser application?

A set of materials and methods were adopted to answer the above questions.

5.2 Materials and methods:

The experiment was laid out as a split-plot with the clones as the main plot factors and shade x manure combinations as sub-plots. There were four blocks each consisting of 24 sub-plots measuring 9m x 12m and having a guard row (shared by adjacent sub-plots) and 6 experimental cacao plants planted at 3m x 3m (Table 4).

Table 4 Layout of the clone * (shade * manure) experiment.

The experiment was re-randomized in each of the four blocks

SCA 6	T 79/501	P 30 [POS]	PA 150
Δ *Gliricidia* shade * Manure	Δ *Gliricidia* shade * No manure	Control shade* No manure	Δ *Gliricidia* shade * No manure
Close plantain shade * Manure	Δ *Gliricidia* shade * Manure	Control shade * Manure	Close plantain shade * Manure
Control shade* No manure	Control shade * Manure	Close plantain shade * No manure	Control shade* No manure
Δ *Gliricidia* shade * No manure	Close plantain shade * Manure	Δ *Gliricidia* shade * Manure	Close plantain shade * No manure
Control shade * Manure	Close plantain shade * No manure	Δ *Gliricidia* shade * No manure	Δ *Gliricidia* shade * Manure
Close plantain shade * No manure	Control shade* No manure	Close plantain shade * Manure	Control shade * Manure

Table 5 Soil fertility of experimental site compared with standard soil ratings for cacao (Smyth, 1966).

Soil fertility parameters	Soil Depth (cm)	pH	% Nitrogen	% O. M	Avail. P	% Carbon	C/N
Medium rating (Standard)	-	5.1-5.5	0.2 - 0.5	2.0 - 4.2	6.5 - 13	1.16-2.44	13 - 20
Low rating (Standard)	-	-	0.1 - 0.2	1.0 - 2.0	3.0 - 6.5	0.58-1.16	> 20
Experimental site	0 – 15	5.7	0.13	1.67	20.55	0.97	7.46
Experimental site	15 – 30	5.5	0.08	1.02	16.69	0.59	7.35

5.2.1 Organic fertiliser treatments:

As the site had soils with lower fertility level than the standard (Table 5) two organic fertiliser treatments - (i) No fertiliser and (ii) 140 g per plant of 'cacao pod husk ash' [CPHA] (equivalent to 125 kg muriate of potash per hectare) plus poultry manure of known nutrient content (1,800 kg/ha per year)were applied (Table 6) .

5.2.1.1 *Application of organic soil amendments*

Due to the approaching dry season, only the cacao pod husk (CPH) ash fertiliser (containing 40 % K) was ring-incorporated into the soil (at 70 g per plant) in October 2007. During the major rainy season of 2008 (in April), the poultry manure (with chemical properties as shown in Table 6) and CPH ash were applied to designated plots at 1,800 kg and 140 kg per hectare, respectively.

Table 6 The chemical properties of applied poultry manure

Total N	Organic C	Organic Matter	Total P	C/N ratio	Moisture Content
2.45%	35.10%	59.67%	1.19%	14.3	12.30%

5.2.2 Soil moisture determination

Soil moisture content was determined by means of the soil moisture probe (Delta-t, U.K; model PR2/4). Due to insufficient probe protection tubes the shade and cacao cultivar treatments were ignored (by restricting measurements to P 30 [POS] under the control shade treatment plots only). One plot per manure treatment in each of two blocks (out of the four) was selected for soil moisture measurement. Soil moisture was measured twice in the dry season on January 20 2007 and February 21 2008.

5.2.3 Gas exchange measurement

Starting from September 2007 the infra red gas analyzer was used to measure stomatal conductance and the rates of transpiration and photosynthesis twice in each of the three seasons (as described in section2.3). For each replication three out of six core plants were tagged and the youngest expanded and hardened leaf of a tagged plant was selected for data collection in the mid-mornings.

Since shade was one of the factors under study no artificial light source was applied.

Leaf Chlorophyll Fluorescence was measured by means of the leaf chlorophyll fluorescence meter (see section 2.3.1 for details). Measurements were carried out between 11:00 am and 12:15 pm on days for gas exchange measurements. Leaves which had been selected for photosynthesis measurement were also used for leaf chlorophyll fluorescence measurements.

5.2.4 Growth data

Growth (girth and height) measurements of experimental plants were made once every two months from September 2007. The stem girth of plants was measured at 20 cm from ground level.

5.2.5 Leaf traits

In March 2008, data on specific leaf weight, relative water content, Leaf chlorophyll concentration and leaf nitrogen content were taken once on a leaf on each of the three plants which were used for photosynthetic data collection in each treatment (see section 2.3.2 for details).

5.3 Results

5.3.1 Soil moisture

Soil moisture content was generally higher in this site than where the mulch * (shade * clone) experiment was conducted. Soil moisture content was only slightly higher in the subplots that had organic fertiliser application than in those that received no fertiliser and the difference was not statistically significant. However, soil moisture was significantly ($p < 0.001$) greater further down the soil profile (Fig 53).

5.3.2 Meteorological data

Air temperature and relative humidity under the different shade treatments across the seasons during the course of the experiment have been shown in Table 7. Under increasing shade slightly higher relative humidity and lower temperature values prevailed particularly in the dry season. As recorded in the shade * clone nursery experiment the dry season had

the highest daytime temperature but the lowest relative humidity values whereas the minor rainy season had the lowest daytime temperature and the highest relative humidity values. Daytime water vapour pressure deficit was, therefore, expected to be lowest in the minor rainy season and highest in the dry season.

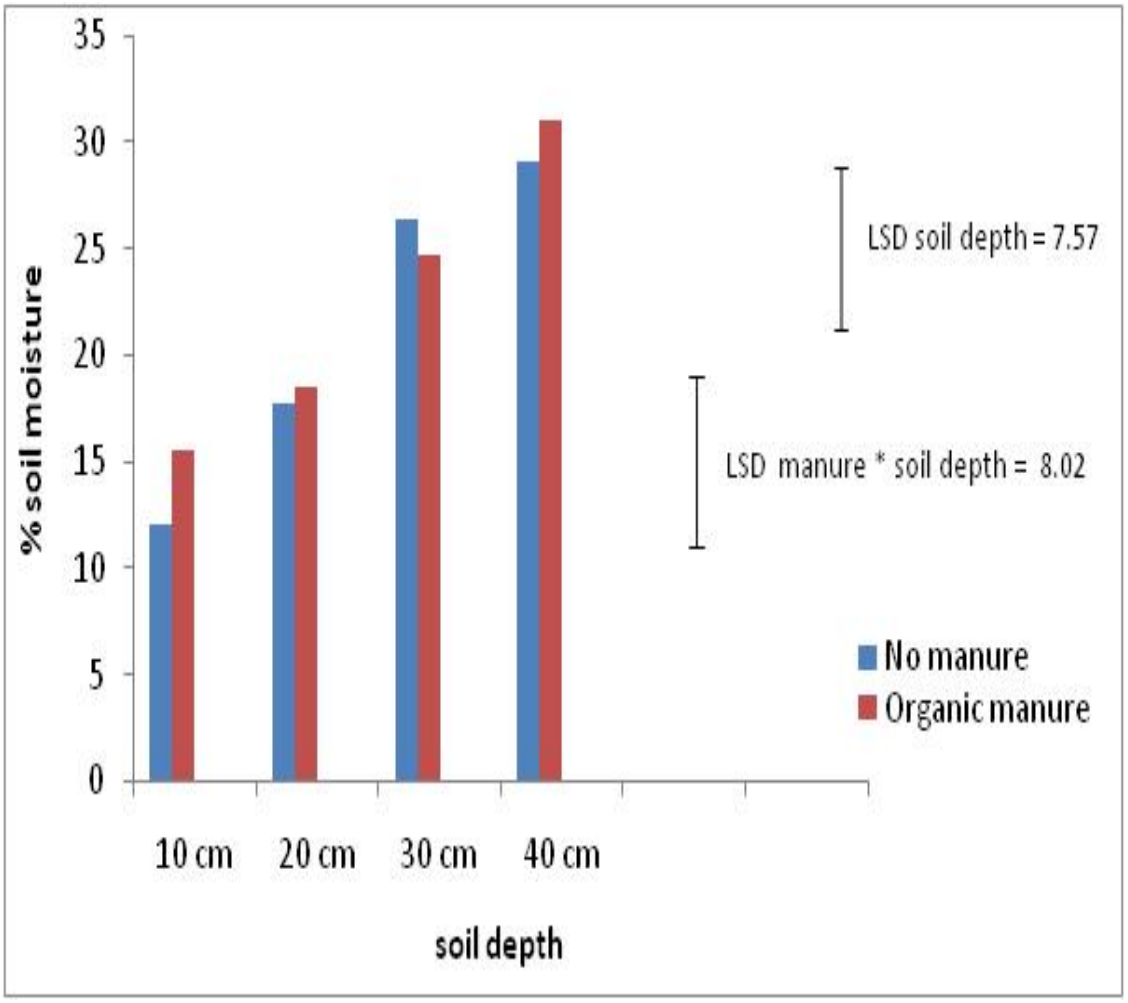

Figure 53 Soil moisture measured under different fertiliser treatments and soil depths in the dry season.

Table 7 Mean relative humidity and daytime temperature under the shade regimes during the seasons.

Each value represents the mean of three readings.

	Major rainy season		**Minor rainy season**		**Dry season**	
Shade Type	R. H. (%)	T °C	R. H. (%)	T °C	R. H. (%)	T °C
Control	64.25	30.50	79.25	28.65	52.25	34.70
Δ Gliricidia	74.50	29.25	82.50	27.00	54.50	33.50
Close plantain	78.50	30.00	83.25	26.80	56.00	33.00

5.3.3 Stomatal conductance

The mean rate of stomatal conductance was 0.43 mol $m^{-2}s^{-1}$ for both the major and minor rainy seasons, being 7.5 times higher than the mean value during the dry season (Fig 54). Significant ($p = 0.018$) differences were observed between cacao genotypes in their rates of stomatal conductance during the major rainy season ranging from 0.381 mol $m^{-2}s^{-1}$ (for P 30 [POS]) to 0.486 mol $m^{-2}s^{-1}$ in PA 150. In the major rainy season stomatal conductance under the shade and fertiliser treatments were significantly different ($p = 0.006$ and $p < 0.001$, respectively). Cacao plants under the triangular *Gliricidia* shade had the lowest rate of stomatal conductance (0.296 mol $m^{-2}s^{-1}$) and plants under the control shade had the highest rate (0.521 mol $m^{-2}s^{-1}$). Cacao plants supplied with organic fertiliser had a mean stomatal conductance rate of 0.534 mol $m^{-2}s^{-1}$, being 63.8% higher than the rate for plants that were

not supplied with manure (Fig 54). None of the interactions were significant.

Unlike the major rainy season no significant differences were observed between the fertiliser or shade treatments during the minor rainy season. However, the clone * shade interaction term was significant ($p = 0.005$). Whereas higher stomatal conductance in PA 150 and P 30 [POS] were recorded under increasing shade, no clear trends were observed for T 79/501 and SCA 6.

In the minor rainy season cacao plants provided with fertiliser had 14.53% higher mean stomatal conductance rate, the difference being on the borderline of significance ($p = 0.053$). The clone * manure and the shade * manure interaction terms were not significant in the minor rainy season.

In the dry season, the cacao clone * shade interaction term was significant ($p = 0.004$). The clone T 79/501 had its lowest rates under the triangular *Gliricidia* shade whereas the other clones had their lowest stomatal conductance under the close plantain shade.

Cacao plants that were treated with fertiliser had a mean stomatal conductance rate that was 25.35% higher than those which were not provided with fertiliser and were significantly ($p = 0.007$) different (Figure 54).

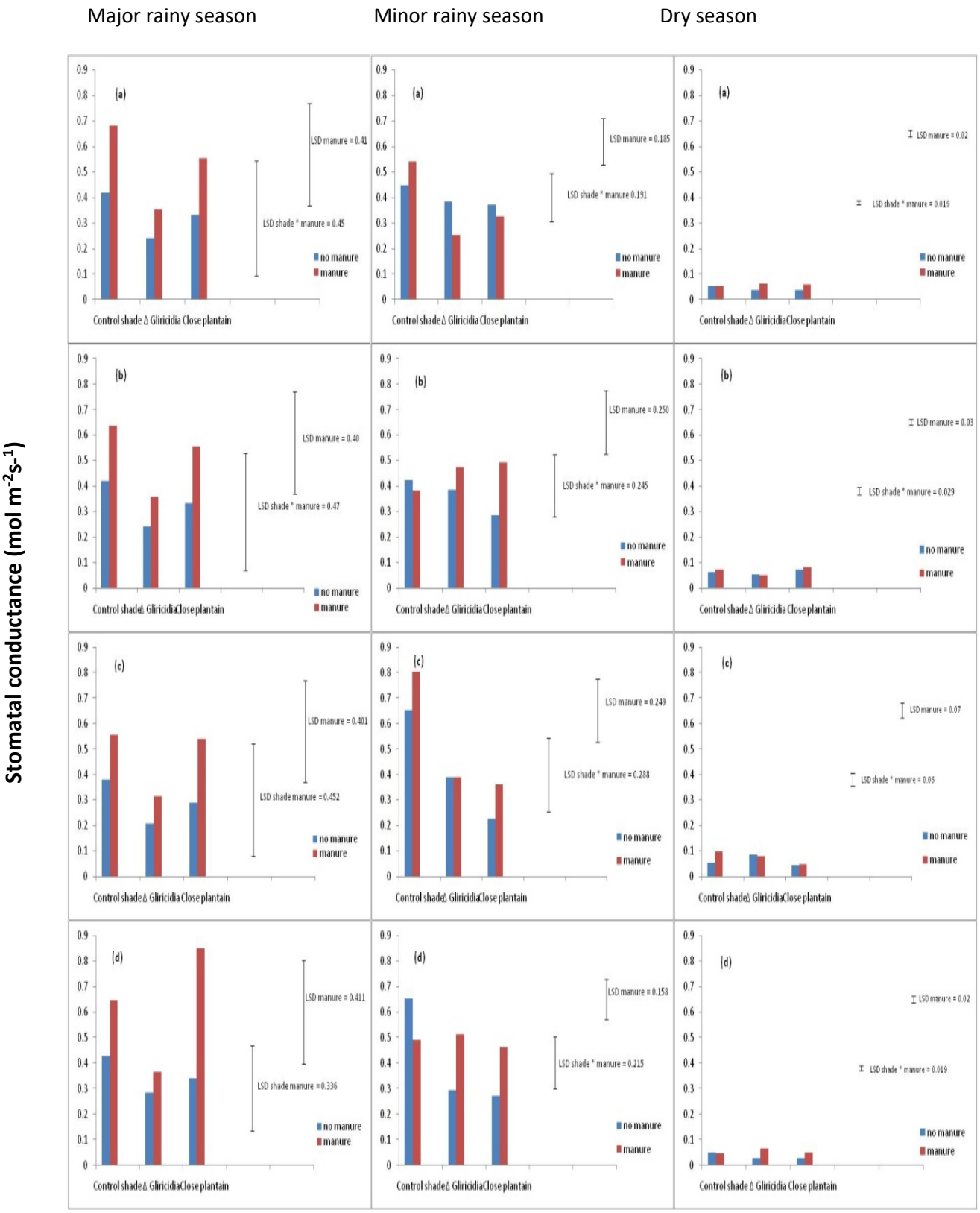

Figure 54 Stomatal conductance of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under the shade and manure treatments and in different seasons.

Each bar represents the mean value for 9 plants.

Transpiration

The mean transpiration rate (4.29 mmol $m^{-2}s^{-1}$) of the plants during the major rainy season was 2.9 times and 4.1 times higher than the mean rates in the minor rainy and the dry seasons, respectively.

In the major rainy season a 12.40% higher transpiration rate was observed under the fertiliser treatment compared with the control ($p = 0.022$). The highest transpiration rates (mean = 4.75 mmol $m^{-2}s^{-1}$) were observed under the control shade treatment. This was 10.1% and 25.0% higher than the value for plants under the close plantain and the triangular *Gliricidia* shade treatments, respectively. The differences were significant at ($p = 0.002$).

Significant ($p = 0.002$) differences were also found among the cacao clones, P 30 [POS] having the lowest rate (3.87 mmol $m^{-2}s^{-1}$) and PA 150, the highest rate (4.51 mmol $m^{-2}s^{-1}$). There were no significant interactions between treatments.

Also, in the minor rainy season differences among the clones were not significant. The shade treatments during this season were significant ($p < 0.001$) with plants under the control shade having the highest rate and those under the triangular *Gliricidia* shade the lowest.

The rate of transpiration was 16.29% higher in the fertiliser treatment compared with the control ($p = 0.004$).

In the dry season differences in the rates of transpiration among the clones were not significant. Also no significant differences were observed between the shade treatments. Differences between the fertiliser treatments were on the borderline of significance ($p = 0.059$), transpiration rates being 14.4% greater in the plants provided with manure than in the control plants.

The clone * shade interaction term was significant ($p = 0.036$). Whereas SCA 6 and PA 150 had their highest transpiration rates under the triangular *Gliricidia* shade, T 79/501 and P 30

[POS] had their highest rates under the close plantain shade. Plants supplied with organic fertiliser had a 14.43% higher rate of transpiration than those under the control, the difference being on the borderline of significance (p =0.059) (Fig 55). The clone * manure and the shade * manure and the 2nd order interaction terms were not significant.

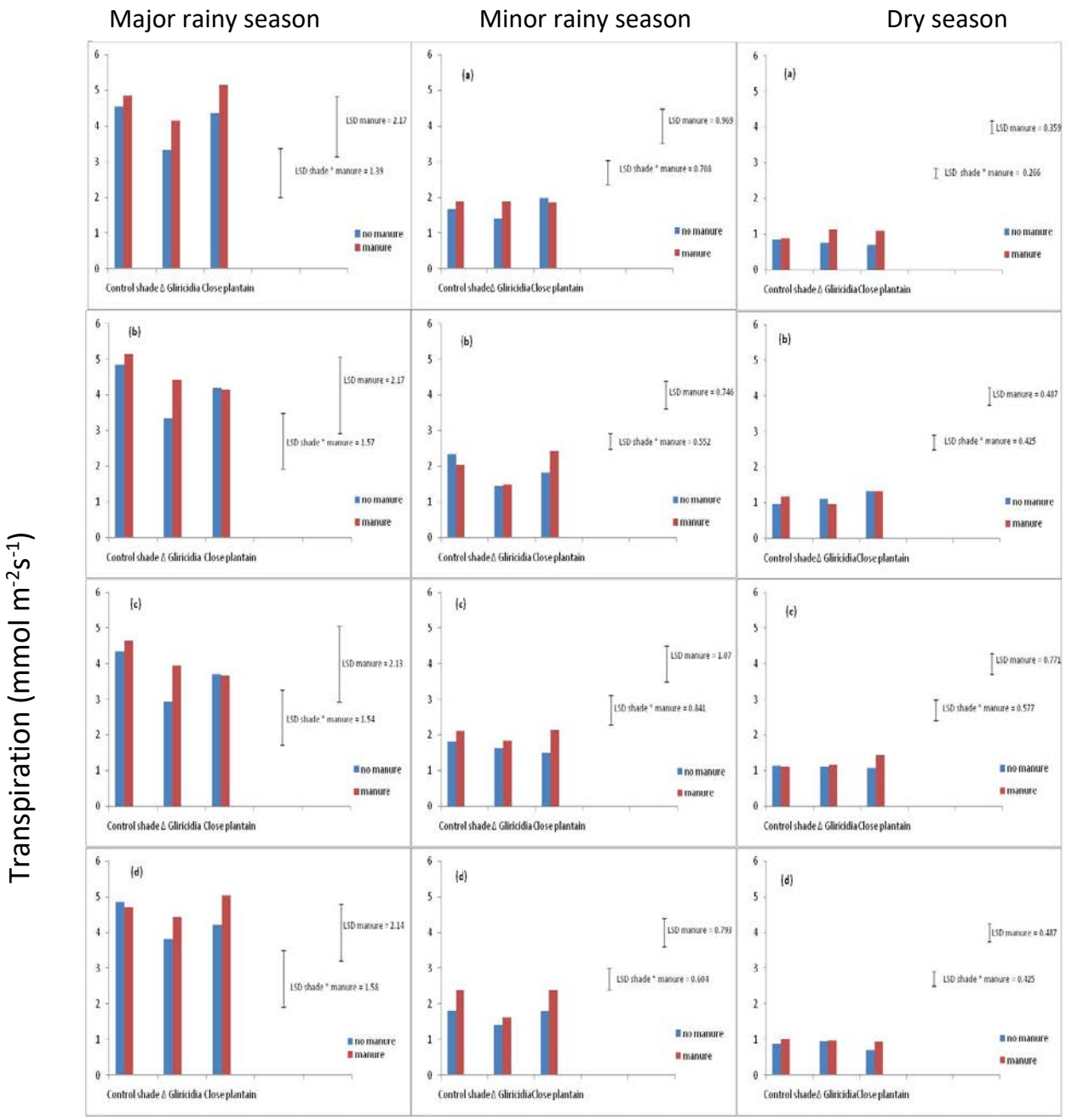

Figure 55 Effect of shade and manure on transpiration of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] 30 and (d) PA 150 in different seasons.

Each bar represents the mean value for 9 plants.

5.3.4 Photosynthesis

Mean photosynthetic rates were higher in the major rainy season than in the minor rainy and dry seasons by 19.26% and 71.86%, respectively (Fig 56). There were no significant differences between the clones in any of the seasons. Likewise, significant (p =0.032) differences were found between the shade treatments during the major rainy season with plants under the control shade having the highest photosynthetic rate and those under the triangular *Gliricidia* shade having the lowest rate.

The shade * manure interaction term was also significant (p = 0.029). Compared with the control treatment, plants that received manure application had 18.11% and 31.62% higher photosynthetic rates under the close plantain and the control shade treatments, respectively, but a 4.11% decrease under the triangular *Gliricidia* shade treatment. The other interaction terms were not significant.

In the minor rainy season, the clone * shade interaction term was significant (p = 0.053). For SCA 6 and T 79/501 the lowest photosynthetic rates were observed under the close plantain shade whereas for P 30 [POS] and PA 150 the lowest rates were observed under the triangular *Gliricidia* shade.

During the dry season another significant (p = 0.012) difference between the shade treatments was found with plants under the triangular *Gliricidia* shade having the lowest mean rate of photosynthesis. The shade * fertiliser interaction term was significant (p = 0.001). There was evidence that plants that were supplied with manure had enhanced photosynthetic rates under all the shade treatments but by considerably different margins. Under the triangular *Gliricidia* and the close plantain shade treatments, manure application caused 40.16% and 47.66% increases in photosynthesis as against an increase of 7.12% under the control shade treatment.

Major rainy season Minor rainy season Dry season

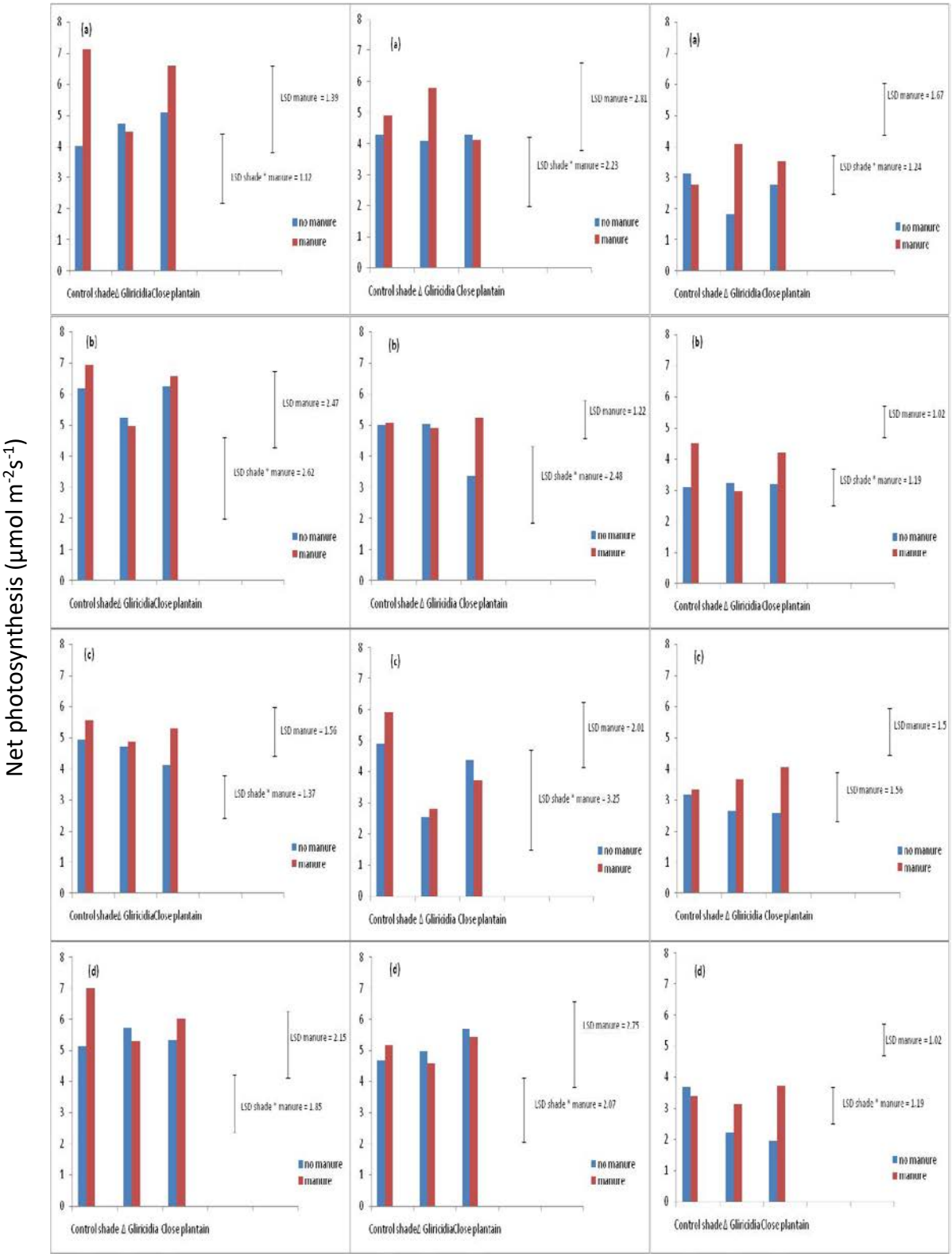

Figure 56 Photosynthesis of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments and in different seasons.

Each bar represents the mean value for 9 plants.

5.3.5 Leaf chlorophyll fluorescence

There were no significant differences in the leaf chlorophyll fluorescence of the genotypes under the shade or manure treatments. The clone * manure interaction term was significant ($p = 0.015$) in the minor rainy season; A 9.39% decrease in the Fv/Fm value was observed in SCA 6 under the fertiliser treatment whilst T 79/501, PA 150 and P 30 [POS] experienced 0.61%, 5.83% and 7.94% higher Fv/Fm, respectively (Fig 57). None of the other interactions were significant.

5.3.6 Water use efficiency

The mean water use efficiency of the cacao plants during the dry season was 3.2 µmol (CO_2) $m^{-2}s^{-1}$ / mmol (H_2O) $m^{-2}s^{-1}$ being 12.0% more efficient than it was during the minor rainy season and over 2 times more efficient than it was in the major rainy season.

