Studies on growth and ecophysiological characteristics of tropical tree species useful in Ghana

(ガーナにおける有用熱帯樹木の生長と生理生態的特性に関する研究)

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The United Graduate School of Agricultural Sciences,
Kagoshima University

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By

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Abstract

Deforestation of African tropical forests as a result of greater dependence on wood resources is a growing concern. However, one of the challenges to successful reforestation efforts in many African countries, including Ghana, is the lack of knowledge on ecophysiological traits of potential plantation species, especially native species, under different site conditions.

A series of experiments were conducted under controlled conditions to examine morphological and/ or physiological responses of some selected tropical tree species to soil water, nutrient and/ or light treatments.

First, soil water availability on seedling height, dry matter production and allocation were investigated for two African mahogany species (*Khaya anthotheca* and *K. ivorensis*) from contrasting semi-deciduous forest sub-types of Ghana. Seedlings of the two species were subjected to three soil watering treatments, which were termed wet, moderate and dry treatment, in a glass house facility. Soil water deficit greatly reduced total dry matter production in *K. ivorensis* and altered proportional allocation to leaves and roots as a possible strategy for acclimation. In the moderate and dry treatments, *K. anthotheca* seedlings had greater total plant dry matter than *K. ivorensis* seedlings, which suggests that *K. anthotheca* have considerable potential for plantation forestry in the humid tropics of Africa where distinct drought periods (4–5 months) occur.

Second, drought resistance in seedlings of *Cedrela odorata, Cassia siamea* and *Khaya senegalensis* were estimated through measurements of transpiration rate, stomatal conductance and chlorophyll fluorescence, and growth responses under drought conditions of a greenhouse experiment. This study was conducted in order to assess the suitability of these species for plantation forestry in semi arid and drier sites of Ghana. Results of this study indicated that *C. siamea* and *K. senegalensis* exhibited stronger drought resistance, whereas *C. odorata* followed a drought-avoiding strategy and was more conservative in its water use. At soil water potential of −0.4 MPa (mild drought condition), *C. odorata* showed restricted growth and lowered physiological traits. In contrast, *C. siamea* and *K. senegalensis* showed reduced growth and physiological traits at −1.0 and −0.8 MPa, respectively, conferring greater advantage to them than *C. odorata* as plantation forestry species in semi-dry environments.

Third, containerized seedlings of three tropical species were grown under four different light treatments [i.e., 100, 45, 22 and 10% sunlight]. Photosynthetic properties and growth analysis reflected the species successional status with *Terminalia superba,* the pioneer species showing largest mean light-saturated photosynthesis (A_{max}), light saturation estimates (LSE), intrinsic water-use efficiency (WUE) and maximal quantum yield of photosystem II (F_v/F_m) at 100% sunlight, whereas at 10% sunlight, it showed the lowest A_{max}, WUE and LSE. At 22% sunlight, *Cedrela odorata,* an intermediate successional species had greater A_{max} and LSE than *Mansonia altissima,* a non-pioneer light demander and *T. superba.* Superior biomass production was found at 45% sunlight for all the species. Allocation to leaf and root followed patterns typical of plants growing in sun or shade conditions. Light requirement for species growth and physiological functions may be specie-specific. This study therefore, suggests that *T. superba* may have greater potential in plantation forestry at exposed sites; *C. odorata* has broad ecological amplitude making it suitable for planting in medium gaps and exposed sites; *M. altissima* may be suitable for enrichment planting in smaller gaps.

Furthermore, growth responses to fertilization in seedlings of *Cedrela odorata* and *Terminalia superba* were studied under 100 and 33% sunlight. Both species attained greatest relative height growth rate at 33% sunlight with fertilization and showed superior biomass production at 100% sunlight with fertilization. N fertilization enhanced foliar N concentration and relative Chl content of both species and without fertilization, 100% sunlight-grown foliage of *C. odorata* showed reduced Chl content. At 100% sunlight, fertilization enhanced F_v/F_m of *C. odorata* and thus, reduced the specie's susceptibility to photoinhibition. Enhancing acclimation of *C. odorata* and *T. superba* seedlings through longer hardening periods plus nutrient additions in an exposed nursery conditions may stimulate their initial growth when planted in the field.

Results from these experimental studies may have practical use in screening tropical tree species for use in reforestation and plantation forestry in degraded forest sites of West Africa where site resources such as soil water, light and nutrient availability may vary considerably.

Abstract (Japanese)

木材資源への過度な依存によるアフリカ熱帯林の減少に対して、近年関心が高まっている。しかし、ガーナを含む多くのアフリカの国々での植林を成功させるための課題の一つは、環境条件の異なる立地で植林に用いる樹種、特に郷土種の生理生態的な特性の情報が不足していることである。

本研究では、ガーナの森林再生に有用と考えられる数種の熱帯樹について土壌水分、土壌養分及び光環境をコントロールした条件下での、生長及び生理生態的反応を調べた。

１．まず、ガーナの半落葉樹林帯の異なる立地に生育する 2 種のアフリカマホガニー（*Khaya anthotheca* and *K. ivorensis*）の実生苗を、ガラス室内で３レベルの土壌水分条件（過湿、適潤及び乾燥）で育て、苗高、乾物重、物質配分に対する土壌水分の影響を調べた。*K. ivorensis* では土壌水分の不足は、乾物重を著しく減少させ、乾燥に対する順応戦略によって葉と根の物質配分を変化させた。*K. anthotheca* の実生苗は、適潤 及び乾燥処理において *K. ivorensis* の実生苗よりも乾物重が多かった。これらの結果から *K. anthotheca* はアフリカ湿潤熱帯の中で 4-5ヶ月の乾期のある立地での造林樹種に、*K. ivorensis* は同地域の低地など土壌の乾燥が起こりにくい立地の造林樹種に適していると考えられる。

２．次に、乾燥半落葉樹林帯からサバンナまでのより乾燥ストレスの大きい地域での多様な植林目的に適する樹種を検討した。*Cedrela odorata*、*Cassia siamea* 、*Khaya senegalensis* の実生苗の耐乾性を、土壌の乾燥過程での蒸散速度、気孔コンダクタンス及びクロロフィル蛍光の変化から比較した結果、*C. siamea* と *K. senegalensis* はより強い耐乾性を示し、*C. odorata* は乾燥回避性を示し土壌水分減少の早い段階から消費水分の減少が見られた。この結果から、この地域の中で強い乾燥ストレスとなる立地では *C. siamea* と *K. senegalensis* を植栽する方が有利である。

３．立地の光環境との関係を検討するために、３樹種の実生苗を相対照度(RLI) 10、22、45、100％の光条件下で育て、乾物重、物質分配、最大光合成速度（$A_{\max}$）、水分利用効率（WUE）、光飽和点（LSE）及び最大光量子収率（F_v/F_m）から比較した。先駆種である *Terminalia superba* は、RLI 100％で $A_{\max}$、WUE、LSE 及び F_v/F_m の平均値が最も高く、RLI 10％では、$A_{\max}$ 、WUE 、 LSE の平均値は最も低くなった。遷移中期

種である *Cedrela odorata* は RLI 22%で *Mansonia altissima* より高い A_{max} 及び LSE を示した。また、３樹種とも RLI 45%で乾物重は最も大きくなった。全光下及び日陰の条件下における葉と根への物質分配は、典型的なパターンを示した。これらの結果から *T. superba* は全光条件となる立地、*C. odorata* は光に関して広い適応域を持ち林内の広いギャップから全光条件下まで、*M. altissima* は林内での小さなギャップへの造林樹種に適していると考えられる。

４．初期生長への施肥の効果を *Cedrela odorata* と *Terminalia superba* の実生苗を用いて光環境（RLI 33%および 100%）との関係で調べた。両種とも施肥、RLI 33%で高い相対成長率を示し、乾物重は施肥、RLI 100%で最も高くなった。施肥で両種の葉の窒素濃度と相対クロロフィル含有量が増加し、無施肥では RLI 100%でクロロフィル含有量が低下した。また施肥は、RLI 100%での *C. odorata* の F_v/F_m を増加させ、光阻害の影響を低減させるなど、初期生長への施肥の大きな効果が認められた。

この研究により、ガーナの森林再生に有望な樹種の光、土壌水分、養分などの立地条件に対する反応特性が明らかになり、研究の成果はこれからの西アフリカ地域での、森林再生、造林の際の立地に応じた樹種選択に実際に適用できると考えられる。

CHAPTER 1.

General introduction

1.1. Background

In recent years, there have been attempts in the tropics to accelerate the recovery of degraded forests and deforested lands in order to restore productivity, biodiversity, and other values through plantation forestry (Evans and Turnbull, 2004). Globally, the annual net rate of deforestation (the balance of loss of natural forest and gain in forest area through afforestation and natural expansion of forest) has decreased slightly from 1980–1990 (13 million ha) to 1990–2000 (9.4 million ha) but it remains high in the tropics with an annual loss of 14.2 million ha converted to other land uses (FAO, 2001). In Africa, the average loss of forest cover over the last decade (i.e., 1990–2000), was 7.8% (Evans and Turnbull, 2004) with estimates for Ghana being 1.3%.

Although reforestation attempts have been carried out using indigenous tree species such as *Terminalia* species, *Triplochiton scleroxylon*, *Khaya* species, *Entandrophragma* species, *Bombax* species etc. in degraded forest sites of West and Central African countries, little success can be attested to these plantations (ITTO, 2006). On the other hand, good results in plantation trials using exotic tree species such as *Tectona grandis*, *Cedrela odorata*, *Gmelina aborea*, *Eucalyptus* species and *Cassia siamea* have been reported (ITTO, 2006). In Ghana, like many other African countries, one of the problems which have hampered reforestation efforts is the lack of knowledge about species performance on different site conditions. In Ghana, native timber species have been previously planted based on intuitive deductions of the species growth in an unshaded environment (Agyeman, 1994). This led to the failure of several plantation schemes in Ghana and a greater dependence of better known exotic species.

Ghana, which is in the western part of the African continent (0–3° W and 5–11° N), has a land area of approximately 239,000 km². Identifiable ecological zones in the country include rain forest (3 %), moist forest (31 %), interior savanna (57 %), coastal savanna (5 %) and Volta Uke or swamp vegetation (4 %), (Ntiamoah-Baidu, 2001). 34 % of the total area of Ghana is covered with tropical forest. One third of this forest, covering an area of about 18,000 km^2, is reserved forest (Hall and Swaine, 1981). The tropical forest of Ghana consists of four major forest types. They are Wet Evergreen, Moist Evergreen, Moist Semi-deciduous and Dry Semi-deciduous forest types (Hall and Swaine, 1981, Figure 1).

The Wet Evergreen is found in areas with the highest annual rainfall in Ghana. Mean annual rainfall in this forest type ranges from 1750–2000 mm. Soils in this forest type are classified as impoverished, strongly desaturated ferrallitic type (Boulet et al., 1971). The soils are severely leached with the pH of the top soil ranging from 3.8−4.3. The tallest trees in the Wet Evergreen are shorter than those occurring in the other forest types, reaching heights less than 40 m. The mean density and basal area of trees in this forest type are 445 trees ha^{-1} and 25.5 m^{-2} ha^{-1}, respectively (Hall and Swaine, 1981). Typical species in the Wet Evergreen forest are *Lophira alata*, *Heritiera utilis* and *Guarea cedreta.*

The Moist Evergreen forest type is located between the Wet Evergreen and the Moist Semi-deciduous forest. The mean annual rainfall in the moist evergreen forest type varies between 1500–1750 mm (Hall and Swaine, 1981). The maximum height of trees attained in the Moist Evergreen (43 m) is slightly greater than that of the Wet Evergreen forest type. Soils in this forest type are not impoverished and strongly desaturated ferrallitic (Boulet et al., 1971). The soils have a higher pH and higher nutrient content than the Wet Evergreen but a lower pH and nutrient content than in the moist semi-deciduous forest type. According to Hall and Swaine (1981), the Moist Evergreen is the second richest forest type in timber. Some of the typical species of this forest type are *Triplochiton scleroxylon* and *Strombosia glaucescens.*

The Moist Semi-deciduous forest type occupies the largest area in the tropical forest (Hall and Swaine, 1981). The mean annual rainfall in this forest type ranges from 1250–1500 mm. The maximum height growth in this forest type is grater than all other forest types, probably because of good balance between rainfall and fertility. Trees attain a height of 50–60 m. Soils here are classified by Boulet et al. (1971) as impoverished, moderately desaturated ferrallitic type. The soil under this forest type has a pH range of 5.0–6.0. The Moist Semi-deciduous forest type has the highest stocking of timber tree species (Hall and Swaine, 1981). Typical species in the moist semi-deciduous forest type include *Terminalia* *ivorensis* and *Etandrophragma utile*.

The fourth major forest type is the Dry Semi-deciduous forest. This forest type forms the boundary between moist forest and the savanna. Mean annual rainfall is between 1000–1250 mm. Trees achieve a height of 30–45 m (Hall and Swaine, 1981). The soils in this forest type are mostly dominated by the impoverished, moderately desaturated ferrallitic type. Other soil types such as slightly desaturated ferrallitic and ferruginous occur in some parts of the forest (Boulet et al., 1971). Typical trees in this forest type include *Milicia excelsa, Celtis Zenkeri, Triplochiton scleroxylon* and *Antiaris toxicaria*.

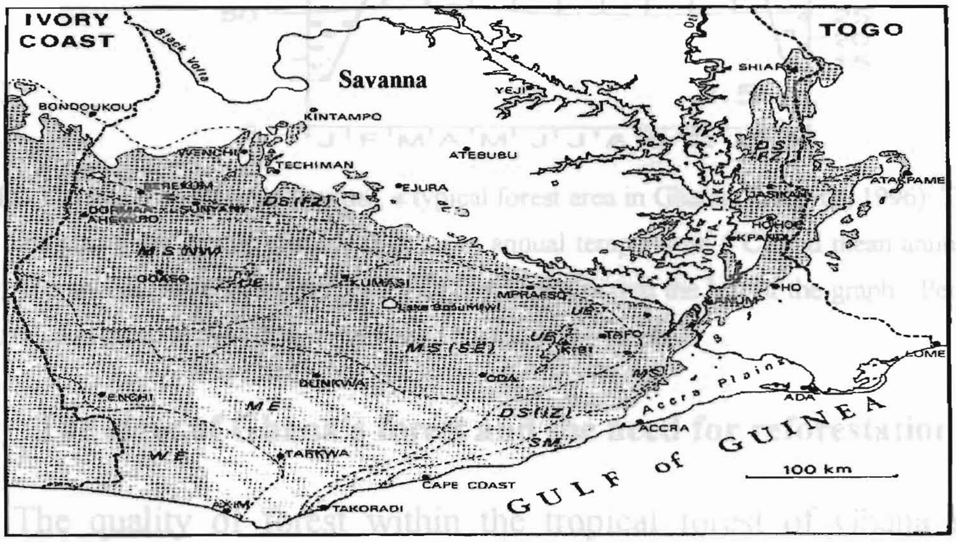

Fig.1. Distribution of forest types (Hall and Swaine, 1976) in southern Ghana. Forest is shaded. **Legend:** WE = Wet Evergreen; ME = Moist Evergreen; MS (SE) = Moist Semi-deciduous South-East Subtype; MS (NW) = Moist Semi-deciduous North-west Subtype; UE = Upland Evergreen; DS (IZ) = Dry Semi-deciduous Inner Zone Subtype; DS (FZ) = Dry Semi-deciduous Fire Zone Subtype; SM = Southern Marginal. Forest type and subtype boundaries shown by broken line (-----).

Throughout the tropical forest zone, rainfall pattern is of the two-peak type with maxima in May–June and September–October. According to Hall and Swaine (1981) the high rainfall in the Wet Evergreen forest type results from higher rainfall amounts in the rainy season, but the total length of the dry season in all the forest types is similar (4–5 months). Temperature variation in the entire tropical high forest zone of Ghana is rather slight; the mean monthly maximum in the hottest months (February–March) is 31-33°C and the mean monthly minimum in the coldest month (December–January in the northern part and August in the south) is 19–21°C. The average daily range is 8–9°C, and the seasonal range of daily mean temperature is 3–4.5°C. Mean temperatures on summits of the highest hills (700 m) in the forest zone may be 3–5°C lower than the surrounding plains (Hall and Swaine, 1981).

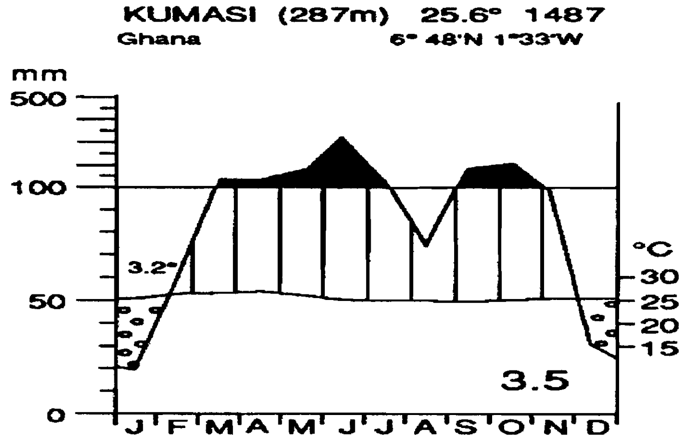

Fig. 1.2. Climate diagrams for Kumasi, a typical forest area in Ghana (Richards, 1996). The station name line also gives station altitude (m), mean annual temperature (°C) and mean annual rainfall (mm). The annual range in temperature (°C) is shown towards the left of the graph. Per humidity index value is given in the bottom right-hand corner of the graph.

1.2. The state of Ghana's forest and the need for reforestation

The quality of forest within the tropical forest of Ghana is quite variable, with conditions worsening with increasing dryness; a disproportionately high amount of good forest is located in hill sanctuaries (Hawthorne and Abu-Juam, 1995). Most of the degradation of the tropical forest is attributable to excessive timber logging, pressures from the demands of agriculture and forest products, and forest fires (Hawthorne, 2001; Ntiamoah-Baidu, 2001; Ghana Forestry Commission, 2002). These exploitative practices have led to small (e.g. a temporary and local disaster of a forest community) and heavy disturbances such as the lost of top soil from log loading bays, main access roads and farms or, in the extreme, where complete deforestation is spread over many hectares, recovery is much less reliable even when such disturbances finally tends to desist (Hawthorne, 2001). Based on criteria such as signs of forest fires, including scorched stems, absence of seedling growth, crown density and the presence of invasive weeds, the state of degradation in reserved forests of Ghana is categorized using scoring description (Table 1) (Hawthorn and Abu-Juam, 1995).

Table 1. State of forest degradation

Score	State of forest	Description
1	Excellent	Rate of disturbance by fires and human activities (cutting and farming) is 2% or less; excellent crown; well developed virgin forest or secondary forest
2	Good	Rate of disturbance is 10% or less with limited damage by cutting. Virtually non-existent bush fire damage
3	Slightly degraded	Clearly disturbed but good stands remain in patches; up to 25% of trees are damaged with minor signs of natural regeneration; up to 50% of the crown is destroyed
4	Mostly degraded	25– 50% of trees are damaged; resulting in unproductive stands; 75% of crown is destroyed
5	Very poor	More than 50% of trees are damaged with no signs of natural regeneration; strong presence of *Chromolaena odorata* weeds; more than 75% of the crown is destroyed
6	No significant forest left	Forest has been destroyed by savannisation, plantation or farm land development; forest survival is 2% or less; trees are scattered and observed in small numbers along rivers

A brief description of the status of tropical forest management in Ghana is outlined in the following Tables.

Table 2a. Total forest area and permanent forest estate (PFE) of Ghana

Total forest area (Range, million hectares)	Total closed natural forest (FAO 2001, '000 hectares)	PFE ('000 hectares)				
		PRODUCTION			PROTECTION	TOTAL
		Natural	Planted	Total		
2.72-6.34	1,634	1,150	97	1,247	353	1,600

Source: ITTO, 2006.

Table 2b. Management of the production PFE of Ghana

NATURAL ('000 hectares)					PLANTED ('000 hectares)		
Total area	Allocated to concession/ under licence	With management plans	Certified	Sustainably managed	Total area	With management plans	Certified
1,150	1,035	1,150	0	270	97	97	0

Source: ITTO, 2006.

Table 2c. Management of the protection PFE of Ghana ('000 hectares)

Total area	Attributed to IUCN categories I–IV	Allocated for soil and water protection	With management plans	Sustainably managed
353	174	n.d.	n.d.	108

Source: ITTO, 2006; n.d. = no data

In addition, attempts have been made to conserve forest species based on their rarity and economic importance. To this end, the species have been rated as Black star, Gold star, Blue star, Red star and Green star, in order of decreasing conservation priority (Hawthorne, 2001). The Red star is the generic term for common species threatened by exploitation for commercial gains, which include the *Khaya* and *Terminalia* species among others. Inference from Tables 2a and 2b indicate that planted forests accounted for approximately, 1.0% of Ghana's forest, an indication that reforestation programs have not been vigorously pursued to mitigate deforestation. These, therefore, affirm the need for regeneration and/ or reforestation in degraded forest lands.

1.3. Influence of site resources on plant growth

Plant growth is determined by environmental factors and the inherent potential of the plant to respond to factors influencing growth (Poorter, 1989). Therefore, species from contrasting habitats and different species in similar environmental conditions will have intrinsic differences in growth rate (Agyeman, 1994).

For instance, the supply of moisture in soils largely controls the type of trees that can grow, and the distribution of forests around the world relates to patterns in precipitation and soil moisture (Fisher and Binkley, 2000). Soil water availability is a main factor in determining plant establishment and productivity. It influences the behavior of a plant at all levels of organization: metabolism, physiology and gross morphology (Kozlowski, 1979; Cowan, 1982; Quero et al., 2006). However, soil water availability becomes a stress factor when the roots of plants are surrounded by excessive or insufficient amount of water, which may lead to reduction in growth of woody plants (Morh and Schopfer, 1995; Osório et al., 1998). Depending on the species, woody plants may display changes in plant structure and function that lead to an enhancement of plant's ability to avoid water stress conditions. This may involve altered relative growth of plant organs and physiological adjustments in transpiration and stomatal conductance at the leaf level of plants (Kozlowski, 1979; Burton et al., 1998).

In addition, tropical tree species exhibit different morphological and physiological response to irradiance (Chazdon, 1986; Lambers and Poorter, 1992; Maruyama et al., 2005) and nutrient supply (Carswell et al., 2000; Webb et al., 2000; Dunisch et al., 2002). Fertilization is often recommendable for tree growth in plantations of tropical humid regions (Fölster and Khanna, 1997). Fertilization, especially nitrogen has a tremendous influence on seedling quality (Mexal and Landis, 1990; Mexal and South, 1991). The different growth responses of tropical tree species to light are characterized by their successional status as pioneers, intermediate successional species and non-pioneers (Denslow, 1980; Whitmore, 1989; Poorter, 1999) and this may also be influenced by nutrient availability (Doley et al., 1988; Reich et al., 1995). Plant size and morphological traits such as height, leaf area ratio, specific leaf area, relative growth rate, biomass production and allocation and foliar nutrient content can be measured to describe the growth of plants (Waring et al., 1985; Poorter, 1989; Hunt, 1990). In addition, physiological responses such as photosynthetic traits, transpiration and foliar chlorophyll fluorescence traits can provide the underlying mechanisms of plant growth in a given light (Fasehun, 1980; Lambers and Poorter, 1992; Mitchell, 1998; Maruyama et al., 2005) and/ or nutrient environment (Peterson et al., 1999; Dunisch et al., 2002).