In the major rainy season no significant differences were observed between the cacao genotypes or shade or manure treatments on water use efficiency. In that season the only significant ($p = 0.012$) difference was observed in the shade * manure interaction term. While plants supplied with organic fertiliser increased their water use efficiency under the close plantain and control shade treatments by 23.92% and 37.55%, respectively, they showed a 37.8% less efficient water use under the triangular *Gliricidia* shade treatment (Fig 58).

In the minor rainy season the clone * shade interaction term was on the borderline of significance ($p =0.067$). Whereas the highest water use efficiencies for SCA 6, T 79/501 and PA 150 were under the triangular *Gliricidia* shade treatment, P 30 [POS] had its lowest water use efficiency under that shade treatment.

In the dry season a significantly ($p = 0.002$) higher water use efficiency was observed under the control shade treatment, the value being 12.45% and 15.17% higher than what was rec-

orded for cacao plants under the close plantain shade and the triangular *Gliricidia* shade treatments, respectively. The shade * manure interaction term was fairly close to being significant ($p = 0.076$). With manure application the water use efficiency reduced under the control shade treatment by 3.52% but increased by 9.63% and 18.08% under the triangular *Gliricidia* and close plantain shade treatments, respectively.

Major rainy season Minor rainy season Dry season

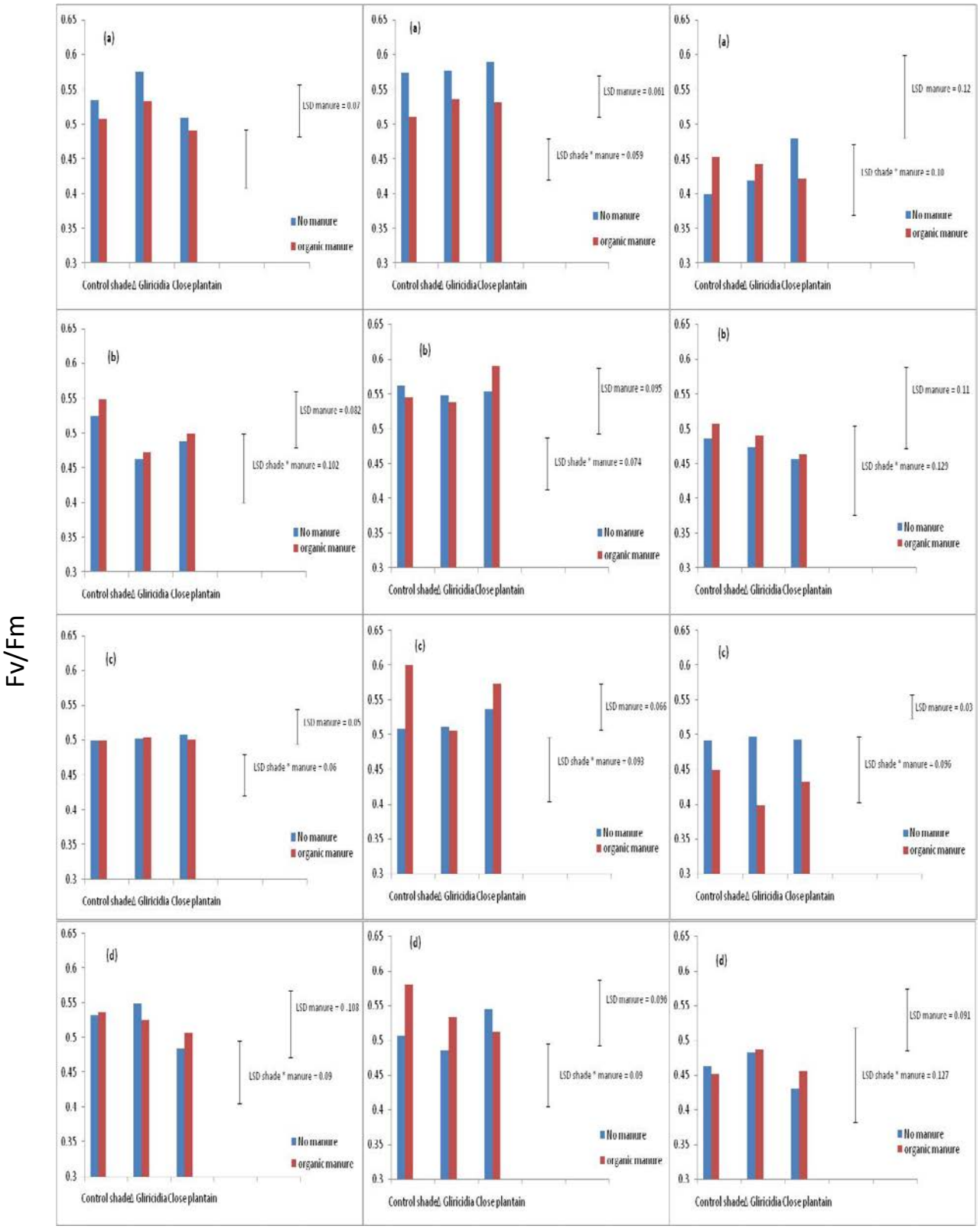

Figure 57 Fv/Fm of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] 30 and (d) PA 150 under different shade and manure treatments and in different seasons.

Each bar represents the mean value for 9 plants.

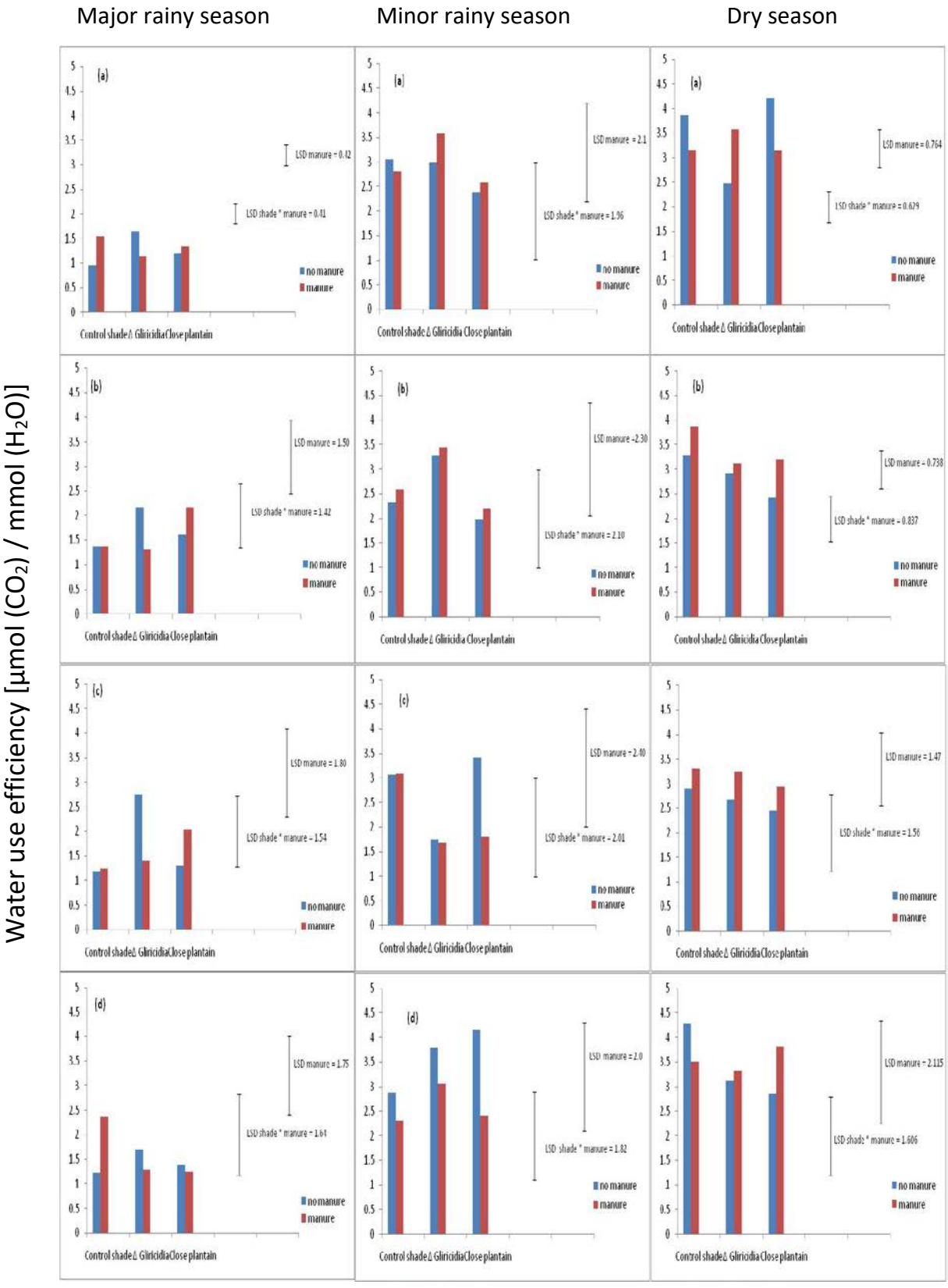

Figure 58 Water use efficiency of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] 30 and (d) PA 150 under different shade and manure treatments and in different seasons.

Each bar represents the mean value for 9 plants.

5.3.7 Leaf traits

Leaf chlorophyll content

None of the treatments were significant on the leaf chlorophyll content. Only slight increases in leaf chlorophyll content were recorded under fertiliser application in the case of SCA 6 and T 79/501. P 30 [POS] experienced a slight decrease and no clear trends were observed for PA 150 (Fig 59). Similarly no clear trends were observed under the shade treatments.

Relative water content

No significant differences were observed in the relative water content of leaves under any of the imposed treatments or their interactions (Fig 60).

Specific leaf weight

There were significant ($p = 0.038$) differences between clones in their specific leaf weights. P 30 [POS] had the highest value (4.53 mg cm^{-2}) and that was 3.01%, 6.08% and 12.52% higher than the values obtained by SCA 6, T 79/501 and PA 150, respectively.

The shade and manure treatments were not significant but the shade * manure treatment interaction component was marginally significant ($p = 0.052$). Plants under the control shade treatment which received no fertiliser had the highest specific leaf weight (4.50 mg cm^{-2}) whilst those that grew under the triangular *Gliricidia* shade and received fertiliser had the lowest value (12.50% lower) (Fig 61).

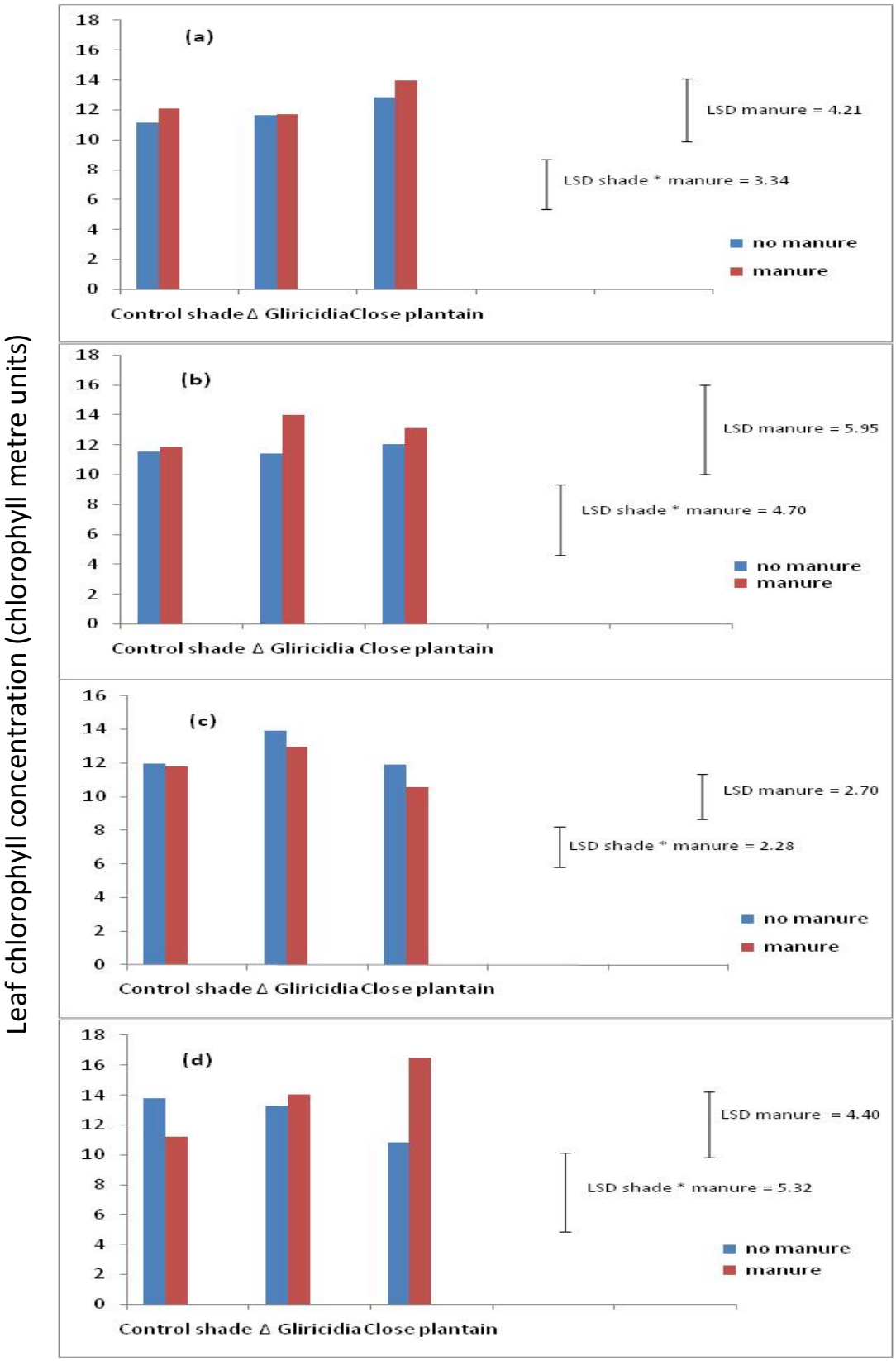

Figure 59 Leaf chlorophyll concentration of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments. Each bar represents the mean value for 12 plants.

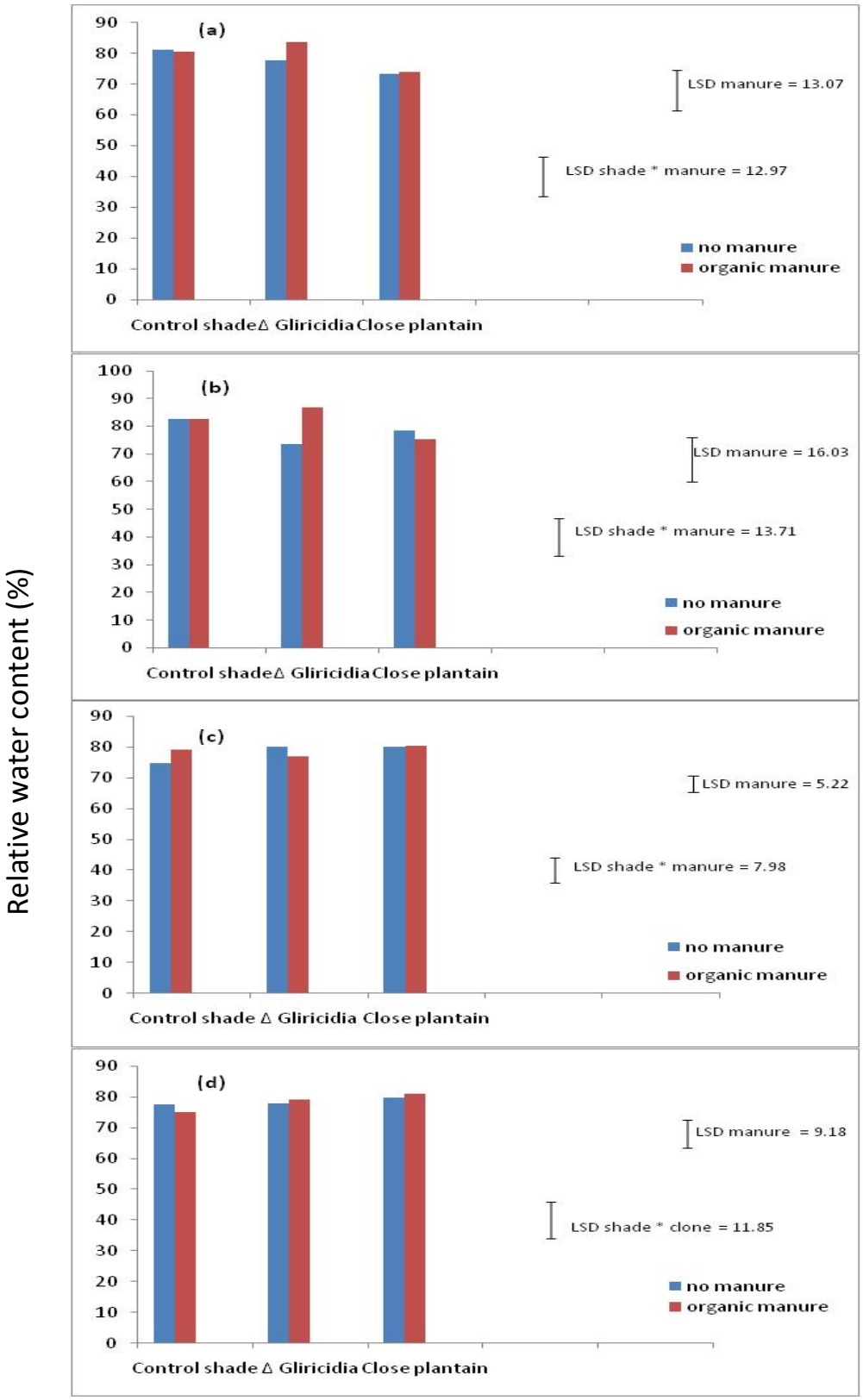

Figure 60 Relative water content of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments.

Each bar represents the mean value for 12 plants.

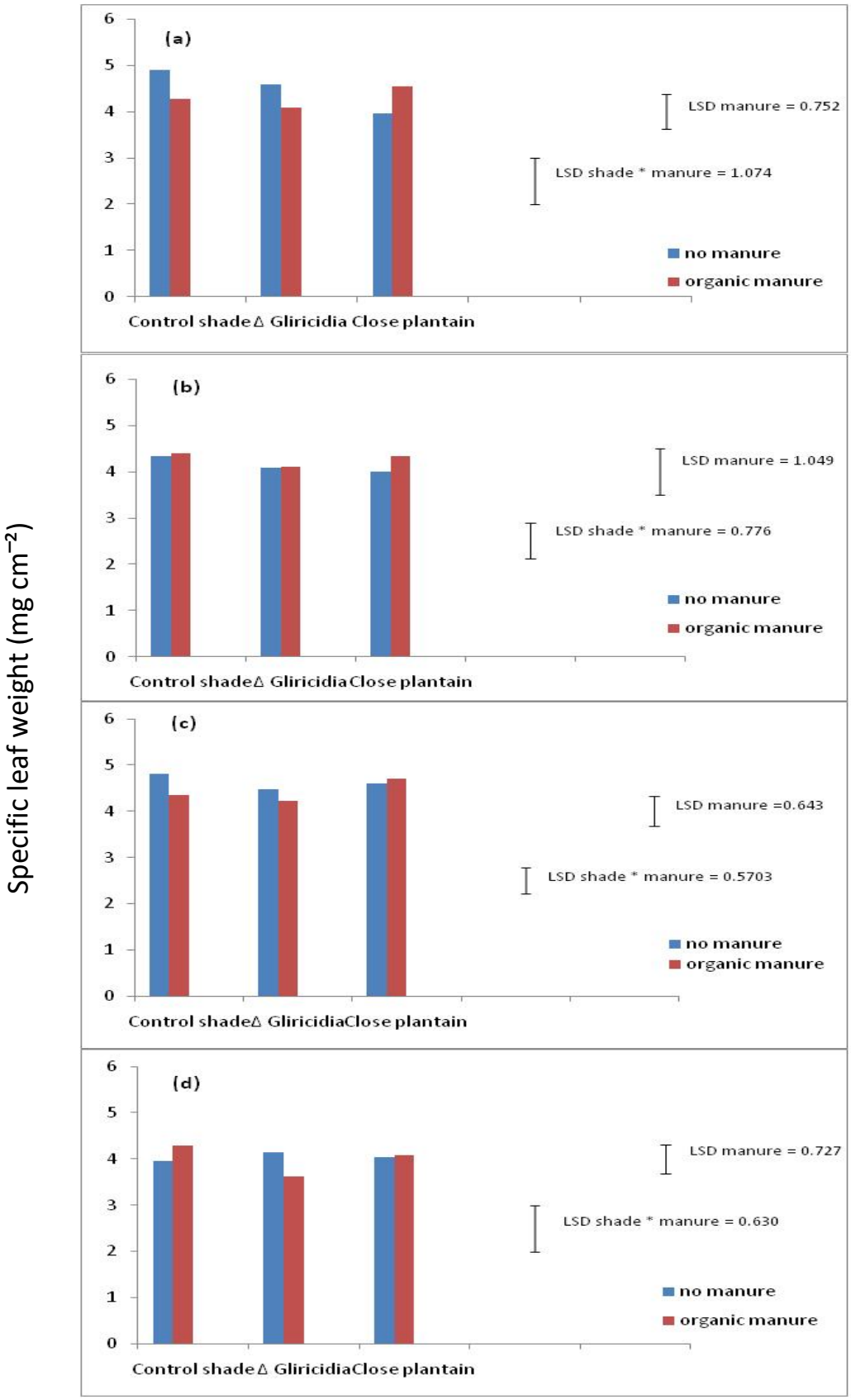

Figure 61 Specific leaf weight of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments. Each bar represents the mean value for 12 plants.

Leaf nitrogen concentration

There were significant (p = 0.012) differences between cacao clones in leaf nitrogen concentration. Their values ranged from 1.78% in SCA 6 to 2.02% in PA 150.

Differences among the shade treatments were on the borderline of significance (p = 0.054) with the plants shaded with the triangularly planted *Gliricidia* having the highest concentration (1.95%) compared with the value (1.83%) for plants grown under the control shade which had the lowest leaf nitrogen concentration.

Leaf nitrogen concentration increased significantly (p = 0.001) with organic manure application. The leaves of cacao plants under the control treatment had 1.81% N as against 1.95% N for the leaves of plants provided with fertiliser (Fig 62). No significant interactions were observed

Leaf phosphorus content

Total leaf phosphorus content did not differ substantially between clones or the shade treatments. Plants that grew under the control shade treatment had a 6.85% higher concentration than those that grew under the close plantain shade (Fig 63). Leaf phosphorus concentration in plants supplied with manure had, on average, an 8.0% increase and that difference was marginally significant (p = 0.047). No significant interactions were observed.

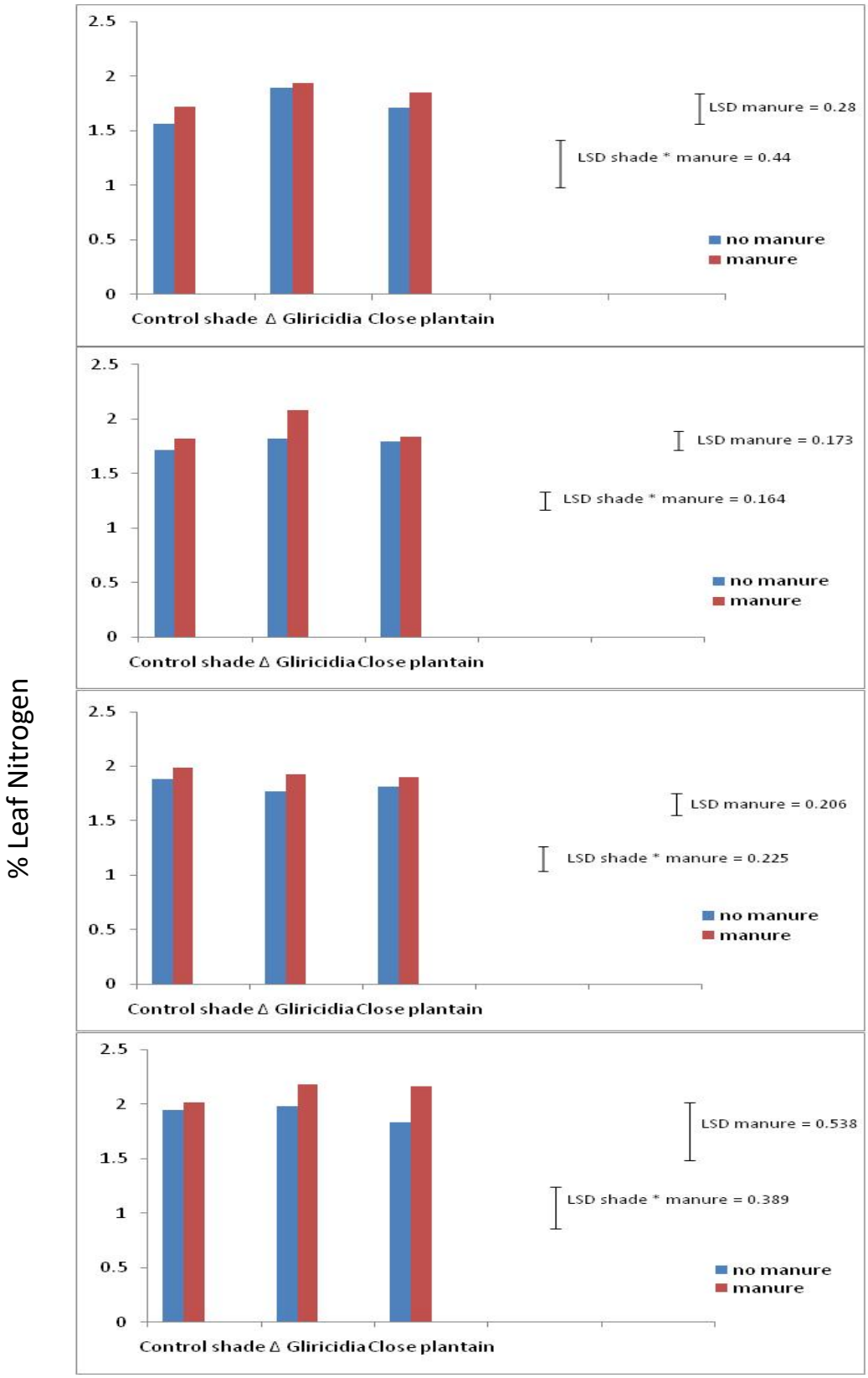

Figure 62 % leaf N of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments.

Each bar represents the mean value for 9 plants.