Thus, it is important to study the physiological and morphological responses of tree seedlings in relation to site resource availability for effective screening of tropical tree species, which have the potential for use in reforestation efforts in African tropics.

1.4. Objectives

The objectives of this study are therefore, to find out (1) the differential morphological and physiological responses of tropical tree species to soil moisture, irradiance and nutrient additions and (2) based on the results of objective (1), make site-species matching recommendations to guide reforestation programs.

1.5. Experimental approach

To meet the above-stated objectives, a series of experiment were conducted to determine the influence of soil water, different irradiances and nutrient additions on tropical tree species growth under green house conditions and at the agricultural field center of the University of Miyazaki, Miyazaki in the southern Japan (32° N and 131° E). This thesis included four different studies, presenting from chapter 2 to chapter 5.

First, in chapter 2, dry matter production and allocation of two African mahoganies (*Khaya* species) in response to water availability was studied in a greenhouse experiment using potted plants. The species were chosen based on seed availability, their economic importance as timber trees and their preference for different forest sub-types in Ghana. Second, in chapter 3, transpiration, chlorophyll fluorescence traits and growth responses in seedlings of three tropical tree species under drought conditions were assessed in a greenhouse experiment using potted plants. The selected species were multipurpose tropical trees, originally from different habitats and grown in plantations of West Africa. Third, in chapter 4, an experiment was conducted to assess the seedling growth and photosynthetic traits of three species, which included a pioneer species, an intermediate successional species and a non-pioneer species under four different irradiances (three shade conditions plus an open site) at a nursery of the University of Miyazaki. Finally, in chapter 5, light-nutrient effects on seedling growth and foliar responses in two of the species used in chapter 4 were studied in a pot trial experiment.

CHAPTER 2.

Dry matter production and allocation of two *Khaya* species in response to water availability

2.1. Introduction

Khaya species is naturally distributed in tropical forest from West to East Africa (Hall and Swaine, 1981). *Khaya anthotheca* (welw.) C. DC. and *K. ivorensis* A. Chev., which are important commercial timber species in Ghana, have been highly exploited over the years. Their prioritized conservation and commercial production are needed (Hawthorne, 2001). The moist semi-deciduous forest type of Ghana shows two floristically distinct subtypes classified by Hall and Swaine (1976) as North-west (NW) and South-east (SE) subtypes. The mean annual rainfall is 1250–1500 mm and 1500–1750 mm for the NW and SE subtypes, respectively. Hall and Swaine (1981) observed that *K. anthotheca* is typical in the NW subtype whilst *K. ivorensis* is typical in the SE subtype and moist evergreen forests, which are wetter than moist semi-deciduous forests. However, plants growing in these habitats are seasonally exposed to distinct drought periods (4–5 months) where mean precipitation is less than 100 mm per month (Hall and Swaine, 1981). Further, during the course of practical forest management, reforested young stands planted after logging may suffer tissue water deficit because of rapid evaporation from the soil surface and undeveloped juvenile root system (Ito et al., 2000). Thus, it is important to investigate the drought tolerance of the two *Khaya* species for successful reforestation.

Water regime is one of the important factors in determining plant establishment and productivity (Burton et al., 1998). Soil water deficits cause reductions in total dry matter, leaf production and rate of shoot expansion of woody plants (Osório et al., 1998). Unless soil water deficits are severe and persistent, woody plants acclimate to water deficit through some major drought avoidance mechanisms. This acclimation involves changes in plant structure and function that lead to an enhancement of plant's ability to avoid dehydration, e.g., an increase in the ratio of root biomass to foliage area (Li et al., 2000) and altered relative growth of leaves, stems and roots (Kozlowski, 1979; Burton et al., 1998; Osório et al., 1998).

There are limited experimental studies on comparative growth response of *K. anthotheca* and *K. ivorensis* to water stress. Since the two tree species grow at different sites in water availability in actual forests, this study expected different responses to water deficits between the two species. The objective of this study was to compare the impacts of water availability on growth, biomass production and allocation patterns for seedlings of the two species.

2.2. Materials and methods

2.2.1. Description of study species

Khaya anthotheca and *Khaya ivorensis* belong to the family Meliaceae. They grow up to 50 m in height as large trees with buttresses. *K. anthotheca* is sometimes briefly deciduous. By contrast, *K. ivorensis* is usually evergreen (Hall and Swaine, 1981). The two species are monoecious and produce wind-dispersed seeds. In Ghana, seed dispersal usually occurs at the start of wet season or end of dry season for *K. ivorensis*, whereas in the case of *K. anthotheca*, it occurs mostly in dry season (October–March) (Hawthorne, 1995). Leaves of both species are paripinnate. In *K. anthotheca*, juvenile leaflets are broadly ovate, but in *K. ivorensis* they are more narrowly oblong, with exceedingly slender drip tips (Hawthorne, 1990).

2.2.2. Plant materials and treatment

Seeds of *K. anthotheca* and *K. ivorensis* were collected from the Brong-Ahafo Regional Forest Services Division's nursery in moist semi-deciduous forest zone of Ghana. Following a standard pre-treatment practice, the seeds were germinated in plastic trays filled with 1:2 volume mixtures of peat and "bora-tsuchi" (light, small porous stones produced from non-weathered substrate of volcanic origin) at a forestry nursery of the University of Miyazaki on April 15, 2003. At two-month old stage, seedlings were transferred into Wagner's pot (19.5 cm in length and 17.5 cm in diameter) and moved to a glass house facility at the University of Miyazaki (32° N and 131° E), Japan. Akahoya soil (silt-loam soil) was used as the substrate because of its moderate texture and nutrient contents (Nagatomo and Tamai, 1993). Over the experiment period, each pot was supplied with a total of 10g of slow release 20:10:10 NPK fertilizer to ensure adequate plant nutrition. The potted seedlings were well spaced and grown under a naturally illuminated glass house. Over the experimental period (July 1 to October 15, 2003) mean daily temperature and humidity recorded in the glass house were 28.5°C and 79%, respectively.

Nine seedlings from *K. anthotheca* and *K. ivorensis* were selected for the study on July 1, 2003. At the beginning of treatment, average height ± (SD) and base diameter ± (SD) of seedlings were 7.0 ± (1.3) cm and 2.0 ± (0.3) mm, respectively for *K. anthotheca* and 7.0 ± (0.8) cm and 2.0 ± (0.2) mm, respectively for *K. ivorensis*. The experiment comprised two species and three watering treatments with three seedlings per treatment. The pots were randomly arranged into three watering treatments which were termed as wet, moderate and dry treatments. Pots were rotated at least once a week to minimize the effect of environmental differences in the glass house.

In the wet treatment, the soil was maintained at –0.001 MPa which was equivalent to 100% or more soil moisture content (SMC) by weight. During rainy periods (6 months), flood-prone banks of water courses in SE subtype and moist evergreen forests where *K. ivorensis* often grows become damp. Thus, the wet treatment was considered to be comparable with site conditions at the SE subtype and moist evergreen forests during rainy season. The NW subtype relatively occupy sites intermediate in moisture (Hall and Swaine, 1981) and this was equated to moderate watering treatment where soil was maintained at –0.01 to –0.014 MPa (i.e., 80–76% SMC). Dry treatment represented a sustained mild water stress approximate to wilting point (–0.70 to –1.42 MPa) equivalent to 40–35% SMC. The watering treatment was maintained for 14 weeks. The water potential of the soil was determined using soil moisture content (SMC) versus water potential curve obtained by the centrifuging procedure. The wet treatment was set by maintaining a water level of 3cm from the bottom of the pot. Moisture conditions in the moderate and dry treatments were set by watering the pots to field capacity and allowed drying to the desired SMC. To keep soil water close to the target value, pots were weighed every three days and rewatered as needed for weight adjustment. Weight of the seedlings was considered negligible and weight of each pot at the target SMC was calculated as described by Haase and Rose (1993), according to the following equation:

$$SMC = (TW - SDW - P) / SDW;\ \textbf{which rearranges as}\ TW = (SMC \times SDW) + SDW + P \quad (1)$$

where SMC is the percentage moisture content on dry weight basis, TW is total weight of dry soil + water + pot weight, SDW is the average weight of dry soil in each pot and P is the pot weight.

2.2.3. Growth measurements

Immediately after transplanting, all seedlings were measured for total height (H). There after, plant height was measured every 21 days until the end of the growing period. Final leaf area (LA) at harvest was determined destructively from scanned leaf images using windows Adobe Photoshop 5.0 software. Biomass was determined at the end of the experiment by harvesting three plants from each treatment. Leaves and stems were separated and soil gently washed from the roots. All plant parts were oven-dried at 70°C for 48 hours and thereafter placed in desiccators at the laboratory for 1 hour before dry weight was measured. Total dry matter consisted of leaves, stems, and roots while leaves consisted of leaf blades and petioles. Dry matter allocation among plant components (leaf, stem and root) and root mass per leaf area ratio were calculated from obtained data.

2.2.4. Data analysis

Height growth measured for both species over time was subjected to repeated measures analysis. Final plant mass production was evaluated using a two-way analysis of variance (ANOVA) including species (2 factors) and treatment (3 factors). Prior to the analysis, final plant mass was transformed to natural logarithms to homogenize variances (Sokal and Rohlf, 1995). Allocation to plant components and root mass per leaf area ratio within species were subjected to univariate ANOVA; and comparisons between species in those variables at each treatment were made by comparing the mean values due to the small sample size.

Comparisons of individual means following ANOVA were done by Fisher's LSD test. All statistical tests were performed using SYSTAT® 10.2 (Statistical software Inc., Richmond, CA, USA). Significant differences are reported as $P < 0.05$.

2.3. Results

2.3.1. Height growth

Height growth (H) over time differed significantly for *K. anthotheca* ($P < 0.0001$) and *K. ivorensis* ($P < 0.0001$). H showed a highly significant linear increase ($F = 212.6$, $P < 0.0001$) over time as shown in Figure 1. The pattern of change across time for the watering treatment differed significantly for both species i.e. Time × Treatment interactions were highly significant for *K. anthotheca* ($P = 0.008$) and *K. ivorensis* ($P = 0.001$). As time progressed, plants in the wet treatment of *K. ivorensis* had greatest height growth than in the moderate and dry treatment (Photo 2.1). *Khaya anthotheca* on the other hand, showed the best growth in the moderate watering treatment than in the wet and dry treatment over time. A comparison made between the two species in each watering treatment at final measurement showed that *K. anthotheca* had significantly better growth than *K. ivorensis* in the moderate ($F = 14.2$, $P < 0.01$) and dry treatment ($F = 5.1$, $P < 0.05$). On the other hand, *K. ivorensis* grew better than *K. anthotheca* in the wet treatment ($F = 5.9$, $P < 0.05$).

Photo 2.1. *K. anthotheca* and *K. ivorensis* seedlings after 14 weeks of irrigation treatments. From left for each species, are samples grown under wet, moderate and dry treatments, respectively.

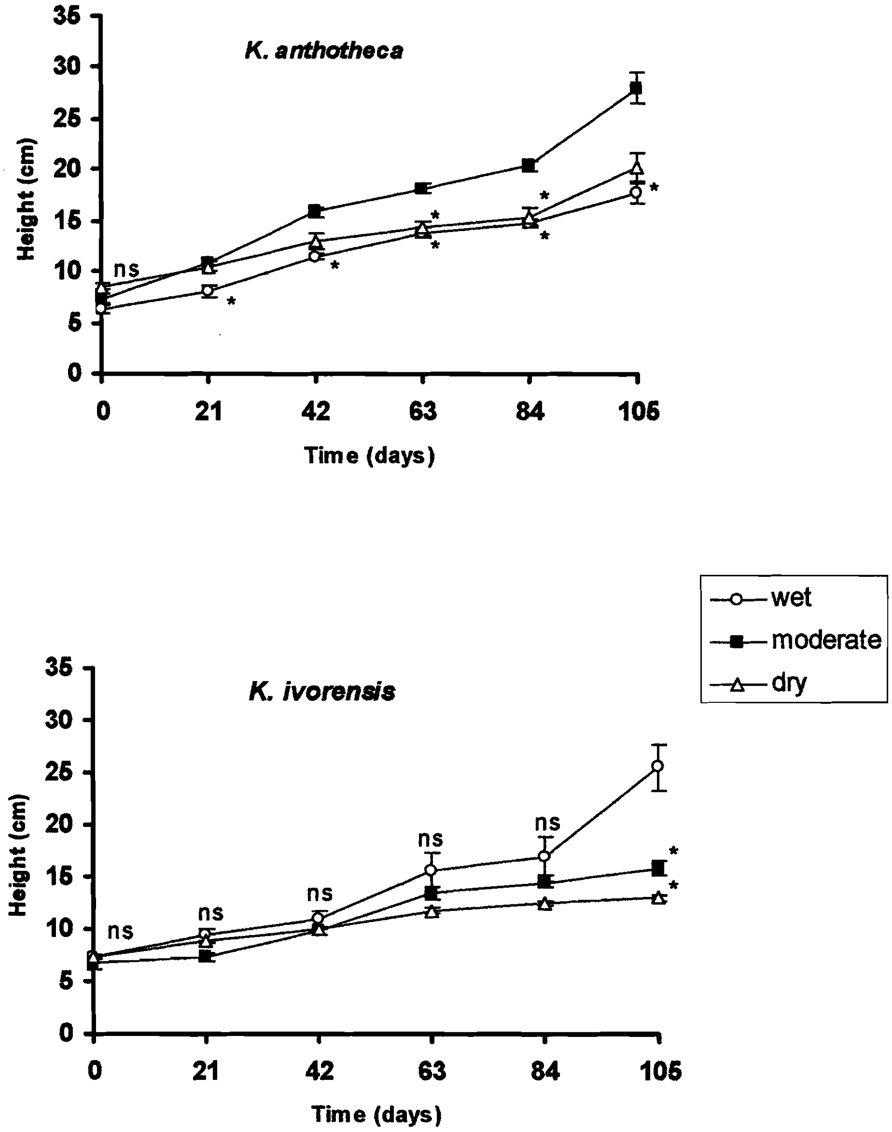

Fig. 1. Height growth of two species subjected to three watering treatments (wet, moderate and dry) over time. Each value is the mean of three plants ± standard error of mean. Within *K. anthotheca* and *K. ivorensis* seedlings, an asterisk (*) indicates significant differences from moderate and wet treatment, respectively at each measurement day (repeat measures ANOVA followed by Fisher's LSD test, $P < 0.05$; ns, $P > 0.05$).

2.3.2. Dry matter production

The results of the two-way ANOVA indicated that total dry matter production was different between the two species ($F = 7.5$, $P < 0.05$) but not among the treatments ($F = 0.8$, $P > 0.05$). Species × Treatment interaction was significant ($F = 5.0$, $P < 0.05$). The coefficient of determination (r^2) was 0.61. Within species, significant differences among the watering treatments in total plant dry mass production was only found in *K. ivorensis* (Table 1). Relative to the wet treatment, subjecting plants to the moderate and dry treatments reduce mean total plant dry mass by 74% for *K. ivorensis*. No significant difference among the watering treatment in total plant dry mass was found in *K. anthotheca*, though the mean total dry mass was largest in the moderate treatment. A probable cause may due to small sample size used in this study. However, a comparison made between the two species in each watering treatment revealed that *K. anthotheca* had greater total dry mass production than *K. ivorensis* in the moderate and dry treatments (Table 1). No significant difference between the two species in total plant dry mass production was found in the wet treatment, though the mean total dry mass was larger in *K.ivorensis*.

2.3.3. Dry matter allocation

Within each species, there was a preferential and proportionate dry matter allocation to plant components due to the different watering treatments. There were no significant differences among the watering treatments in *K. anthotheca* in the proportion of assimilate allocated to plant components (Table 1). The proportion of assimilate allocated to leaves in *K. ivorensis* decreased significantly in the moderate and dry treatments whereas the proportion allocated to roots increased with increasing water deficits. For both species, the proportion of assimilate allocated to the stem did not show significant difference among the watering treatments. Comparatively, mean dry matter allocation to leaves in wet treatment of *K. anthotheca* (52%) and *K. ivorensis* (49%) were similar. However, in the moderate and dry treatments, *K. anthotheca* showed greater percent mean leaf dry matter allocation than *K. ivorensis* (Table 1). In contrast, mean root dry matter allocation in the moderate and dry treatments were 29% and 31%, respectively for *K. anthotheca* and 33% and 38%, respectively for *K. ivorensis*.

Table 1. Effect of watering treatments and species differences in plant dry matter production and the relative proportion of dry matter allocation to leaf, stem and root of *K. anthotheca* and *K. ivorensis* seedlings. Each value is the mean of three plants (±SE). Within each species, means in a column followed by same lower case letters are not significantly different and; different upper case letters in a row represents significant difference between species (Fisher's LSD test, $P < 0.05$).

Parameter	Watering regime	***K. anthotheca***	***K. ivorensis***
Plant dry matter (g)	Wet	1.38 (0.41) a A	2.41 (0.83) a A
	Moderate	3.03 (1.16) a A	0.62 (0.14) b B
	Dry	2.40 (0.85) a A	0.62 (0.25) b B
Leaf dry matter (%)			
	Wet	52.43 (0.54) a	**48.60 (2.19) a**
	Moderate	48.16 (6.75) a	34.40 (1.28) b
	Dry	46.23 (2.11) a	34.85 (4.67) b
Stem dry matter (%)			
	Wet	21.97 (1.23) a	26.10 (2.20) a
	Moderate	22.94 (2.26) a	32.22 (1.33) a
	Dry	22.52 (1.70) a	26.50 (1.52) a
Root dry matter (%)			
	Wet	25.60 (1.44) a	**25.32 (2.97) a**
	Moderate	28.90 (4.60) a	33.40 (1.27) ab
	Dry	31.25 (2.77) a	**38.19 (2.96) b**

2.3.4. Root mass/ foliage area ratio

For both species, root mass: leaf area ratio showed increasing mean values with increasing water deficits. The increase in the ratio was significant in only *K. ivorensis* (Table 2). A comparison in mean values of the ratio between the two species in each treatment showed that *K. ivorensis* had 33 and 50% proportional increase in the moderate and dry treatments, respectively.

Table 2. Effect of watering treatment on root mass: leaf area ratio of *K. anthotheca* and *K. ivorensis* seedlings. Each value is the mean of three plants (±SE). Within each species, means in a column followed by same letters are not significantly different (Fisher's LSD test, $P < 0.05$).

				Between species
Parameter	Watering regime	***K. anthotheca***	***K. ivorensis***	Proportional change
root mass: Leaf area ratio				
(x 10^{-3}g cm^{-2})	Wet	2.3 (0.0003) a	**2.4 (0.0002) a**	7%
	Moderate	3.0 (0.0006) a	4.0 (0.0001) ab	33%
	Dry	4.0 (0.0004) a	**6.0 (0.001) b**	50%

2.4. Discussion

Several investigators have reported for many woody species that biomass accumulation, growth and physiological processes of carbon fixation were highly sensitive to stress conditions caused by shortage of water in soil (Bahari et al., 1985; Osório et al., 1998; Ngugi et al., 2003). Within a local area, the strength of genetic control of height growth of trees within a species tends to be low and site factors such as soil water and nutrients are major determinants of height growth (Burton et al., 1998). In this study, *Khaya ivorensis* attained highest height growth and largest biomass production under wet treatment indicating that water deficit impacted negatively on height growth and biomass production (Figure 1 and Table 1). Watering treatments only influenced significantly height growth of *K. anthotheca*. The decrease in height growth of *K. anthotheca* in the wet treatment may be attributed to excess standing water maintained at the base of the pots which caused poor aeration. This might have led to an inhibition of oxygen supply to roots and concomitantly reduction of water and mineral conductivity (Kramer and Jackson, 1954). The absence of marked treatment effects on dry mass production and allocation in *K. anthotheca* in this study may have resulted from the small sample size and from shorter duration of the experiment as reported for other plants (Ledig, 1983). Hawthorne (1994) suggested that *K. ivorensis* is suitable for plantation in low-lying areas with moist site conditions (1500-1750 mm of annual precipitation). Inference from Hall and Swaine (1981) indicate that *K. anthotheca* thrive best at sites intermediate in moisture where forest ochrosols (less leached soil, better supplied with nutrient and slightly acidic to neutral) dominates. As expected, *K. ivorensis* grew best in the wet treatment, whereas *K. anthotheca* grew best in the moderate treatment.

Water stress has been recognized to alter the allocation of dry matter to leaves and supporting biomass. An increase in mass of roots and a decrease in carbon allocation to leaves are considered typical acclimation mechanism in response to water shortage (Pereira and Chaves, 1993). In this study, *K. ivorensis* from wetter site showed large increase in root mass: foliage area ratio than *K. anthotheca* under water stress (Table 2). A species showing this acclimation is less drought-tolerant because it may not have any physical mechanism to avoid drought stress. In contrast, *K. anthotheca* which is typically found in the NW subtype (drier site), showed similar root mass: foliage area ratio in the moderate and dry treatments suggesting that *K. anthotheca* is more tolerant to drought. In a field guide to forest trees of Ghana, Hawthorne (1990) describes the leaflets of *K. anthotheca* as being dry on the lower surface with leathery appearance and with or without impressed larger veins enhancing the ability of *K. anthotheca* to tolerate desiccation of leaf tissue better than *K. ivorensis*.

2.5. Conclusion

These characteristic traits have partly shown that seedlings of *K. anthotheca* have considerable potential for use in plantation forestry in the humid tropics of Africa, which has a wide rainfall distribution range and distinct drought periods (4–5 months). This is shown by the species remarkable height growth under moderate water stress condition. Seedlings of *K. ivorensis* on the other hand, are more suited for planting on moist site (low-lying areas with 1500–1750 mm of annual precipitation) as demonstrated by the species ability to grow best in wet condition. The result of this study may have implications in matching the two *Khaya* species to sites different in water availability but further research in other aspects, such as growth of larger juveniles and adults under field conditions, mortality by water stress as well as information on physiological traits are recommend.

CHAPTER 3.

Transpiration, chlorophyll fluorescence traits and growth responses in seedlings of three tropical species under drought conditions

3.1. Introduction

Cedrela odorata L., *Cassia siamea* Lam. and *Khaya senegalensis* (Desr.) A. Juss. are important multipurpose tropical tree species grown in plantations of West Africa. *C. odorata*, a forest species native to Central and South America, is mainly used for round wood production and furniture; *Cassia siamea*, a semi-arid multipurpose tree, native to South-east Asia is used for protection planting (windbreaks, ornamentals, agroforestry) and also used as building poles, fuel/charcoal and fodder; whereas *K. senegalensis*, a tree, which typically occurs in savanna vegetation of tropical Africa, is utilized as fuel wood, tannins and medicines (Pancel, 1993). In Ghana, these three species are cultivated in the dry semi-deciduous forest zone, which forms a peripheral boundary around the moister forest type and is adjacent to the Guinea savanna zone in the north. Mean annual precipitation in the dry semi-deciduous forest zone is 1250 mm (Hall and Swaine, 1981). During the dry periods (4–6 months), precipitation in most of the cultivation areas is less than 50 mm per month and mean monthly maximum temperature is 32°C (Climatological Data Annual Report, B/A region Meteorological Services Department, Ghana). Hence, plants are often subjected to drought as a result of high temperature and irradiance, high vapor pressure deficit and soil water deficit in their growing environment. From descriptive ecology of West African vegetation, savanna and semi-arid plants are not only adapted to surviving recurrent fires, but they also differ from forest plants in their response to drought (Richards, 1996). However, in Ghana few experimental studies on comparative water usage of forest, savanna and/

or semi-arid species focusing on their physiological responses have been reported in literature. This kind of study is important for species selection in plantation forestry programs of African tropics where there is lack of information on potential plantation species.

Plant strategies to cope with drought normally involve a mixture of stress avoidance and tolerance strategies that vary with species (Chaves et al., 2002). One of the first responses to a mild water stress in leaves is stomatal closure (Kozlowski, 1979), which leads to a decrease in carbon uptake by the leaves (Chaves, 1991; Cornic and Massacci, 1996). A previous study showed that soil water deficit led to decreased stomatal conductance and hence lower transpiration rate of container grown *C. odorata* seedlings (Nagashiro, 2000). In another study, Okali and Dodoo (1973) found that *Khaya senegalensis* is more drought-resistant than the forest species *K. ivorensis*, apparently because it is able to check transpiration when under water stress and has a greater tolerance to desiccation of the leaf tissues. Physiological responses such as transpiration and electron transport rate were suppressed in *Cassia siamea*, a semi-arid tree species in a progressive drought study (Oo et al., 2003). Trees are relatively sensitive to moisture stress during the establishment stage compared with their ability to withstand drought once their root systems are fully developed. In general, shoot growth is more sensitive to water deficits than root growth (Sharp and Davies, 1989).

The measurement of chlorophyll fluorescence emitted from leaves in response to actinic irradiation can be used to detect stress caused by water deficits at photosystem II (PS II) (Flexas et al., 1999; Li and Kakubari, 2001; Fleck et al., 2004). The ratio of variable to maximum fluorescence (F_v/F_m) emitted from PS II may serve as a quantitative indicator of the efficiency of the photochemistry of PSII (Kitajima and Butler, 1975).

It was hypothesized that *C. odorata* from a mesic habitat is less tolerant to drought than *C. siamea* from semi-arid regions and *K. senegalensis* from savanna habitats. If this is true, the three different species will show differences in their rates of transpiration, stomatal conductance and photochemical efficiencies in a way consistent with their natural distribution. Therefore, I subjected the species to progressive water stress and investigated some physiological characteristics and other growth traits (shoot growth, leaf expansion and allocation to plant organs). In addition, I examined linear relationships between the physiological traits and soil water potential for each species. This study was conducted to further enhance the understanding of water stress mechanisms associated with these species and the findings may help evaluate the species potential for multi-purpose plantation forestry in drought-prone areas in West Africa.

3.2. Materials and methods

3.2.1. Plant materials, drought treatment and growing conditions

In early July 2004, six 1-year-old *C. odorata* plants (52.5 ± 2.0 cm high, 8.8 ± 0.2 mm root collar diameter) and six 3-month-old *K. senegalensis* plants (13.0 ± 1.2 cm high, 3.0 ± 0.1 mm root collar diameter) were lifted from a nursery and transferred to individual 11-litre pots filled with silt loam soil and grown under natural light at a greenhouse facility of the University of Miyazaki (32° N and 131° E), Japan. The plants were irrigated every three days with 250 ml of water, which is equivalent to the average rainfall amount on rainy days in a dry semi-deciduous forest. One week after transplanting, a total of 6g fertilizer (N: P: K = 5: 10: 6, plus trace elements; Hyponex solution, Japan) was gradually added to each pot over a 2-month period.

As seedlings grew for 2 months, the induction of drought was undertaken by withholding irrigation. Each individual first measurement was taken after irrigation; then as irrigation was withheld (mid September), a series of measurements were taken until mid November of 2004. This 2004 drought treatment with *C. odorata* and *K. senegalensis* was termed year 1 experiment. A similar experimental design with the same species plus six 15-month-old *C. siamea* plants (89.0 ± 3.6 cm high, 9.5 ± 0.3 mm root collar diameter) was started in early July, 2005. The drought treatment of 2005 spanned approximately the same period as in 2004, and was termed year 2 experiment. Thus, for *C. odorata* and *K. senegalensis*, two drought cycles, each 2 month long, was completed. After year 1 drought treatment, the plants were well-watered and kept at the green house facility for the interval until the start of year 2 drought treatment.

Temperature and relative humidity during the experimental period in each year at the greenhouse were measured with a digital thermo recorder TR-72S T&D Corporation, Japan from which vapor pressure deficit (VPD) was calculated. Mean temperature and humidity ranges over the experiment period were 22.1–28.0°C and 69.2–80.4%, respectively for year 1, and 20.0–29.6°C and 61.0–74.3%, respectively for year 2. The mean ± standard error photosynthetic photon flux density (PPFD) measured randomly at several points in the greenhouse with a luminance meter T–1M (Minolta Co. Ltd. Japan) around midday during the period of experiment was 700.0 ± 34.0 μmol m^{-2} s^{-1} (range, 540–1200 μmol m^{-2} s^{-1}) and 800.0 ± 66.0 μmol m^{-2} s^{-1} (range, 600–1300 μmol m^{-2} s^{-1}) for year 1 and 2, respectively. Withholding water for approximately 60 days in each drought cycle resulted in a small range in the degree of soil dryness.

3.2.2. Study species

Cedrela odorata (family Meliaceae) reaches 40 m and develops a pyramidal to elliptical crown volume. It has compound leaves 25–50 cm long, and opposite leaflets numbering 10–22 and 5–15 cm long (Tropical Agriculture Research Center, 1977). *C. odorata* has strong, but variable, dry season deciduousness depending on the intensity of the dry season and the local site (Poorter and Hayashida-Oliver, 2000). Fewer standing leaf numbers have been observed for the species during the dry season compared to the rainy season (Poorter and Hayashida-Oliver, 2000). Occurs naturally in latitudes 27° S–24° N; forest habitats characterized by 1600–2500 mm rainfall; mean annual temperature range of 22–32°C (Pancel, 1993).

Cassia siamea (family Caesalpiniaceae) rarely exceed 20 m in height and 50 cm in diameter at breast height. It has a dense evergreen spreading crown and smooth grayish bark. Young branches are finely haired. Leaves are pinnately compound with even leaf arrangement of 7–10 pairs. Leaflets are ovate-oblong, 7–8 cm long and 1–2 cm wide (Smitinand and Larsen, 1981). Occurs naturally in latitudes 1–5° N; dry low land forests with mean annual temperature ranging from 20 to 28°C; most common in areas with mean annual rainfall range of 650–950 mm and a dry season of 4–6 months (Smitinand and Larsen, 1981; Pancel, 1993).

Khaya senegalensis (family Meliaceae), on the other hand, grows up to 30m. The leaves are 12–25 cm long; feather-like with 2–6 pairs of glabrous leaflets measuring up to 10cm in length (Hutchinson and Dalziel, 1954). *K. senegalensis* shed its leaves early in the dry season, often replacing them long before the early rains begin (Okali and Dodoo, 1973). Occurs naturally in latitudes 8–15° N; savanna habitats with mean annual temperature range of 19–29°C and a mean maximum hottest month of

37–40°C; most common in areas with mean annual rainfall range of 700–1500 mm and a dry season of 5–7 months (Smitinand and Larsen 1981; Pancel, 1993).

3.2.3. Soil water content, Leaf transpiration and chlorophyll fluorescence measurements

Soil water content measurements of the pots were made every five days for the year 1 and 2 experiment periods with 10-cm ECH$_2$O di-electric aquameter probes (Decagon Devices, Inc., USA) which were wholly inserted in the pots from the soil surface. The soil water potential (Ψ_s) was determined with soil water content versus soil water potential curve obtained by a centrifuging procedure in a laboratory experiment.

All the species used in this study have compound leaves. In year 1, only *C. odorata* and *K. senegalensis* plants were used for the drought experiment. The third or fourth branch from the shoot apex that was well exposed was selected for each species for measurements. In year 1, transpiration rate (E) was measured around midday on two attached and fully developed leaves of six plants per species with a steady state porometer (LI-1600 Li-Cor, Lincoln, NE). From the primary measurements, stomatal conductance (g_s) was computed according to the Porometer handbook of operation (LI-1600 Li-Cor, Lincoln, NE, Revision 6, 1989).

The measurements of chlorophyll fluorescence on leaves were made with a portable pulse-modulated chlorophyll fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany). The same leaves from the third or fourth branch of the species were used for both transpiration and chlorophyll fluorescence measurements on the same day and period of time (1130–1500 h). Maximum fluorescence yield of light adapted samples ($F_{m'}$), steady-state yield (F) and quantum yield of photosystem II (Φ_{PSII}; equivalent to $(F_{m'} - F)/ F_{m'}$) (Genty et al., 1989) were measured. Apparent electron transport rates (ETR, μmol electrons m^{-2} s^{-1}) through PSII at a given PPFD were then estimated using the equation: ETR = $(F_{m'} - F)/ F_{m'} \times 0.84 \times 0.50 \times$ PPFD. The following assumptions are made: transport of one electron requires absorption of two quanta, because two photosystems are involved (factor 0.5); 84% of the incident quanta are absorbed by the leaf (factor 0.84) (Mohammed et al., 1995). A miniature 8 V/20 W halogen lamp (Bellaphot, Osram) served as the light source for continuous actinic illumination until steady-state was approached (about 2 min) at each light intensity settings. Light curves were produced from plots of ETR versus PPFD (Schreiber et al., 1994). Leaves of seedlings were then dark-adapted for 30 min with dark leaf clips (DLC-8) to obtain minimum fluorescence yield (F_0), maximum fluorescence yield (F_m) and potential quantum yield of PSII photochemistry (F_v/F_m) (equivalent to $(F_m-F_0)/F_m$) was measured at leaf temperature of 24.0°C. Physiological measurements (E, g_s and F_v/F_m) were conducted approximately every 10 days for 2 months on the selected leaves, whereas Φ_{PSII} and ETR measurements were made at the beginning and end of drought treatment. Same physiological parameters were measured during the year 2 experiment except measurements were conducted on three plants of *C. odorata*, *C. siamea* and *K. senegalensis*, respectively.

3.2.4. Shoot and root measurements and dry mass determination

Only leaf area and height growth were assessed in year 1 experiment. To assess total leaf area expansion, the average leaf area of 10 randomly selected leaves per species obtained with a portable area meter (LI-3000A, Li-Cor Inc., Lincoln, NE) was multiplied by total leaf count at the beginning and at the end of the study. Shoot height was measured with a rigid tape at the beginning and end of drought treatment to assess shoot elongation. In year 2 experiment, six, 15-month-old plants of *C. siamea* of similar sizes were included in the study and similarly, total leaf area expansion and shoot elongation was assessed for the species. In year 2 experiment, the following parameters were further investigated:

(i) Three plants of similar height and leaf numbers were selected per species for leaf expansion measurement. Leaf expansion during irrigation stage (i.e. September 18–24, 2005) and immediately after the inception of drought (September 25–October 9, 2005) was followed separately by manually drawing the outline of each selected leaf every second or third day. A pair of youngest leaves was chosen per plant. The paired leaf was positioned centrally or at the end of the 2nd/3rd lateral branch close to the shoot apex for ease of measurement.

(ii) At the end of year 2 experiment, four plants from each species were destructively harvested. Leaves and stems (including branches and petioles) were separated, and the soil washed gently from the roots.

The length of the longest root of each plant was measured with a rigid tape. In addition, coarse roots ($\geq$ 2 mm in thickness) and fine roots (< 2 mm in thickness) were determined with a caliper. The plant components were then oven-dried for 48 h at 70°C after which dry mass was determined. In addition, the root-shoot ratio of each plant was determined.

3.2.5. Data analysis

Data for year 1 and 2 experiments were subjected to statistical analyses. We compared trends in E, g_s and F_v/F_m in each year using analysis of variance (ANOVA) with repeated measures where days of drought was the within-subject factor and species was the between-subject factor. Similarly, leaf expansion rate during the year 2 experiment were subjected to repeated measures analysis. Linear associations between pairs of the measured variables (E, g_s, F_v/F_m and Ψ_s) were analyzed by Pearson correlation for each species. The Φ_{PSII}/PPFD and ETR/PPFD curves, whole-plant leaf area assessment and shoot height at the beginning and end of drought treatment were analyzed with paired *t*-tests (two-tailed) for each species. Mean values of the final plant organ dry mass, root length and root-shoot ratio of each species was determined and the values compared among the species. All statistical tests were performed using SYSTAT® 10.2 (Statistical software Inc., Richmond, CA, USA). Significant differences are reported as $P < 0.05$.

3.3. Results

3.3.1. Growing conditions

Variations in mean midday VPD during the first and second year's experimental period are shown in Figs. 1a and 2a, respectively. Figures 1b and 2b illustrate changes in soil water potential in pots of each species as irrigation was withheld (days of drought) in year 1and 2 experiments, respectively. In year 1 experiment, an initial period of constant soil water potential (Ψ_s) was followed, both in *C. odorata* and *K. senegalensis* pots, by comparatively lower soil water potential (Fig. 1b). After 25 days of drought, Ψ_s remained constant for *K. senegalensis* at −0.15 MPa, whereas Ψ_s declined to −0.20 MPa for *C. odorata*. At the end of the first year's drought treatment, Ψ_S in pots of *C. odorata* and *K. senegalensis* were similar (Fig. 1b). Similarly, in year 2, an initial period of constant soil water potential (Ψ_S) was followed, in pots of the three species, by comparatively lower soil water potential (Fig. 2b). However, until 35 days of drought, Ψ_S remained constant for *C. odorata* at −0.18 MPa, whereas Ψ_S declined to −0.33 MPa for *C. siamea* and *K. senegalensis* plants after 30 days of drought. At the end of the year 2 drought treatment, Ψ_S in pots of *C. odorata*, *C. siamea* and *K. senegalensis* were −0.4, −1.0 and −0.8 MPa, respectively (Fig. 2b).

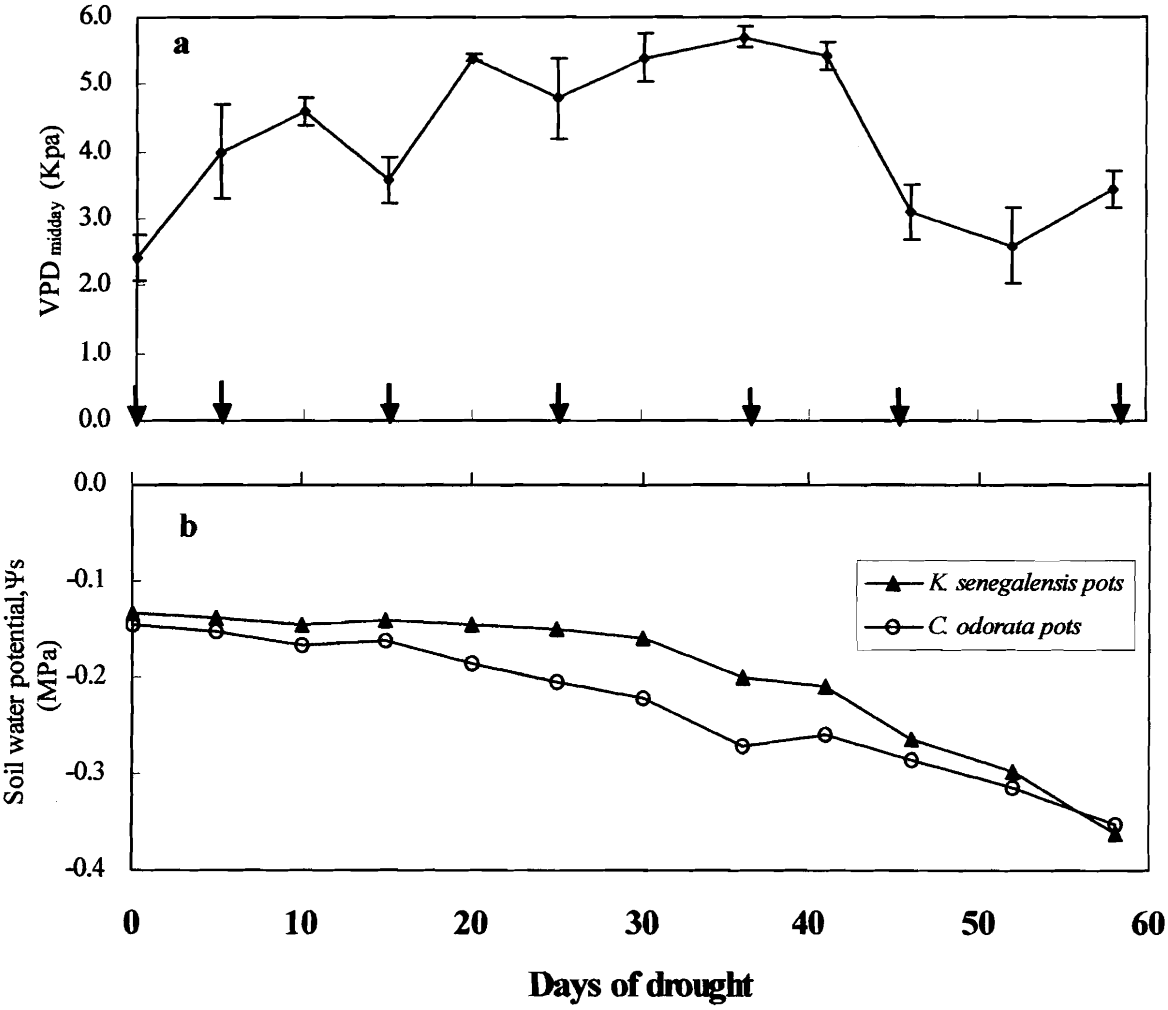

Fig. 1. Vapor pressure deficit (VPD) at midday (**a**), and soil water potential (Ψ_S) (**b**) during year 1 drought treatment. In (**a**), each value represents the mean ± standard error (SE) of five 30-min measurement periods during the midday. In (**b**), values are averages of Ψ_S values derived from two aquameter probes readings in two pots per species. Arrows indicate the days of measurement.

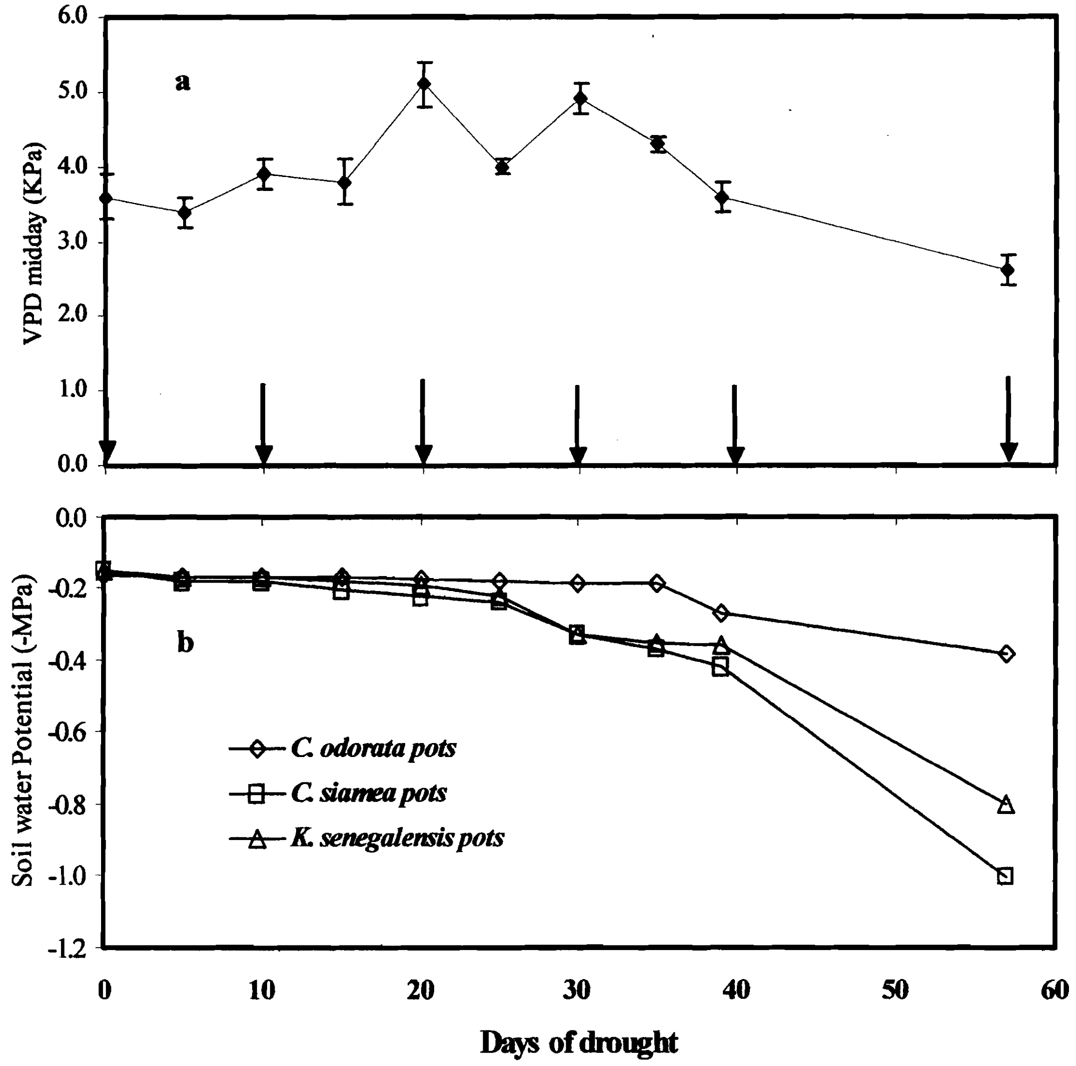

Fig. 2. Vapor pressure deficit (VPD) at midday (**a**), and soil water potential (Ψ_s) (**b**) during year 2 drought treatment. In (**a**), each value represents the mean ± standard error (SE) of five 30-min measurement periods during the midday. In (**b**), values are averages of Ψ_s values derived from two aquameter probes readings in two pots per species. Arrows indicate the days of measurement.