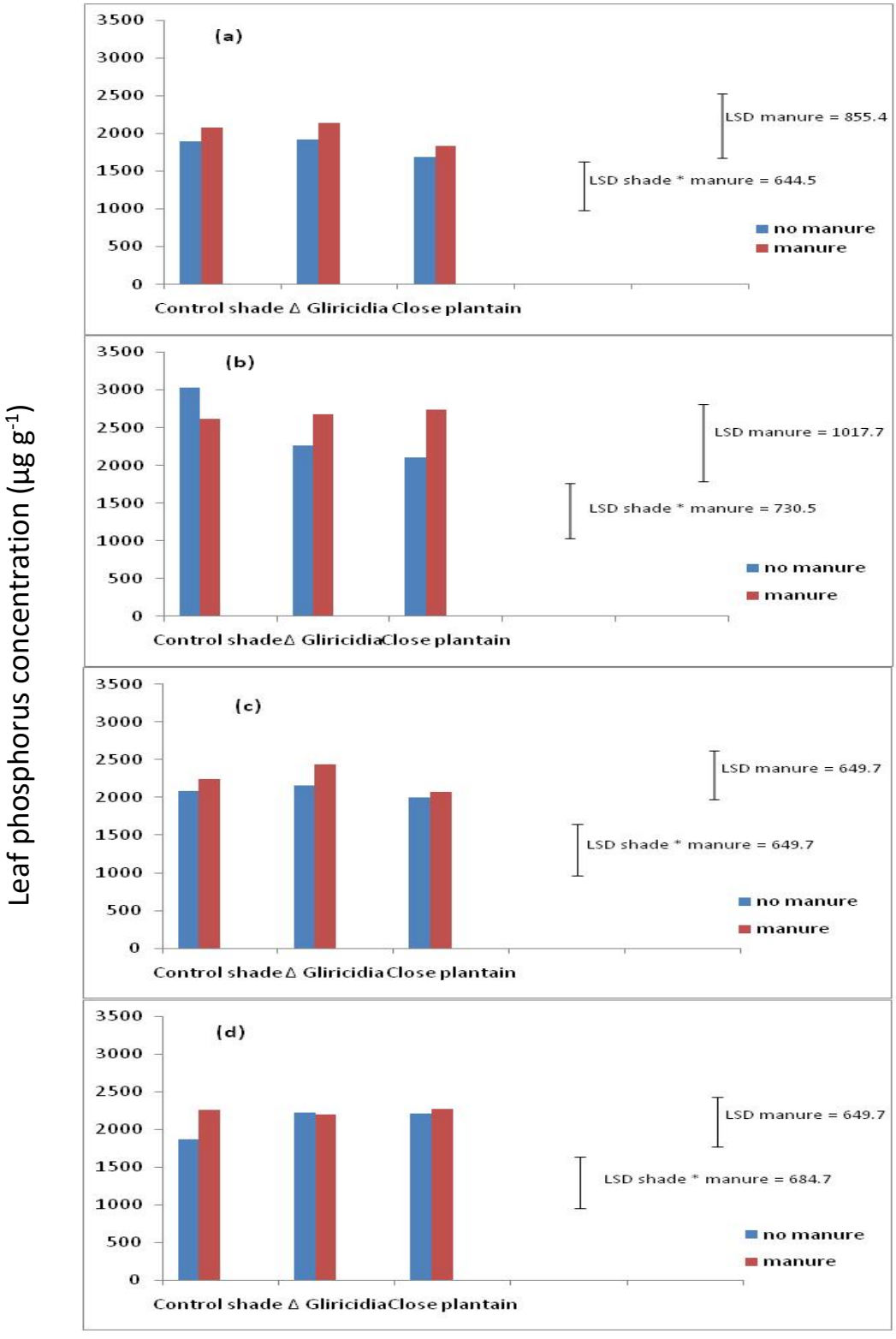

Figure 63 Leaf P concentration of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments.

Each bar represents the mean value for 9 plants.

5.3.8 Leaf K^+ concentration

Whilst no differences were noticed between clones in their leaf potassium ion concentrations the clone * shade interaction term was significant (p = 0.018). Plants that grew under heavier shade had lower leaf K^+ concentration in SCA 6, T 79/501and P 30 [POS] whereas plants of the clone PA 150 had higher leaf K^+ concentration under increasing shade (Fig 64). Plants which were provided with fertiliser had a 13.7% higher leaf K^+ concentration compared with the control (p = 0. 001)

The clone * manure and shade * manure interaction units were not significant.

5.3.9 Plant survival

Survival rates of the clones were significantly (p = 0.027) different, ranging from 79.86% for SCA 6 to 96.53% for PA 150 during the first year.

There were no significant differences between the shade treatments on plant survival.

Cacao plants provided with manure had a significantly (p = 0.02) higher survival rate (Fig 65).

No significant interactions were observed.

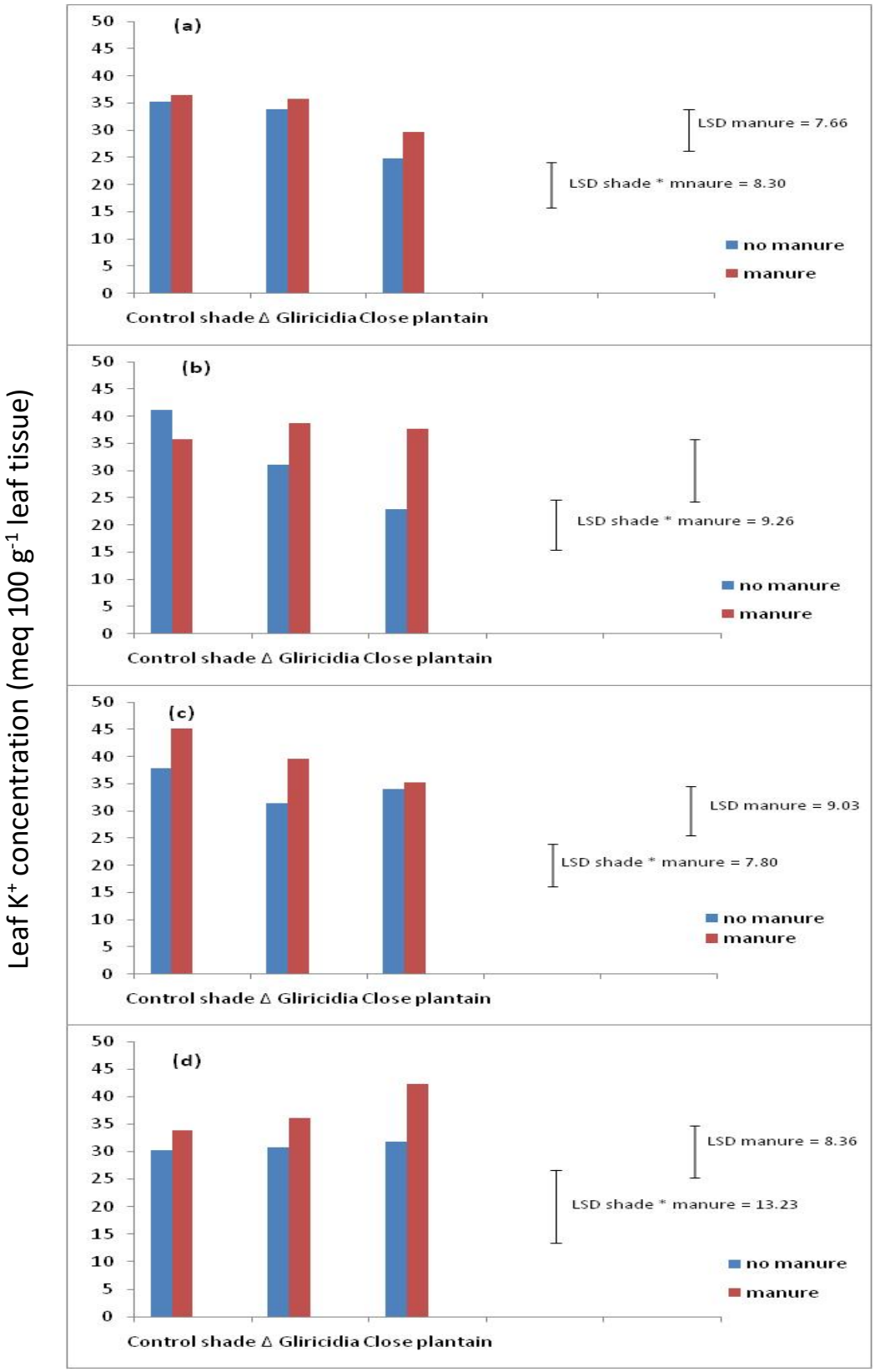

Figure 64 Leaf K^+ concentration in (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments.

Each bar represents the mean value for 9 plants.

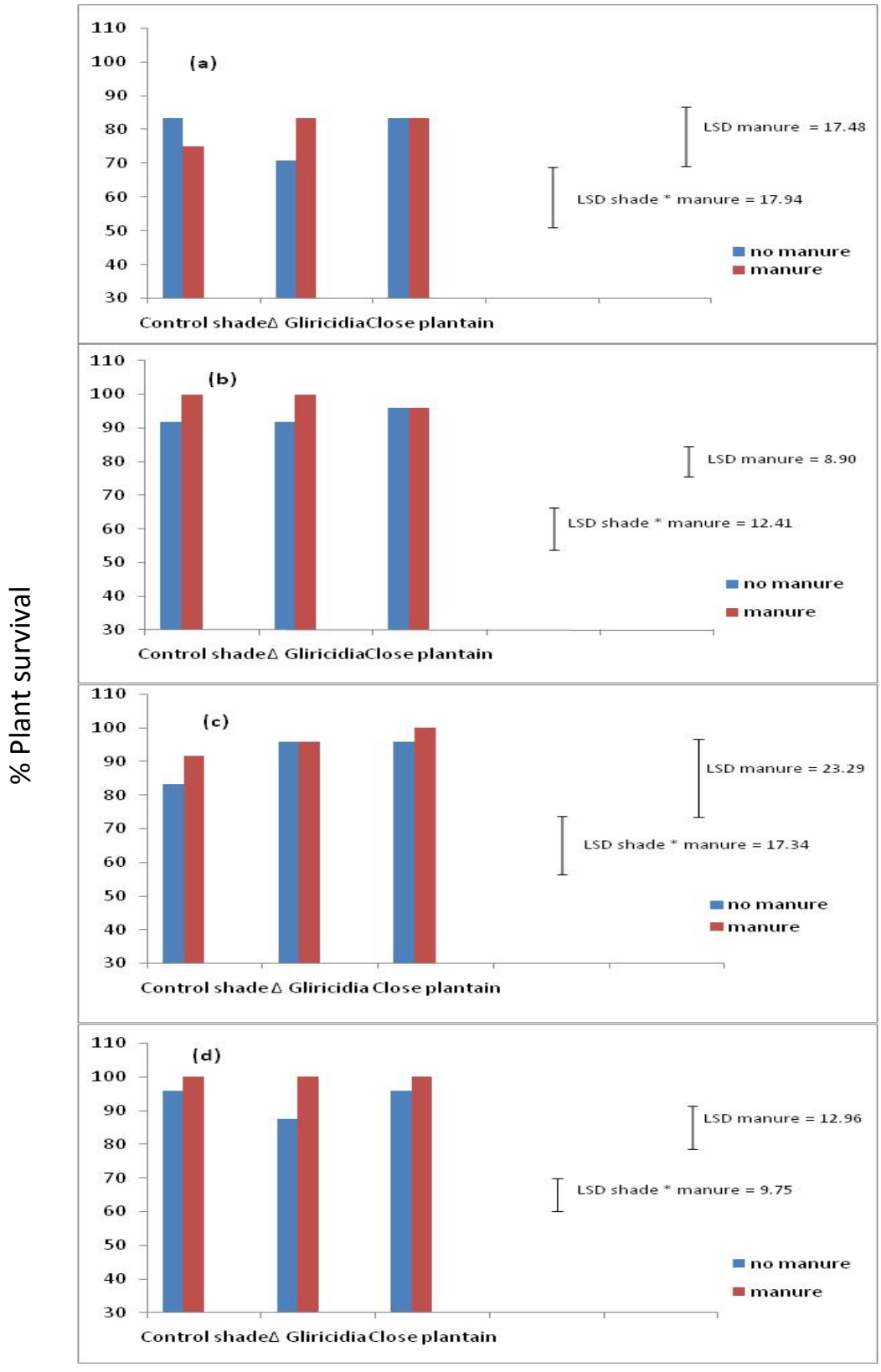

Figure 65 % Plant survival of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments.

Each bar represents the mean value for 24 plants.

Stem diameter

Mean stem diameter of the clones ranged from 2.30cm (for SCA 6) to 2.99 cm (for T 79/501). The difference was significant at ($p = 0.029$).

No significant differences were noticed between the shade treatments and plants that were treated with the organic fertiliser did not experience increases in plant stem girth (Fig 66).

Plant height

The cacao clones had different ($p = 0.034$) plant heights. The clone PA 150 was the tallest (140.30 cm) and this was 20.53% taller than SCA 6 (the shortest).

Whereas no significant differences were observed between the fertiliser treatments the clone * fertiliser interaction term was marginally significant ($p = 0.091$). With fertiliser application SCA 6 and P 30 [POS] experienced 14.65% and 1.62% reductions in plant height, respectively whereas PA 150 and T 79/501 became 3.80% and 4.57% taller, respectively, compared with control plants (Fig 67).

Plants growing under the different shade treatments did not have significant differences in their heights.

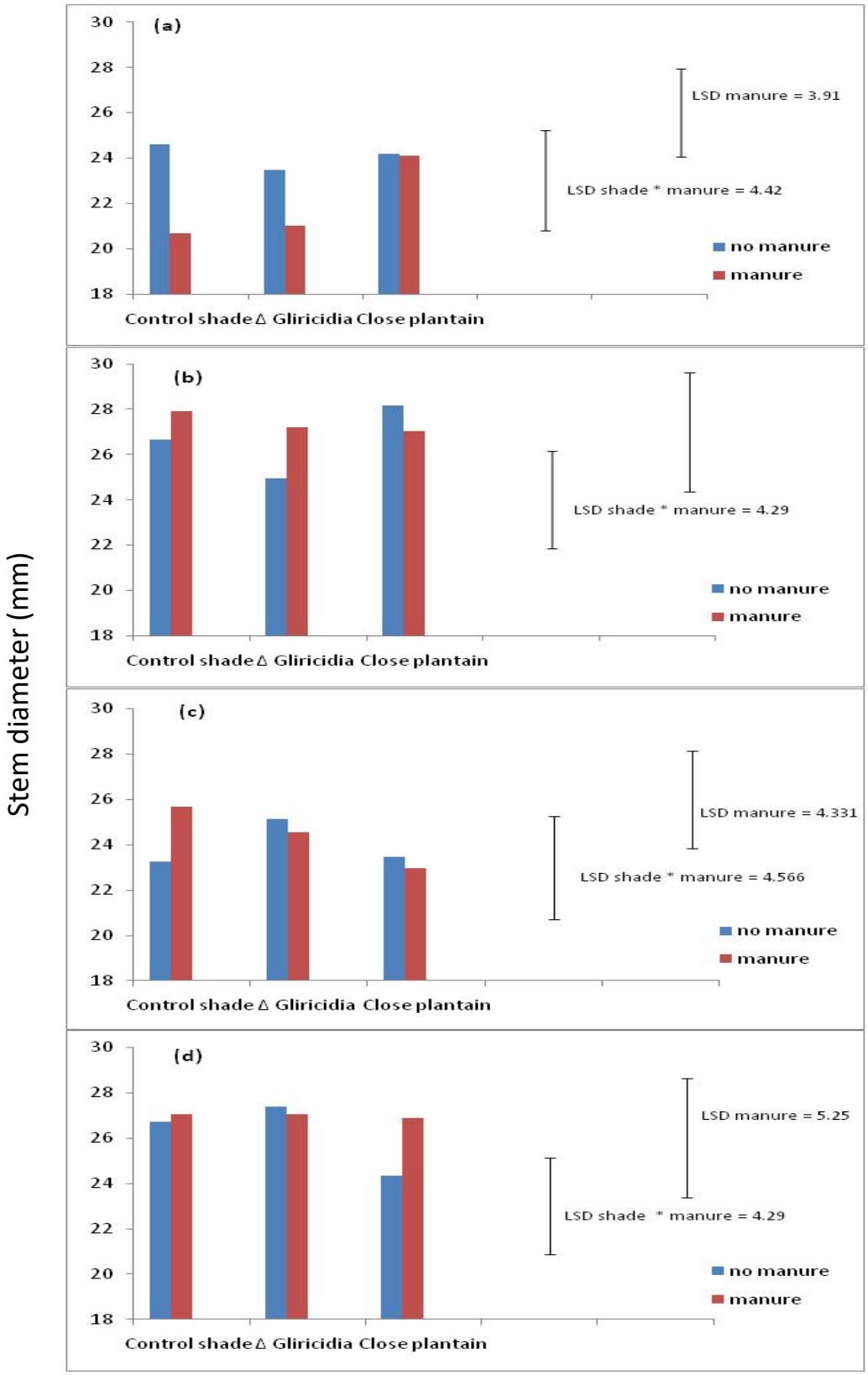

Figure 66 Stem diameter of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments.

Each bar represents the mean value for 12 plants.

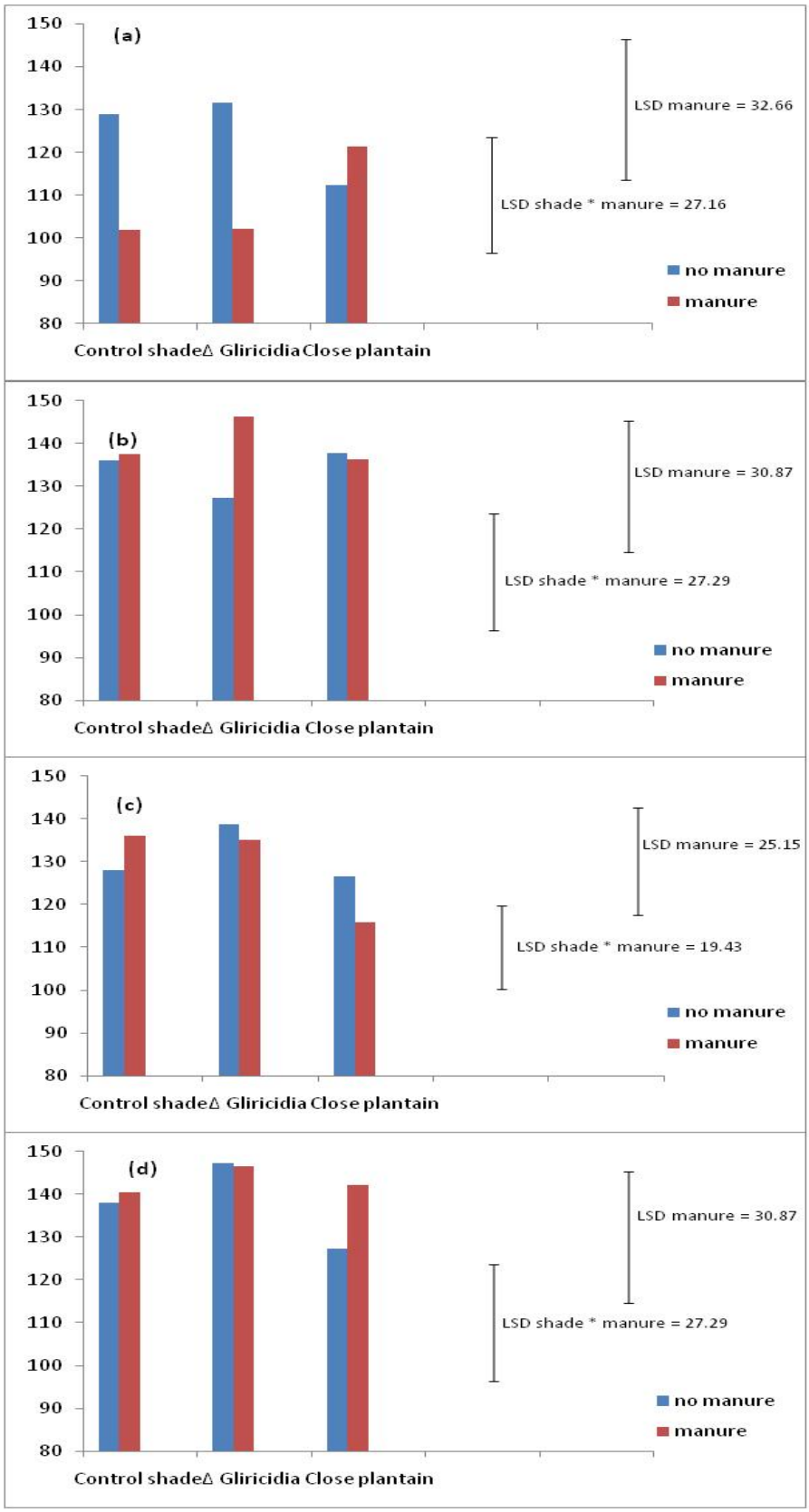

Figure 67 Height of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments.

Each bar represents the mean value for 12 plants.

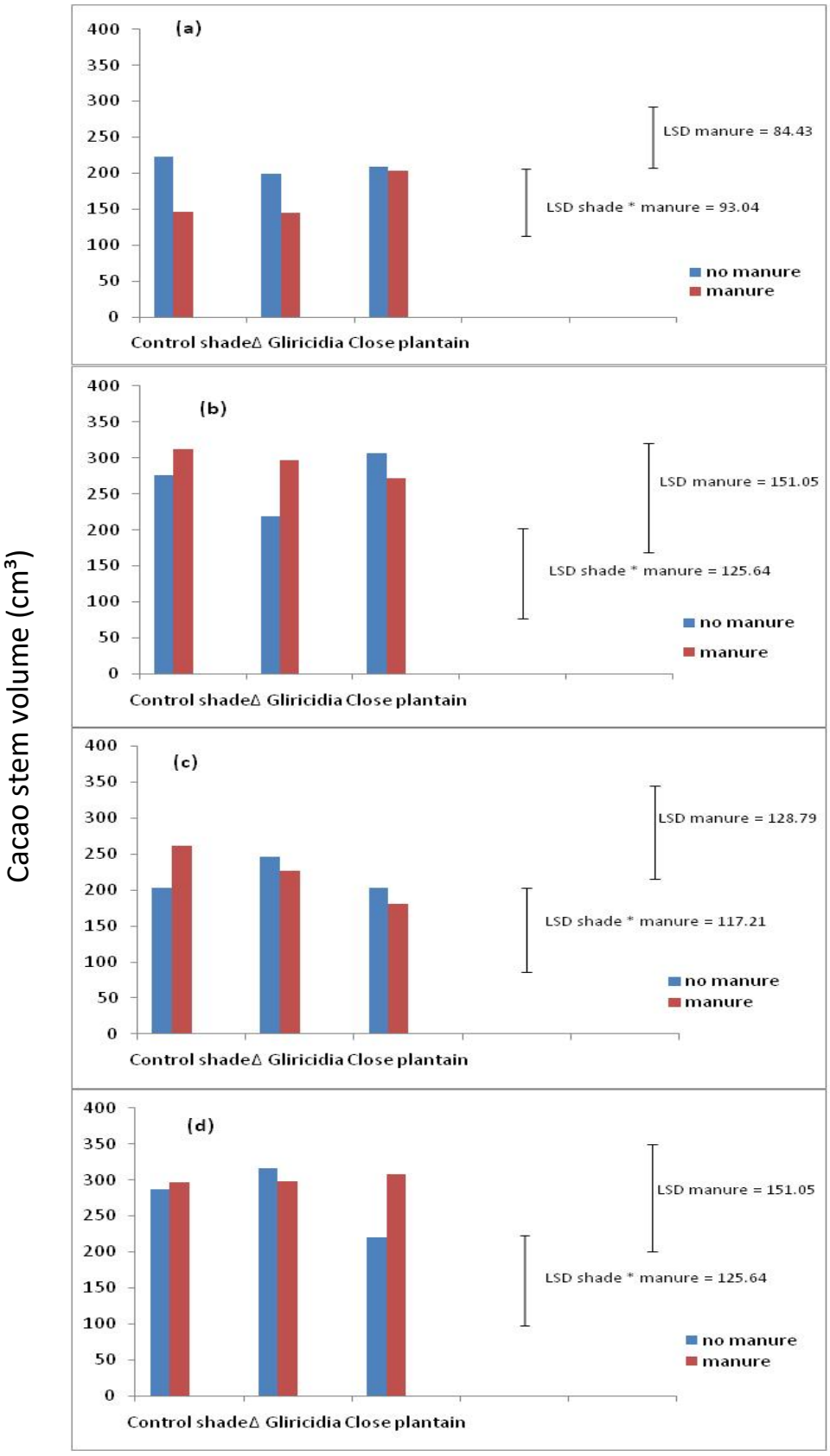

Figure 68 Stem volume of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different shade and manure treatments.

Each bar represents the mean value of 12 plants

Stem volume

The cacao clones had statistically different (p = 0.024) stem volumes. The clone PA 150 which had the largest stem volume (mean = 287.58 cm^3) was 2.60%, 23.46% and 53.61% bigger than T 79/501, P 30 [POS] and SCA 6, respectively.

No substantial differences were recorded between the shade or fertiliser treatments (68).

5.4 Discussion

5.4.1 Stomatal conductance and transpiration

The observation that plants under the control shade had the highest stomatal conductance, transpiration and CO_2 assimilation rates in the rainy seasons but the lowest rates in the dry season is similar to the observation made in the shade * clone experiments. Plants under increased shade experienced reduced atmospheric stress during the dry season as a result of a reduced water vapour pressure deficit. As explained in Chapter 4 more light energy was needed during the rainy seasons to drive water uptake and transpiration.

During the major rainy season stomatal conductance and transpiration rates were substantially different for the cacao genotypes, with PA 150 having the highest rates and P 30 [POS] the lowest but in the dry season there was little difference between clones. Thus the data in this experiment do not show which cacao genotypes can better tolerate a condition of drought by means of a more effective stomatal regulation. However, they show differences in the degree of plasticity among the clones suggesting that P 30 [POS] and SCA 6 have an intrinsically lower stomatal conductance under non-limiting conditions.

The significant increase in stomatal conductance and transpiration rates observed under fer-

tiliser application in all the seasons seems to suggest that the amounts of nutrients supplied were reasonable and not excessive considering the soil's water status. It has been noted in a few plants that application of some soil nutrients must be commensurate with available soil water. Working on the interaction of nitrogen supplied and water stress on photosynthetic parameters of rice plants Otoo et al. (1989) observed that the internal water relations of the plants under water stress conditions could be worsened by high soil nitrogen. Similar observations have been made on other crops including banana (de Melo et al., 2009) and *Cassia streata* L. and *Sesbania rostrata* L. (Boruah et al., 2008). In a review of the effects of soil nutrients and soil water on plants in the dry land areas of Northern China Li et al. (2009) observed increased plant water uptake with appropriate N supply and improved stomatal regulation with K supply. Thus fertilisation with N and K in their right amounts is associated with increases in transpiration rates when soil water is adequate but with a more effective stomatal control in a situation of soil water deficit. These authors reported that if total leaf area increases with fertiliser application the greater ground coverage by plants reduces soil surface evaporation which makes more soil water available for use in transpiration. In a similar observation Turnbull et al. (2007) recorded a higher stomatal conductance rate in *Eucalyptus globules* L. plants after nitrogen fertilisation.

Orchard (1978) showed, in cacao seedlings, an inverse relationship between K doses and leaf transpiration and Bosshart and Uexkhull (1987) observed mature cacao trees that had been supplied with reasonably high doses of K to be more tolerant to soil moisture stress.