3.3.2. Transpiration, stomatal conductance and F_v / F_m ratio

3.3.2.1. *Year 1 experiment*

As days of drought progressed, the tests of within-subjects effects (Sphericity assumed) indicated significant reduction in E ($P < 0.0001$), g_s ($P < 0.0001$) and F_v/F_m ($P < 0.0001$) for both species. Significant differences between species in E, g_s and F_v/F_m were ($F = 306.56$; df = 1, 22; $P < 0.0001$), ($F = 250.22$; df = 1, 22; $P < 0.0001$) and ($F = 9.73$; df = 1, 22; $P < 0.01$), respectively (Figs. 3a, 3b and 3c). Species × days of drought interactions were highly significant ($P < 0.0001$) for E and g_s but not F_v/F_m ($P = 0.056$). Linear correlations between pairs of the measured variables are shown in Table 1 for *C. odorata* and *K. senegalensis* plants. For both species, Pearson correlation between E and g_s; E and Ψ_s; E and F_v/F_m; g_s and Ψ_s; g_s and F_v/F_m; F_v/F_m and Ψ_s were positive and significant ($P < 0.01$). The correlation coefficient for E and Ψ_s; E and F_v/F_m; g_s and Ψ_s; g_s and F_v/F_m were higher in *K. senegalensis* than *C. odorata.*

Table 1. Pearson correlation matrix selected variables (Ψ_s = soil water potential; E = transpiration rate; g_s = stomatal conductance and F_v / F_m = potential quantum yield of PSII photochemistry) for the two species used in year 1 experiment.

	Ψ_s	E	g_s	F_v / F_m
C. odorata				
Ψ_s	1.00	-	-	-
E	0.41**	1.00	-	-
g_s	0.45**	0.86**	1.00	-
F_v / F_m	0.65**	0.37**	0.44**	1.00
K. senegalensis				
Ψ_s	1.00	-	-	-
E	0.72**	1.00	-	-
g_s	0.68**	0.85**	1.00	-
F_v / F_m	0.63**	0.58**	0.52**	1.00

**** Correlation is significant at the 0.001 level (2-tailed)**

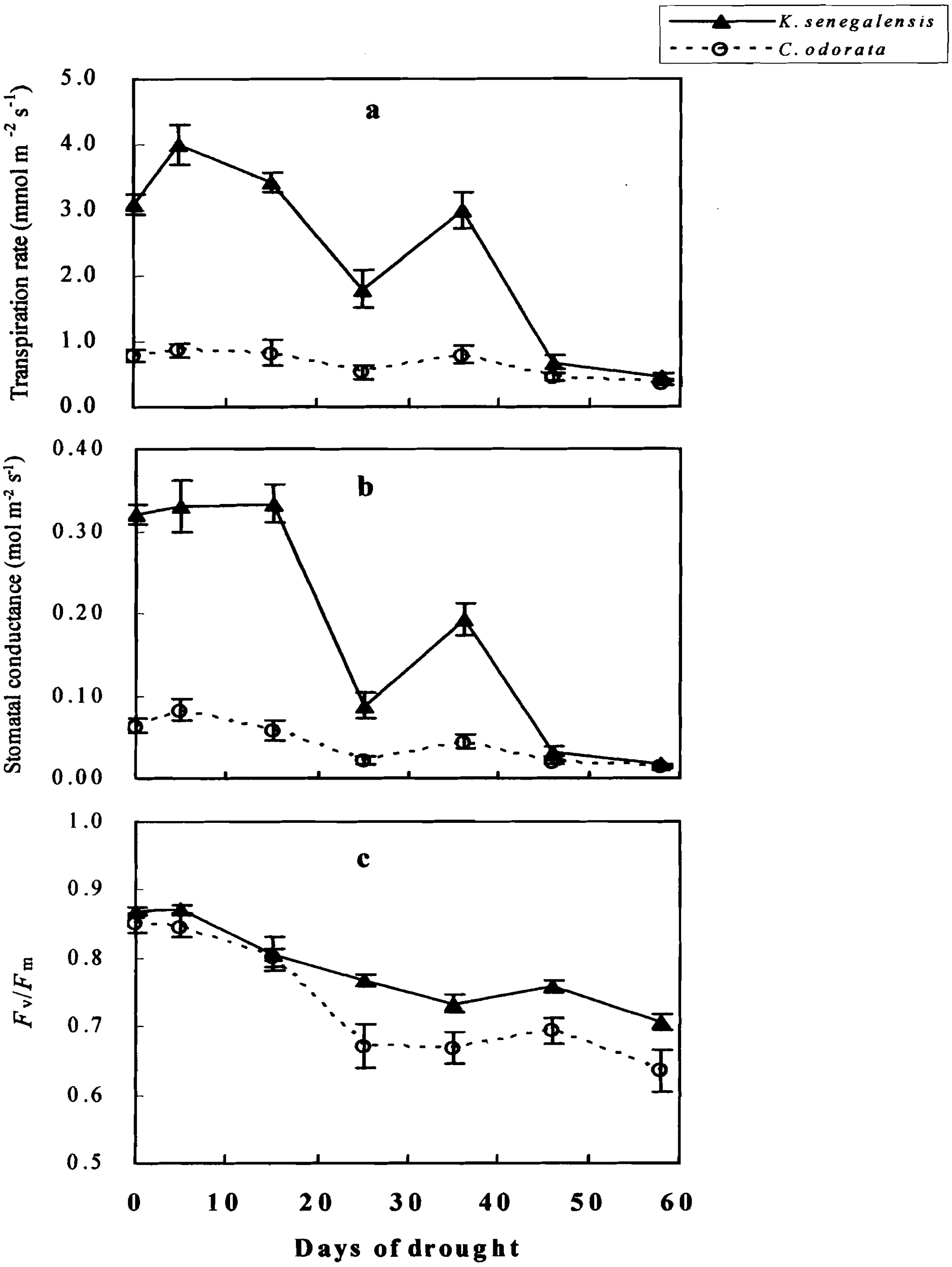

Fig. 3. Mean (±SE) transpiration rate (a), stomatal conductance (b), and potential quantum yield of PSII photochemistry (F_v/F_m) in leaves of *C. odorata* ($n = 12$) and *K. senegalensis* ($n = 12$) seedlings during year 1 drought treatment.

3.3.2.2. *Year 2 experiment*

As the days of drought progressed, the tests of within-subjects effects

(Sphericity assumed) indicated significant reduction in E ($P < 0.0001$) for *C. odorata*, *C. siamea* and *K. senegalensis*, respectively. Similar trends in g_s for *C. odorata* ($P < 0.01$), *C. siamea* ($P < 0.0001$) and *K. senegalensis* ($P < 0.0001$) were observed. Trends in F_v/F_m for *C. siamea* and *K. senegalensis* were similar ($P < 0.0001$), respectively, whereas the trend of decreasing F_v/F_m with days of drought for *C. odorata* was not significant ($P = 0.165$) (Figs. 4a, 4b and 4c). Significant differences between species in E, g_s and F_v/F_m were ($F = 6.94$; df = 2, 15; $P < 0.01$), ($F = 4.52$; df = 2, 15; $P < 0.05$) and ($F = 5.4$; df = 2, 9; $P < 0.05$), respectively. Species × days of drought interactions effect were significant for E ($P = 0.001$), g_s ($P = 0.008$) and F_v/F_m ($P < 0.0001$). Linear correlations between pairs of the measured variables are shown in Table 2 for *C. odorata*, *C. siamea* and *K. senegalensis* plants, respectively. For *C. siamea* and *K. senegalensis* plants, Pearson correlation between E and g_s; E and Ψ_s; E and F_v/F_m; g_s and Ψ_s; g_s and F_v/F_m; F_v/F_m and Ψ_s were positive and significant ($P < 0.05$). However, in *C. odorata* plants, only E and g_s; E and Ψ_s; F_v/F_m and Ψ_s showed positive and significant ($P < 0.05$) correlation during the second year of drought treatment.

Table 2. Pearson correlation matrix selected variables (Ψ_s = soil water potential; E = transpiration rate; g_s = stomatal conductance and F_v / F_m = potential quantum yield of PSII photochemistry) for the two species used in year 1 experiment.

	Ψ_s	E	g_s	F_v / F_m
C. odorata				
Ψ_s	1.00	–	–	–
E	0.47*	1.00	–	–
g_s	0.34	0.94**	1.00	–
F_v / F_m	0.41*	0.27	0.23	1.00
C. siamea				
Ψ_s	1.00	–	–	–
E	0.48*	1.00	–	–
g_s	0.49*	0.93**	1.00	–
F_v / F_m	0.85**	0.58**	0.58**	1.00
K. senegalensis				
Ψ_s	1.00	–	–	–
E	0.48*	1.00	–	–
g_s	0.50*	0.97**	1.00	–
F_v / F_m	0.87**	0.41*	0.43*	1.00

Asterisks * and ** indicate correlation is significant at the 0.05 and 0.001 level (2-tailed), respectively.

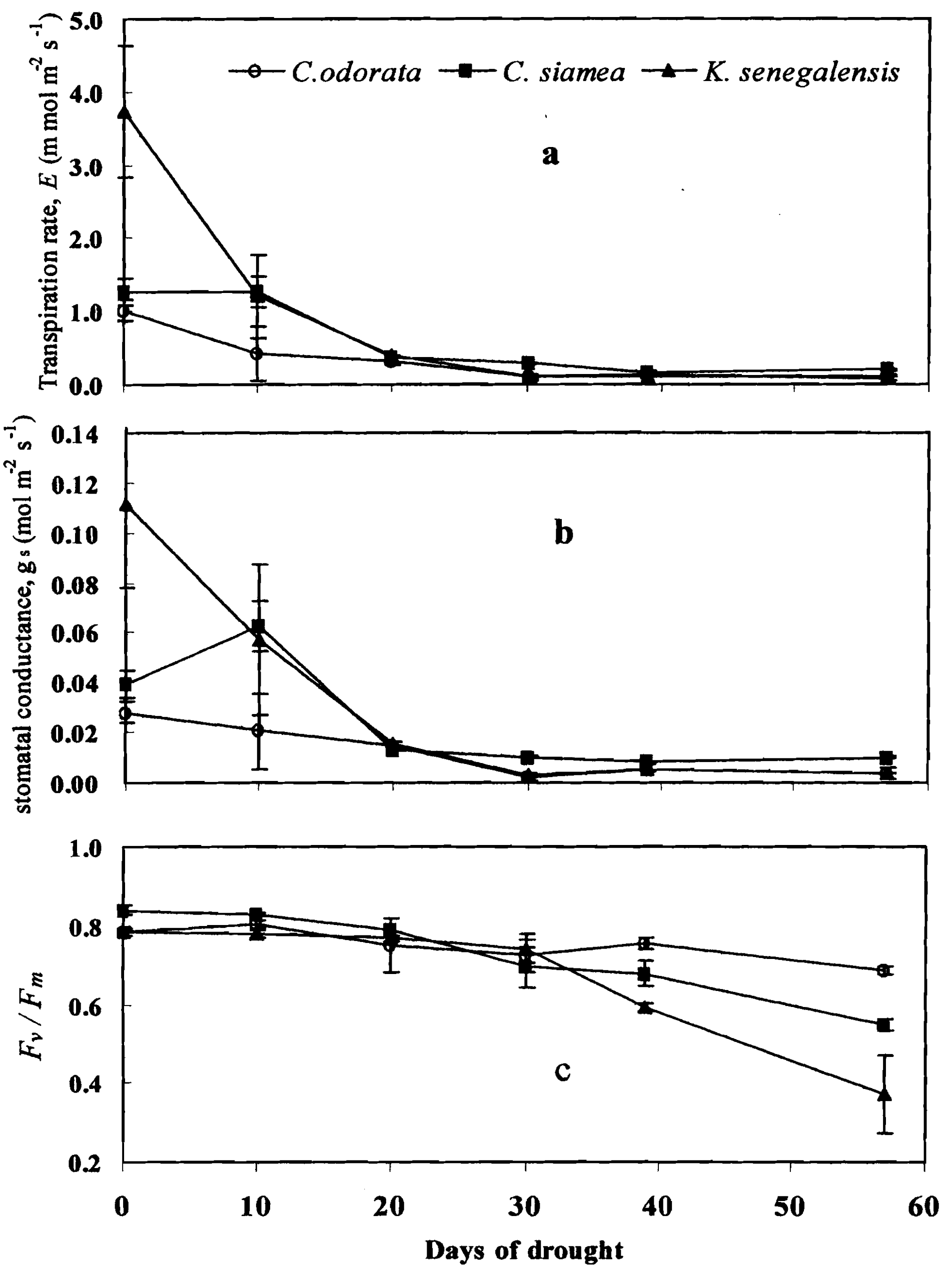

Fig. 4. Mean (±SE) transpiration rate (a), stomatal conductance (b), and potential quantum yield of PSII photochemistry (F_v/F_m) in leaves (n = 6) of *C. odorata*, *C. siamea* and *K. senegalensis* seedlings during year 2 drought treatment.

3.3.3. Quantum yield of photosystem II (Φ_{PSII}) and Electron transport rate (ETR)

3.3.3.1. *Year 1 experiment*

The Φ_{PSII} at PPFD<150 µmol m^{-2} s^{-1} was significantly lower in leaves of *C. odorata* plants measured after drought than in leaves measured before drought, whereas for *K. senegalensis* plants, the Φ_{PSII} at any given PPFD was significantly lower in leaves measured after drought treatment than in leaves measured before drought treatment (Figs. 5a and 5b). Hence, in *C. odorata* plants, ETR at PPFD>150 µmol $m^{-2} s^{-1}$, light saturated ETR, and the PPFD required to saturate ETR were all significantly reduced in leaves after drought treatment compared with the leaves before drought treatment. In contrast, in *K. senegalensis* plants, ETR at PPFD>300 µmol $m^{-2} s^{-1}$, light saturated ETR, and the PPFD required to saturate ETR were significantly reduced in leaves after drought treatment compared with the leaves before drought treatment (Figs. 5c and 5d).

3.3.3.2. *Year 2 experiment*

The Φ_{PSII} at PPFD<100 µmol $m^{-2} s^{-1}$ measured in leaves of *C. odorata* plants before and after drought was not significantly different. This was in contrast with the results from year 1 of the same species (Fig. 6a). In contrast, the Φ_{PSII} at any tested PPFD for *C. siamea* and *K. senegalensis* plants was significantly lower in leaves measured after drought treatment than in leaves measured before drought treatment (Figs. 6b and 6c). Hence, in *C. odorata* plants, ETR at PPFD>160 µmol $m^{-2} s^{-1}$, light saturated ETR, and the PPFD required to saturate ETR were all significantly reduced in leaves after drought treatment compared with the leaves before drought treatment (Fig. 6d). Consequently, in *C. siamea* and *K. senegalensis* plants, ETR at PPFD>350 µmol $m^{-2} s^{-1}$, light saturated ETR, and the PPFD required to saturate ETR were all significantly reduced in leaves after drought treatment compared with the leaves before drought treatment (Figs. 6e and 6f), respectively.

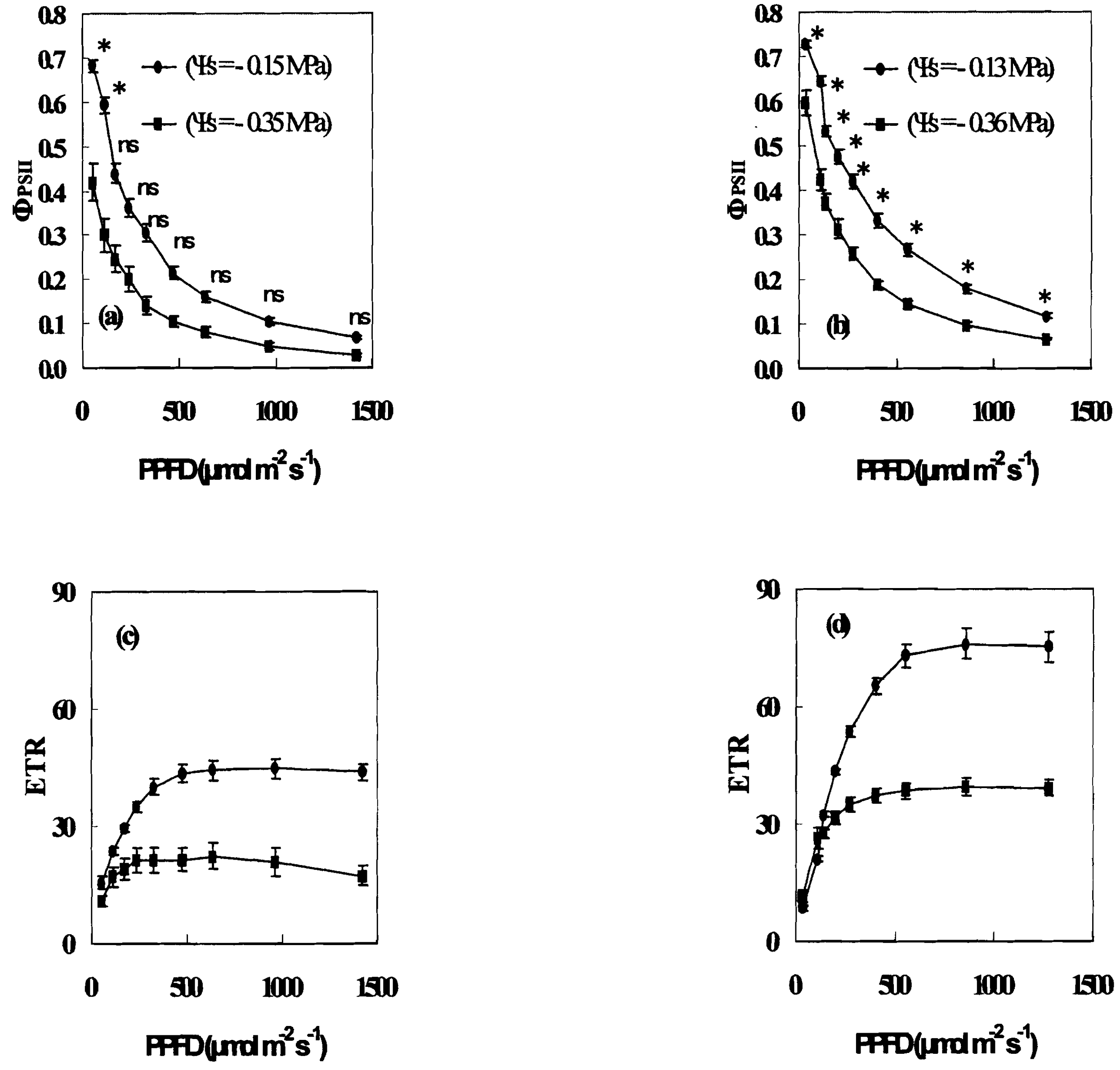

Fig. 5. Response of quantum yield of photosystem II (Φ_{PSII}) and electron transport rates (ETR, µmol electrons m^{-2} s^{-1}) to photosynthetic photon flux density (PPFD) in leaves of *C. odorata* (**a, c**) and *K. senegalensis* (**b, d**) measured at soil water potential (Ψ_s, −MPa) before (●) and after (■) year 1 drought treatment. Small bar denotes the standard error (SE). Some SEs are too small to display. An asterisk indicates significant differences ($P < 0.05$; ns, $P > 0.05$, paired *t*-tests) in Φ_{PSII} between soil water potential at a given PPFD.

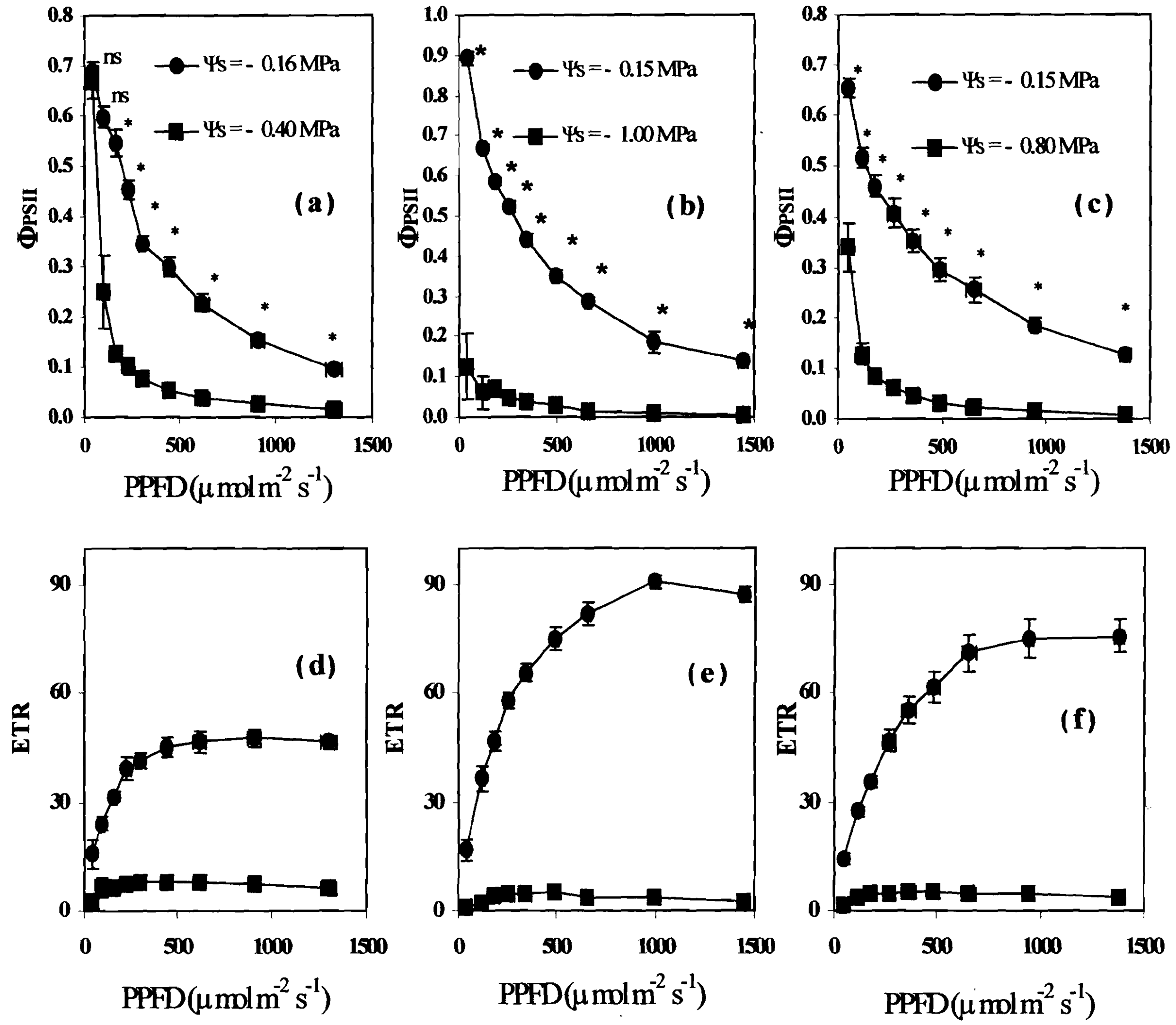

Fig. 6. Response of quantum yield of photosystem II (Φ_{PSII}) and electron transport rates (ETR, μmol electrons m^{-2} s^{-1}) to photosynthetic photon flux density (PPFD) in leaves of *C. odorata* (**a, d**), *C. siamea* (**b, e**) and *K. senegalensis* (**c, f**) measured at soil water potential (Ψ_s, −MPa) before (●) and after (■) **year 2** drought treatment. Small bar denotes the standard error (SE). Some SEs are too small to display. An asterisk indicates significant differences ($P < 0.05$; ns, $P > 0.05$, paired *t*-tests) in Φ_{PSII} between soil water potential at a given PPFD.