In an experiment to determine whether transpiration could be partially controlled by the soil nutrients 'status' Cramer et al. (2008) made a slow release fertilizer N and K either available in the root zone of *Ehrharta calycina* plants without any hindrance or partially restricted and made available only by diffusion and /or mass flow. Transpiration was observed to be 60%

higher in the plants with a restricted flow of nutrients. This was explained as an adaptation by the plants with a restricted nutrient flow to increase the transport of nutrients from the soil to the roots so as to ensure plant survival. Therefore combining organic materials that were good sources of N and K would be associated with improved water relations of the young cacao plants.

The mean water use efficiency was highest in the dry season and is consistent with the observation in the clone * (quantity of water * frequency of watering) in which plants that grew under conditions of reduced soil water had a more efficient water use. Rada et al. (2005) observed, in young cacao that under conditions of soil water stress stomatal closure reduced water loss through transpiration much more than it reduced the rate of photosynthesis resulting in an increase in water use efficiency. This also explains the higher water use efficiency recorded under the control shade than the heavier shade treatments during the dry season.

5.4.2 Leaf chlorophyll fluorescence and photosynthesis

The cloudy situation and low temperatures of the minor rainy season would have been less conducive for photosynthesis than the major rainy season [Raja Harun and Hardwick (1988); Hadley (1994); Galyuon et al. (1996b); Daymond and Hadley (2004)] . As explained in the discussion of the shade * clone experiment (Chapter 4) light saturation in young cacao is only achieved at 400 µmol $m^{-2}s^{-1}$ or at a higher light intensity (Okali and Owusu 1975; Raja Harun and Hardwick, 1988). The efficiency of photosystem II (Fv/Fm) being highest in the minor rainy season was probably an indication that photoinhibition was minimal in that season. As explained previously, other species have been noted to react similarly. For example, Volkova et al. (2009) conducted a study on the tree fern *Dicksonia antartica* L. under climate cham-

ber conditions with three moderate sequential temperatures (between 15 ^{o}C, 30 ^{o}C and back to 15 ^{o}C) and two light levels (170 µ mol $m^{-2}s^{-1}$ and 900 µ mol $m^{-2}s^{-1}$) and recorded lower Fv/Fm and maximal Rubisco activity under higher light intensity and increased temperature. The similar response which was observed in cacao in the present work may indicate an adaptation by the plants to increase their efficiency of light use when available light levels were low in order to maintain a good level of photosynthetic activity.

The lower stomatal conductance in the dry season resulted in reduced photosynthetic rates in that season. Furthermore, light use efficiency was reduced under increased light availability. A similar response was observed by Yeo and Lee (2009) on two rose cultivars who found their Fv/Fm to decrease with increasing light in order to protect the photosynthetic apparatus from being damaged.

Fertilisers applied as soil amendments have been associated with increased photosynthetic rates in several plant species including *Eucalyptus globules* L. (Turnbull et al., 2007) sweet corn (Efthimiadou et al., 2009), cotton (Ahmed et al., 2009) and *Brassica napus (Liu et al., 2010).* According to Cakmak and Engels (1999) mineral nutrients influence photosynthetic electron flow either as "constituents of the light-harvesting complex" or as "ions facilitating electron flow". As rational amounts of N and K are also linked to improved internal water balance of plants they are likely to support increased photosynthetic rates. Nitrogen is also associated with an increase in the function of enzymes and protein formation and is involved in the synthesis and transfer of energy (Rana et al., 2009, Liu et al., 2010). Phosphorus would improve the conversion of light energy to chemical energy (ATP) during photosynthesis (Bown et al., 2009).

In young cacao a positive relationship between improved nutrition and photosynthesis was demonstrated by Lockard and Asomaning (1963) although later Ahenkorah et al. (1974) ob-

served that cacao does not respond to N application until after the second year of cropping when soil fertility begins to decline. As the initial N and K levels in the soil used for the current experiment were below the critical values for successful establishment [See Table 5 in the General Materials and Methods Chapter] fertiliser application was expected to stimulate photosynthesis. In conformity with Liebig's "Law of the Minimum", plants that grow on soils that contain lower than the critical value of a particular nutrient tend to have lower concentrations of the nutrient concerned in their tissue and give the highest response to fertilisation with that nutrient (Salisbury and Ross, 1992).

The fact that increased photosynthesis was found in fertilised plants in the dry season more than in the rainy seasons may be attributed to its ability to modulate soil temperatures and improve plant water status. The benefits associated with such interventions become more manifest under harsher conditions such as were prevalent in the dry season. Experiencing more stress in the dry season plants that had not been provided with manure would exhibit greater stomatal closure in order to conserve plant tissue water inevitably causing reductions in CO_2 uptake and consequently lower rates of photosynthesis. In addition to improved plant water status that was observed with the supply of plant nutrients to rice plants, Otoo et al. (1989) also noted that the rate of photosynthesis was relatively higher even when water supply was restricted.

The observation made in the current experiment is consistent with an earlier one made by Alvim (1977) who reported that under conditions of adequate soil moisture low light intensity reduces the photosynthetic response of cacao seedlings to fertilizer application. In mature cacao yield response to fertilizer has been reported as having a positive relationship with light interception (Vernon, 1967, Vernon and Sundaram, 1972, Zuidema et al., 2005).

Organic manures increase soil organic matter, reduce soil bulk density and thus facilitate

root penetration down the soil profile (Li et al., 2009). Although there was only a small increase in soil moisture content following manure application, root growth and deeper root penetration could have occurred. Li et al. (2009) report that increased availability of plant nutrients improves the ability of the roots of many plants to absorb plant nutrients and water.

The significant shade * manure interaction in which plants that received manure had higher increases in photosynthesis under increasing shade in the dry season may be explained as a result of the ameliorating effect of shade by reducing the water vapour pressure deficit and reducing evaporation of soil water by increasing ground coverage by plants.

5.4.3 Leaf traits

Unlike leaf phosphorus and potassium contents which were not different, the significant difference in leaf nitrogen content among the cacao clones indicates that there is considerable variability in the genotypes of cacao as regards nitrogen uptake and/or translocation into leaf tissue. The insignificant effect of shade on leaf N was similar to the observation made in the shade * clone experiment in which the range of shade levels was greater. Changing the spatial arrangement of plantains or introducing additional *Gliricidia* shade plants was not found to be associated with a significant change in leaf N or P. Similarly, Isaac et al. (2007) observed that nutrient uptake in young cacao was sustained for N and P when the cacao was intercropped under *Terminalia superba* and *Newbouldia laevis* shade plants, suggesting low inter-specific competition for the nutrients.

The observed increase in leaf N and K in plants following organic fertiliser application is consistent with an earlier finding of Ahenkorah et al. (1981). Turnbull et al. (2007) found in their experiment with *Eucalyptus globules* L. that plants which were supplied with NPK fertilizer

annually had increased leaf nitrogen content and Isaac et al. (2007) observed that young cacao plants that experienced increased nutrient supply had higher NPK uptake. Agele and Agbona (2008) found in an experiment involving cashew seedlings that leaf NPK increased with increasing quantities of applied powdered cacao pod husk as a soil amendment. In the present experiment leaf P did not increase significantly with the application of the organic fertiliser suggesting that the amount of P supplied was below the threshold quantity that was needed for increased leaf P. Initial soil analysis showed that the soil already contained more than the critical level of P for cacao establishment [= 0.013 mg kg^{-1}, Akirinde and Ayegboyin (2006); compare with Table 5 in section 2.6.1]. In such a case higher levels of application are required to lead to appreciable changes in uptake of the nutrient in question (Salisbury and Ross, 1992).

With increased leaf N in response to manure application, it was expected that the leaf chlorophyll content would also increase significantly, as has been noted by other workers in some plant species including peanuts (Sheshshayee et al., 2006) and cacao (Costa et al., 1998). The fact that a corresponding increase in leaf chlorophyll content was not detected may be a result of masking by other pigments (Thomas, 2000). Lee et al. (1987) reported that changing levels of other pigments including anthocyanins and phenols are capable of masking the chlorophyll content of cacao leaves. Whatley (1992) found similar effects in a range of tropical plants including cacao and coffee. Another possible cause would be a slowing down of chlorophyll synthesis by the presence of hormones as a result of organic fertiliser application. Baker and Hardwick (1973) observed that some plant hormones such as auxins, cytokinins and gibberellins can hamper chlorophyll synthesis in cacao.

Unlike the observation made in the shade * clone experiment, the shade * clone interaction term in this experiment was not significant. The difference in the two results may be due to

the differences in the ranges of applied shade or in other environmental conditions under which the two experiments were carried out, one being a nursery experiment and the other, a field experiment.

Li et al. (2009) reported increases in the relative water content of some plants when organic fertilisers were applied in the dry land areas of Northern China. However, in the current work there was only an insignificant increase in relative water content with the application of the organic fertiliser and that may have been due to the timing of data collection. Clearer results may have been obtained if the measurement was made before the dry season of 2007/2008 ended unexpectedly. The observation that plants which grew under the lightest shade and received no manure had the highest specific leaf weight was probably, a reflection of the lower leaf nitrogen concentrations in the in those plants. The observation agrees with results of other studies. For example, Steinbauer (2000) found greater specific leaf weights in seedlings of *Eucalyptus globules* L. which were low in nitrogen and high in fibre.

Plant survival

The significantly different survival rates for the cacao genotypes reflected their different growth rates. With the annual dry spell in West Africa, progenies with greater seedling vigour establish better. As mentioned in Chapter 1 such genotypes are more likely to develop deeper root systems that have greater access to soil water before the dry season begins (Balasimha 1999).

Although organic fertiliser application was not found to be significantly associated with plant growth within the period of study it was clearly linked with higher plant survival. The connection cannot be clearly described with certainty as enhanced plant survival could have been caused not only by improved plant nutrition but also by indirect benefits such as reduced soil temperature and increased root and stem water content during the dry season.

That higher plant nutrition is associated with increased cacao seedling survival has been demonstrated by Rajagopal and Balasimha (1994) who reported higher cacao seedling survival under water stress with the foliar application of potassium and proline under nursery conditions. Furthermore, the higher N and K content in plant tissue (as evidenced by the higher leaf concentrations) was likely to help plant osmoregulation as Mikkelsen (2007) and Orlova et al. (2009) suggest. Although Premachandra and Joly (1991) did not detect osmoregulation in cacao seedlings, de Almeida et al. (2002) reported evidence of the phenomenon in some genotypes. Assessing the response of eight 5-month old genotypes that had been grafted on *Cacau comun* rootstock de Almeida et al. (2002) noted that drought tolerance was attained by way of osmotic adjustment resulting in considerable increases in leaf concentrations of P and K as water stress increased to -1.5MPa. Later Rada et al. (2005) detected the phenomenon in seedlings of the cacao variety Guasare. Therefore, the increase in plant survival that was found with organic fertiliser application could have been achieved through greater osmoregulation.

A faster plant growth was expected under manure application. Bonaparte (1975) and Ahenkorah et al. (1987) emphasized the importance of soil nutrients to plant growth and suggested that it was only when there were enough plant nutrients that the positive relationship between light and growth becomes clearly seen in cacao. Expressing a similar view on mature cacao, Cunningham and Arnold (1962) theorised that the dependence of cacao yield on light interception becomes important once the required nutrients are obtainable by the plant. It was not determined why organic fertiliser was not clearly associated with a significantly faster plant growth. It has been noted in cacao that excessive application of potassium is often correlated with a slower magnesium and zinc uptake due to nutrient antagonism which can limit plant growth (Hardy, 1960). With the increased plant survival and assimila-

tion rates that were observed, nutrient antagonism is not likely to have occurred. Formation pruning was carried out in the experiment to remove basal chupons and unwanted developing branches and that may have confounded the results on growth to some extent. Also the time period within which the growth data were taken was, probably, too short to record any substantial difference in growth between the fertiliser treatments.

The significant shade * fertiliser interaction term in which fertiliser application was associated with a 31% increase and an 18% increase in photosynthetic rates under the close plantain and the control shade treatments, respectively, seems to confirm a long-held view in Ghana that without the use of fertilizers good establishment is unlikely in areas where cacao would have to be re-planted (Hardy 1960).

This experiment has shown a positive link between the application of organic fertilisers and the field establishment (survival) of cacao in a site that previously carried moribund cacao. Although differences in shade were not linked with plant survival, the significant shade * fertiliser interaction on photosynthetic activity in the dry season shows that during the dry season shade increases the benefits that cacao plants get from fertiliser application. On the other hand, the significant interaction between shade and fertiliser on photosynthetic activity suggests that shade would have to be lighter in the major rainy season to facilitate fertiliser usage.

Since only a non-significant increase in soil moisture level was observed with the organic fertiliser application, the question remains unanswered by this experiment whether simple agronomic interventions can be put in place to maintain adequate soil moisture levels and whether doing that will improve the field establishment of young cacao. That is considered in the next chapter.

CHAPTER 6

Photosynthesis, survival and growth of cacao genotypes grown under different 'mulch' and shade treatments

6.1 Introduction

Cacao has commonly been grown under thinned forests to satisfy the expected shade requirement (Bergman, 1969). It is also intercropped with other cash crops such as coconuts (*Cocos nucifera*) and rubber (*Hevea brasiliensis*), in planned associations (de Almeida and Valle, 2007). Thus although very little quantitative data is available for shade in the establishment phase of cacao, it has usually been provided on the assumption that it provides physiological benefits for cacao. Wood (1985) cites Lee (1978) as showing that a level of shade is needed to get young cacao plants with the right "shape and geometry".

As a matter of preference, however, some cacao farms and plantations have been planted without shade. According to Padi and Owusu (1998) 50% of cacao farms in West Africa are under mild shade but about 10% of cacao farms in Ghana and 35% in Cote d'Ivoire have been cultivated without shade.

As mentioned in Chapter 1, a major reason for the preference shown for the no-shade situation is the avoidance of inter-species competition, not only for soil nutrients (which may be corrected by the use of fertiliser) but also for soil water (Ahenkorah et al., 1974).

In spite of the mentioned unattractive attributes of shade plants shade it is still recommended for the establishment of cacao by others who explain that the advantages of good shade outweigh the benefits of a no-shade situation (Beer et al., 1998).

In view of the foregoing and also of the significant differences observed under different shade levels when soil moisture contents were held constant (in the shade * clone nursery

trial) and under different soil water levels when incident light was even (in the genotype * quantity of water supplied * frequency of watering greenhouse experiment) it was of considerable interest to investigate the response of cacao under varying soil water levels in relation to varying shade. It was expected to provide useful information as most previous studies undertaken on the responses of cacao to water stress have been conducted under greenhouse conditions.

The experiment aimed to answer the questions: Can simple mulching practices be designed to maintain enough soil water for young cacao whilst shade is provided by other plant species? Are there differences between the cacao genotypes under different shade and mulch treatments in relation to field establishment?

Regarding the different soil moisture levels a 'control' treatment (devoid of any intervention towards soil moisture conservation) was compared with the application of an organic and an inorganic mulching material as well as with irrigation.

6.2 Materials and methods

6.2.1 Site selection

Since irrigation was involved in this field trial a six-acre (2.4 hectares) site near a dam was selected. Similar to the site for Experiment 3 [the Clone * (Shade * Fertiliser)] trial this site carried moribund cacao trees, *Gliricidia* and forest trees used as shade for previous trials.

6.2.2 Soil analysis

A soil nutrient analysis was done (as described in section 2.6.1) prior to the beginning of the trial and determined values of soil fertility parameters have been compared with standard values in Table 8.

Table 8 Soil fertility of experimental site compared with standard soil ratings for cacao (Smyth, 1966)

Soil fertility parameters	Soil Depth	pH	% Nitrogen	% O. M	Avail. P ($\mu g\ g^{-1}$)	% Carbon	C/N
Medium rating (Standard)	-	5.1- 5.5	0.2 - 0.5	2.0 - 4.2	6.5 - 13	1.16-2.44	13 - 20
Low rating (Standard)	-	-	0.1 - 0.2	1.0 - 2.0	3.0 - 6.5	0.58-1.16	> 20
Experimental site	0 - 6"	5.7	0.11	1.53	7.5	0.88	8.07
Experimental site	6 - 12"	5.3	0.07	0.81	4.7	0.47	7.01

6.2.3 Field planting

After lining and pegging the fields, establishment of the 'control' and the 'close plantain' shade treatments was done in May 2007, and that of the 'triangular *Gliricidia*' shade treatment was done in August, 2007 (See section 2.7.1). Field planting of the cacao clones was done in June 2007.

6.2.4 Layout

The experiment was laid out as a split-plot (shown in Table 9) with mulch as the main plot factor, and shade level * cacao genotype combinations as the sub-plot factor. There were four blocks each containing 48 sub-plots measuring 9 m x 12 m and containing a guard row of cacao plants (shared by adjacent sub plots) and six experimental cacao plants planted at 3 m x 3 m spacing. The total plot size for this experiment was 2.4 ha including access roads.

Table 9 Layout of the mulch * (shade * clone) experiment.

The design below was replicated four times

No mulch	**Irrigation**	**Plastic Mulch**	**Coffee Husk**
Δ *Gliricidia* shade * P 30 [POS]	Control shade * SCA 6	Control shade * PA 150	Δ *Gliricidia* shade * T 79/501
Close plantain shade * SCA 6	Δ *Gliricidia* shade * SCA 6	Close plantain shade * T 79/501	Δ *Gliricidia* shade * P 30 [POS]
Control shade*T 79/501	Close plantain * P 30 [POS]	Control shade * P 30 [POS]	Close plantain * P 30 [POS]
Close plantain shade * PA 150	Control shade * PA 150	Close plantain shade * PA 150	Control shade*T 79/501
Control shade * SCA 6	Δ *Gliricidia* shade * PA 150	Δ *Gliricidia* shade * SCA 6	Close plantain shade * SCA 6
Close plantain shade * T 79/501	Control shade*T 79/501	Δ *Gliricidia* shade * P 30 [POS]	Close plantain shade * T 79/501
Δ *Gliricidia* shade * PA 150	Δ *Gliricidia* shade * T 79/501	Close plantain shade * P 30 [POS]	Control shade * P 30 [POS]
Δ *Gliricidia* shade * T 79/501	Control shade * P 30 [POS]	Δ *Gliricidia* shade * PA 150	Close plantain shade * PA 150
Close plantain shade * P 30 [POS]	Close plantain * T 79/501	Control shade * SCA 6	Δ *Gliricidia* shade * SCA 6
Control shade * P 30 [POS]	Δ *Gliricidia* shade * P 30 [POS]	Close plantain shade * SCA 6	Control shade * PA 150
Δ *Gliricidia* shade * SCA 6	Close plantain shade * PA 150	Control shade*T 79/501	Δ *Gliricidia* shade * PA 150
Control shade * PA 150	Close plantain shade * SCA 6	Δ *Gliricidia* shade * T 79/501	Control shade * SCA 6

6.2.5 Mulch treatments:

Considering irrigation as a mulch treatment (by reason of its enhancing effect on soil moisture as is the case with mulches in general) four mulch treatments were applied. They are:

1) No mulch.
2) Irrigation of experimental plants.
3) Black polythene sheet (as plastic mulch) spread at the base of test plants in October of each year and removed the following year (at the end of the dry season around April)
4) 15 kg decomposed coffee husk applied around the base of test plants in October of each year.

6.2.5.1 *Imposition of the mulch treatments:*

Irrigation

An irrigation system which consisted of a water pump which pumped water from a reservoir (dammed river) through an inlet pipe (Plate 16) into a 3,825 litre-capacity plastic tank mounted on a 2.5 metre high concrete platform (Plate 17) was set up in December 2007.

Water from the reservoir was distributed under gravity through an outlet system. This consisted of distribution lines (Plate 18) which served individual experimental plants in designated plots as well as a fountain (Plate 19) which indicated the pressure in the outlet system and served as a vent for trapped air. The pipes were buried (in a sub-surface system) to a depth of 15cm beneath the soil surface (Plates 16 and 18) to avoid damage during cultural operations. Water from the lines formed a continuous wetted area around the base of watered plants. The outlet from the overhead tank was fitted with a meter (Plate 20) to enable an estimation of water supplied to the experimental plants. Each plant was supplied with 2.7 litres of water (equivalent to about 12.5 mm of rainfall) twice a week between December

22nd 2007 and the end of February 2008 when the rains resumed.

Plate 16 Inlet pipe from the dam

Plate 17 Water tank mounted on a raised concrete platform

Plate 18 Distribution pipes to serve individual plants

Plate 19 Fountain as part of the outlet system

Plate 20 Meter for estimating quantity of water supplied through the outlet system

Plastic mulch

After clean-weeding the plots in October 2007 a meter-wide black polythene sheet (thickness = 0.13 mm) was spread at the base on either side of experimental plants (both cacao and shade plants) as an inorganic mulch in designated plots to conserve soil water around the plants. Short wooden pegs were used to hold the sheet in place (Plate 21). The polythene sheet was removed after the rains had resumed in March 2008 and then re-applied in October 2008.

Coffee husk mulch

15 kg of decomposed coffee husk was split-applied in equal doses as a mulch to core cacao plants in appropriate plots in October and late November 2007 (Plate 22) and repeated in October 2008.

Plate 21 Cacao and plantain provided with plastic mulch

Plate 22 Cacao provided with coffee husk mulch

6.2.6 Soil moisture determination

Soil moisture content was determined by means of the soil moisture probe (Delta-T, U.K; model PR2/4). The shade and cacao genotype treatments were ignored (by restricting data collection to the control shade with P 30 [POS] treatments only). One plot per mulch treatment in each of two blocks (out of the four) was selected for soil moisture measurement. The measurement was done twice in the dry season on January 20 2007 and February 21 2008.

6.2.6.1 *Gas exchange measurement*

Starting from September 2007 the infra red gas analyzer (IRGA) was used to collect data on stomatal conductance and the rates of transpiration and photosynthesis twice in each of the three seasons (as described in section 2.3). For each replication three out of six core plants

were tagged and used for gas exchange measurement. No artificial light source was applied. Leaf Chlorophyll Fluorescence was measured by means of the leaf chlorophyll fluorescence meter (as described in section 2.3.1). Data were collected between the mid mornings and early afternoons (between 11:00 am and 12:15 pm) on the same days as the gas exchange measurements. Leaves which had been selected for photosynthesis measurement were also used for leaf chlorophyll fluorescence measurements.

6.2.7 Growth data

Growth (girth and height) measurements of all experimental plants were made once every two months from September 2007. The stem diameter of plants was measured at 20 cm from ground level.

6.2.8 Leaf traits

In March 2008, data on specific leaf weight, relative water content, Leaf chlorophyll concentration and leaf nitrogen content were taken once on a leaf on each of the three plants which were used for photosynthetic data collection in each treatment (see section 2.3.2 for details). Young, fully expanded and hardened leaves of selected plants were used.

6.2.9 Plant survival

Plant survival rate was assessed in September 2007 and during the last week of March 2008 by counting surviving plants. The numbers surviving were expressed as a percentage of the total number planted per mulch * (shade * clone) treatment. The data were arc–sine transformed before analysis.

6.3 Results

Table 10 shows the mean relative humidity and temperature in the different seasons and under the shade treatments at the times of data collection. The mean relative humidity value was 56.5% in the dry season compared with 74.3% and 83.7% in the major rainy and the minor rainy seasons, respectively. The mean seasonal relative humidity under the control shade was 68.1% as against 71.7% and 74.8% under the triangular *Gliricidia* and the close plantain shade treatments, respectively (Table 10).

Temperatures were 25.6% higher in the dry season than they were in the major rainy season and 36.1% higher than they were in the minor rainy season (Table 10). Also modifications were noted in mean daytime temperatures under the shade treatments: Whereas the mean seasonal temperature under the control shade was 30.2 °C, it was 29.7 °C under the triangular *Gliricidia* shade treatment and 29.1°C under the close plantain shade treatment.

Table 10 Mean relative humidity and air temperature under different shade regimes and in different seasons.

Each value represents the mean of three readings taken in each season.

Seasons	**Major rainy (mid-March to mid-July year 2008)**		**Minor rainy (mid-July to October year 2007)**		**Dry season (November to February 2007/8)**	
Shade Type	R. H. (%)	T °C	R. H. (%)	T °C	R. H. (%)	T °C
Control	66.5	31.35	82.50	25.15	55.25	34.20
Δ Gliricidia	75.25	30.50	83.50	24.85	56.25	33.80
Close plantain	81.25	30.80	85.00	23.90	58.00	32.60

6.3.1 Soil water content under the mulch treatments

Figure 69 shows the mean soil moisture contents at varying soil depths and under the different mulch treatments. There was evidence (p = 0.04) of higher soil water under the mulch treatments but not with changing soil depth. Generally the soil at this site was a sandy loam and was likely to have a lower water holding capacity than the soil at the site for the clone * (shade * manure) experiment. Usually it takes more supplied water to increase the moisture content of more loose soils as water more easily percolates down the soil profile or evaporates into the atmosphere.

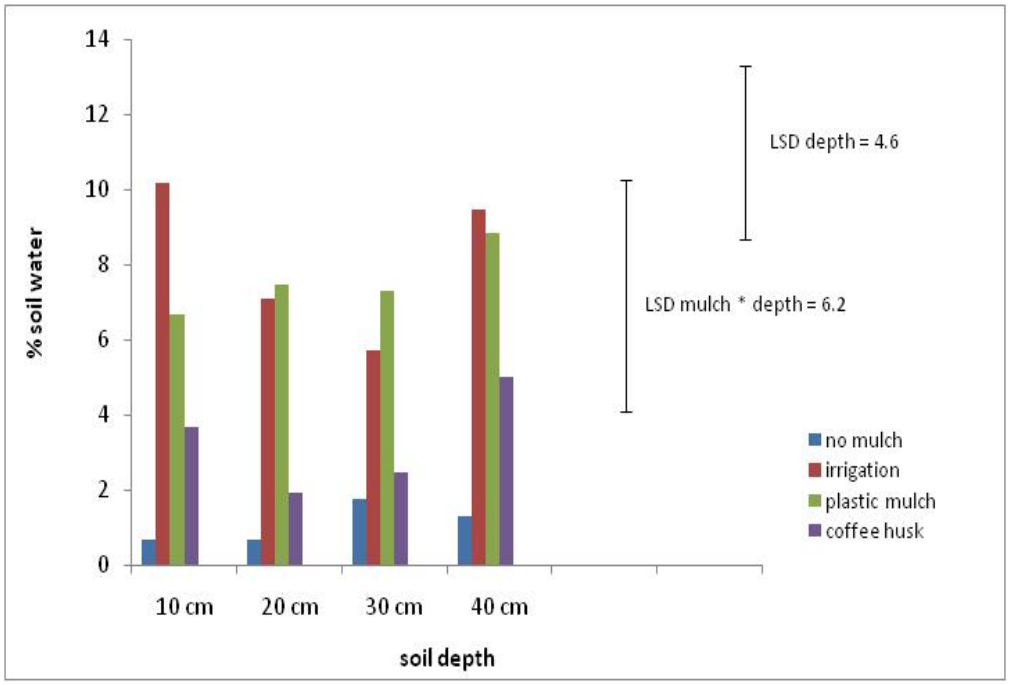

Figure 69 Soil water status under the mulch treatments and at different depths during the dry season of 2007/2008.

Each bar represents the mean value for two plots per treatment each measured twice.

6.3.2 Photosynthetic activity

Stomatal conductance

Stomatal conductance rates of the cacao plants were significantly (p = 0.006) different between the seasons, the rates during the minor rainy and the major rainy seasons being 3.21 times and 6.47 times higher than the rate during the dry season, respectively.

There were no significant differences between cacao genotypes in their rates of stomatal conductance. This was the case across the seasons and under the different mulch treatments (Fig 70). The season * mulch interaction term was on the borderline of significance (p = 0.083). Stomatal conductance rates were not significantly different under the mulch treatments during two rainy seasons. During the dry season plants on which the irrigation and plastic mulch treatments were imposed had a 17.9% and 41.9% increase over the zero mulch treatment, respectively. This indicates, however, that stomatal conductance rates during the dry season were still lower (under the irrigation and plastic mulch treatments) than they were in the rainy seasons. The season * mulch * clone interaction term was not significant.

Transpiration

Significant (p =0. 001) differences were observed between the seasons on the rate of transpiration in the cacao plants. A mean transpiration rate of 5.152 mmol $m^{-2}s^{-1}$ was observed in the minor rainy season. That was 35.58% and 6.36 times higher than the mean rate at which the plants transpired in the major rainy and the dry seasons, respectively.

Over the entire period no significant differences were noted between the mulch treatments were observed on rates of transpiration (Fig 71). However, the season * mulch interaction component was significant (p = 0.018). During the rainy seasons the zero mulch treatment

had plants with the highest transpiration rate (mean = 4.8 mmol $m^{-2}s^{-1}$) which was 6.2%, 11.35% an 14.45% higher than the transpiration rates recorded in plants under the irrigation, coffee husk and plastic mulch treatments, respectively. During the dry season, however, plants provided with no mulch transpired at a rate of 0.607 mmol $m^{-2}s^{-1}$ which was 16.15%, 19.82% and 48.12% lower than the rate observed for plants provided with the coffee husk mulch, irrigation and the plastic mulch, respectively. There were no significant differences between cacao genotypes in their rates of transpiration. The season * clone interaction term was close to being significant ($p = 0.069$). During the dry season SCA 6 had the highest transpiration rate (0.859 mmol $m^{-2}s^{-1}$) which was 13.92% greater than that of PA 150 (which had the lowest rate). The mulch * clone interaction element was not significant and no clear trends were observed. The season * mulch * clone interaction term was also not significant.

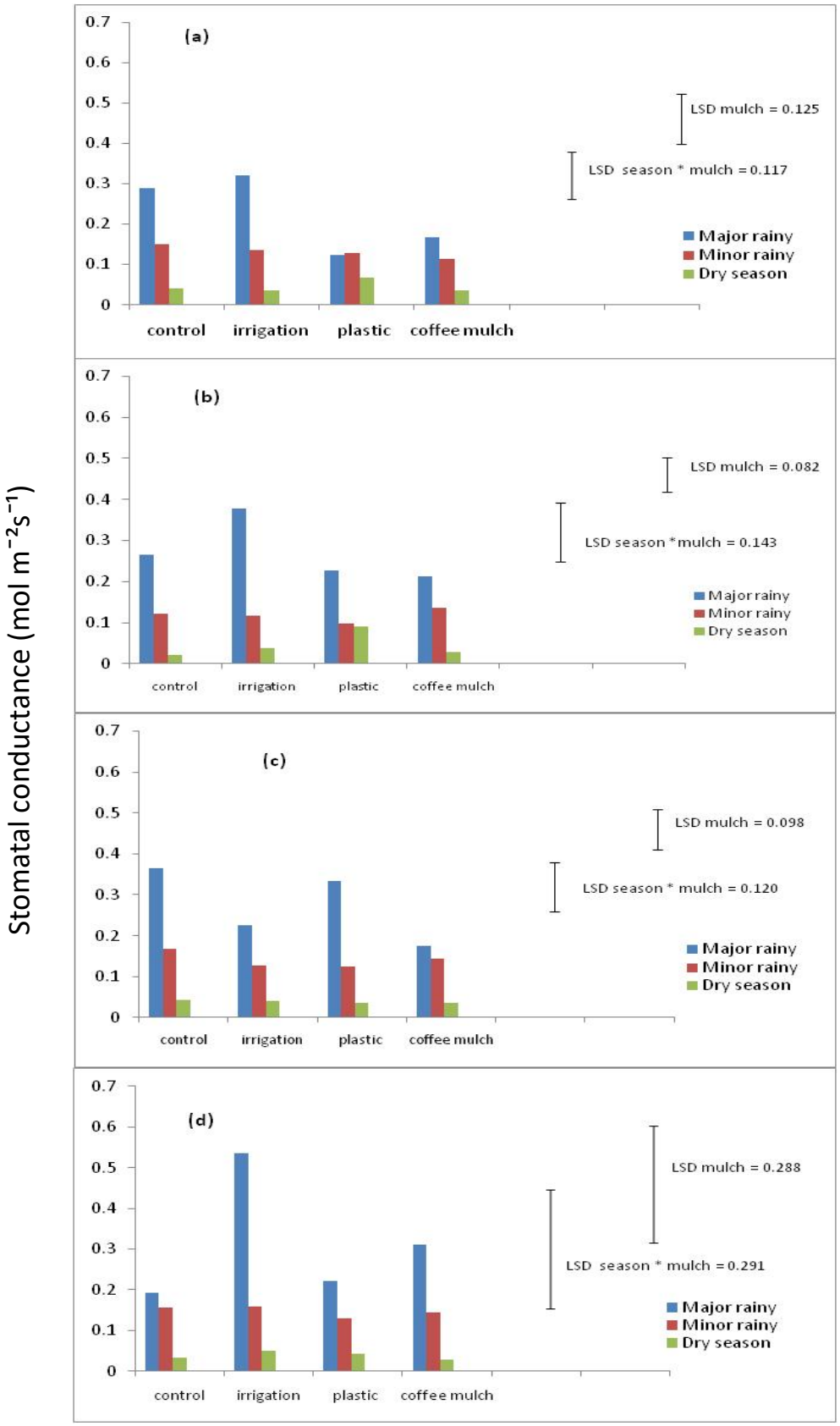

Figure 70 Stomatal conductance of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 in different seasons and under different mulch treatments.

Each bar represents the mean value for 12 plants.

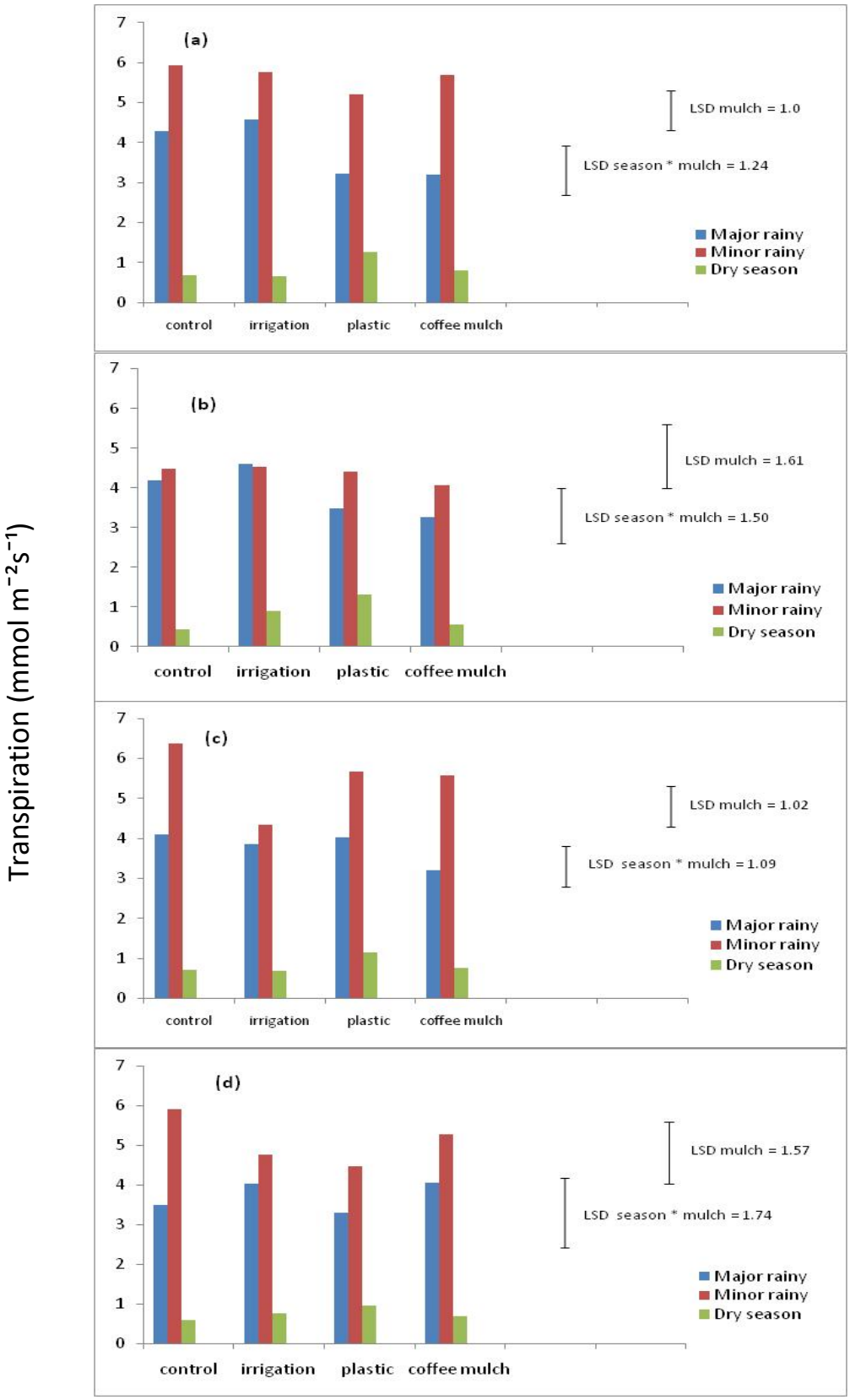

Figure 71 Transpiration of (a) SCA 6 (b) T79/501 (c) P 30 [POS] and (d) PA 150 in different seasons and under different mulch treatments.

Each bar represents the mean value for 12 plants.

Photosynthesis

Significant ($p < 0.001$) differences were observed on net photosynthesis of the cacao plants between the seasons, the mean rate in the major rainy season being 4.8 µmol $m^{-2}s^{-1}$. That was 2.17 and 3 times greater than the rates during the minor and dry seasons, respectively. Considered over the whole period, significant differences were not detected between the mulch treatments. However, the season * mulch interaction term was significant ($p = 0.019$). In the two rainy seasons the zero mulch treatment had plants with the highest rate of photosynthesis (mean = 3.689 µmol $m^{-2}s^{-1}$) and that was 3.8%, 5.47% and 7.83% greater than the mean rates for plants under the irrigation, plastic and coffee husk mulch treatments, respectively. In the dry season the lowest photosynthetic rate (mean = 1.26 µmol $m^{-2}s^{-1}$) was observed in the zero mulch treatment. That was 9.1%, 32.65% and 68.14% lower than the mean photosynthetic rates of plants provided with the coffee husk mulch, irrigation and plastic mulch treatments, respectively.

No differences were observed between the cacao genotypes in their rates of photosynthesis. The season * clone interaction element was not significant; highest photosynthetic rates were observed for all clones in the major rainy season and the lowest rates in the dry season. The mulch * clone interaction term was significant ($p = 0.025$). The main reason for this could probably be attributed to the response of SCA 6 which had its highest photosynthetic rate (3.193 µmol $m^{-2}s^{-1}$) under the irrigation treatment and its lowest rate (2.598 µmol $m^{-2}s^{-1}$) under the plastic mulch whilst the other clones had their highest photosynthetic rates under the plastic mulch treatment (Fig 72).

Water use efficiency

The water use efficiency was significantly ($p < 0.001$) different with season, the plants expe-

riencing the lowest efficiency of water use [0.685 μmol (CO_2) mmol (H_20)$^{-1}$] in the minor rainy season (Fig 73). That value was 69.31% lower than that of the dry season during which the plants had the highest water use efficiencies.

There were no significant differences between the mulch treatments or the cacao genotypes or interactions between them.

Leaf chlorophyll fluorescence

There was evidence that the Fv/Fm values of plants differed ($p = 0.002$) between the seasons, the ratio being 18.3% and 25.46% higher in the minor rainy season than in the major rainy and the dry seasons, respectively.

There were significant ($p = 0.034$) differences between the mulch treatments on the Fv/Fm values of plants; those that received the irrigation treatment having an Fv/Fm ratio of 0.489 which was 3.16%, 5.40% and 6.51% higher than the values obtained by other plants which were provided with the plastic mulch, coffee husk mulch and no mulch, respectively.

The season * mulch interaction term was not significant (Fig 74).

There were no significant differences between the cacao clones in their Fv/Fm values and no significant interaction of clone with season or mulch was observed.

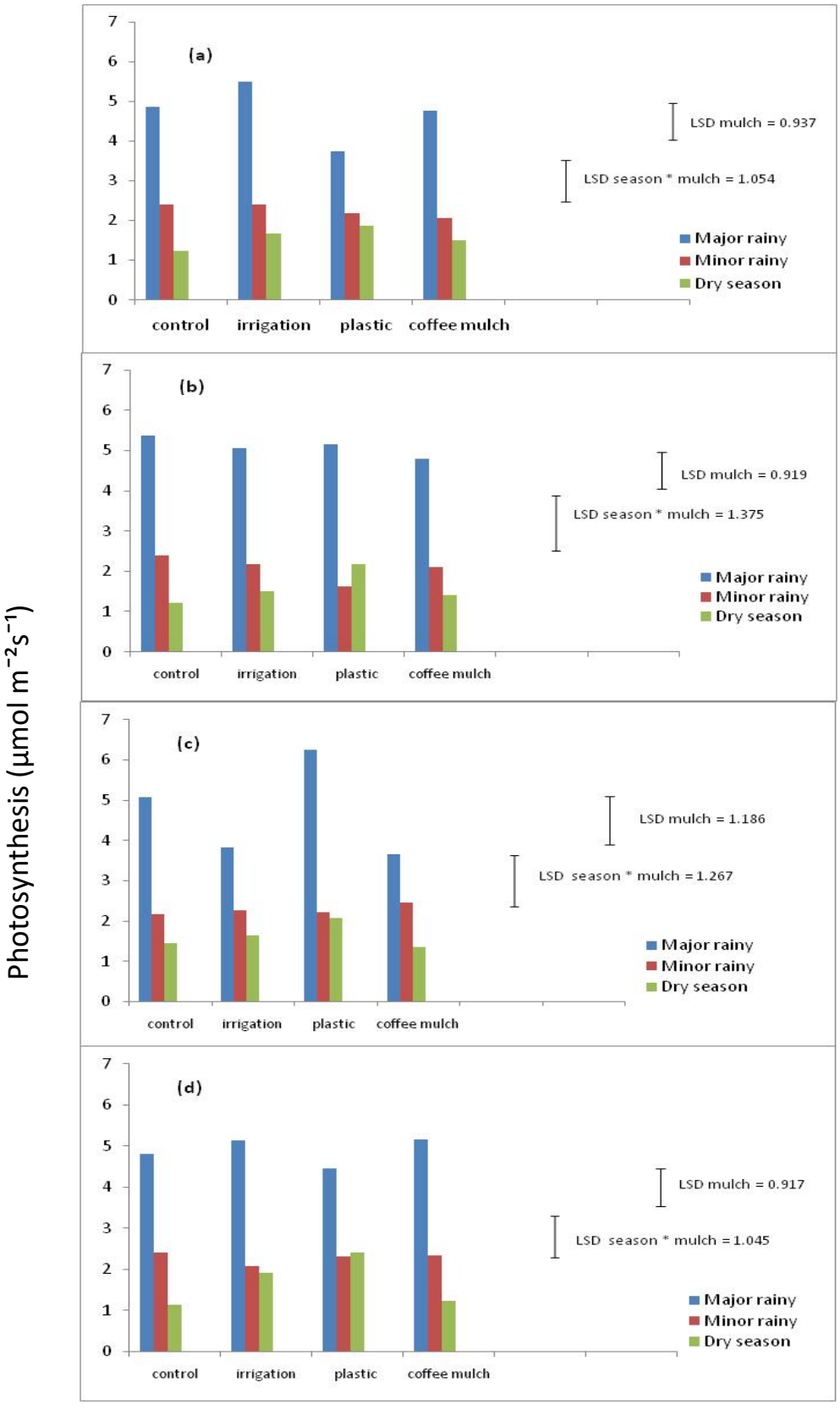

Figure 72 Net photosynthesis of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 in different seasons and under different mulch treatments.

Each bar represents the value mean for 12 plants

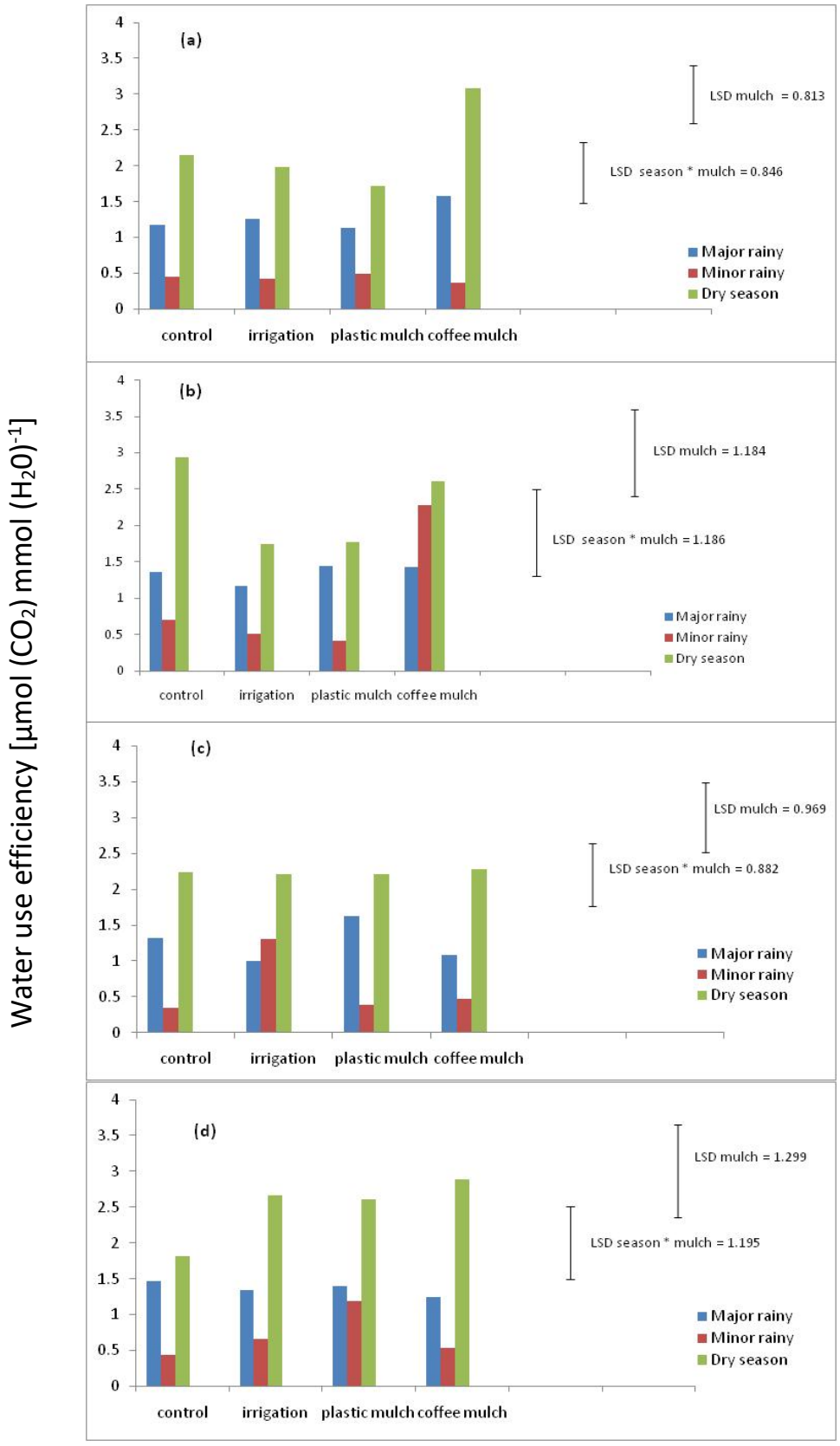

Figure 73 Water use efficiency of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] (d) PA 150 in different seasons and under different mulch treatments.

Each bar represents the mean value for 12 plants.

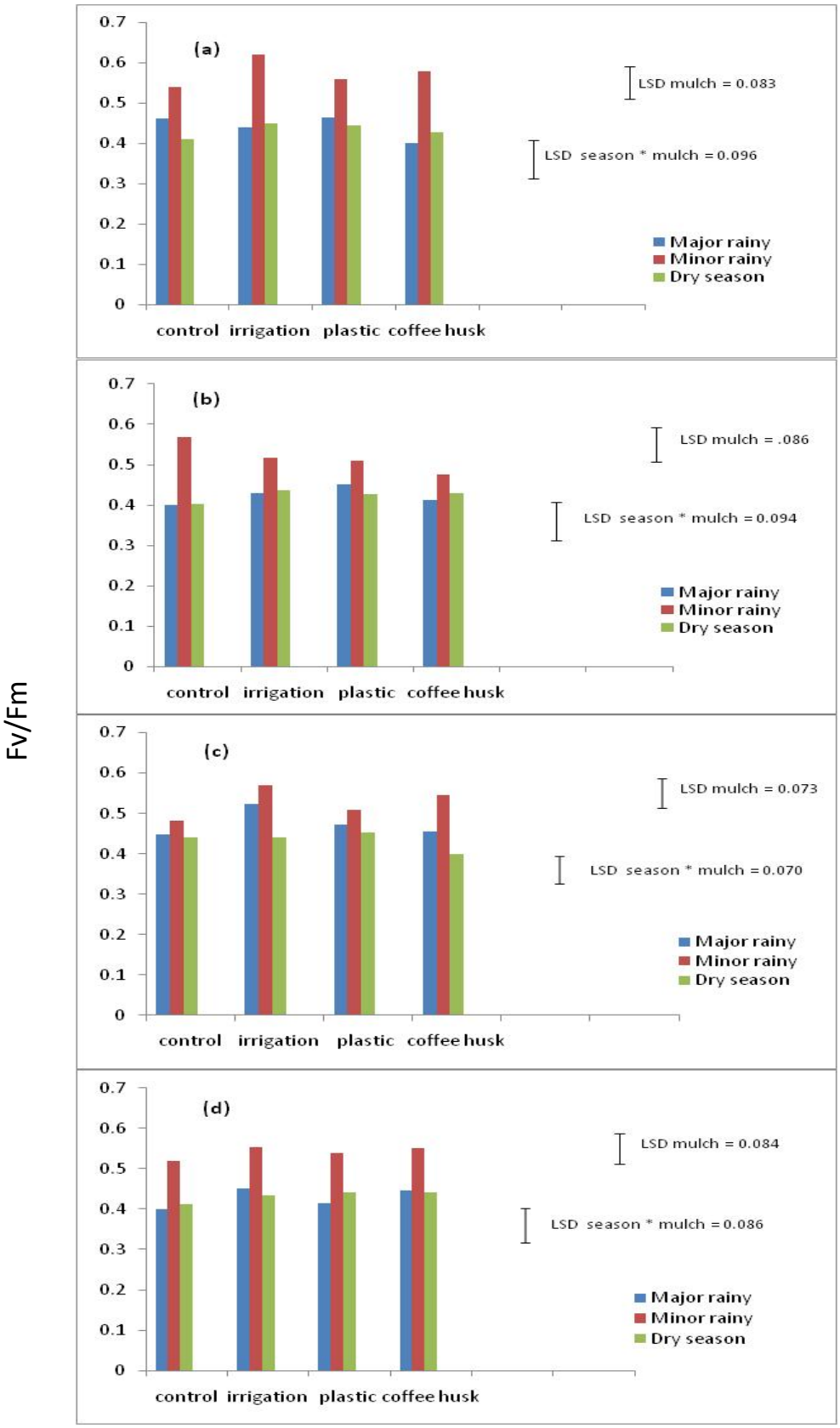

Figure 74 Fv/Fm of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 in different seasons and under different mulch treatments.

Each bar represents the mean value for 12 plants.

6.3.3 Leaf traits

Leaf chlorophyll content

Leaf chlorophyll contents of plants differed significantly ($p = 0.05$) between the mulch treatments. The highest leaf chlorophyll concentration was observed with the plastic mulch treatment. The coffee mulch and the irrigation treatments had plants with 14.92% and 22.79% lower leaf chlorophyll contents, respectively, whilst the zero mulch treatment had plants with 35% lower leaf chlorophyll content (Fig 75).

Plants under the different shade treatments also had significant ($p < 0.001$) differences between them on leaf chlorophyll content. The close plantain shade treatment had plants with a mean chlorophyll content of 10.67 chlorophyll meter units which was 7.61% and 16.85% more concentrated than that of plants that grew under the triangular G*liricidia* shade and the control shade treatments, respectively. The mulch * shade interaction term was not significant.

There were significant ($p = 0.002$) differences between cacao genotypes in their leaf chlorophyll contents. SCA 6 had the highest value (10.35 chlorophyll meter units) and that was 0.89%, 3.26% and 26.94% higher than the mean chlorophyll content of PA 150, P 30 [POS] and T 79/501, respectively. None of the interaction terms were significant.