3.3.4. Shoot and root growth characteristics and dry mass

3.3.4.1. *Year 1 experiment*

Khaya senegalensis had large increases in leaf numbers (178 %) and total leaf area (172 %) at the end of drought treatment. In contrast, *C.*

odorata showed reduced leaf numbers (–20 %) and total leaf area (–30 %) at the end of drought treatment (Table 3). Shoot length increased by 80 % at the end of drought treatment for *Khaya senegalensis*, whereas *C. odorata* showed an increase of only 19 % at the end of drought treatment (Table 3).

Table 3. Leaf count, total leaf area and shoot length of *C. odorata* and *K. senegalensis* plants before and after year 1 drought treatment. Mean ± SE, (n = 6) values within the same row followed by different letters are significantly different at $P < 0.05$, paired *t*-tests.

	Before drought	After drought	Proportional change
C. odorata			
Leaf count	132 ± 10.8 a	105 ± 9.0 a	-20%
Total leaf area (cm^2)	1729.1 ± 140.6 a	1202.8 ± 164.0 b	-30%
Shoot length (cm)	65.3 ± 3.6 a	77.8 ± 4.9 b	19%
K. senegalensis			
Leaf count	19 ± 1.0 a	53 ± 5.0 b	178%
Total leaf area (cm^2)	628.3 ± 35.2 a	1711.7 ± 156.0 b	172%
Shoot length (cm)	40.5 ± 1.2 a	72.7 ± 2.1 b	80%

3.3.4.2. *Year 2 experiment*

At the end of year 2 drought treatment, the reduction in leaf numbers of *C. odorata*, *C. siamea* and *K. senegalensis* were 68, 31 and 37%, respectively (Photo 3.1 and Table 4). Total plant leaf areas were reduced in *C. odorata*, *C. siamea* and *K. senegalensis* by 82, 35 and 35%, respectively. Statistically, reductions in leaf numbers and leaf areas of *C. odorata* were significant ($P < 0.05$). *C. siamea* showed similar trends in leaf numbers ($P = 0.07$) and leaf areas ($P = 0.03$) reductions. In contrast, *K. senegalensis* did not show significant reductions in leaf numbers ($P = 0.10$) and leaf areas ($P = 0.14$) (Table 4). At the end of year 2 drought treatment, shoot length increased by 1 and 4% for *C. odorata* and *K. senegalensis*, respectively, whereas a decrease of –0.4% was found in *C. siamea*.

Table 4. Leaf count, total leaf area and shoot length of *C. odorata*, *C. siamea* and *K. senegalensis* plants before and after year 2 drought treatment. Mean ± SE, (n = 4) values within the same row followed by different letters are significantly different at $P < 0.05$, paired *t*-tests.

	Before drought	After drought	Proportional change
C. odorata			
Leaf count	84 ± 11 a	27 ± 15 b	−68%
Total leaf area (cm^2)	977.0 ± 132. 5 a	177.6 ± 103. 4 b	−82%
Shoot length (cm)	122.3 ± 4.1 a	123.3 ± 4.5 a	1%
C. siamea			
Leaf count	533 ± 14 a	369 ± 57 a	−31%
Total leaf area (cm^2)	5223.4 ± 132. 4 a	3409.3 ± 473. 6 b	−35%
Shoot length (cm)	139.3 ± 2.7 a	138.8 ± 2.8 a	−0.4%
K. senegalensis			
Leaf count	125 ± 21 a	78. ± 10. a	−37%
Total leaf area (cm^2)	2768.0 ± 461. 1 a	1786.3 ± 447. 2 a	−35%
Shoot length (cm)	131.8 ± 8.3 a	137.6 ± 10.2 a	4%

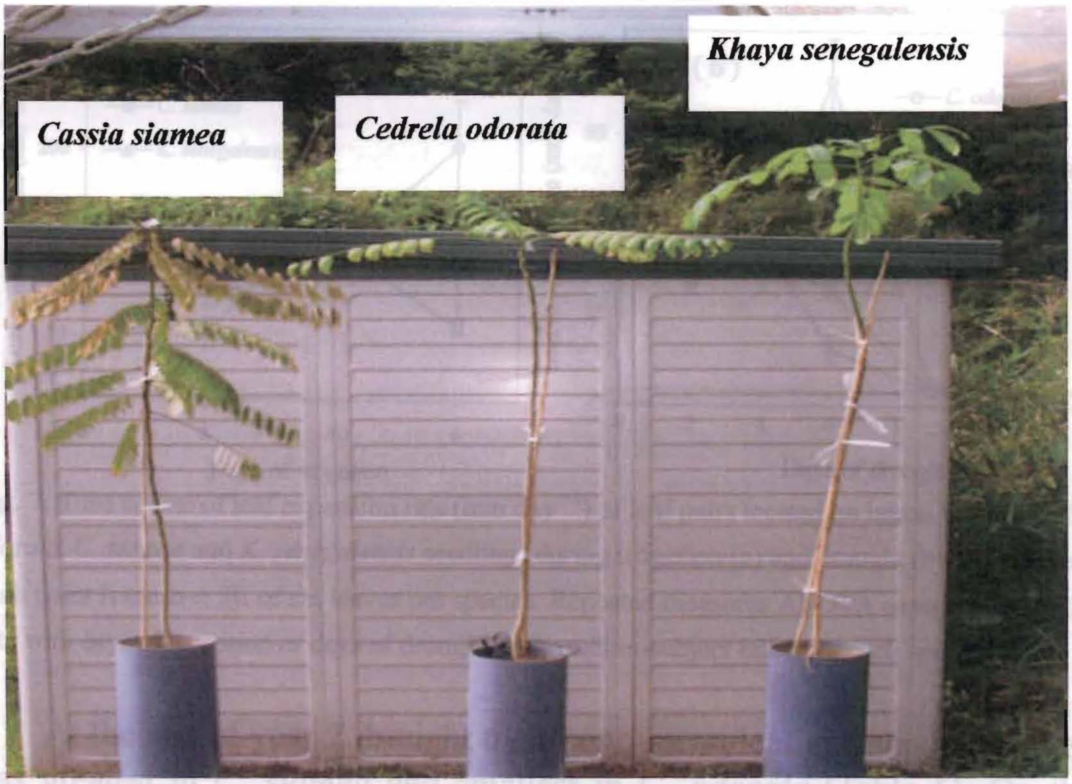

Photo 3.1. Plant samples at the end of year 2 drought treatment. From left, are *C. siamea*, *C. odorata* and *K. senegalensis* seedlings.

During the irrigation period (i.e., September 18–24, 2005), the pattern of leaf expansion rate differed for the species (Fig. 7a). *C. odorata* displayed a linear increase in leaf expansion rate during the irrigation period ($P < 0.05$). *C. siamea* on the other hand, displayed almost constant leaf expansion rate at day 2 and 4 before showing a reduction at day 6 ($P < 0.0001$). In contrast, *K. senegalensis* showed a trend of increasing leaf expansion rate from day 2 to 4 before declining slightly at day 6 ($P = 0.11$). The mean leaf expansion rate during the irrigation period ranged from 81–205 mm^2 day^{-1}, 59–127 mm^2 day^{-1} and 54–156 mm^2 day^{-1} for *C. odorata*, *C. siamea* and *K. senegalensis*, respectively. During the drought period (i.e., September 25–October 9, 2005), leaf expansion rate was significantly reduced for all the species ($P < 0.001$) (Fig. 7b). Tests of between-subject effects for the drought period showed that leaf expansion rate differed significantly among the species ($df = 2, 15$; $F = 4.6$; $P < 0.05$). At the end of year 2 drought treatment, mean leaf expansion rate had decreased to 0.06, −0.02 and −0.07 mm^2 day^{-1} in *C. odorata*, *C. siamea* and *K. senegalensis*, respectively (Fig. 7b).

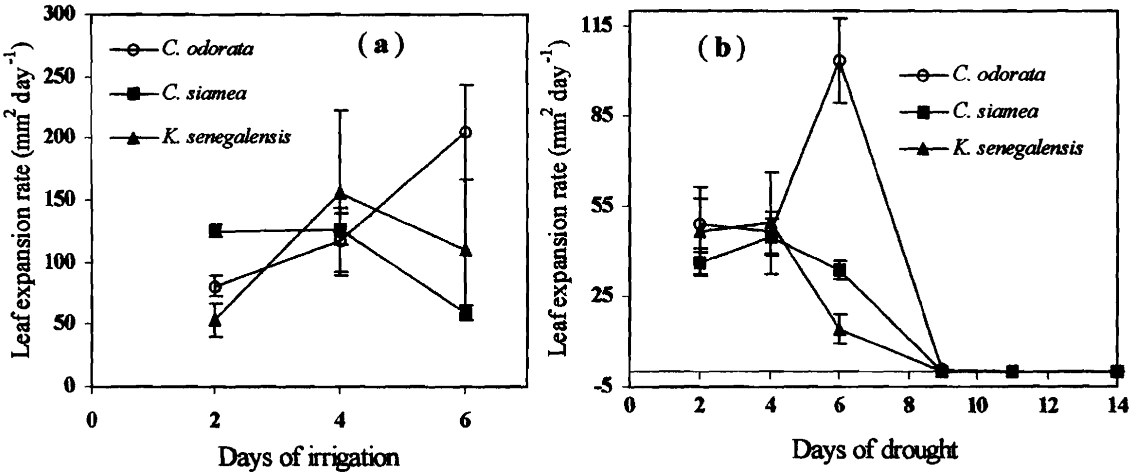

Fig. 7. Time course of leaf expansion rate (mm day^{-1}) of leaf pairs located on lateral branches of *C. odorata*, *C. siamea* and *K. senegalensis* seedlings during irrigation (a) and drought (b), respectively. Each point is mean ± SE of six leaves per species. Repeated measures ANOVA were applied to the data with days of irrigation or days of drought as the within-subject effect for each species.

At the end of year 2 drought treatment, mean leaf and shoot dry mass was greatest in *C. siamea* and smallest in *C. odorata* (Table 5). On the other hand, mean total root dry mass was greatest in *K. senegalensis* and smallest in *C. odorata*. However, mean root length was similar for all the species at harvest.

Table 5. Plant organ dry mass, root-shoot ratio and final root length of *C. odorata*, *C. siamea* and *K. senegalensis* seedlings at the end of year 2 drought treatment. For a given parameter, mean values (SE) of four plants are shown.

Growth parameter	*C. odorata*	*C. siamea*	*K. senegalensis*
Leaf dry mass (g)	0.3 (0.2)	13.4 (2.0)	8.3 (2.8)
Shoot dry mass (g)	19.6 (2.4)	45.4 (3.0)	34.7 (5.3)
Coarse root dry mass (g)	5.4 (0.7)	7.4 (0.7)	17.1 (4.3)
Fine root dry mass (g)	5.9 (0.7)	7.8 (0.4)	7.0 (0.5)
Total root dry mass (g)	11.4 (1.0)	15.1(0.8)	24.1 (4.5)
Root-shoot ratio (g g^{-1})	0.6 (0.08)	0.3 (0.01)	0.7 (0.03)
Root length (cm)	80.5 (6.1)	83.3 (2.3)	80.0 (2.2)

3.4. Discussion

Transpirational water loss from leaves of plant growing in wet soil progressively reduced soil water content and hence Ψ_s (Figs. 1b and 2b). As a consequence, *Cedrela odorata*, *Cassia siamea* and *Khaya senegalensis* plants showed a decline in stomatal conductance (g_S) and hence decreased transpiration rate (E) in response to increasing days of drought during year 1 and year 2 drought treatments (Figs. 3a, b and 4a, b), respectively. This phenomenon was more pronounced in *K. senegalensis* than in *C. odorata* in the year 1 drought treatment as shown by the higher correlation coefficients values between Ψ_s and g_S, and Ψ_s and E (Table 1). However, in year 2 drought treatment, *C. siamea* and *K. senegalensis* showed higher sensitivity than *C. odorata* to drought. This was a reflection in the higher correlation coefficients values between Ψ_s and g_S, and Ψ_s and E of *C. siamea* and *K. senegalensis*, respectively (Table 2). During drought, plants avoided desiccation through stomatal closure during midday when light intensity was high, as reported elsewhere (Baker and Pérez-Salicrup, 2000; Chaves et al., 2002). Various experiments have shown that stomatal responses are often more closely linked to soil moisture content than to leaf water status (Davies and Zhang, 1991; Jackson et al., 1995). In addition, stomata may close in response to either a decline in leaf water potential (Ludlow, 1980) or to a low-humidity atmosphere (Schulze, 1986). Higher VPD increases the transpirational demand, influencing how much moisture from plant tissues is transferred into the surrounding air. More specifically, the extent of midday stomatal closure depends on air humidity and soil moisture availability (Kozlowski, 1979). In the first drought cycle of *C. odorata* and *K. senegalensis*, Ψ_s had decreased for both species 36 days after drought treatment (Fig. 1b). However, midday VPD (Fig. 1a) on the same measurement day was relatively high, allowing some appreciable transpiration to take place, but at lower rates when compared with early days of drought (Fig. 3a).

The apparent slower water depletion during the first 25 days of year 1 drought treatment in *K. senegalensis* compared with *C. odorata* may be due to the initial smaller transpiring surface (i.e. total leaf area) of the former (Table 3). At the end of the first drought cycle, *K. senegalensis* plants showed greater shoot growth (leaf area expansion and shoot elongation) than *C. odorata*, which might have caused the rapid depletion of water from the soil towards the end of drought treatment. However, at the end of the first drought cycle, (i.e. a mild drought condition), hydraulic conductivity of the soil in pots of *C. odorata* and *K. senegalensis* was found to have decreased at almost the same rate (Gyimah and Nakao, 2006a). In contrast, by the end of the year 2 drought treatment, differential water uptake by the species might have led to different hydraulic conductivity of the soil in pots of the three species, which reflected in the different mean Ψ_s values in the pots of the three species (Figs. 1b and 2b). In terms of plant architecture, the largest mass of fine root (Table 5) and total leaf area (Table 4) maintained by *C. siamea* might have contributed to the species rapid soil water depletion from the pots. It is also important to emphasize that under high VPD, plants growing in drying soil may show increased internal water deficits, which could restrict plant growth (Gyimah and Nakao, 2006a). This is attributable to the fact that absorption from roots could not keep pace with transpiration. Consequently, shoot growth of *C. odorata* plants was restricted when compared with *K. senegalensis* plants during the first drought cycle (Table 3). However, in year 2 drought treatment, shoot growth of *C. siamea* was the most restricted (Table 4). Thus, the largest drop in soil water potential of *C. siamea* to approximately −1.0 MPa at the end of year 2 drought treatment, made soil water less available for root absorption and transpiration demand.

An increase in mass of plant roots and a decrease in carbon allocation to shoot are considered typical acclimation mechanism in response to water shortage (Pereira and Chaves, 1993; Gyimah and Nakao, 2006b). At the end of year 2 drought treatment, mean root-shoot ratio (R/S) of the species decreased in the following order: *K. senegalensis* > *C. odorata* > *C. siamea* (Table 5), suggesting superior acclimation of *K. senegalensis* to drought. The lowest R/S displayed by *C. siamea*, a semi-arid species, was a result of the highest shoot biomass maintained by the species under the drought condition. In contrast, *C. odorata*, a forest species, displayed the lowest shoot mass under drought condition through greater leaf shedding.

This experiment partly revealed that *C. siamea* and *K. senegalensis* seedlings exhibited both drought avoidance and tolerance as a means to resist drought. Drought avoidance often involves more than one adaptation and varies for different species of trees growing side-by-side (Kozlowski, 1979). *C. siamea* and *K. senegalensis* avoided drought through rapid stomata closure which resulted in a sharp decline in stomatal conductance and transpiration rate during year 1 and 2 drought cycles (Figs. 3a, b and Figs. 4a, b), respectively. The drought tolerance strategy of *C. siamea* and *K. senegalensis* plants was associated with reduced leaf expansion rate, fewer leaves withering and falling off and hence increased total leaf area when compared to *C. odorata* plants during the drought cycles (Fig. 7b, Tables 3 and 4). In contrast, drought-avoiding adaptations showed by *C. odorata* plants were characterized by a lower transpiration rate and lower stomatal conductance, even at the initial stages of drought when soil water potential was relatively high, and reduced total leaf surface area through leaf shedding when soil water potential was lowest. This result indicates a conservative water use strategy in the *C. odorata* plants. Poorter and Hayashida-Oliver (2000) in a study of some seedlings in a Bolivian moist forest, reported that not a lot of water is lost through transpiration in facultative deciduous species, such as *Cedrela*. Many tropical rain forest trees lose all their leaves in response to even mild drought, with their pattern of leaf shedding not necessarily linked to an annual cycle (Richards, 1996).

Furthermore, drought conditions caused significant decreases in quantum yield of PSII and ETR in all the species, but at different PPFDs for *C. odorata*, *C. siamea* and *K. senegalensis* during the year 1 and 2 drought treatments (Figs. 5 and 6, respectively). The decreases in Φ_{PSII} for all the species indicate that the fluorescence-derived ETR, which reflects the relative rate of energy conversion in PSII (Genty et al., 1989; Schreiber et al., 1994) was likely down-regulated during midday (Fleck et al., 2004). Hence, photochemical work, which is a normal means of energy dissipation in photosynthesis, was limited through the closure of stomata (Figs. 3b and 4b) to reduce transpirational loss of water in response to soil drying and high irradiance associated with summer season. Ishida et al. (1999) showed that a tropical tree exhibited higher photosynthetic rate early in the morning than at midday at a given ETR, suggesting a high rate of photorespiration at midday. Light-saturated ETR was significantly reduced in the plants after the drought treatments, and the reduction in ETR varied with the species and the extent of soil dryness. For instance, in the first drought cycle when Ψ_s in pots of *C. odorata* and *K. senegalensis* reached approximately −0.4 MPa, *C. odorata* displayed lower mean ETR values than *K. senegalensis* in the given range of PPFDs. However, in the year 2 drought treatment, when Ψ_s in pots of *C. siamea* and *K. senegalensis* reached approximately −1.0 and 0.8 MPa, respectively, mean ETR values were slightly lower in *C. siamea* in the given range of PPFDs. The drought conditions affected photosynthetic capacity by reduction of electron transport in the PSII of the chloroplasts (Potter and Boyer, 1973), inactivation of the primary photochemistry of the PSII reaction center complex, and reduction of ATP (Powles and Critchley, 1980). Other studies have also suggested that a significant flux of electrons may occur through alternative routes under conditions of drought, high temperature and light of the greenhouse that both species were subjected to (Fleck et al., 2004). The potential quantum yield of PS II (F_v/F_m) for all the species decreased with increasing soil drying in both year 1 and 2 drought treatments. The importance of stomatal control in photosynthetic activities of plants is highlighted by the strong correlations between decreasing g_s and decreasing F_v/F_m during the drought conditions (Tables 1 and 2). These results are consistent with those reported in a covered nursery for Holm oak (Fleck et al., 2004). The decrease in F_v/F_m of *C. odorata* was greater than *K. senegalensis* during the first drought cycle indicating that *C. odorata* was more susceptible to photodamage of PSII centers under mild drought conditions. However, during year 2 drought treatment when Ψ_s of *K. senegalensis* and *C. siamea* were further decreased, F_v/F_m of the species decreased greatly, showing the susceptibility of the two species to photodamage of PSII centers as water stress became severe.

3.5. Conclusion and implication for plantation forestry

In this study, plants of the three species had reduced physiological activities under the given range of drought conditions. This study supports the assertion that drought tolerance and drought-avoiding adaptations often vary for different species. Results from this study indicate that *C. siamea* and *K. senegalensis*, which show xerophytic traits, is more drought resistant than *C. odorata*, a mesophytic species. This assertion was reflected in the higher sensitivity of *C. siamea* and *K. senegalensis* to the transpiration and chlorophyll fluorescence parameters, and the root-shoot growth characteristics under the drought conditions. Observations indicated that *C. siamea* and *K. senegalensis* showed better leaf characteristics associated with drought tolerant species than *C. odorata*. These characteristics included small and/ or leathery and thick leaves, and highly reflective, small vein islets which enhance the two specie's ability to resist drought.

Results of this study suggest that *C. odorata* followed a drought-avoiding strategy (greatest leaf shedding) and was more conservative in its water use when compared to either *C. siamea* or *K. senegalensis*. Thus, under drought conditions, growth of the forest species, *C. odorata* was restricted and its physiological functions lowered. In terms of growth performance, *C. odorata* may not be a suitable candidate species for plantation forestry in semi-dry environments where drought is a common occurrence. In contrast, the ability of *C. siamea* and *K. senegalensis* to maintain relatively higher mass of foliage and/ or root growth under drought conditions confers greater advantage to them as plantation forestry species in semi-dry environments. In addition, greater tolerance of *C. siamea* and *K. senegalensis* to drought conditions by way of high sensitivity to stomatal closure and hence, transpiration control and xerophytic leaf characteristics make them suitable for cultivation in savannah and semi-arid climates of Africa as multi-purpose trees.

CHAPTER 4.

Early growth and photosynthetic responses to light in seedlings of three tropical species differing in successional status

4.1. Introduction

The annual net rate of deforestation remains high in the tropics with an annual loss of 14.2 million ha converted to other land-uses (FAO, 2001). In Africa, the average loss of forest cover over the last decade (i.e., 1990–2000), was 7.8% (Evans and Turnbull, 2004). Rapid deforestation in Ghana through over exploitation of commercial timber has necessitated the establishment of plantation forests, which at present, accounts for merely 0.8% of Ghana's forest (FAO, 2001; Ghana Forestry Commission, 2002). Reforestation success with *Terminalia* species in degraded forest sites of West and Central African countries have been reported, though with little success in the case of Ghana (ITTO, 2006). In Ghana, *Cedrela odorata* has gained importance in reforestation programs because of its presumed broad ecological amplitude (Pancel, 1993; Evans and Turnbull, 2004). Compared to pure plantation, enhanced growth and survival of *C. odorata* seedlings has been reported when grown in shade conditions in agroforestry systems (Navarro et al., 2004).