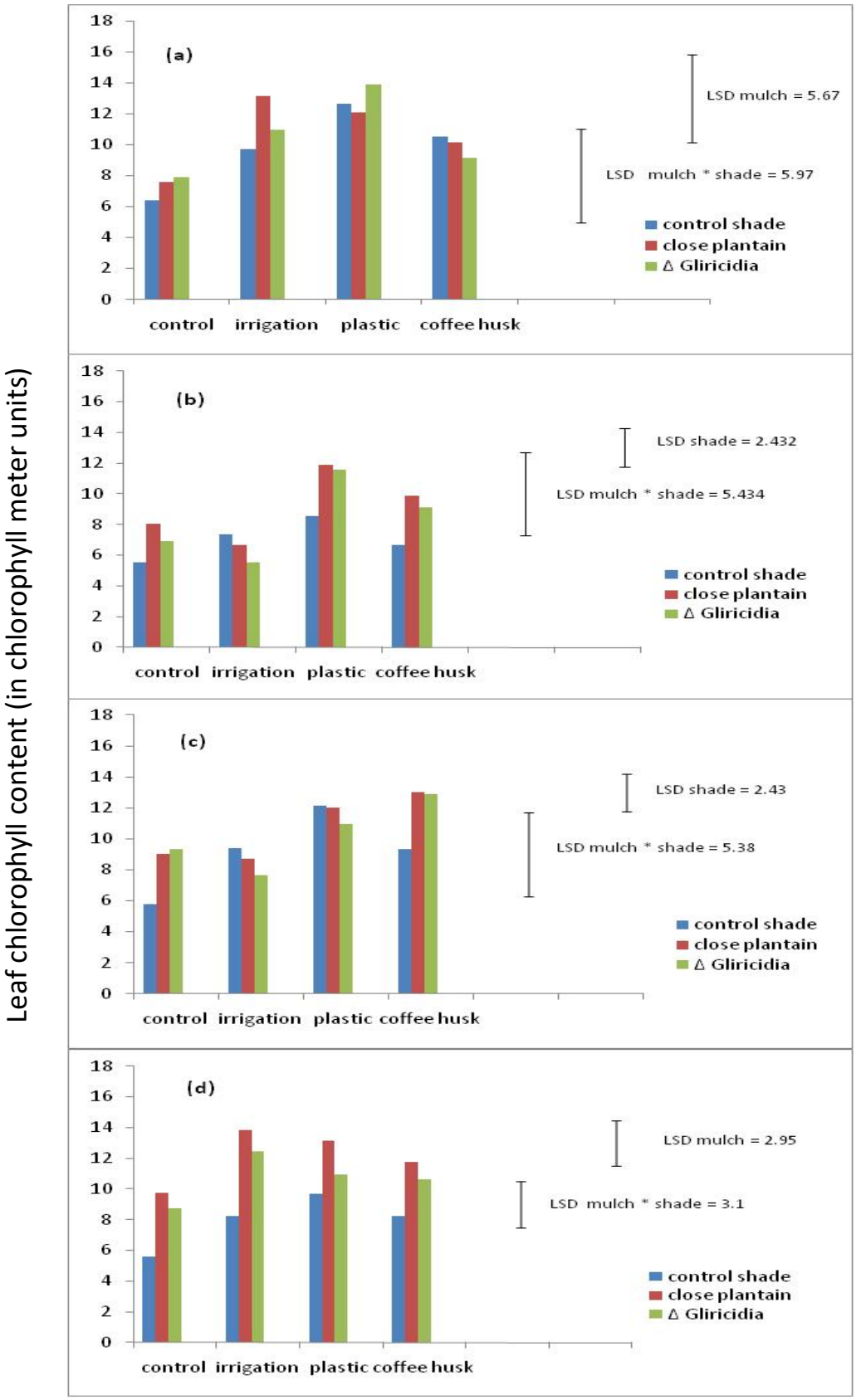

Figure 75 Leaf chlorophyll content of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different mulch and shade treatments.

Each bar represents the mean value for 12 plants

Relative water content

The relative water content values fell within a narrow range between clones (59.00 ± 0.52) shade (58.98 ± 1.18) % and mulch (59.30 ± 1.32) %, treatments. There were no significant differences between the different sets of factors or their interactions with other factors and no clear trends were observed (Fig 76).

Specific leaf weight

Significant ($p = 0.001$) differences were noted in the specific leaf weight of plants under the different mulch treatments. The highest value (4.68 mg cm^{-2}) was observed for plants grown with the plastic mulch and that value was 2.55%, 6.44% and 12.08% greater than the specific leaf weight of plants that received the irrigation, zero mulch and the coffee husk mulch treatments, respectively.

Significant ($p = 0.027$) differences were observed on specific leaf weight between the shade treatments (Fig 77), with cacao plants growing under the close plantain shade regime having the highest specific leaf weight (4.57 mg cm^{-2}) which was 3.29% and 4.76% greater than that of plants that grew under the triangular *Gliricidia* shade and those that grew under the control shade, respectively. The mulch * shade interaction factor was not significant.

Significant ($p < 0.001$) differences were observed among the genotypes. SCA 6 had the highest mean value (4.68 mg cm^{-2}) and that was 6.2% greater than P 30 [POS] and PA 150 and 8.64% greater than that of T 79/501.

The mulch * clone interaction and shade * clone interaction terms were not significant.

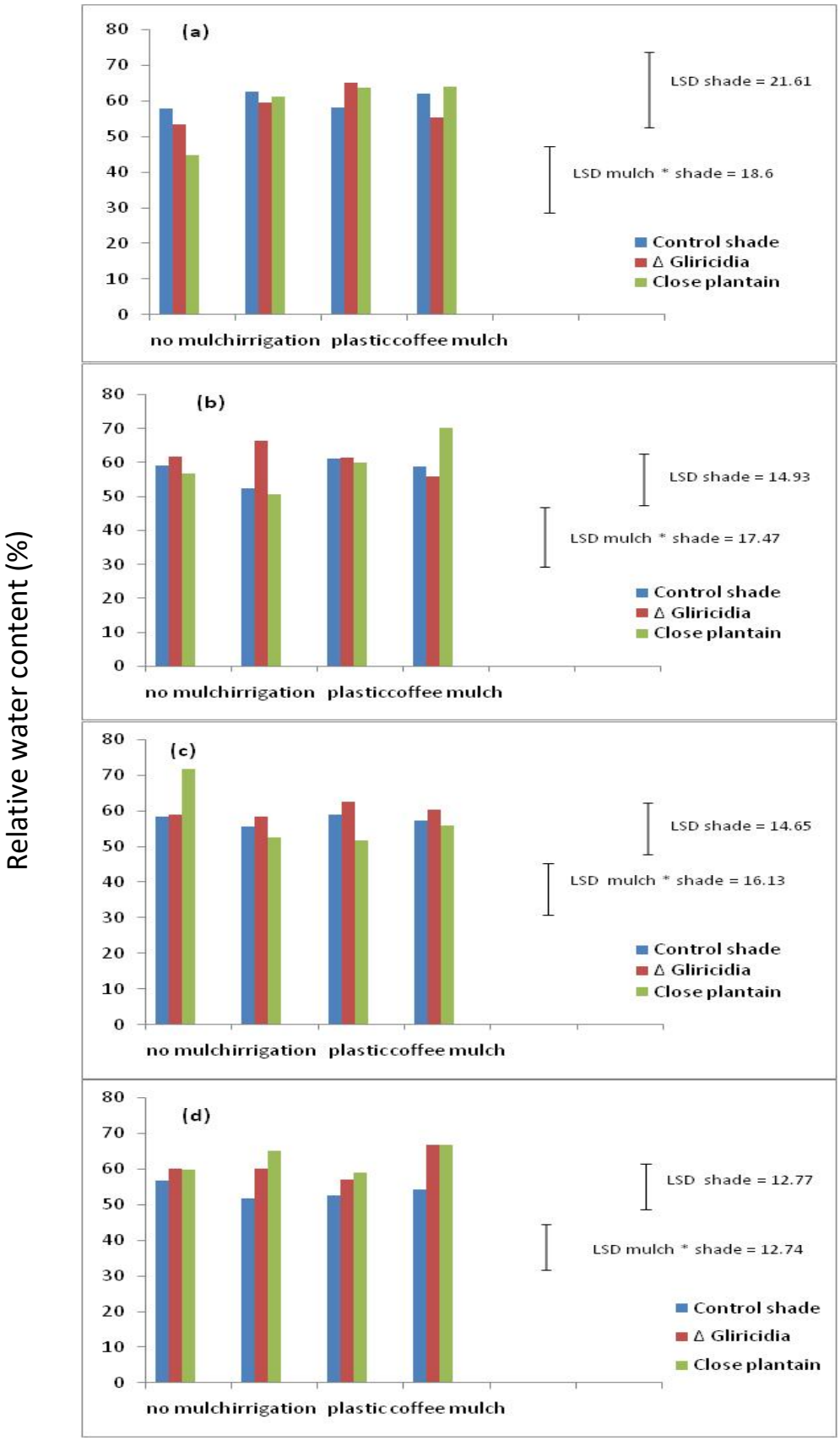

Figure 76 Relative water content of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different mulch and shade treatments.

Each bar represents the mean value for 12 plants.

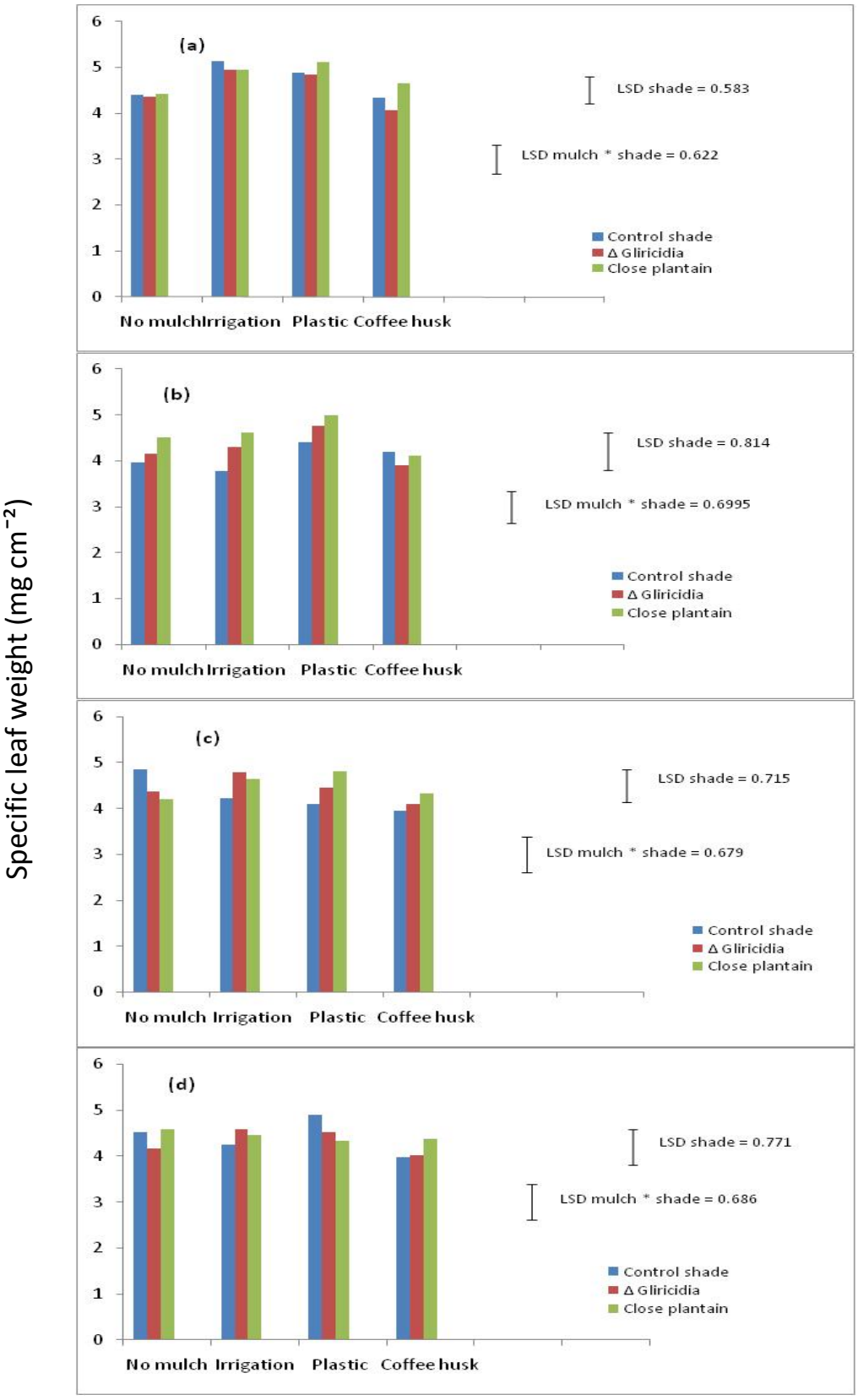

Figure 77 Specific leaf weight of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different mulch and shade treatments.

Each bar represents the mean value for 12 plants.

6.3.4 Plant growth

Stem girth

Stem diameter differed significantly (p = 0.013) between the mulch treatments. The irrigated plants had a mean stem girth of 22.40 mm. They were 3.9%, 9.62% and 11.83% thicker than plants which were treated with the plastic mulch, coffee husk mulch and the zero mulch, respectively (Fig 78).

There were no significant differences between shade treatments. Furthermore, the mulch * shade interaction unit was also not significant.

There were significant (p < 0.001) differences between cacao genotypes in their stem girths. T 79/501 had the thickest stems (22.01cm) which were 1.32%, 4.58% and 11.60% thicker than the stems of PA 150, P 30 [POS] and SCA 6, respectively. There were no significant interactions between clone and mulch or clone and shade. The mulch * shade * clone interaction term was not significant.

Plant height

Significantly (p = 0.042) different plant heights were recorded between the mulch treatments. The irrigated plants were 112.45 cm tall on average which made them 10.78%, 11.61% and 12.40% taller than plants that grew under the coffee husk mulch, plastic mulch and zero mulch treatments, respectively (Fig 79).

There were no substantial differences between shade treatments and the mulch * shade interaction term was also not significant. However, there were significant (p < 0.001) differences between clones in plant height, PA 150 being 109.44 cm tall on average and 15.23% taller than SCA 6 which was the shortest.

The mulch * clone and the shade * clone interaction terms were not significant.

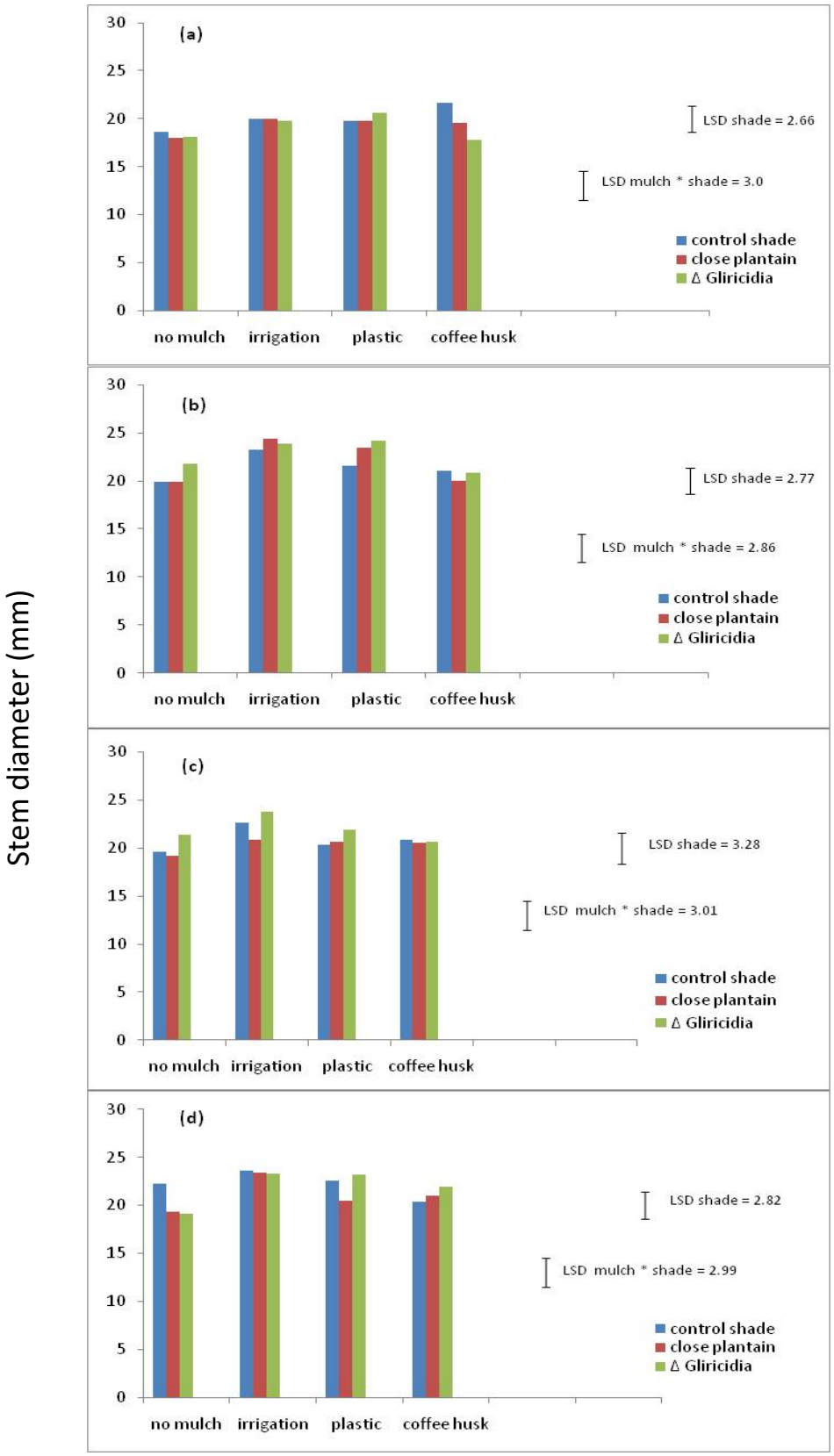

Figure 78 Stem diameter of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different mulch and shade treatments.

Each bar represents the mean value for 24 plants.

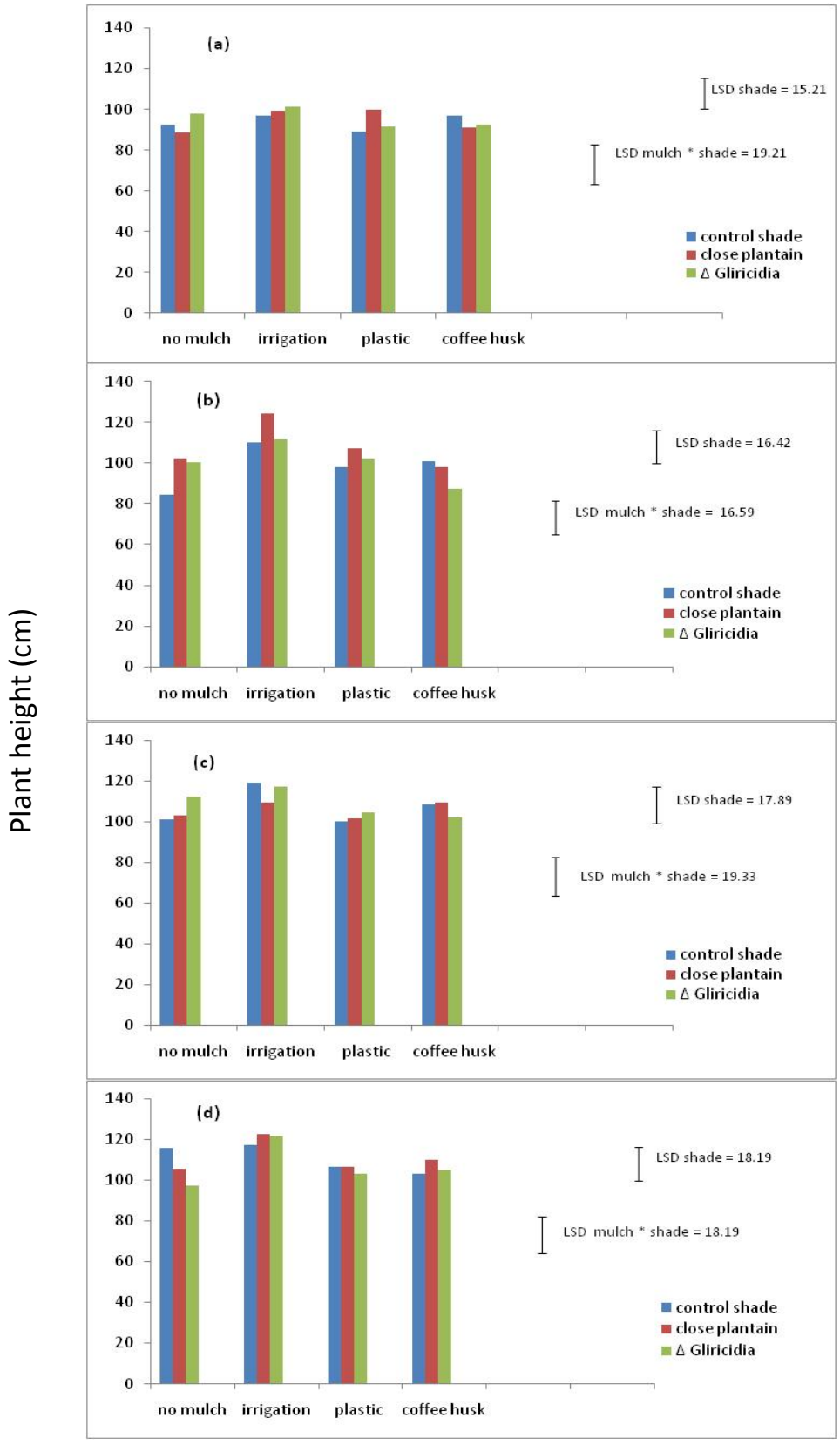

Figure 79 Mean plant height of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different mulch and shade treatments.

Each bar represents the mean value for 24 plants.

Stem volume

Plants that were subjected to the mulch interventions had significantly (p = 0.006) bigger stems than those under the control treatment. Plants that received the irrigation treatment were the biggest. They had a mean stem volume of 164.87 cm^3 which was 24.79%, 39.65% and 48.36% greater than plants that received the plastic mulch, coffee mulch and zero mulch treatments, respectively.

There were no significant differences observed between shade treatments in stem volume. However, there was a trend in which plant growth decreased under increasing shade intensity so that plants that grew under the close plantain shade were the smallest.

The mulch * shade interaction term was not significant. There were significant (p = 0.001) differences between cacao clones in their stem volume (Fig 80). The clone PA 150 had the biggest stems (147.12 cm^3). It was 3.38%, 7.84% and 29.74% bigger than T 79/501, P 30 [POS] and SCA 6, respectively.

The mulch * clone and the shade * clone interaction terms were not significant. The mulch * shade * genotype interaction term was also not significant.

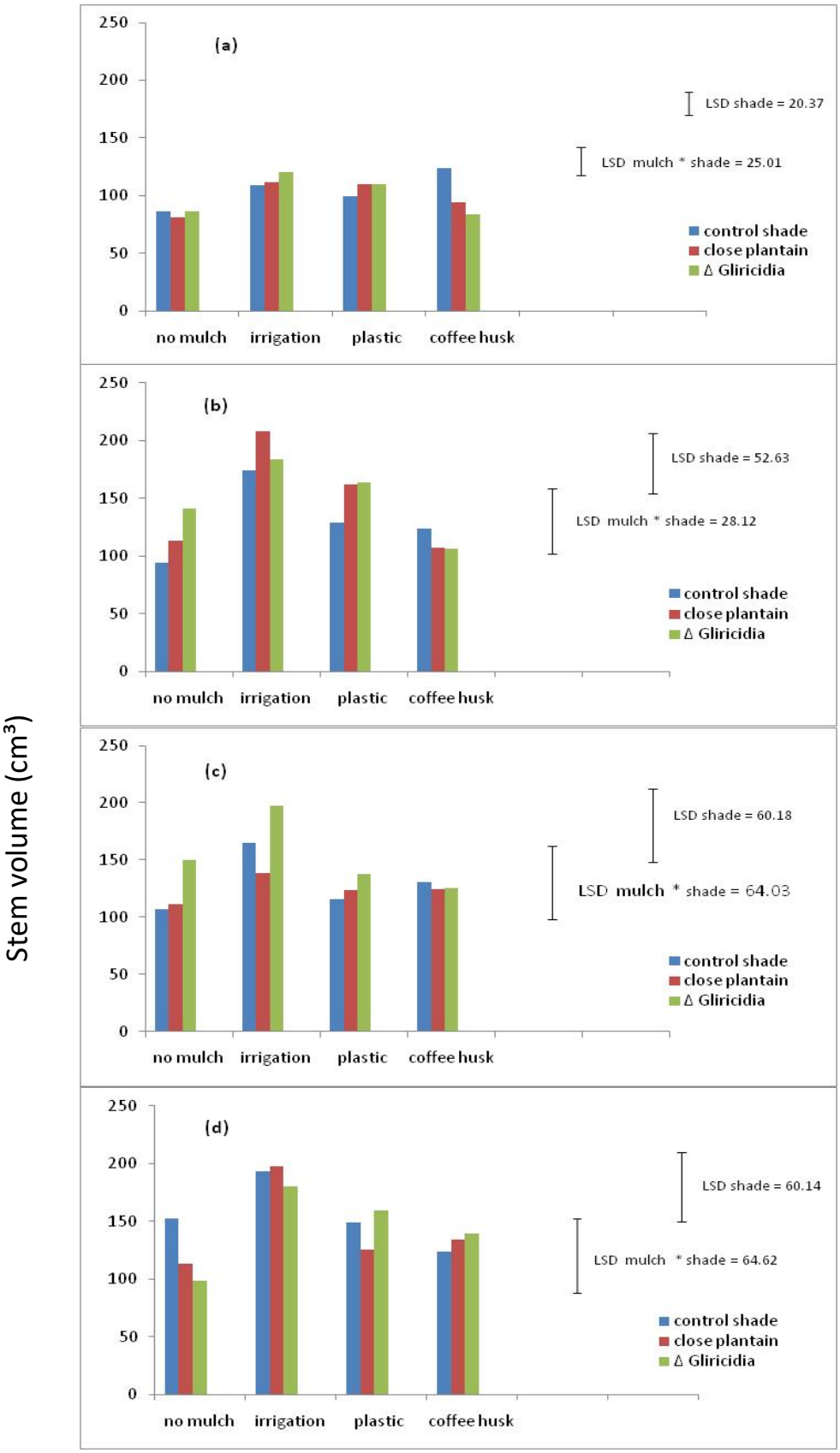

Figure 80 Stem volume of (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different mulch and shade treatments.

Each bar represents the mean value for 24 plants.

Plant survival

Plant survival rates were significantly (p = 0.04) different for the mulch treatments, the irrigation treatment having the highest plant survival (93.5%). Respectively, 90.7%, 81.0% and 69% of plants which were grown under the plastic mulch, coffee husk mulch and the zero mulch treatments survived.

Plant survival was also different (p = 0.011) between the shade treatments. Under the close plantain shade treatment 87.1 % of the cacao plants survived as against 85.94% and 74.72% survival rate under the triangular *Gliricidia* shade and the control shade treatments, respectively (Fig 81). The mulch * shade interaction term was not significant.

The average survival rates of the clones were not significant, however the mulch * clone interaction term was significant (p = 0.028). All the clones except SCA 6 survived better under the irrigation treatment than the other treatments. In the case of SCA 6 94.4% of the plants survived under the plastic mulch, 88.90% survived under the irrigation and 65.00% under the zero mulch treatments, respectively. T 79/501 had high survival rate under the irrigation and plastic mulch treatments but not under the coffee husk mulch. The lowest survival rate (53.70%) was obtained by P 30 [POS] under the zero mulch treatment.

The shade * clone interaction term was not significant. For all the clones the lowest plant survival rate was recorded under the control shade treatment. The mulch * shade * clone interaction term was not significant.