Light availability plays an important role in regenerating tropical tree seedlings (Agyeman, 1994; Poorter, 1989). An understanding of desired species' growth and photosynthetic traits when planted in either shade or exposed sites is required for successful reforestation. *Cedrela odorata*, *Terminalia superba* and *Mansonia altissima* are among tree species that are frequently used in regeneration and/ or reforestation efforts in Ghana. Seedlings of *C. odorata* and *T. superba* are reported to thrive best in medium and large gaps (Maruyama et al., 2005 and Hawthorne, 1995), respectively. Seeds of *M. altissima* are known to germinate in shade but their seedlings are light demanding (Veenendaal et al., 1996). Growth rate differences among seedlings may be determined by several plant responses. For example, plants may invest highly in photosynthetic efficiency in low irradiance, whereas, a higher investment in the photosynthetic efficiency is favored in high irradiance or optimum conditions (Lambers and Poorter, 1992). The photosynthetic and respiratory capabilities of plants are controlled by several traits, which include, leaf area ratio (LAR), specific leaf area ratio (SLA), net assimilation rate (NAR) and biomass allocation (Poorter, 1989). Generally, pioneers and light demanders tend to have a higher SLA compared to shade-tolerants in low irradiance due to smaller amount of energy used in producing thinner leaves (Poorter, 1989).

When compared to shade-intolerant species, tolerant species have greater low-light growth rates and also physiological traits such as lower respiration rates, higher photosynthetic rates in low light, lower light compensation and lower light saturation points at low light intensity (Walters and Reich, 1999). These traits enhance such low-light growth. In addition, seedlings for plantation in tropical areas are normally grown in a nursery under shaded and well-watered conditions (Maruyama et al., 2005). Therefore, the ability for sun-grown seedlings to adjust physiologically to high irradiance may indicate a species acclimation to high light (Powles, 1984) and may enhance reforestation success in the field. The influence of light on growth and/ or photosynthesis of *C. odorata* have been reported (Poorter, 1999; Poorter and Hayashida-Oliver, 2000; Ricker et al., 2000; Maruyama et al., 2005). However, for *M. altissima* and *T. superba* studies have concentrated on the effect of light on growth traits (Agyeman, 1994 and Sokpon, 1995; Veenendaal et al., 1996, respectively) with little attention paid to physiological traits of the species. Photosynthetic parameters have been used in a growth model to assess biomass production of tropical tree species, including *C. odorata*, for plantation success in sites differing in resource availability (Dunisch et al., 2002). Nevertheless, there are still some open questions to be addressed, such as the association of individual plant species responses with their ecological strategy.

Hence, apart from comparative growth and photosynthetic study of the species to light, it was hypothesized that *C. odorata* and *T. superba* (intermediate and pioneer species, respectively) will exhibit a stronger relationship between mean maximum photosynthetic rate and mean net assimilation rate than *M. altissima* (a non-pioneer) at increased light availability. In addition, the differences in short term acclimation to 100 and 10% sunlight among these three species were determined by comparing intrinsic water use efficiency, photochemical efficiency and root system of their seedlings. Results from this work will provide further information on the study species, which may be used in screening tropical tree species for plantation forestry.

4.2. Materials and methods

4.2.1. Plant material and study species

Seeds of *Cedrela odorata* L., *Mansonia altissima* A. Chev. and *Terminalia superba* Engl. and Diels collected from the Brong-Ahafo Regional Forest Services Division's nursery in a semi-deciduous forest zone of Ghana were sown in plastic containers (60 × 25 × 15 cm) filled with 10 liters of river sand and forest soil, respectively. The seeds were germinated in the plastic containers under natural conditions at the University of Miyazaki forest nursery with partial shading in late March 2005. At two-month in age, seedlings were transplanted into individual pots (20.0 cm in length and 17.5 cm in diameter) containing a 2:1 mixture of forest soil and river sand by volume ratio, respectively. The forest soil was a moderately moist brown soil, B_D subtype (Japanese soil classification system, JAFTA, 1999a) obtained from a foot slope in the University of Miyazaki forest. Five grams of slow release granular fertilizer (Luxebrown; 5:10:15:4 – N, P, K, Mg plus trace elements) were added to the potting mix to ensure adequate plant nutrition. The release time for the fertilizer was approximately 1 month. The pots were then kept in a glass house facility at the nursery briefly. The pot used for the experiment is comparable to the medium sized pot used in Agyeman (1994), which did not show adverse effect of pot-binding for fast-growing species. The seedling height (SD), root collar diameter (SD) and leaf numbers (SD) at transplant were 7.8 (0.6) cm, 1.8 (0.1) mm and 6 (0.0), respectively, for *C. odorata*; 5.1 (1.0) cm, 2.8 (0.2) mm and 2 (0.0), respectively, for *M. altissima*; and 3.8 (1.0) cm, 2.1 (0.1) mm and 2 (0.0), respectively, for *T. superba*. A detailed description of the study species is shown in Table 1.

Table 1. Description of the three species used in the experiment (nomenclature follows Hall and Swaine, 1981; Pancel, 1993) and their average seed mass recorded.

Species	Family	Origin of seeds	Average seed mass (g, $n = 500$)	Natural distribution	Strategy	Other characteristics
Cedrela odorata L.	Meliaceae	Sunyani, Ghana (semi-deciduous forest zone)	0.02	Mesoamerica (27° S−24° N)	Intermediate and mid successional [1]	Moderate to fast growing species
Mansonia altissima A. Chev.	Sterculiaceae	Sunyani, Ghana (semi-deciduous forest zone)	0.2	African tropics (6° S−10° N)	Non-pioneer light demander [2]	Survive better under forest canopy than pioneers
Terminalia superba Engl. & Diels	Combretaceae	Sunyani, Ghana (semi-deciduous forest zone)	0.09	African tropics (6° S−10° N)	Late seral pioneer [3]	Shows traits of secondary rain-forest trees [3]

[1]Maruyama et al. 2005; [2]Hawthorne 1995; [3]Richards 1996

4.2.2. Growth conditions and experimental design

The study was conducted at the Agricultural experimental field center of the University of Miyazaki, Miyazaki in the southern Japan (32° N, 131° E). Three pairs of aluminum metal frames, each approximately 2.0 m^2 and 1.8 m high and facing each other at equidistance (1.2 m apart), were draped with black polypropylene shade fabric of different grades to provide three different levels of paired shade frames. The tunnel-shaped shade frames with a semicircular cross-section at both ends were positioned perpendicular to the daily track of the sun. This arrangement of shade frames was done to reduce temporal variation in irradiance within the paired shade frames and to accommodate space constraints. Percent mean (SD) sunlight in the shade frames were measured at several points with a luminance meter (MINOLTA T-1M) at noon, on clear sky days (June 27, and August 30, 2005). Minimal variation in SD sunlight between each of the three paired frames was observed (45.3% (0.8) versus 44.7% (0.8); 23.0 % (I.0) versus 22.0% (0.5); and 10.0% (0.05) versus 10.1% (0.05)). Thus, a paired shade frame in this experiment was considered to have same light treatment judging from their similar percent sunlight extinction values. An additional open site with the same area coverage as the paired frames served as the 100% sunlight treatment. Hence, the four light treatments used in this experiment were described as 100, 45, 22 and 10% sunlight. These light levels represent a range of light environments from clear-cut forests sites to different canopy openings observed in some degraded semi-deciduous forests of Ghana (Agyeman, 1994; Hawthorne and Abu-Juam, 1993). A 20-cm gap was left between the bottom of the shade fabrics and the ground to permit free air movement. Adjacent shade frames were about 2.5 m apart to reduce possible treatment overlap. During the experimental period (June 30–October 30, 2005), mean, maximum and minimum air temperatures at the site were 25.3°C, 29.3°C and 22.0°C, respectively. Mean relative humidity and mean monthly rainfall were 75.7% and 336.4 mm, respectively (Miyazaki Meteorological Agency, 2005). Average monthly air temperature between the open site and shade frames varied less than 1.0°C. Mean relative humidity in the 10 and 45% sunlight treatments were 80.0% and 75.0%, respectively.

In late June 2005, ten individuals of 3-month-old *C. odorata* and *T. superba* and eight individuals of 3-month-old *M. altissima* were transferred into each of the four light treatments (Photo 4.1). Pots were positioned randomly in four rows × three seedlings per shade frame or four rows × six seedlings per light treatment. Inter seedling distance was 30 cm, and each pot was at least 20 cm from the edge of the shade frame. The pots were periodically repositioned to prevent shading and to randomize any effect of position across all pots within each light treatment. The seedlings were watered at least twice a week and hand-weeded as necessary. To prevent insect attacks, seedlings were sprayed regularly with an aerosol insecticide (Kadan - D ®). Two harvests were conducted. For each species and light treatment, an initial harvest was carried out on August 24, 2005, when the seedlings had exhausted their cotyledon reserves as detected by the falling-off or shriveling of the cotyledons. Photosynthetic-type cotyledon was observed in *C. odorata*, whereas storage-type cotyledon was observed in *M. altissima* and *T. superba*. At this harvest, pairs of conspecific individuals of the same size were identified of which one was harvested and the second was left for the second harvest. The second harvest was conducted on November 7, 2005. The second harvest was timed to limit seasonal variation effect on the seedling growth and also limit the size range over which biomass and growth rates of species would be compared (e.g. fast growing species may grow faster in high light environment). In this way, the time at which plants would be compared was reasonably objective. Thus, the two-factor experiment contained 3 species × 4 light treatments × 2 harvest × 4 individuals = 96 seedlings.

Photo 4.1. Shade frames at the experiment site draped with black polypropylene fabric of different grades to create the three paired shade levels. The irradiances in the paired frames are 45%, 22% and 10% from front to back, respectively.

4.2.3. Seedling morphology and growth analysis

At each harvest, stem length, length of longest root, leaf area and dry mass were measured. Leaves and stem (including branches and petioles) were separated, and the roots were washed gently to get rid of the potting mix. Leaf area of each plant at harvest was measured using leaf area meter (LI-3000A, Li-Cor Inc., Lincoln, NE). Plant components were then oven-dried for 48 h at 70°C for dry mass determination.

From the primary data, the following variables were derived; leaf mass ratio (LMR, leaf mass per unit plant mass g g^{-1}); stem mass ratio (SMR, stem mass per unit plant mass g g^{-1}); root mass ratio (RMR, root mass per unit plant mass g g^{-1}); leaf area ratio (LAR, leaf area per unit plant mass specific cm^2 g^{-1}); and specific leaf area ratio (SLA, leaf area per unit leaf mass cm^2 g^{-1}). The average values of LMR, SMR, RMR, and SLA from the two harvests were used for the growth analysis, whereas stem length, length of the longest root and plant dry mass at second harvest, were used for final plant size responses.

Mean relative growth rate (RGR, g g^{-1} day^{-1}), mean net assimilation rate (NAR, g cm^{-2} day^{-1}) and mean leaf area ratio (LAR, g cm^{-2}) were calculated with formulas given by Hunt (1978) as follows:

$$\mathrm{RGR} = (\ln W_2 - \ln W_1)\,(t_2 - t_1)^{-1} \tag{1}$$

$$\mathrm{NAR} = (W_2 - W_1)\,(t_2 - t_1) \times [(\ln A_2 - \ln A_1) / (A_2 - A_1)] \tag{2}$$

$$\mathrm{LAR} = [(A_2 - A_1)\,(\ln W_2 - \ln W_1)] / [(W_2 - W_1)\,(\ln A_2 - \ln A_1)] \tag{3}$$

where W_2 and W_1 is the dry mass at second and first harvest, respectively; t_2 and t_1 represent time (days) at second and first harvest, respectively; A_2 and A_1 represent leaf area at second and first harvest, respectively. The formula for the calculation of the NAR is only valid if leaf area and plant dry mass are linearly related (Hunt, 1978). For all the species-light treatment combinations, this condition was met; mean r^2 of the linear regression of leaf area against plant mass was 0.75 (range, 0.60–0.87).

4.2.4. Foliar physiology

Approximately mid-way through the experiment (mid summer), a series of foliar measurements was made. Maximal quantum yield of photosystem II (PSII) was measured as the ratio of variable and maximal fluorescence (F_v / F_m) with pulse amplitude modulated fluorimeter (Mini-PAM, Heinz Waltz, Effeltrich, Germany) on four randomly selected seedlings per species in each shade treatment and the open site. F_v / F_m were made after sunset on one fully expanded leaf (following 30 min of leaf dark adaptation) at standard measuring intensity of 0.15 µmol m^{-2} s^{-1}; modulation frequency of 0.6 kHz and saturating light pulse about 1800 µmol m^{-2} s^{-1} for 0.8 s (Mini-PAM Handbook of operation, 2nd edition, 1999).

Net photosynthetic rate (A) and transpiration rate (E) were measured at PPFDs of 0, 50, 100, 200, 400, 600, 800, 1000 and 1500 µmol m^{-2} s^{-1} with a portable open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE). The PPFDs were obtained by using a quartz halogen light unit coupled to leaf chamber of the instrument. One fully expanded leaf near the seedling apex was placed in the cuvette of the LI-6400 under the following cuvette conditions: block temperature, 30 ±1.5 °C; relative humidity, 49 ± 5%; leaf-to-air vapor pressure deficit (VPD), 2 ± 0.4 kPa; reference CO_2, 400 ± 19.8 µmol CO_2 mol^{-1}. The instrument was adjusted for automatic light curve recording. The leaf was kept at each of the PPFDs for approximately 2 min 30 sec and a constant CO_2 flow through the chamber was maintained. On each measurement date, gas exchange was measured from 0900 to 1800 hours.

Photosynthetic light response curves were constructed for four seedlings from each treatment and species. Light-saturated net photosynthesis rates (A_{max}), day respiration (R_{day}), light compensation point (LCP) and light saturation estimates (LSE) were then estimated with a Photosyn Assistant software for windows version 1.2 (Dundee Scientific, Scotland, UK). This software determines the photosynthetic parameters by fitting data to the model function, expressed as a quadratic equation by Prioul and Chartier (1977). In addition, stomatal conductance (g_s) was measured and intrinsic water use efficiency (WUE) was calculated from the primary data as follows:

$$\mathrm{WUE} = A/E \qquad (4)$$

4.2.5. Data analysis

Plant responses were analyzed using a two-way ANOVA, with light and species as variable factors. With the exception of plant biomass, stem length and root length at final harvest, the average values of the other examined growth variables from the two harvests were used in the growth analysis. One-way ANOVA tests were performed for the light and species combination where a significant difference was detected by the two-factorial ANOVA (SYSTAT, Statistical software Inc., CA, USA). This was followed by a *post hoc* multiple comparisons of means test using Tukey HSD test. E, g_s and WUE were compared within and between species in the open site (100% sunlight) and shade (10% sunlight) by ANOVA. Significant differences are reported as $P < 0.05$. Relationships between mean A_{max} and mean NAR were analyzed by linear regression for each species across the light treatments.

4.3. Results

4.3.1. The relative importance of light and species

The results of the two-way ANOVA indicated that light and species had strong influence on growth traits and biomass allocation patterns as shown by the proportion of explained variance (Table 2). Significant light × species interactions were observed for only biomass, SMR, SLA, LAR and NAR. The explained variation by the ANOVA model was high (mean 0.80, range 0.49–0.93).

Table 2. Results of the two-way ANOVA with species and light as factors, degrees of freedom (*df*) and coefficient of determination (r^2). The proportion of the explained variance (SS_x / SS_{total}) and the level of significance ($P > 0.05$, NS; $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***) for each factor and the interaction are indicated.

Sources of Variation	*df*	H	Root length	Biomass	LMR	SMR	RMR	SLA	LAR	NAR	RGR
Species	2	54.5***	22.2***	60.3***	73.5***	25.1***	71.6***	61.2***	33.1***	15.2***	1.0 NS
Light	3	14.8**	5.1**	16.7***	15.3***	4.3*	94.7***	48.9***	75.5***	41.9***	6.8**
Species ×Light	6	1.7 NS	1.7*	3.2*	2.7*	1.8 NS	2.5*	6.1**	9.1***	3.7**	2.0 NS
Residual	36										
Total [(3×4×4)-1]	47										
ANOVA r^2		0.83	0.66	0.84	0.85	0.67	0.93	0.89	0.91	0.83	0.48

Variables: Height (H), Mean leaf mass ratio (LMR), Mean stem mass ratio (SMR), Mean root mass ratio (RMR), Mean specific leaf area (SLA_{mean}), Mean leaf area ratio (LAR_{mean}), net assimilation rate (NAR), relative growth rate (RGR).

4.3.2. Size and morphology

In general, plants attained highest biomass under intermediate light conditions. Total plant mass at final harvest was highest at 22 and 45% light (Photo 4.2 and Table 3). Stem length showed a close relationship to biomass and hence, for each study species, stem length attained the highest mean value at 22 and 45% sunlight (Table 3). For all the species, plant responses were typically those found along a light gradient: generally, LMR, SLA, LAR decreased with increasing irradiance, whereas RMR increased with increasing irradiance. In *C. odorata* and *T. superba*, allocation to stem (SMR) increased significantly at lower light ≤ 22% sunlight, whereas in *M. altissima* there was no clear trend of higher SMR values with decreasing light treatments ($P = 0.502$) . In contrast, at final harvest, *M. altissima* had significantly reduced root length at lower light ≤ 22% sunlight, whereas in *C. odorata* and *T. superba*, there was a trend of shorter root length with decreasing light treatment ($P = 0.980$ and 0.096, respectively; Table 3). However, *T. superba* had a greater root length at 45 and 100% sunlight compared to either *C. odorata* or *M. altissima* at those light treatments (Table 3). Most of the plant variables examined for *C. odorata* and *T. superba* were significantly different at the extremes of the light treatments (i.e., 10 and 100% sunlight).

4.3.3. Responses of RGR, NAR and LAR to light

T. superba showed a trend of increasing RGR values with increasing light treatment, with highest value at 45% sunlight, and declined thereafter at 100% sunlight ($P = 0.003$; Table 3). A Similar trend among the light treatments was observed for RGR of *C. odorata* ($P = 0.025$). *M. altissima*, on the other hand, did not show a distinct trend of higher RGR with increasing light treatment ($P = 0.807$). For all the species, there was a trend of lower LAR values with increasing irradiance ($P = 0.0001$). In contrast, there was a trend of higher NAR values with increasing light treatments in *C. odorata* ($P = 0.0001$), *M. altissima* ($P = 0.011$) and *T. superba* ($P = 0.0001$) (Table 3). At 10% sunlight, RGR decreased among the species in the following trend: *M. altissima* > *C. odorata* > *T. superba* ($P = 0.050$). In contrast, at 100% sunlight, NAR decreased among the species in the following trend: *T. superba* > *C. odorata* > *M. altissima* ($P = 0.004$).

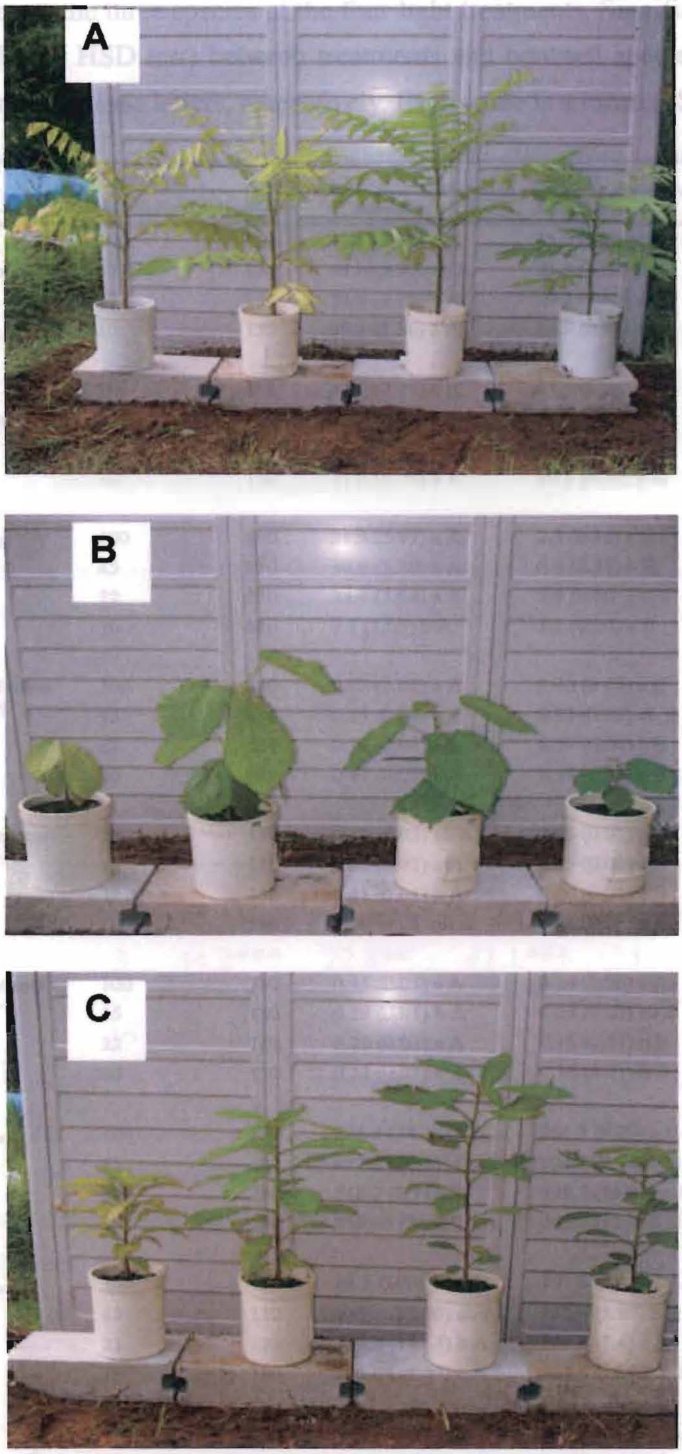

Photo 4.2. Seedlings of *C. odorata* (A), *M. altissima* (B) and *T. superba* (C) after 120 days of growth in the four light treatments. From left to right, are samples of each species grown at 100, 45, 22 and 10% sunlight, respectively.

Table 3. Responses of the three species at the four light treatments. Significant differences ($P < 0.05$, Tukey HSD test) between treatments and between species are indicated by lower and upper case letters, respectively. Each value is a mean (1SE) of four plants.