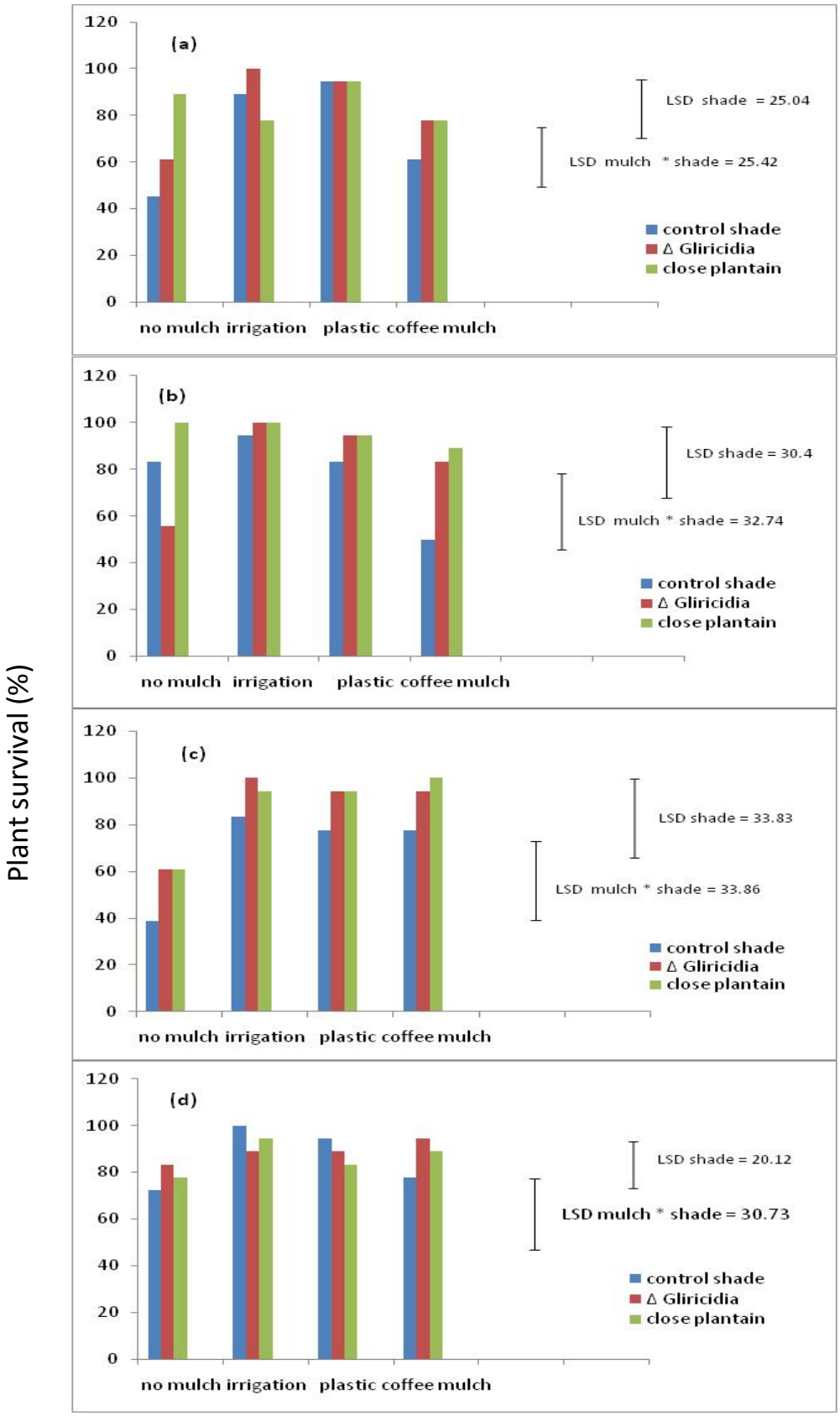

Figure 81 Field survival of young (a) SCA 6 (b) T 79/501 (c) P 30 [POS] and (d) PA 150 under different mulch and shade treatments.

Each bar represents % plants surviving out of 48 (= 100%).

6.4 Discussion

Soil moisture

Organic mulches have long been noted to improve soil water retention (Jordan and Opoku, 1966). Therefore, the observation that soil water content was higher under mulches in this work is consistent with such earlier ideas. The use of plastic mulches for this purpose is more recent. However, recent publications show that plastic mulches are effective in retaining soil water for agricultural purposes (Chakraboyty et al., 2008, Liu et al., 2009).

Stomatal conductance and Transpiration

The fact that plants that were subjected to the irrigation and plastic mulch treatments had higher stomatal conductance and hence higher photosynthesis in the dry season but not in the two rainy seasons suggests that soil moisture, if adequate, is partially able to ameliorate the suppressive effect of atmospheric drought on stomatal conductance and transpiration, presumably, by improving the plant water status (Rada et al., 2005, Hutcheon, 1977, Joly and Hahn, 1989a). This shows that during the dry season, although soil water might have been improved adequately by means of the 'mulching' methods, plant water uptake did not measure up to the rate of transpiration, causing the plants to restrict stomatal opening so as to control the creation of an internal water deficit.

The non-significant mulch * clone interaction indicates that stomatal conductance and transpiration were similar under the mulches irrespective of plant material. It confirmed results of the genotype * (quantity of water * frequency of watering) greenhouse experiment in showing that differences in soil water reflect on stomatal opening of all the cacao genotypes. The results also agree with a few other findings by other workers. Date palms exhibit a similar but much more outstanding response as, according to Smith (1989) stomatal conductance in the palms seems to be relatively insensitive to the high water vapour pressure defi-

cits experienced in the arid regions of the world, "provided soil water was not limiting". Similar observations on the effect of soil water on stomatal conductance have been made under field conditions in maize (Tardieu et al., 1992), carambola trees (Al-Yahyai et al., 2004), Arabica coffee (Tesfaye et al., 2008), cassava (Elsharkawy et al., 1984) and almond trees (Romero and Botia, 2006).

In the rainy seasons the soil water content would not be very different for the mulch treatments. So differences in stomatal conductance would not be expected under the different mulch treatments during those seasons. The temperatures and relative humidity values recorded in this experiment fall within the normal ranges for the cacao growing area of Ghana. The differences observed in stomatal conductance and transpiration between the seasons follow the same trend as those observed in the shade * clone experiment which was conducted using the same genotypes but under nursery conditions. Seasonal changes in environmental conditions explain the significantly lower stomatal conductance and transpiration rates in the dry season than in the rainy seasons. With high relative humidity and low temperatures as was observed in the minor rainy season vapour pressure deficit would be low - permitting wide stomatal opening and high stomatal conductance and transpiration as reported on by Slatyer (1966) and (Raja Harun and Hardwick, 1988).

The borderline significance in the season * clone interaction (in which PA 150 had the lowest transpiration rates in the dry season) suggests modest genetic variability in stomatal regulation. This confirms results of the shade * clone nursery experiment where a similar observation was made.

Photosynthesis

Although stomatal conductance was high in the minor rainy season, the cloudy situation and

low temperatures in that season must have reduced the rate of CO_2 fixation. Consequently the highest photosynthetic rates were observed in the major rainy season when soil water was not limiting and also light levels and temperatures were higher. These observations are in agreement with the results obtained in the shade * clone experiment described earlier (in chapter 4) in this thesis and with earlier ones such as those obtained by Müller et al. (1992). These authors observed higher rates of photosynthesis in cacao until a saturating irradiance for photosynthesis was reached. Low temperatures below the optimum have been noted by Raja Harun and Hardwick (1988), Alvim (1977), Hadley (1994) and Daymond and Hadley (2004) to limit photosynthesis and vegetative growth.

Photosynthesis was lowest in the dry season. The significant reduction in the Fv/Fm observed in this work in the dry season is dissimilar to an observation made by Praxedes et al. (2006) on field-grown Robusta coffee that a dry period had little effect on leaf chlorophyll fluorescence. The low photosynthetic rates in the dry season could largely be explained by stomatal closure. Under water stress conditions Perez-Martin et al. (2009) observed limited diffusion of CO_2 into the leaf chloroplast of *Vitis vinifera* L. Cv. Tempranillo and *Olea europae* L. cv. Manzanilla not only by reducing stomatal conductance but also mesophyll conductance of the leaves. Guang-yu et al. (1991) observed in field-grown soya bean that water stress conditions caused restricted stomatal opening which, in turn, caused further increases in leaf temperature.

Consistent with the observation made in the shade * clone trial (in chapter 4) and with earlier observations made by Balasimha and Daniel (1995) significantly lower leaf chlorophyll fluorescence was observed during the drier months of the year which may have partially reflected higher photoinhibition as a result of higher light levels (Galyuon et al., 1996a). Thus the low rates of photosynthesis observed in the current work during the dry season could

have been a reflection of several physiological impediments including low stomatal conductance, low mesophyll conductance as well as photoinhibition of photosynthesis.

The significant season * mulch interaction in which the zero mulch treatment had plants with the highest rate of photosynthesis in the wet season but the lowest rate in the dry season reflected the pattern observed with the mulches, in relation to stomatal conductance and the efficiency of photosystem II . This suggests that net photosynthetic rates at the leaf level were restored after the dry season when soil water would be about the same across the mulch treatments. This was similar to a report by Galle et al. (2010) who observed the restoration of photosynthetic activity of *Nicotiana sylvestris* at the leaf level two days after an imposed drought stress was alleviated. Similarly Xu et al. (2009) examined the effects of soil drought and re-watering on plant biomass, relative growth rate (RGR), and photosynthetic potential of a perennial grass (*Leymus chinensis*). These authors reported that recovery of plant biomass following re-watering was lower for the plants that had experienced previous drought compared with the control. However, the plant RGR, leaf photosynthesis, and light use potential were strikingly stimulated by the previous drought, depending on drought intensity. It was explained that following re-watering the plants might have tried to overcompensate the loss of the plant's net primary production due to the drought effect. In a similar way a greater stimulation in photosynthetic activity could have occurred in the zero-mulched cacao plants of the current experiment.

Compared with the changes noted in the Fv/Fm as soil water was varied in the greenhouse clone * (quantity of water supplied * frequency of watering) trial, it can be reasoned that the soil water levels obtained by the application of the mulches were satisfactory.

The observation that the season * clone interaction term was not significant corroborated results obtained under nursery conditions in the shade * clone experiment (see Chapter 4). It

is also significant that in this experiment, as in the genotype * (quantity of water * frequency of watering) experiment, no differences were observed in the Fv/Fm of the genotypes (Fig 74). That probably suggests a major reason why the rates of net photosynthesis were not very different among the clones even under the different seasons.

Water use efficiency

In contrast with the dry season the minor rainy season (which had the highest relative humidity and the lowest temperatures) is regarded as the period when the water table is highest in Ghana [(Entwistle, 1972, Wood, 1985); Appendix 2]. Those environmental factors may explain the lowest water use efficiency in the minor rainy season and the highest in the dry season when the efficiency of plant water conservation becomes more important (Ben Rouina et al., 2007, Liao et al., 2008, Rada et al., 2005).

Leaf traits

At the end of the dry season plants provided with the mulch treatments (notably the plastic mulch treatment) had a higher mean leaf chlorophyll content than plants under the zero mulch treatment. Comparing these results with those obtained in the clone * (quantity of water * frequency of watering) experiment that involved potted plants of the same cacao clones and of the same age under greenhouse environments it would, again, appear that the quantity of irrigated water supplied to the plants was not excessive. Too wet (or too dry) a soil medium would be associated with lower leaf chlorophyll concentrations. Alternatively the water used in irrigating the plants could have replenished some of the nutrients that would have been lost through leaching and plant uptake depending on its nutrient content (Akirinde and Ayegboyin, 2006). The results were dissimilar to some results that have been obtained in other species than cacao. Working on 12 peanut genotypes Arunyanark et al.

(2008) observed, in two field trials, that plants which experienced a reduction in soil water from 'field capacity' to one third available water, had increases in chlorophyll content per unit leaf area. Evaluating the influence of water stress on photosynthetic pigmentation of two sugar beet varieties, Hussein et al. (2008) recorded higher leaf chlorophyll *a* concentration by withholding water supply down to 40% of the soil's water holding capacity (W.H.C.) than by supplying the 'normal quantity' of water (= 80% W.H.C.) although the highest values were obtained when the soil water was 60% of the soil's water holding capacity. In Quinoa (*Chenopdium quinoa* Willd.) plants Gonzalez et al. (2009) found that a waterlogged situation rather than drought conditions caused significant reductions in leaf total chlorophyll content. In this current experiment, however, cacao plants that grew under soil water conservation by means of mulching had higher leaf chlorophyll concentrations. Probably that was an indication of higher nutrient uptake during the dry season.

The observation that plants that grew under heavier shade had higher leaf chlorophyll content agrees with those made earlier by Daymond (2000) which showed that in cacao shaded leaves often exhibited greater total chlorophyll concentrations than sun leaves. That probably is an adaptation to enhance light utilization efficiency with decreasing incident light as, according to Baker and Hardwick (1973) greater chlorophyll development in cacao is associated with higher photosynthetic capacity.

In the greenhouse clone * (quantity of water supplied * frequency of watering) experiment (in Chapter 3) differences were found between cacao genotypes that were similar to the ones obtained in this field experiment. The significant differences among the cacao genotypes suggested genetic differences in their rates of chlorophyll development.

The relative water contents of cacao leaves were lower than those observed by Joly and Hahn (1989a) but fell within the range of values obtained by Daymond (2000).

During the second week of December 2007 when the relative water content was measured the soil was probably not dry enough to detect differences in relative water contents under the different factors. A report by de Almeida et al. (2002) on cacao showed that different dehydration levels are not always clearly expressed by the relative water contents of leaves. The observation that the specific leaf weight was higher in plants provided with the plastic mulch and irrigation treatments suggests that conserved soil moisture increased leaf density (Fig 76). This observation contradicts the observation made in the clone * (quantity of water supplied * frequency of watering) experiment conducted under greenhouse conditions. This is probably because under field conditions- unlike in pot experiments- plants can better utilise higher levels of soil moisture for CO_2 assimilation resulting in greater starch production and a higher specific leaf weight. The results on specific leaf weight are similar to the observation made by Gonzalez et al. (2009) on quinoa plants in which both relative water content and specific leaf weight were increased as a result of increasing soil moisture. Also under field conditions Chakraboyty et al. (2008) reported rice husk mulch as improving the soil moisture status and the specific leaf weight of wheat (*Triticum aestivum* L.). The observation also agrees with an earlier one in which de Almeida et al. (2002) found, in cacao, about 25% increase in specific leaf area (and therefore a decrease in specific leaf weight) when soil water potential fell between -0.5MPa and -0.3MPa. Differences among the clones were clearer in this experiment with SCA 6 having the highest specific leaf weight as it did in the clone * (quantity of water supplied * frequency of watering) experiment. The results suggest the existence of genetic variability with respect to specific leaf weight.

Plant survival and growth

Cacao plants that experienced the soil water conservation interventions had higher plant

survival and growth rates which were a reflection of the higher rates of photosynthesis and probably plant cell survival during the dry season, the critical period for the survival of young, field-grown cacao (Balasimha, 1999, Meyer and Boyer, 1972). According to Joly and Hahn (1989a) water stress of sufficient intensity is capable of causing lasting disruption to the photosynthetic apparatus of young cacao.

In addition to conserving soil moisture the mulch treatments could also have reduced stress by reducing root zone temperature which (independently of air temperature) is known to influence the survival, growth and dry matter partitioning of field plants (Smith, 1989).

Although data were not taken in support of it, leaf abscission in the dry season was noticeably lower and leaf area higher in plants that grew under the mulch treatments than in those that grew under the zero mulch treatment. Previous studies in cacao have shown that conserved soil moisture had a positive influence on leaf area duration (Balasimha, 1999, Klepper et al., 1984, Zuidema et al., 2005).

The observation that plants that were subjected to heavier shade survived better may be explained in terms of the benefits that shade provides for young cacao (with little self shading) especially in the dry season. As outlined by Beer et al. (1998) the provided shade must have ameliorated the effect of stressful conditions in the microenvironment through the reduction of day-time air and soil temperatures, maintenance of a higher relative humidity and reduction of wind speed. Other benefits of shade include protection against high photoinhibition, excessive transpiration and leaf abscission (Galyuon et al., 1996a).

Whereas differences observed in plant survival under the shade intensities were significant only non significant differences were noted in plant growth. Although not statistically different, the observed trend in which the close plantain shade treatment (which provided the heaviest shade) had cacao plants with the smallest stem volumes was noteworthy as a simi-

lar trend was seen in the shade * clone nursery experiment. It cannot be said with certainty whether a direct light limitation to photosynthesis or indirect influences of shade were more important to plant growth. The triangular *Gliricidia* plants had the tallest cacao plants with the thinnest stems. This was probably due to the fact that the cacao plants grew in the midst of three *Gliricidia* plants located equidistantly at 0.60 m around the cacao and provided shade from three angles. To compete for sunlight the cacao plants must have had the tendency to invest more photosynthate towards increases in plant height at the expense of stem girth.

The fact that plants survived the dry season better under increasing shade implies that the greater amelioration of aerial stresses as a consequence of increased shade more than compensated for the negative effect of possible competition from the shade plants.

The significant mulch * clone interaction in which there were greater plant losses of some cacao genotypes compared with others under the zero mulch treatment suggests that under field conditions the soil water requirements of the genotypes differ. Under the mulch treatments P 30 [POS] and SCA 6 experienced increased survival with a higher margin over the zero mulch treatment than T 79/501 and PA 150 which seem to be more drought-tolerant.

The experiment has shown that plant survival and growth increase with reasonable increases in soil water levels which may be obtained through the direct supply of water (sub-surface irrigation) or through soil water conservation by means of both organic (coffee husk) and inorganic (plastic) mulching. Plants under higher soil moisture experienced decreases in water use efficiency but higher CO_2 uptake, leaf chlorophyll fluorescence and net photosynthesis as plant water supply increased. The significant season * mulch interaction showed that with young cacao it may be superfluous to apply those interventions with the purpose of in-

creasing soil water during the rainy seasons. This observation is important in the case of irrigation considering the cost implication associated with its usage.

The non significant mulch * shade interaction term suggests that the benefits that can be obtained from providing both shade and soil water may be 'additive'. However, the observation that plants under heavier shade tend to experience slower growth confirms the need for good shade management in order to achieve higher plant survival and also good growth. As observed more clearly in the shade * clone nursery experiment, reduced shade may be useful during the rainy seasons to promote faster plant growth.

CHAPTER 7
General discussion

7.1 Environmental impact

The data in this thesis show that it is feasible to significantly improve cacao establishment in the deteriorating planting environment (caused by depleting soil nutrients and poorer annual rainfall distribution) of Ghana by some of the agronomic interventions employed in these experiments. They also underscore the benefit of conducting most of the experiments over a time-course of one year, giving an idea about how the different seasons influence plant reaction under the factors considered.

In both the nursery (shade * clone) and the field experiments [the mulch * (shade *clone) and clone * (shade * manure) experiments] noticeable differences were observed between the seasons in most of the physiological processes considered. The dry season was the most unfavourable with its high water vapour pressure deficit (due to high daytime temperature and low relative humidity), increased soil moisture stress (in the case of the field experiments) and higher light intensity (due to the associated cloudless sky). As both atmospheric and soil stresses were highest in that season most of the recorded seedling losses occurred at that time. Restrictions and constraints in important physiological processes including stomatal conductance, transpiration, light use efficiency and net photosynthesis were highest under the dry condition.

Of the three seasons, the minor rainy season which had the lowest vapour pressure deficit, high soil water table (in the case of the field experiments), and low light intensities (due to cloudiness), was the least stressful. The highest efficiency of photosystem II was observed in that season but net photosynthesis was still less than that recorded in the major season in

which CO_2 assimilation was highest. As explained in chapters 3, 5 and 6, a combination of factors existed in the major rainy season that must have contributed to the realisation of the highest net photosynthesis in that season. These include the higher light intensity, low water vapour pressure deficit and increased soil water (in the case of the field experiments).

The observations (in the various seasons) made on photosynthetic activity, growth and establishment underlined the importance of the agronomic interventions that were applied in the different experiments of this work, namely, the provision of shade, application of organic manure and the direct supply or the conservation of soil water by means of irrigation, organic and inorganic mulching.

7.2 Photosynthetic activity, survival and growth of young cacao under different soil water levels

The [clone * (quantity of water supplied * frequency of watering)] experiment was used to determine whether important physiological processes of growth and survival of young cacao plants increase as soil water increases towards 'field capacity' and whether there are genotypic differences in the processes between the cacao genotypes as water stress changes.

The significant interaction between the quantity of water supplied and frequency of watering observed in the experiment indicated higher photosynthetic activity as soil water increased up to 50 - 62% of field capacity. It was, therefore, reasoned that although Öpik et al (2005) recommend a soil's 'field capacity' of water as being the ideal for most plants (owing to the fact that the soil will have a high water potential but also enough air-filled spaces) other factors such as the interaction of soil water with soil density and soil nutrient status can influence the optimal soil moisture content for young cacao.

It was also observed that the optimal soil water for leaf chlorophyll development (30 - 36%

field capacity) fell below the optimum soil water for photosynthetic activity. The observed reductions in leaf chlorophyll concentration as soil water increased above the optimum for photosynthesis may also have been an indication of a possible nutrient dilution in plant tissue (Maier and Chvyl, 2003, Arunyanark et al., 2008, Hussein et al., 2008). It was suggested that probably increased soil water needed to be supported with a supply of plant nutrients. This seemed to be corroborated by the observation that the highest leaf nitrogen content was also recorded between 30 - 36% field capacity.

With reducing water supply, all the genotypes became more efficient in their water use both in the field and under greenhouse conditions showing a more stringent stomatal control which reduced transpirational water loss more than it reduced the rate of photosynthesis. However, the extent to which water use efficiencies improved in the various genotypes as water stress increased varied significantly. Genotypic differences existed among the cacao clones with SCA 6 having the highest and PA 150 having the lowest water use efficiency in that experiment which was conducted during the rainy seasons. Higher water use efficiencies were recorded when soil water content was reduced by reducing the frequency of watering than by reducing the quantity of water supplied at a time. That observation suggested that under rain-fed conditions rainfall distribution would exert a stronger influence on CO_2 uptake than the intensity of precipitation.

The experiment also demonstrated that a faster plant growth and a higher plant survival rate are more likely to be obtained when soil water is maintained between 50% - 62% field capacity. If this was the case under field conditions it would imply that for optimum field establishment less irrigation water might be required by young cacao than by many other plant species. However, there are differences between pot and field hydrology due primarily to differences in the way soil water is dispensed under the two conditions.

As stated in the General Introduction (chapter 1) the strategy adopted in this work was to conduct experiments under greenhouse and nursery conditions expecting results from them to provide background information that could facilitate the understanding of similar experiments conducted under field conditions. It was, therefore, interesting to observe more similarities between the greenhouse / nursery experiments and the [mulch * (shade * clone)] field experiment (than there were dissimilarities) in the response of the cacao plants to soil water and shade.

The significant season * mulch interaction on stomatal conductance in which stomatal conductance rates were similar under the different mulch treatments during the rainy seasons reflected the uniform soil moisture conditions during these seasons. During the dry season plants provided with the mulch treatments (including irrigation) were found to have significantly higher stomatal conductance and transpiration rates reflecting the higher soil moisture content. Photosynthetic rates were up to 68% higher in mulched plants during the dry season. These observations were explained as indicating that the mulch treatments were effective in conserving soil water, answering one of the questions that the thesis sought to answer. They also demonstrated the importance of soil water conservation to the physiological processes of young cacao plants in the dry season.

Mulched plants also had higher Fv/Fm ratios suggesting that the resulting increased soil moisture ameliorated any suppressive effects of water vapour pressure deficit and high light intensity. The greater plant survival rate and the overall faster plant growth were probably a reflection of the mentioned benefits that were experienced in the dry season. This corroborated Joly and Hahn's (1989a) observation that water stress was capable of causing lasting disruption to the photosynthetic apparatus of cacao and demonstrated the feasibility of employing the agronomic interventions (mulching /irrigation) used to conserve or supply soil

water. That provided, in part, an answer to the question posed at the outset of this thesis as to what agronomic practices could be used to satisfy the water demand of young cacao and improve field establishment. There was also a suggestion that the suitability of a soil water conservation method may be genotype specific. This was deduced from the observation that whereas the irrigation treatment was associated with increased photosynthetic activity in all the genotypes, photosynthetic rates were higher under the plastic mulch treatment for all the clones except SCA 6.

Thus in many respects the photosynthetic activity of young cacao under increased soil water was similar under both greenhouse and field conditions and the adopted approach to expect results from the greenhouse experiment to provide useful insights into the mulch * (shade * clone) experiment conducted under field conditions worked quite well.

It must be mentioned, however, that the biological advantages notwithstanding, the cost implications of the various agronomic interventions used either to supply or conserve soil water must be weighed against the biological advantages they offer. For example the cost of haulage and application of the coffee husk, and plastic mulch amounted to one hundred and fifty (150) and three hundred and twenty (320) pounds sterling per hectare per year, respectively. The irrigation system (capable of serving up to 10 hectares of young cacao) cost two thousand one hundred pounds sterling (£2,100) to install and eighty pounds sterling (£80) to run per hectare per dry season. The biological benefits may be realised in a higher crop survival , a faster crop growth rate with its indirect benefits of weed control, reduced soil erosion, reduced evaporation, less insect pest (especially capsid) infestation and early yield. With good maintenance an irrigation system can remain a permanent feature of a farm to sustain higher yields especially if it is decided to use it as a means by which fertigation will be practised.

7.3 Photosynthesis, growth and establishment of young cacao under different shade regimes.

In the shade * clone nursery experiment a significant season * shade interaction in which lower stomatal conductance and photosynthetic rates were observed in the wet seasons but higher stomatal conductance and photosynthetic rates were recorded in the dry season under increasing shade showed that increasing shade was needed to modulate the stressful effects of a high water vapour pressure deficit during the dry season and that less shade was required in the rainy seasons. These responses were in line with Balasimha's (1999) observation that stomata of intact leaves generally open in response to increasing light but the effect of low relative humidity can interfere with that response to light. It was reasoned that although soil water was not in short supply during the dry season an internal water deficit developed in the plants under the lightest shade (32.5% shade) as water uptake did not match the rate of transpiration, causing a restricted stomatal opening in order to conserve plant tissue water. The cacao under heavier shade received benefits associated with reduced stress in the dry season. Thus the provision of appropriate shade was found as an answer to the question of what agronomic practices would reduce the water demand of cacao during the establishment phase.

The diurnal study showed plants that grew under heavier shade to have a lower photosynthetic capacity. It was reasoned, however, that although absolute photosynthetic rates were lower in heavier shaded plants, a greater reduction in photosynthetic efficiency must have occurred (as a result of photoinhibition) in the plants that grew under higher light intensities. It was noted, however, that any depressions caused by high photosynthetic photon flux density were dynamic in nature rather than chronic and the stressed plants recovered overnight

and their photosynthetic efficiencies were restored by the beginning of the following day.

When considering biomass partitioning it was found that plants which grew under the medium shade (55% shade) had a greater shoot: root ratio than those which grew under light (32.5%) shade. Thus the heavier shaded plants translocated a greater proportion of assimilate into leaf production to maximize light interception in order to maintain a functional equilibrium at the whole plant level. Plants that grew under lighter shade had smaller individual leaf sizes but the greater number of leaves produced under that condition more than compensated for the smaller leaf sizes causing those plants to have a greater total leaf area. Coupled with the higher absolute photosynthetic rates those plants experienced a greater total dry matter production than heavier shaded plants. This nursery experiment showed that provided other requirements such as soil water are met young cacao plants can be grown under 32.5% shade, throwing light on the question of what the shade requirement of young clonal cacao is.

Observations made in the shade * clone nursery experiment, and especially from the data gathered from the destructive harvesting carried out in that trial aided our understanding of plant reactions under varying shade under field conditions although a much narrower range of shade levels (50 -63% shade) was used in the field experiments.

The influence of shade on temperature and relative humidity in the field experiments followed the trends observed under nursery conditions, higher relative humidity and lower temperatures occurring under heavier shade. In the clone * (shade * manure) field experiment the highest rates of photosynthesis were recorded under the lightest (the control) shade treatment in the two rainy seasons. In the dry season, however, the highest photosynthetic rates were obtained under the heaviest (close plantain) shade treatment. Thus whereas in the rainy seasons more light energy (as obtained under lighter shade) was need-

ed to drive water uptake and transpiration, in the dry season increasing shade was needed to ameliorate the stressful effects of the dry season by sustaining a higher relative humidity and reduced aerial temperature. In the dry season, plants under the lightest shade had the highest water use efficiencies.

Similar to the observation in the shade * clone nursery experiment, increasing shade was associated with increased leaf chlorophyll concentration in the [mulch * (shade * clone)] trial but no clear differences in chlorophyll concentration were noticed in the clone * (shade * manure) experiment. Also a greater specific leaf weight was observed under heavier shade in the [mulch * (shade * clone)] trial. These observations would, probably, have been better understood if repeated leaf analysis was done and seasonal differences considered. It was, however, observed in the [clone * (shade * manure)] trial that leaf N, P and K concentrations were not reduced under increasing shade showing that there could only have been very little inter-specific competition (between the shade and the cacao plants) for soil nutrients.

Unlike the results obtained under the shade * clone nursery experiment total plant biomass was not different under the shade treatments under field conditions. However the plant survival rate was higher under increased shade in the [mulch * (shade * clone)] experiment which was conducted at a site which had soils with a lower water holding capacity than the [clone * (shade * manure)] trial. The differences observed in plant growth under different shade was not as striking under field conditions as they were in the nursery experiment, which may partly be attributed to the narrowness of the range of shade levels used in the field experiments. Also whereas plants in the nursery experiment experienced stress from only one source (water vapour pressure deficit) the field plants experienced higher levels of stress from vapour pressure deficits as well as from reduced soil water.

It was worthy of note that the spacing and planting arrangement that gave the lightest shade level in the field experiments has been the standard for cacao establishment in Ghana. The decision to test heavier shade using the 'standard' as the control was based on the fact that the growing environment of cacao is becoming more hostile and the supposition that young cacao plants may require higher shade levels to survive.

In spite of the less dramatic response to shade (as observed in the field experiments) a few definite conclusions can be drawn about the provision of shade during the establishment phase of the cacao genotypes (SCA 6, T 79/501, P 30 [POS] and PA 150) used in this work.

1. The young cacao plants had a dynamic shade requirement that was time and season dependent.
2. The shade requirement is affected by other factors including soil water and relative humidity.
3. Plantains and *Gliricidia sepium* stakes can be used in different planting arrangements to provide the required shade under field conditions.
4. Whilst the upper limit of the requirement was around 55% shade the plants had better growth at 32.5% shade when soil water was not limiting.
5. The physiological basis of the shade requirement was expressed in the changes that occurred in physiological and morphological characters of the plant including, net photosynthesis, the efficiency of water and light use, leaf area development, assimilate partitioning, plant survival and growth.
6. Shade ameliorated the stressful effect of water vapour pressure deficit, high soil temperature and excessive light.

7.4 Photosynthetic activity and growth of cacao with or without fertiliser

A significant shade * manure interaction term was observed in the clone * (shade * manure)

field experiment. In the major rainy season photosynthetic rates were highest in fertilised plants growing under the control shade. Probably increased light provided greater energy to facilitate nutrient uptake and utilization in photosynthesis in that season when soil moisture was not limiting. In the critical dry season, however, a diminished rate of photosynthesis was observed under reducing shade. The results were consistent with the observation of Adams and McKelvie (1955) who reported that under heavier shade, nutrient uptake is reduced because of reduced transpiration and photosynthesis. These authors explained that, when photosynthesis is greater under higher light, the uptake of nutrients should also increase so as to make use of the synthesized carbohydrate by transforming it into protein. This seemed to be similar to an idea expressed by Isaac et al. (2007) that when fertilisers are not available or not wanted appropriate shade trees can be used to regulate the nutritional status of young cacao.

Another significant shade * manure interaction was detected in the specific leaf weight of the plants. Plants which grew under the lightest shade but received no manure had the highest specific leaf weight probably as a sign of low nitrogen and high fibre content.

Increased leaf nitrogen and potassium contents were observed with manure application suggesting an increase in uptake of N and K in response to increased availability. Although within the period of study no significant increase in plant growth rate was observed with manure application, a higher survival rate was recorded in fertilised plants. This signified the potential which exists for organic fertilisers as a soil amendment in the present circumstance of unrelenting soil degradation which is rendering the field establishment of cacao increasingly difficult. Exploiting this potential will have the added benefit of relieving poultry and cacao farmers of the drudgery of manure disposal which is a requirement of farm sanitation. According to Agele and Agbona (2008), in Nigeria alone, about eight (8) million tons of cacao

pod husks are discarded from which between sixty-four thousand (64,000) and ninety-four thousand (94,000) tons of K, Ca and P could be obtained. Evidently a similar situation exists in all the cacao producing countries of West Africa. As mentioned in the case of the mulches, however, any biological gains must be weighed against the monetary cost involved. In the present work the cost of manure acquisition and application amounted to an equivalent of two hundred and twenty British pounds (£220) per hectare per year.

Genetic variation in physiological traits and growth of cacao

A major interest of the thesis was to identify cacao varieties which are well adapted to a condition of reduced soil moisture and high water vapour pressure deficit and can respond positively to the agronomic interventions aimed at improving establishment. To that end parameters such as net photosynthesis, water use efficiency, growth and survival rates were considered to be variates of importance. Results from the shade * clone nursery experiment suggested that PA 150 and T 79/501 were more sensitive to vapour pressure deficit than P 30 [POS] and SCA 6. Under field conditions, however, no such significant differences were observed due, probably, to differences in the experimental environments. However, in the [clone * (shade * manure)] field trial, whereas no differences were observed among the genotypes in stomatal conductance and transpiration rates during the dry season, significant differences were observed in the rainy seasons with PA 150 having the highest rates and either P 30 [POS] or SCA 6 having the lowest rates. This seemed to suggest that the observed differences (in the shade * clone nursery experiment) were mere indications of different levels of plasticity in the parameters rather than of drought tolerance by means of better stomatal regulation. A much slower transpiration rate in some of the clones than the others during the dry season would have implied greater drought tolerance.

Throughout the work no clear differences were observed in the photosynthetic efficiencies of the genotypes. This suggested that their rates of resource capture and utilization were about equal. This was not surprising as no consistent differences were found in their leaf traits. Differences in the average leaf area per plant of the different genotypes (as measured in the shade * clone nursery experiment) were on the borderline of significance ($p = 0.05$) and plant growth rates (measured by stem volumes) were different for the clones. These differences in stem growth are likely a reflection of differences in the proportion of assimilate that is partitioned into different organs of the plant.

The significant difference in shoot: root ratios in which PA 150 and T 79/501 had the smallest values and SCA 6 had the greatest value suggests that with the faster-growing clones a bigger proportion of assimilate is translocated into root growth. A faster root growth would help access soil water and nutrients to promote growth at the whole plant level. That trait improves plant survival and establishment.

In the [clone * (quantity of water supplied * frequency of watering)] experiment which was conducted on greenhouse benches where root growth was restricted to a larger extent than in the other experiments, differences in the growth rate of the genotypes were not significant. This observation probably indicated the importance of root growth to total plant growth in the early stages of a cacao plant. These observations suggest that differences in the growth of the cacao plants were a result of differences in the sink strengths of the various organs rather than of genetic variability in their photosynthetic efficiencies. It may be interesting to confirm this explanation and possibly attempt to take advantage of differences in partitioning strategies in breeding programmes. Figure 82 shows, schematically, the link between the agronomic interventions employed in this study and plant growth rate (vigour) and establishment. Under optimal soil water, light (depending on the season) and soil nutri-

ent levels (all of which may be achieved by means of a good, integrated application of the interventions) a higher rate of CO_2 assimilation as well as the most beneficial assimilate partition strategy is likely to be observed that can enhance plant growth and field establishment.

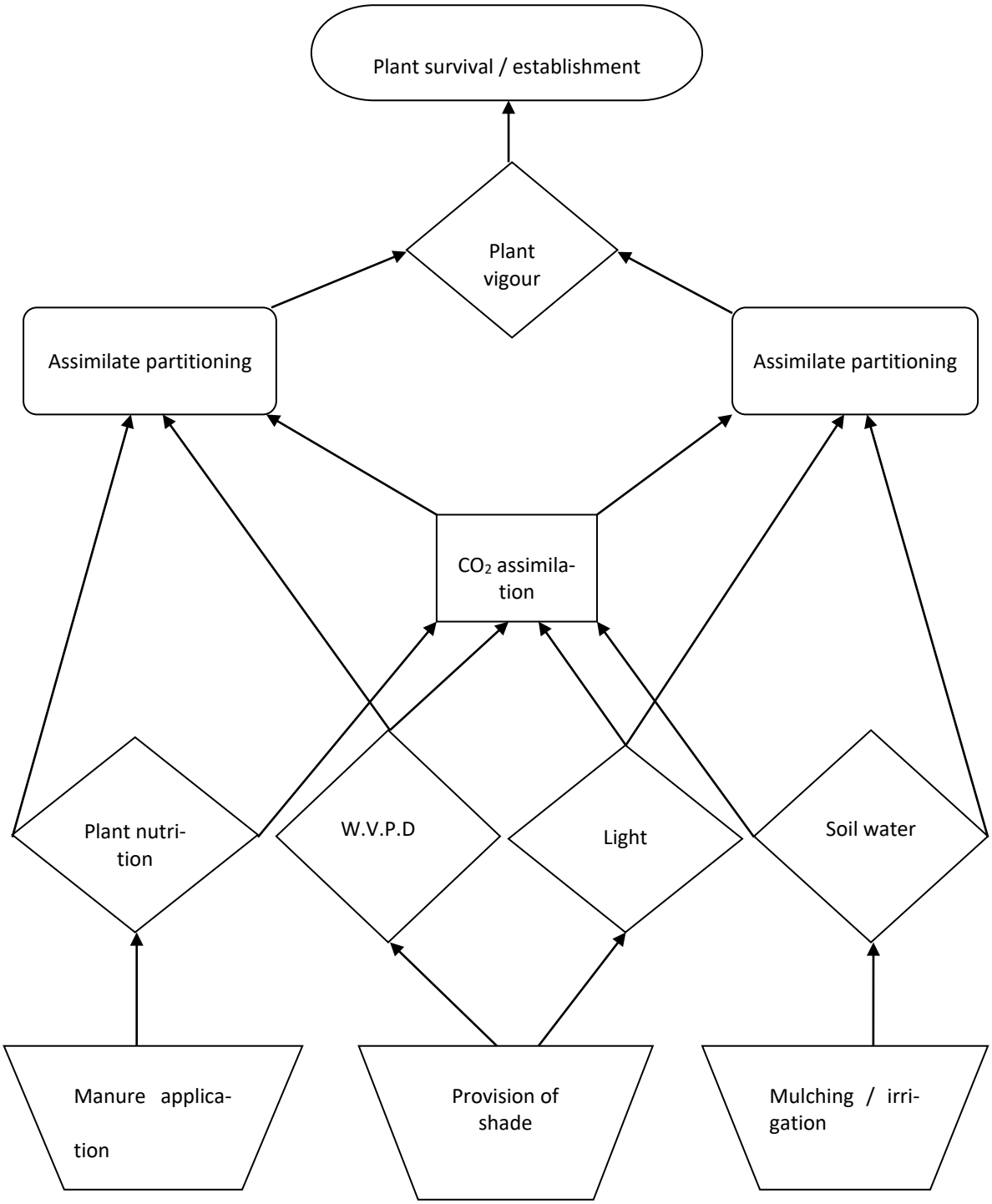

Figure 82 A schematic diagram showing the link between agronomic interventions (the provision of shade, manure and soil water) and the survival and growth of young cacao plants

7.4.1 Implications and recommendations

In cacao nurseries around Ghana watering has generally been done without any informed and up–to–date guidance. The general practice for container plants has been to wet the soil thoroughly every other day. Evidence coming from this thesis shows that apart from its, obviously, being less economical, it is also likely to cause the leaching out of soil nutrients, as well as create hypoxic conditions (if sufficient drainage is not provided) which will not give the best plant growth (Nematia et al., 2001, Sharp et al., 2009, da Silva et al., 1993). It has been shown that for potted plants the ideal is to determine the 'field capacity' of the soil medium and then water the plants with half the soil's 'field capacity' of water every other day. This gives the best plant survival and growth. In a situation where that is not affordable the alternative will be to water with the full 'field capacity' of water every 5th day. Differences between pot and field situations may, however, require this recommendation to be limited to young cacao plants growing in pots.

Under field conditions irrigation of cacao is practically nonexistent in cocoa producing countries. Apart from the envisaged cost involved another difficulty that has put many stakeholders off has been the fear that water bearing pipes would become an impediment in the farm and obstruct farm operations. It is, therefore, reassuring that by means of the sub-surface irrigation system that difficulty can be circumvented. That system has other advantages over the conventional surface irrigation systems. These include a smaller maintenance cost (as the pipes are much less likely to be damaged during farm operations, and are protected from ultra violet rays that cause more wear and tear in them) and less salinity problems as a smaller quantity of water is usually applied to get a desired soil moisture level. There is little doubt that such a simple irrigation system has the potential to facilitate the establishment of cacao even in the marginal and denuded areas of West Africa. Data from this thesis also

show that the use of mulches helps cacao establishment. A difficult problem that is associated with mulching has been with haulage of organic mulches to farm sites since most farms are not accessible by transport. For such farms plastic mulching, which has been found in this work to be very effective in conserving soil water for cacao plants may be used (depending on the cacao genotype planted). Currently, a few pineapple farmers in the savannah areas of Ghana have adopted the use of plastic mulch as it offers the benefits of being portable, checking weed growth, reducing soil temperature and conserving soil water (Awuah and Adzim, 2004) and that practice may be applied for cacao.

The problem that is associated with haulage to applies to the use of organic manures too. The process of 'ashing' (as used in this work) has made the use of cacao pod husk much cheaper and more practicable as it reduces the bulkiness of the organic substance making it much more applicable in most farm situations. The use of unprocessed poultry manure (as was used in this work) may be limited to farms that are reachable by transport as well as nurseries.

The importance of shade during the early stages of the growth of a cacao plant was clearly demonstrated in the experiments that involved shade as a treatment. The recommendation of about 50% shade (Wood 1985) for young cacao has been adhered to by organizations in Ghana [mostly subsidiaries of the Ghana Cocoa Board (The COCOBOD)] that establish plantations. The peasant Ghanaian farmer has always provided shade for both young and old cacao but has not shown much interest in how much shade is provided at a given time. During the establishment phase a major concern centres on what percentage of the establishment cost can be recouped through the use of arable and annual food crops including maize, cocoyam, cassava and plantains (Asare and Sonii, 2010). The result has often been that during the rainy seasons of the first year of establishment (when data from this thesis show that not so

heavy shade is required by the cacao plant) the cacao is heavily shaded. During the dry season of the first year of establishment (when the cacao plant requires heavier shade) the shade available is scanty as the maize, for example, will have been harvested. This partly explains the high seedling losses encountered during the dry season.

In the interest of cacao it may be good to give more attention to a few longer-lasting temporary shade plants such as plantains that can be managed to provide the right amount of shade all year round. The close plantain shade treatment provided the best shade (in terms of cacao plant survival but not cacao growth rate) under field conditions. Planting the plantains much closer to the cacao (0.6 m instead of the recommended 1.5 m away from the cacao plant) ensured greater shade when the plantains were young and had only a few leaves. In the dry season although some of the leaves of the plantains dried out there was still enough shade for the cacao due to the closeness of the shade plant to the cacao. Also positioning the plantains to the west of the cacao plants took the diurnal shade requirement into consideration. In the early hours of the day when the cacao plants needed more sunlight for higher photosynthetic activity (as they would have recovered from any photoinhibition they might have experienced the previous day and the available sunlight would not be so inhibitory) the plantains provided little shade for the cacao plant. At midday and in the afternoon when the cacao would be stressed by the high light intensity and increased vapour pressure deficit shade provided by the plantains was more available for the cacao. This strategic positioning of the plantains is novel and has little added requirement. Having proved to be so successful, the idea can be given to the farmer and other stakeholders for possible adoption.

It was learned from the shade * clone nursery experiment that in cacao nurseries where adequate water supply is assured and often wind speeds are reduced through sheltering the shade level need not be maintained around 50% all year round. In the minor rainy season

when the raising of nurseries is often started, the shade level can be reduced to about 32.5% and in the dry season increased to about 55%. Shade levels around 76% did not support the highest photosynthetic activity at any time.

It was observed that the water use efficiencies of PA 150 and T 79/501 were not different from those of the other genotypes of cacao used in this work. However they were noted to have a greater plasticity in stomatal control (consuming more water in the rainy seasons when atmospheric water pressure deficit was low but being no more demanding of soil water in times of high water vapour pressure deficit). Thus they appeared to have a more effective "water use" than the other genotypes (Blum, 2009). Probably as a result of their mode of assimilate partitioning, PA 150 and T 79/501 had the ability to grow faster, a character that has been exploited in drought resistance breeding programmes for cacao (Balasimha 1999). As mentioned earlier a faster growth rate confers many establishment advantages. Although the two clones had significantly higher survival rates (in two of the three experiments in which plant mortality was experienced) it may be overambitious to recommend them (at this stage of experimentation) for planting in the marginal areas of cacao production in Ghana. They can, however, be recommended for inclusion in future work that will subject them to further scrutiny.

Future work

It is seen from the contributions of shade, manure, and soil water across different seasons that it takes more than a single factor to improve the survival and growth rates of cacao. There are a number of questions related to these factors that could be investigated further. For example, is it possible, in the rainy seasons, to further reduce the shade levels for young cacao (to less than 32.5%) under similar conditions as were used in the nursery work in order to improve plant growth? It was not determined why in the greenhouse work it was ob-

served that the container-grown cacao had higher photosynthetic activity and plant growth at about 62% field capacity of water compared with the highest watering regime (about 87% of field capacity). It would be interesting to know whether under the highest watering regime there was much greater soil nutrient leaching or soil compaction or due to a possible inadequate drainage, this observation was influenced by occasional hypoxic soil conditions. It may be useful to confirm this observation in future experiments. If confirmed it may be important to determine why in the case of cacao, unlike other plants (Öpik et al., 2005, Smith, 1989), maximum photosynthetic activity and growth may not be obtained around field capacity.

In addition to the water use efficiency (WUE) it may also be worthwhile to determine the effectiveness of water use (EUW) since, in recent times, crop growth improvement programmes for dry areas consider this as a more important target (Blum 2009). Joly and Hahn (1989a) explain that water use efficiency for a cacao genotype may be a poor strategy as it could mean that the genotype with a higher water use efficiency fails to capture and utilise available soil water, surrendering it to competitors. Such genotypes, according to these authors, are likely to have a slow growth.

The indication that the frequency of watering has a greater impact on photosynthetic activity and plant growth than the quantity of water applied needs a field verification since it is hoped that the simple nature of the irrigation system will promote its adoption by capable farmers. Having seen the physiological activity of the young cacao under the separate treatments of mulch, it may also be interesting to evaluate those activities under the combined treatments of irrigation and plastic mulch, for example, during the dry season. The differences in photosynthetic activity of the young cacao in the different seasons suggest that the field planting of cacao close to the end of the major rainy season (as the practice has been)

may not be the ideal and that it may be better to plant earlier on in that season to take greater advantage of the more conducive aerial condition of that season. It would therefore be useful to study the effect of varying planting time within the major rainy season to determine the best time for field planting.

Finally, it may also be beneficial to study (in a single experiment and by means of destructive harvesting) the root and shoot development of young cacao under varying shade, water and soil nutrient levels under field conditions. Combining those important factors in such an experiment is likely to provide further insight into the link between improved agro-ecological conditions and the field establishment of cacao.

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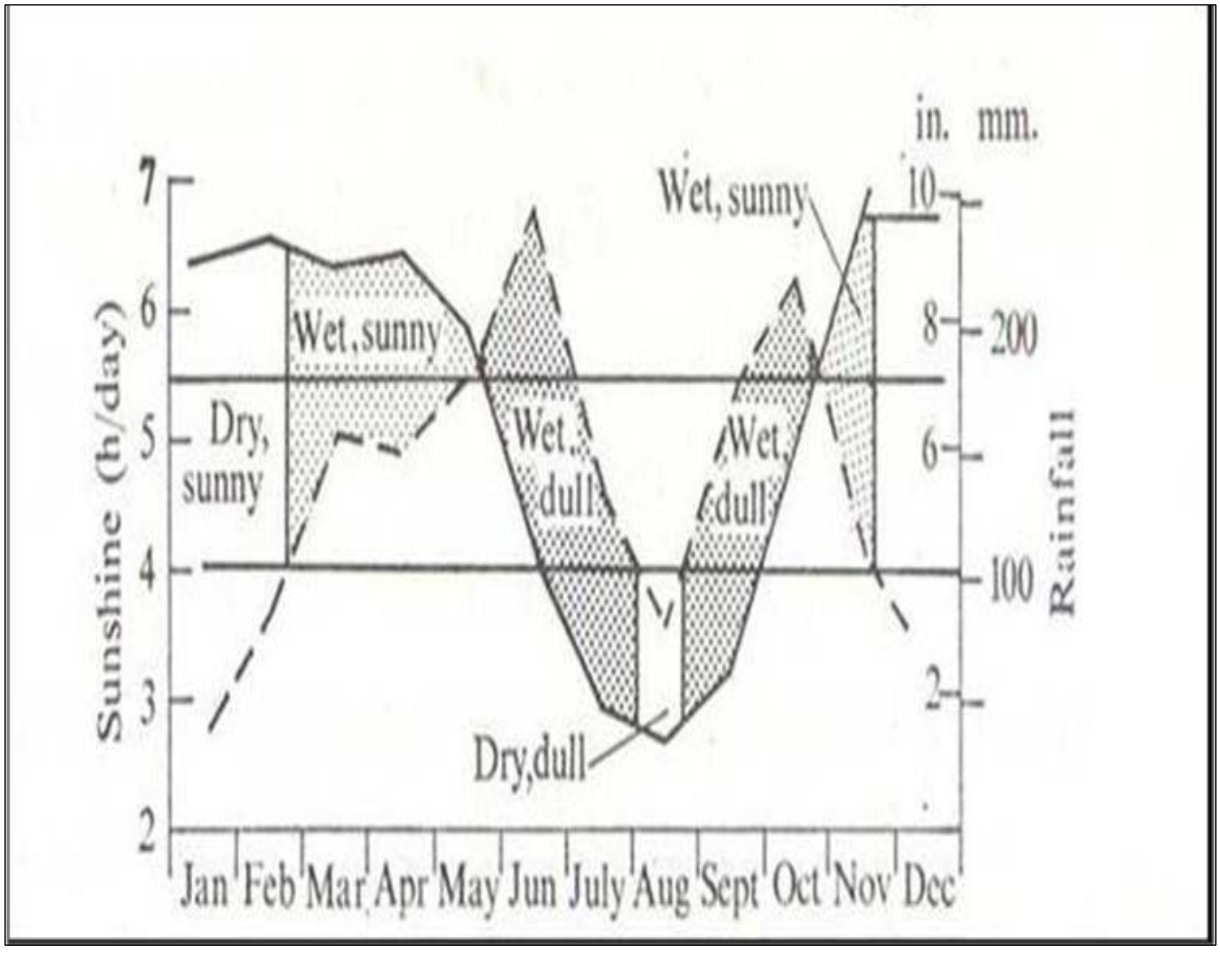
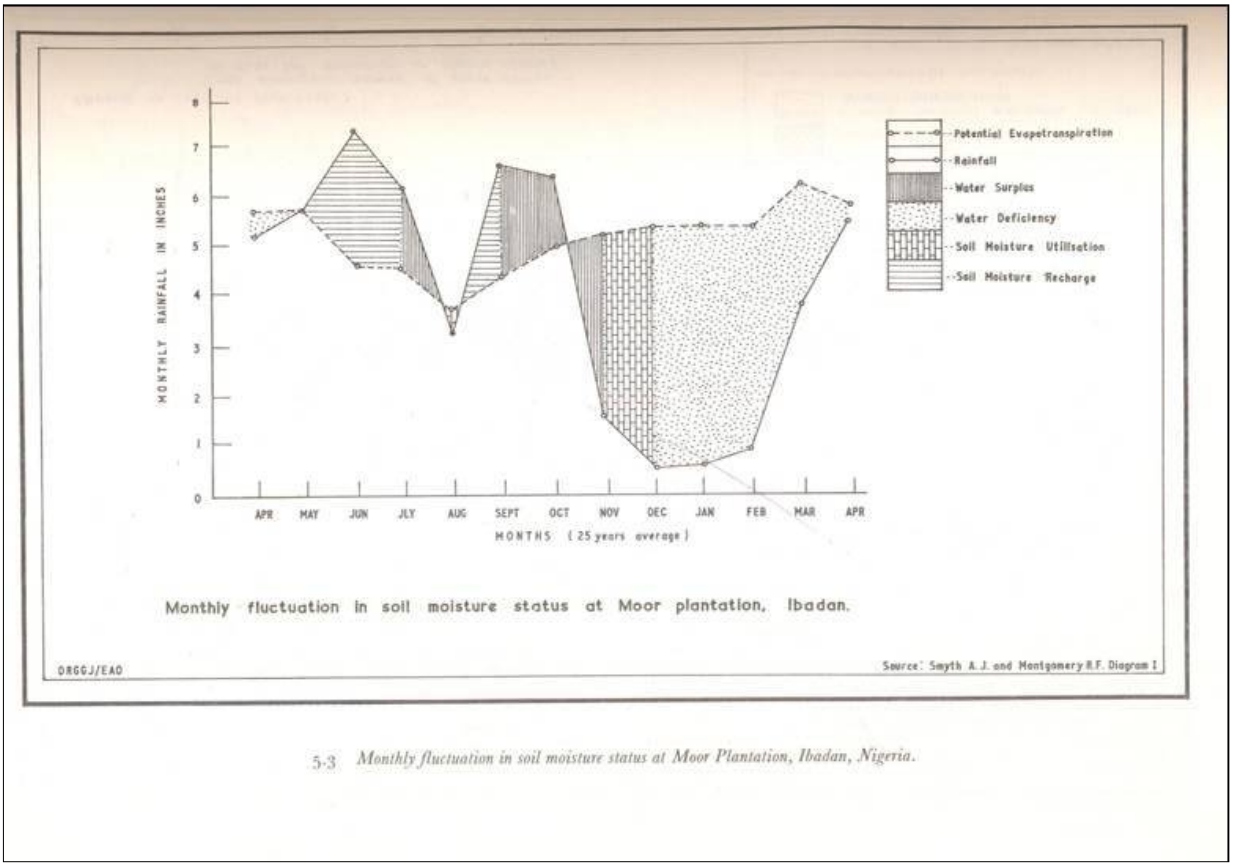
Appendices



Appendix 1 Monthly weather pattern in the cacao growing area of Ghana

Rainfall (dotted line) based on 30-year means and sunshine (continuous line) on 20-22-year means at Tafo, Ghana.
Source: Entwistle (1972), p 33.



5·3 *Monthly fluctuation in soil moisture status at Moor Plantation, Ibadan, Nigeria.*

Appendix 2 Monthly fluctuations in soil moisture at Moor plantation, Ibadan, Nigeria
Source: Are and Jones (1973), p35