		Treatment	Period	Species		
Group	Variable	(% sunlight)	(days)	*C. odorata*	*M. altissima*	*T. superba*
Plant size	Stem length, cm	100	130	32.5 (2.99) a A	15.0 (0.35) a B	19.6 (1.75) a B
		45	130	43.8 (3.60) ab A	23.5 (4.02) b B	33.1 (4.56) ab AB
		22	130	53.0 (4.12) b A	20.1 (2.50) ab B	42.3 (4.37) b A
		10	130	39.1 (1.36) a A	13.9 (0.97) a B	28.8 (3.60) ab C
	Root length, cm	100	130	35.0 (2.87) a A	31.5 (0.96) a A	42.5 (1.19) a B
		45	130	34.5 (2.01) a A	26.8 (0.43) b B	43.4 (2.84) a C
		22	130	36.8 (1.49) a A	19.0 (0.71) c B	41.8 (6.39) a A
		10	130	31.0 (3.76) a A	20.5 (0.29) c A	29.0 (6.40) a A
	Biomass, g	100	130	21.9 (3.97) a A	2.3 (0.16) a B	9.8 (2.97) a B
		45	130	30.9 (3.38) a A	6.5 (2.27) b B	14.4 (2.99) a B
		22	130	23.9 (2.64) a A	3.4 (0.60) ab B	9.8 (1.71) ab C
		10	130	7.3 (1.05) b A	1.3 (0.20) a B	3.6 (1.60) b AB
Allocation	Mean leaf mass ratio, g g^{-1}	100	130	0.27 (0.02) a A	0.40 (0.05) a B	0.41 (0.01) a B
		45	130	0.35 (0.01) b A	0.51 (0.03) ab B	0.41 (0.03) a C
		22	130	0.38 (0.01)b A	0.59 (0.00) b B	0.45 (0.02) ab C
		10	130	0.41 (0.01) b A	0.57 (0.03) b B	0.48 (0.01) b C
	Mean stem mass ratio, g g^{-1}	100	130	0.39 (0.02) ab A	0.26 (0.02) a B	0.24 (0.01) a B
		45	130	0.35 (0.01) a A	0.25 (0.01) a B	0.29 (0.01) b C
		22	130	0.41 (0.01) b A	0.26 (0.01) a B	0.30 (0.01) b C
		10	130	0.40 (0.01) ab A	0.28 (0.03) a B	0.28 (0.02) ab B
	Mean root mass ratio, g g^{-1}	100	130	0.35 (0.01) a A	0.34 (0.06) a A	0.36 (0.01) a A
		45	130	0.29 (0.01) b A	0.24 (0.03) ab B	0.30 (0.02) b A
		22	130	0.22 (0.01) c A	0.16 (0.01) b B	0.25 (0.01) bc C
		10	130	0.21 (0.01) c A	0.15 (0.01) b B	0.24 (0.01) c A
Growth analysis	SLA_{mean}, cm^2 g^{-1}	100	130	371.3 (34.18) a A	264.3 (8.20) a B	205.9 (9.97) a B
		45	130	437.6 (20.76) ab A	324.7 (8.34) b B	267.5 (10.60) b C
		22	130	513.7 (9.7) b B	399.7 (13.00) c A	318.0 (11.66) b C
		10	130	815.45 (31.20) c A	465.2 (13.8) d B	426.4 (18.80) c B
	LAR_{mean}, cm^2 g^{-1}	100	130	88.5 (10.03) a AB	107.6 (3.02) a A	72.4 (3.64) a B
		45	130	115.3 (13.00) ab A	152.6 (5.89) b B	93.3 (2.62) ab A
		22	130	153.5 (14.65) b A	216.3 (7.6) c B	117.7 (8.51) b A
		10	130	295.0 (11.57) c A	233.6 (19.10) c B	190.8 (10.15) c B
	NAR, mg cm^2 day^{-1}	100	130	0.267 (0.01) a A	0.212 (0.03) a A	0.350 (0.01) a B
		45	130	0.246 (0.02) a AB	0.165 (0.01) ab A	0.340 (0.05) a B
		22	130	0.134 (0.03) b AB	0.110 (0.00) b A	0.196 (0.03) b B
		10	130	0.068 (0.00) b A	0.112 (0.02) b A	0.077 (0.00) c A
	RGR, g g^{-1} day^{-1}	100	130	0.025 (0.00) ab A	0.025 (0.00) a A	0.026 (0.00) a A
		45	130	0.030 (0.00) a A	0.028 (0.00) a A	0.033 (0.00) a A
		22	130	0.019 (0.00) b A	0.024 (0.00) a A	0.023 (0.00) ab A
		10	130	0.020 (0.00) b AB	0.030 (0.01) a A	0.015 (0.00) b B

4.3.4. Chlorophyll fluorescence and photosynthetic responses to light availability

Light and species significantly influenced maximal quantum yield of PSII (photochemical efficiency, F_v / F_m) (Table 4).The Light × species interaction effect on F_v / F_m was not significant. There was a trend of higher F_v / F_m values with decreasing light treatments in *C. odorata* (P = 0.050), *M. altissima* (P = 0.002) and *T. superba* (P = 0.001) (Table 5). Sun-grown *T. superba* had significantly higher F_v / F_m than either sun-grown *C. odorata* or *M. altissima.* At all the shade treatments (≤ 45% sunlight), F_v / F_m did not differ significantly among the species.

Table 4. Results of the two-way ANOVA with species and light as factors, degrees of freedom (*df*) and coefficient of determination (r^2). The proportion of the explained variance (SS_x / SS_{total}) and the level of significance ($P > 0.05$, NS; $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***) for each factor and the interaction are indicated.

Source of Variation	*df*	A_{max}	LCP	LSE	R_{day}	F_v / F_m
Species	2	16.7***	11.2*	12.6***	7.0 NS	7.9*
Light	3	44.2***	22.8**	41.1***	23.0**	43.6***
Species X Light	6	14.8 **	9.0 NS	24.4**	12.3 NS	6.1 NS
Residual	36					
Total [(3x4x4)-1]	47					
ANOVA r^2		0.76	0.43	0.78	0.42	0.58

Parameters: Light-saturated maximum photosynthetic rate (A_{max}), light compensation point (LCP), light saturation estimate (LSE), day respiration (R_{day}), maximal quantum yield of PSII (photochemical efficiency, F_v / F_m).

Table 5. Effects of light on photosynthetic parameters of three species grown in pots after 2 months of treatments. Significant differences ($P < 0.05$, Tukey HSD test) between treatments and between species are indicated by lower and upper case letters, respectively. Each value is a mean (1SE) of four plants.

		Species		
Parameter*	% Light	*C. odorata*	*M. altissima*	*T. superba*
A_{max} (μmol CO_2 m^{-2} s^{-1})	100	7.54 (0.9) a AB	4.70 (0.7) a A	7.67 (2.4) a B
	45	7.84 (0.6) a A	4.44 (1.8) a B	6.20 (0.8) a AB
	22	6.05 (0.4) b A	3.94 (1.5) a B	2.40 (0.6) b B
	10	3.54 (0.7) c A	3.22 (0.4) a A	1.97 (0.4) b B
LCP (μmol m^{-2} s^{-1})	100	21.73 (6.6) a A	11.27 (5.2) a A	19.40 (5.7) a A
	45	12.81 (4.5) b A	7.50 (3.0) a A	12.01 (6.8) a A
	22	7.50 (3.8) b A	7.70 (4.0) a A	15.30 (4.0) a B
	10	6.36 (1.8) b A	7.60 (7.3) a A	10.94 (8.8) a A
LSE (μmol m^{-2} s^{-1})	100	144.0 (24.8) a AB	100.0 (18.2) a A	189.0 (35.3) a B
	45	140.0 (13.0) a B	70.0 (25.3) a A	135.0 (8.9) b B
	22	117.0 (16.7) ab A	67.4 (38.6) a B	58.0 (15.0) c B
	10	78.40 (11.3) c A	78.51 (19.0) a A	38.20 (20.7) c B
R_{day} (μmol CO_2 m^{-2} s^{-1})	100	1.32 (0.3) a A	0.70 (0.4) a A	0.99 (0.6) a A
	45	0.80 (0.3) b A	0.60 (0.3) a A	0.61 (0.3) a A
	22	0.41 (0.20) b A	0.47 (0.14) a A	0.87 (0.22) a B
	10	0.40 (0.2) b A	0.34 (0.3) a A	0.60 (0.4) a A
F_v/F_m ratio	100	0.64 (0.09) a A	0.66 (0.02) a A	0.74 (0.003) a B
	45	0.71 (0.07) ab A	0.77 (0.04) b A	0.77 (0.02) ab A
	22	0.78 (0.01) b A	0.79 (0.02) b A	0.80 (0.03) b A
	10	0.79 (0.01) b A	0.80 (0.02) b A	0.80 (0.01) b A

*See Table 4 for abbreviated parameter meanings.

The proportion of explained variance due to light effect on all the photosynthetic parameters examined was greater than that due to species effect (Table 4). The light × species interaction effect was found for only light-saturated maximum photosynthetic rate (A_{max}) and light saturation estimates (LSE). In *C. odorata* and *T. superba*, there was a trend of higher

$A_{\max}$ values with increasing light availability (P = 0.0001 and 0.0001), respectively. In contrast, there was no distinct trend of higher $A_{\max}$ with increasing light availability for *M. altissima* (P = 0.512). Between species comparisons showed that *C. odorata* had higher $A_{\max}$ than either *M. altissima* or *T. superba* at 22% sunlight, whereas, *T. superba* had the lowest mean $A_{\max}$ at 10% sunlight. This pattern of $A_{\max}$ response to the light treatments in seedlings of *C. odorata*, *M. altissima* and *T. superba* were similar to the pattern of LSE response to light. For instance, *C. odorata* and *T. superba* grown at 100 and 45 % sunlight had greater mean LSE than *M. altissima* grown at 100 and 45 % sunlight, and furthermore, *T. superba* showed the lowest LSE at 10% sunlight. In *C. odorata*, there was a trend of lower light compensation point (LCP) and day respiration (R_{day}) values with decreasing light availability (P = 0.005 and 0.002), respectively. In contrast, there was no distinct trend of lower LCP and R_{day} values with decreasing light treatments in *M. altissima* (P = 0.765 and 0.580), respectively and *T. superba* (P = 0.427 and 0.576), respectively (Table 5). The relationship between PPFD and net photosynthetic rate, A of *C. odorata*, *M. altissima* and *T. superba* is shown in Fig. 1a–d. In general, A was more variable in sun-grown foliage than in shade-grown foliage of the three species.

A plot of mean $A_{\max}$ versus mean NAR showed that *C. odorata* and *T. superba* had a strong linear relationship between mean $A_{\max}$ and mean NAR (Figs. 2a and 2c), respectively, whereas *M. altissima* showed a less strong linear relationship between mean $A_{\max}$ and mean NAR (Fig. 2b).

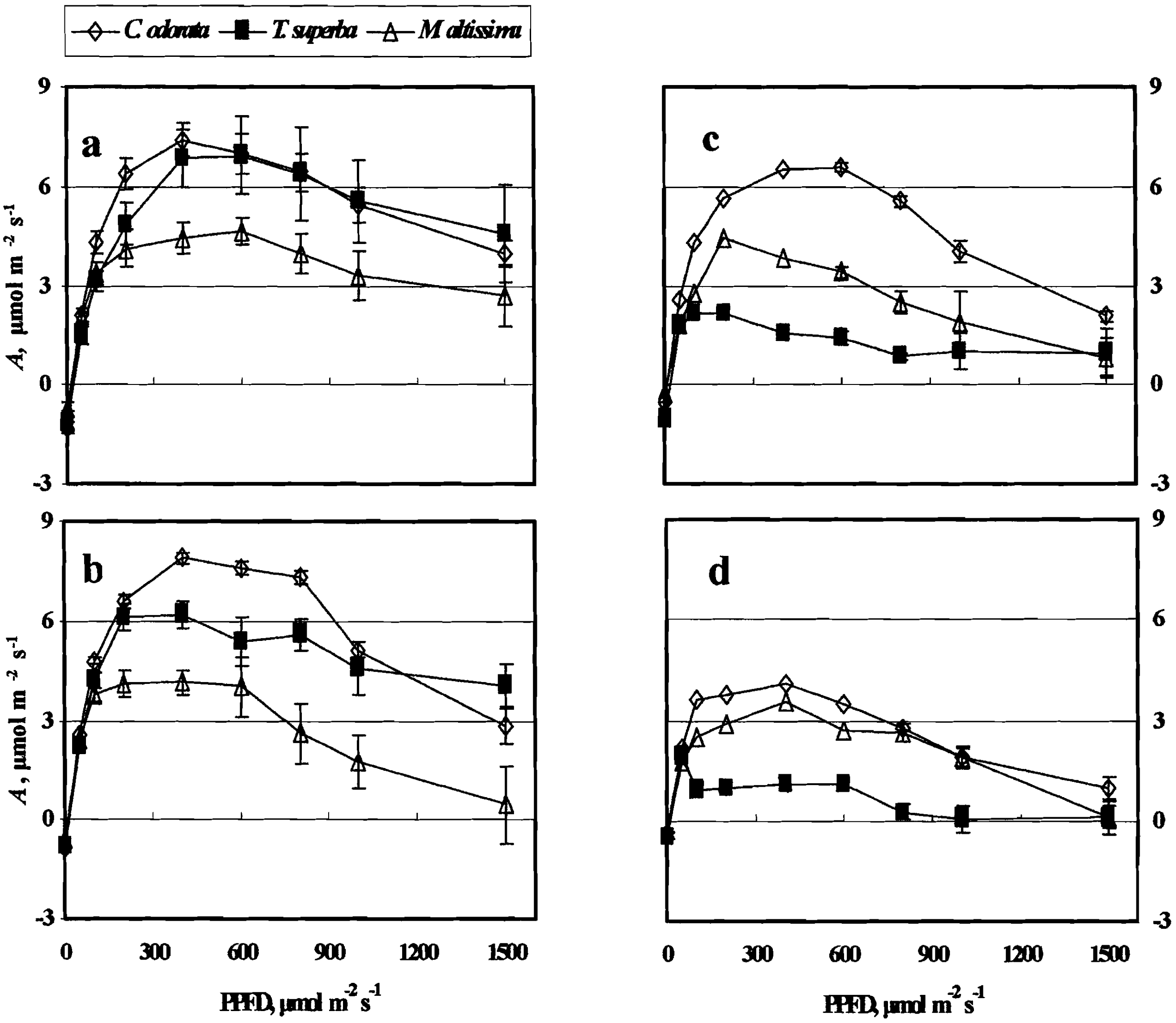

Fig. 1. Relationships between photosynthetically active photon flux density (PPFD) and net photosynthetic rate (*A*, µmol m^{-2} s^{-1}) of *C. odorata* (open diamonds); *M. altissima* (open triangles); and *T. superba* (closed squares) at open site (a), 45% sunlight (b), 22% sunlight (c) and 10% sunlight (d) respectively. Bars indicate 1SE of mean (*n* = 4 plants). Some SEs are too small to display.

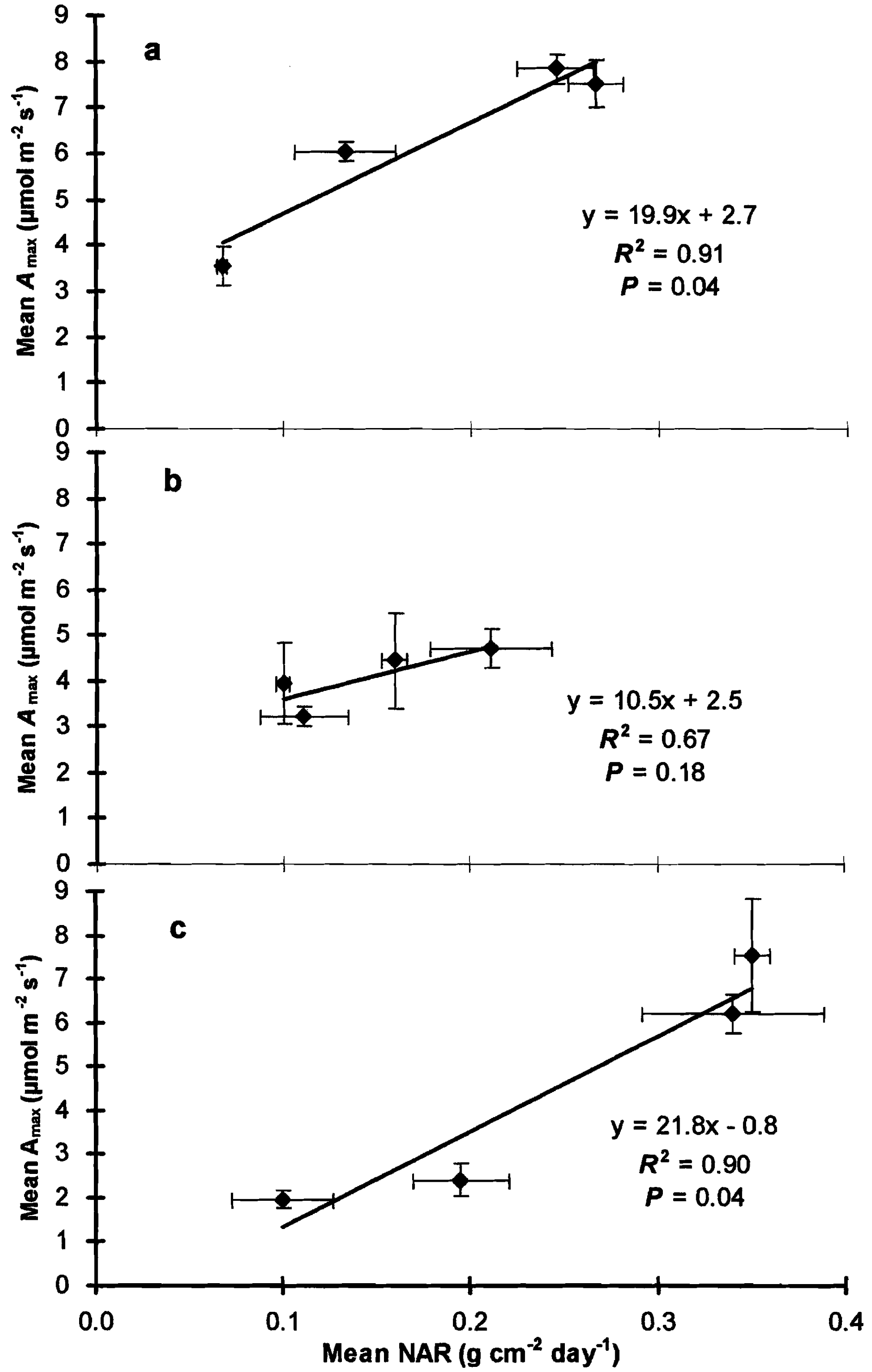

Fig. 2. Linear relationship between mean light-saturated maximum photosynthetic rates (A_{max}) and mean net assimilation rate (NAR) for the light treatments of *C. odorata* (a), *M. altissima* (b), and *T. superba* (c), respectively. Vertical and horizontal bars at each point indicate 1SE of mean (n = 4 plants) for A_{max} and NAR, respectively.

4.3.5. Transpiration, stomatal conductance and water use efficiency

Generally, transpiration rate (*E*), stomatal conductance (g_s) and water use efficiency (WUE) responses to PPFD in the open-site (Fig. 3a, c and e) were higher than in shade conditions (10% sunlight, Fig. 3b, d and f).

For the plants grown under open-site conditions, *C. odorata* had significantly higher *E* than either *M. altissima* or *T. superba* at all the PPFDs tested (Fig. 3a). Within species, there was a distinct trend of lower stomatal conductance (g_s) values with increasing PPFD in only *C. odorata* ($P = 0.0001$). In contrast, *T. superba* and *M. altissima* did not show distinct trend of lower g_s with increasing PPFD ($P = 0.965$ and 0.467), respectively (Fig. 3c). However, g_s did not differ significantly among the three species at any of the PPFDs tested at the open site. At the open site, *T. superba* had significantly higher water use efficiency (WUE) than either *C. odorata* or *M. altissima* at PPFDs > 200 µmol m^{-2} s^{-1} (Fig. 3e).

E, g_s and WUE responses of plants grown at 10% sunlight contrasted sharply with the same responses of open site-grown plants. For example, from PPFDs of 200–800 µmol m^{-2} s^{-1} both *C. odorata* and *M. altissima* showed higher *E* and g_s responses than *T. superba* (Fig. 3b, d), respectively. At PPFD >200 µmol m^{-2} s^{-1}, *C. odorata* and *M. altissima* displayed an almost constant WUE, whereas *T. superba* showed a sharp decline in WUE from 600 µmol m^{-2} s^{-1} with significant reduction at 1000 µmol m^{-2} s^{-1} (Fig. 3f).

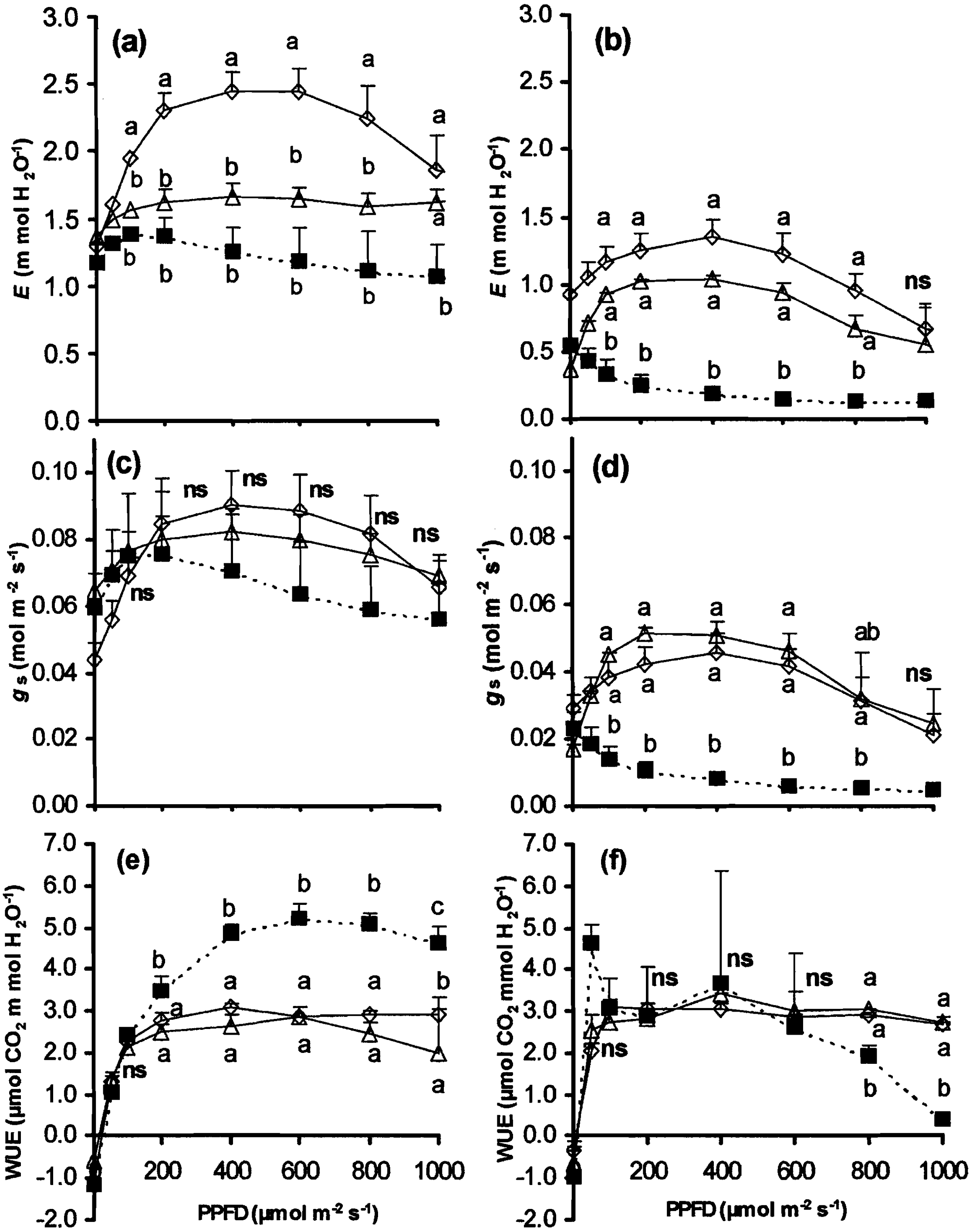

Fig. 3. Relationships between photosynthetically active photon flux density (PPFD) and transpiration rate (*E*, mmol H_2O^{-1}), stomatal conductance (g_s, μmol m^{-2} s^{-1}) and intrinsic water use efficiency (WUE, μmol CO_2 mmol H_2O^{-1}) of *C. odorata* (open diamonds); *M. altissima* (open triangles); and *T. superba* (closed squares) at open site (a, c and e) and 10% sunlight (b, d and f), respectively. Lower case letters indicate significant differences between species at the selected PPFD levels.

4.4. Discussion

4.4.1. Seedling size and morphology

At final harvest, *M. altissima* seedlings grown at extremes of the light treatments (i.e., 10 and 100% sunlight) had significantly reduced biomass, whereas *C. odorata* and *T. superba* had reduced biomass at only 10% sunlight. All the three species had the highest biomass at 45% sunlight. Nursery seedlings are susceptible to environmental stresses, such as strong radiation loads, when planted in the open (Ang, 1991; Iwasa et al., 1994) or in limited light environment (Poorter and Hayashida-Oliver, 2000), which can cause stunted growth and may result in seedling mortality. Another probable reason is that the plants were only in the exposed site for a relatively short period and thus, they had not fully acclimated to 100% sunlight. Some important features of pioneer trees are that they are light-demanding, intolerant of shade and have capacity for rapid growth, especially in height when young (Richards, 1996). However, studies with some tropical tree seedlings have shown that under intermediate light conditions (25–50% sunlight), many species, including pioneers, attained their highest values of biomass and stem length (Agyeman, 1994), which may be due to optimal light for seedling growth at those light conditions irrespective of species' successional status at the seedling stage.

Leaf mass ratio (LMR) and root mass ratio (RMR) of the three species was consistent with findings from (Agyeman, 1994; Poorter, 1999) confirming that allocation patterns of the plants were generally typical of those observed along a light gradient. However, stem mass ratio (SMR) response to light availability was specie-specific. For example, at 22 and 45% sunlight, *C. odorata* had a greater SMR than either *M. altissima* or *T. superba* probably due to larger allocation to stem growth to enhance the species' light competitive advantage at those shade treatments. In addition, SLA was sensitive to low light condition (10% sunlight) in all the species probably due to the fact all the species are light demanders. High SLA resulting from the production of thin leaves may allow seedlings to harvest light more effectively at low irradiance (Loach, 1970). However, the longevity of such thin leaves can not be guaranteed as they may be prone to herbivory (Coley, 1983). On the contrary, low SLA is associated with adaptation that increases leaf life-span. For this reason, the pioneer species, *T. superba* was probably superior in this trait at higher light conditions.

Hence, in the pioneer species, SLA showed a trade-off with leaf longevity at higher irradiance.

4.4.2. Whole-plant C assimilation

Differences in species' successional status revealed differences among the species in RGR at low light (10% sunlight). *Mansonia altissima* had a greater RGR than *T. superba* at 10% sunlight. *Cedrela odorata* and *T. superba*, both described as high light-demanding species, displayed a trend with peak RGR at 45% sunlight after which it declined slightly at 100% sunlight (P = 0.003 and 0.025, respectively). Observed differences in RGR response to light availability may be explained by underlying patterns in LAR and NAR. With an increase in light, NAR increases, basically due to an increase in photosynthesis per unit leaf area. At the same time LAR decreases, however, resulting in a simulation of RGR far less than expected from the photosynthetic rate values (Poorter and Van der Werf, 1998). *C. odorata* and *T. superba* followed this trend. Furthermore, some studies have shown that a decrease in LAR may be due to a decrease in SLA (Pons, 1977; Hunt and Halligan, 1981). This relationship was observed for all species in this experiment. However, unlike that reported by Poorter and Van der Werf (1998), there was a trend of higher LMR values with decreasing light availability for *C. odorata* (P = 0.0001), *M. altissima* (P = 0.007) and *T. superba* (P = 0.033) .

4.4.3. Photosynthetic traits and NAR responses vis-à-vis specie's successional status

Generally, at higher irradiances, net photosynthetic rate responses to PPFDs were higher in *C. odorata* and *T. superba* than *M. altissima*, whereas at lower irradiances, *C. odorata* and *M. altissima* showed a higher response than *T. superba*. Our observation that the trend of higher A_{max} values with increasing light treatments of *M. altissima* was not distinct (P = 0.512) partly fit the specie's description as a non-pioneer light demander. Further support for this classification is the less strong linear relationship between mean A_{max} and mean NAR (R^2 = 0.67, P = 0.18) and similar relative growth rates at all the light treatments of *M. altissima*. Agyeman (1994) reported that *M. altissima* had almost same RGR at 10, 27, 42 and 65% sunlight and attributed this to the fact that the species has a high plasticity in different irradiances. These findings from *M. altissima* contrasted sharply with those found for *C. odorata* and *T. superba* at intermediate light and high light, respectively. At 45 and 22% sunlight, $A_{\max}$ of *C. odorata* was significantly greater than $A_{\max}$ of *M. altissima*, whereas at 100% sunlight, $A_{\max}$ of *T. superba* was significantly greater than $A_{\max}$ of *M. altissima*. In addition, the strong linear relationship between mean $A_{\max}$ and mean NAR for *C. odorata* ($R^2 = 0.91$, $P = 0.04$) and *T. superba* ($R^2 = 0.90$, $P = 0.04$) indicated that differences in leaf photosynthetic rates of the species were reflected in their NAR (Poorter and van der Werf, 1998), which ultimately affected their relative growth rates at the different light availabilities. Hence from 10–100% sunlight, light availability increased, which led to increased photosynthetic rates and consequently, an increased NAR for *C. odorata* and *T. superba*.

Higher light saturation estimates associated with strongly light-demanding species in open-grown than shade-grown plants (Lüttge, 1997) were well exhibited in *C. odorata* and *T. superba*, but not in the non-pioneer species, *M. altissima*. This might have reduced the ability of *M. altissima* to obtain maximum rates of net photosynthesis at higher irradiances. Generally, high growth potential of strong light-demanding species might require high whole-plant or leaf respiration rates, traits that might be disadvantageous in low light. In this study, lower R_{day} per unit leaf area in shade-grown (10% sunlight) than open-grown *C. odorata* are similar to values quoted by Walters and Reich (1999) for intermediate tropical evergreen species. Thus, *C. odorata* exhibited a trade-off in the lowest light condition by replacing unused growth potential with low R_{day}. Although light compensation point (LCP) showed a decreasing trend at lower irradiances in only *C. odorata* ($P = 0.005$), *T. superba*, the pioneer species, showed larger maximum LCP values at irradiances $\leq$ 22% sunlight than *C. odorata* and *M. altissima*. This finding was consistent with those reported by Walters and Reich (1999) for intolerant tropical evergreen species at 4–12% light.

4.4.4. Short-term acclimation of seedlings to open site and shade (10% sunlight)

Foliar acclimation of the photosystem II (PS II) may be mechanisms by which plants can survive under high and low light conditions. Open-grown plants of *T. superba* had significantly greater photochemical efficiency (F_v / F_m) than open-grown plants of either *M. altissima* or *C. odorata*, suggesting that open-grown foliage of *C. odorata* and *M. altissima* are more susceptible to photoinhibition than *T. superba*. Within species, F_v / F_m was lower in sun-grown foliage than in shade-grown foliage, suggesting that quantum yield increased in shade-grown foliage, thereby allowing more efficient energy transfer from chlorophyll to PS II (Groninger et al., 1996).

At low PPFD (200 µmol m^{-2} s^{-1}) and moderate PPFD (400 µmol m^{-2} s^{-1}), sun-grown *C. odorata* had greater transpiration rate (*E*) than either sun-grown *M. altissima* or *T. superba* and this contributed to the reduced water-use efficiency (WUE) in leaves of *C. odorata* at those tested PPFDs. In this study, WUE was measured under standardized conditions (see Materials and methods) and thus it represents an intrinsic WUE. At high PPFD (1000 µmol m^{-2} s^{-1}), *T. superba* grown at 100% sunlight had the lowest mean *E*, which enhanced greatly, the species' water-use efficiency. The lowest *E* and highest WUE at high irradiance for *T. superba* is an indication of the species' ability to acclimate better than *C. odorata* and *M. altissima* to high light conditions. With respect to mean WUE, the decreasing order of acclimation to higher irradiances among the species at the open site was *T. superba* > *C. odorata* > *M. altissima*. Stomatal conductance (g_s) of open-grown foliage of the three species were not significantly different at the irradiances tested, which suggest a common range of PPFDs to which stomatal conductance can acclimate in these three species. This finding contrasted with Maruyama et al. (2005), who reported interspecies differences in g_s among open-planted seedlings. In open-grown seedlings of *C. odorata*, *E* was limited by g_s at high irradiance. Hence, for the same mean VPD, open-grown *C. odorata*, which generally had a higher transpiration rate than open-planted *M. altissima* and *T. superba*, avoided excessive water loss at higher PPFDs by reducing its stomatal conductance. The apparent low WUE of *C. odorata* and *M. altissima* compared to *T. superba* at the PPFDs tested suggest that *C. odorata* and *M. altissima* may require more water than *T. superba* in order to maintain their growth during establishment stage in open fields. In addition, plants in high light are faced with high radiation loads. Therefore, they invest in root mass in a way that compensates for higher transpiration losses by water uptake (Poorter, 1999; Poorter and Hayashida-Oliver, 2000). At final harvest, a similar trend was observed, with *T. superba* showing larger root system (RMR and root length) than *C. odorata* and *M.*

altissima at high light > 45% sunlight, indicating a competitive advantage of *T. superba* in terms of soil water uptake from deeper parts of the pots.

In contrast, at the same tested PPFDs, shade-grown (10% sunlight) *T. superba* was inferior to either *C. odorata* or *M. altissima* in E, and g_s responses. Furthermore, as a consequence of the lowest net photosynthetic rate displayed by *T. superba* at 10% sunlight, it followed that intrinsic mean WUE was lowest for the species at 10% sunlight particularly at high PPFD of 1000 μmol m^{-2} s^{-1}.

4.5. Conclusion and implications for reforestation efforts in forest gaps and/ or exposed sites

The hypothesis that *C. odorata*, an intermediate successional species and *T. superba*, a pioneer species will exhibit a stronger linear relationship between mean maximum photosynthetic rates and mean net assimilation rate than *M. altissima*, a non-pioneer as light availability increases, was supported by the results obtained in this study.

Physiological traits such as A_{max}, LSE and NAR and RGR formed the basis for biomass production in the shade and open site conditions of *C. odorata*, *M. altissima* and *T. superba* seedlings. Similar accounts have been reported for *Terminalia* species (Fasehun, 1980) and *C. odorata* (Walters and Reich, 1999; Dunisch et al., 2002). At the lowest light condition (10% sunlight), all the species had reduced biomass production and showed a trend of greater biomass production with increasing light. However, *M. altissima*, showed a significant biomass reduction at 100% sunlight, making the species less suitable for planting in exposed sites when compared to the other two species. At 10% sunlight, *T. superba* showed the lowest A_{max} and LSE, and at 100% sunlight, it showed a tendency to attain maximum A_{max} and LSE, a trait typical of most pioneers. Consequently, *T. superba* had the lowest RGR at 10% sunlight, and highest NAR at 100% sunlight, although that did not lead to a higher RGR at this light level. In contrast, *C. odorata* showed a superior A_{max} and LSE at 22% sunlight (intermediate light conditions) when compared to either *T. superba* or *M. altissima* and RGR generally increased with light availability. *M. altissima*, tolerated shade (10% sunlight) through enhanced RGR, but leaf-level photosynthetic traits were not significantly influenced by the different light treatments. In addition, it was found that a high LAR (LMR + SLA) is an advantage at lower light environments, such as in smaller forest gaps, where interception of light is of primary importance. However, as light increases a high NAR is more important, to enable plants to fully benefit from a high photosynthetic capacity. Inferences made from intrinsic WUE, F_v / F_m and root length and RMR of open-grown plants indicated that short-term acclimation to high light was generally superior in *T. superba*, but at low light the same species was inferior when compared to the other two species. In addition, this may also imply that in exposed sites where conditions of low soil water availability may arise from high rates of evapo-transpiration, species such as *T. superba*, which displayed a high intrinsic WUE, may be more suitable for open-field planting. This is because they have the capacity for smaller water lost per unit of carbon gained.

Screening tropical tree species for successful reforestation programs in degraded forest sites with different canopy sizes is important. In this study, treatment differences accounted only for variance in light associated with different canopy gap sizes. However, under actual tropical field conditions, temperature, water and nutrient variation associated with different gap sizes may play an equal or greater role in determining actual growth response. Light availability, biomass allocation and physiological characteristics play a critical role in determining the success of seedlings in enrichment planting or plantation. 10% sunlight-grown seedling may be considered more efficient than sun-grown seedling in using low levels of light because of lowered mean values of light compensation point and higher photochemical efficiency (Lüttge, 1997; Walters and Reich, 1999). In addition, shade condition may suppress shade-intolerant competitors (Lieffers and Stadt, 1994) and reduce moisture and temperature stress. However, these stated advantages may be offset by decreased biomass production and shifts in allocation patterns at 10% sunlight. From this study, the implications for reforestation efforts in African tropics are that *T. superba* may have greater potential in plantation forestry at exposed sites; *C. odorata* has broad ecological amplitude making it suitable for planting in medium gaps and exposed sites; *M. altissima* may be suitable for enrichment planting in smaller gaps.

CHAPTER 5.

Growth responses to fertilization in seedlings of *Cedrela odorata* and *Terminalia superba* grown under varied irradiance

5.1. Introduction

Intensive nursery cultivation of quality and commercially important tropical timber seedlings, capable of establishing in the field during early stages of planting, is important in plantation forestry (Evans and Turnbull, 2004), especially in African tropics where efforts are currently being made to establish plantation forests in abandoned pasture and degraded forest sites. *Cedrela odorata* and *Terminalia superba* are among the major species selected for reforestation efforts in Ghana (Ghana Forestry Commission (GFC), 2002). However, information about the two species' responses to nutrient availability at the seedling stage when grown in a shaded or open nursery, typical of planting conditions in large clearings, remains inadequate.

Some potential reforestation sites in degraded semi-deciduous forest of Ghana are characterized by soils, which are naturally low in fertility (Hall and Swaine, 1981; JAFTA, 1999b). In addition, traditionally produced seedlings from GFC nurseries are grown, usually on forest soil with no additional fertilizer (Brong-Ahafo Regional Forestry Manager, *pers. comm.*). Nursery seedlings are susceptible to environmental stresses when planted in the open, and as a result, their growth may be impaired compared to those planted under shade (Ang, 1991). Enhanced growth and survival of *C. odorata* seedlings have been reported when grown in shade conditions in agroforestry systems (Navarro et al., 2004). *T. superba* seedlings are also known to thrive best in medium to large gaps (Hawthorne, 1995). Nitrogen (N) and phosphorus (P) fertilization have been reported to have a great effect on biomass production and hence, improve seedling growth and quality (Mexal and Landis, 1990; Webb et al., 2000). In another study, fertilization had a minor influence on seedling morphology of *C. odorata* but shade had a strong influence on the seedling morphology in a nursery (Mexal et al., 2002). The level of light required for optimal growth is species-specific and differences in growth responses of tropical forest tree seedlings to light and nutrient inputs could be influenced by their successional status (Thompson et al., 1992; Reich et al., 1995). Some typical features of pioneer species and light-demanding trees are that they have capacity for rapid growth, especially in height when young (Richards, 1996). Pioneers tend to exhibit higher plasticity than non-pioneers in biomass allocation patterns in different irradiances (Pons, 1977; Swaine and Whitmore, 1988).

The ability of open-field-grown seedlings to adjust physiologically to high irradiance exposure may indicate a species acclimation to high light (Powles, 1984), which may enhance reforestation success in the field. Chlorophyll, which contains high amounts of nitrogen, is a major component of the light harvesting complex of photosystem II (PSII) (Lüttge, 1997). Since PSII has been shown to acclimate to changing light conditions (Mitchell, 1998; Griffin et al., 2004), it may imply that nitrogen supply is important for light acclimation process (Lüttge, 1997). The ratio of variable fluorescence to maximum fluorescence (F_v/F_m) of dark-adapted leaves is used commonly to assess the relative state of PSII. F_v/F_m is often used as an expression of photoinhibition, which results from excess irradiance (Critchley, 1998; Kitao et al., 2000). In addition, species tolerant to high light are able to maintain high concentration of chlorophyll when grown in a high light environment (Griffin et al., 2004). However, I am not aware of the effect of nutrient additions on F_v/F_m at high light for juvenile seedlings of *C. odorata* and *T. superba*. The influence of site resources (i.e., nutrient and light) on tropical tree seedling growth responses is important for screening potential species for reforestation success (Veenendal et al., 1996; Dunisch et al., 2002).

Hence, the objective of study in this chapter was to compare and contrast early morphology and foliar physiological responses of *C. odorata* and *T. superba* seedlings to nutrient addition in either shade or open site condition using basic growth data, chlorophyll fluorescence and foliar nutrient concentration and relative chlorophyll content.

5.2. Materials and methods

5.2.1. Plant material and study species

Seeds of *Cedrela odorata* L. and *Terminalia superba* Engl. and Diels were collected from the Brong-Ahafo Regional Forest Services Division's nursery in a semi-deciduous forest zone of Ghana. 120 seeds of *C. odorata* and *T. superba*, respectively, were then sown in plastic containers filled with equal proportions of forest soil, peat and river sand on April 27, 2004. The seeds were germinated under natural conditions at the University of Miyazaki forest nursery under partial shading from a tree. Germination percent (%) recorded for *C. odorata* (21 days after sowing) and *T. superba* (32 days after sowing) was 67% and 51%, respectively. At two-month, in age, approximately 85 and 80% of the germinated seedlings of *C. odorata* and *T. superba*, respectively, survived. From June 28–30, 33 individuals of similar height from each species were sampled from the surviving two-month-old seedlings and then transplanted into individual plastic pots (19.5 cm in length and 17.5 cm in diameter) containing a 2:1:1 mixture of forest soil and river sand and peat by volume ratio, respectively. Organic-based mixtures were used because they are light and possess good texture, water holding-capacity and nutrient retention (Pancel, 1993). Mixtures with peat are commonly used in Africa for some container-grown tropical tree seedlings (Napier and Willan, 1983). The pot used for the experiment is comparable to medium sized pot used in Agyeman (1994), which did not show adverse effect of pot-binding for fast growing species.

Cedrela odorata is described as a moderate to fast growing intermediate species (Poorter, 1999; Maruyama et al., 2005), whereas *T. superba* is described as a late seral pioneer species, which grow less fast when compared with typical rain-forest pioneers such as *Cercropia* and *Musanga* species (Richards, 1996). *T. superba* may dominate middle stages of secondary succession. At maturity, *C. odorata* and *T. superba* can attain 30–40 m and 40–60 m in height, respectively (Pancel, 1993). More information about the species is outlined in Table 1, chapter 4 of this thesis.

5.2.2. Growth conditions and experimental design

The study was conducted in a nursery at the Agricultural experimental field center of the University of Miyazaki, Miyazaki in the southern Japan (32° N, 131° E). Aluminum metal frame, 2.0 m square and

1.8 m high, was draped with black polypropylene shade fabric. A similar frame without shade fabric served as 100% sunlight treatment. Mean irradiance in the shaded frame measured at several points with a luminance meter (MINOLTA T-1M) on clear skies/ sunny days (2004/7/29, 2004/8/6 and 2004/8/19) was 33% sunlight. This moderate light (33% sunlight) measured in the shade frame was considered to be within the range of shade conditions for most pioneers and light demanding species in nurseries of the tropics (Evans and Turnbull, 2004). A 30-cm gap was left between the bottom of the shade fabric and the ground to permit free air movement (Loach, 1967). Frames were about 1.8 m apart to reduce possible treatment overlap. During the experimental period, maximum and minimum air temperatures at the site were 37.1°C and 11.0°C, respectively. The mean relative humidity and mean monthly rainfall were 76.1% and 448.3 mm, respectively (Miyazaki Meteorological Agency, 2004). Mean air temperatures varied less than 1.0°C between the unshaded and shaded frame. Mean relative humidity was 1.0% higher in the shaded frame.

The transplanted seedlings were conditioned under a partial shade for 8 days after which they were moved into the shade frames on July 9, 2004. Before the start of treatment, an initial random sampling of five seedlings each of *C. odorata* and *T. superba* was conducted from the 33 two-month-old seedlings. Therefore, the study design for the remaining plants were 2 nutrients × 2 sunlights, which included seven seedlings of each species randomly located within each frame. Half of each of these sets of pots was fertilized, and the other half (non-fertilized) was kept as a control. A 6:10:5 (N_2: P_2O_5: K_2O) Hyponex nutrient solution plus trace elements were applied to each pot at an increasing rate as the plant grew. Initially, nutrient solution was applied bi-weekly at a rate of 100 ml per pot using a diluted 0.2% nutrient concentration from July 8–August 4. Subsequently, from August 5–19, the nutrient solution applied to the pots was increased to 200 ml, and from August 20–September 25, the nutrient solution applied was further increased to 250 ml bi-weekly at the same nutrient concentration. The control pots were supplied with equal quantity of water each time the nutrient solution was administered to the fertilized pots. Additional water, when necessary was supplied to the pots, especially those in 100% sunlight to prevent the occurrence of water stressed condition. The potting mix allowed free draining of excess nutrient solution from the pots. The weight equivalent of N, P and K applied to each fertilized pot over the treatment period was 1.20, 0.91 and 0.86g, respectively. The pots were periodically repositioned to prevent shading and to randomize any effect of position across all pots within each frame. Each pot was placed at least 30cm from the edge of the shade frame. The experiment period, July 10 to October 28, represented summer and fall climatic conditions of Miyazaki, which are suitable for growing tropical trees.

5.2.3. Morphological measurements

Five plants per species harvested at transplant (before treatment) were measured for seedling height (H), leaf area (LA) and dry mass of each organ (Table 1).

Table 1. Size and dry mass of plant organs of the transplants (two-month in age) used in this study. The values represent mean (1SE) of five plants per species.

Species	Seedling height (cm)	Total leaf area (cm^2)	Leaf mass (g)	Stem mass (g)	Root mass (g)	Total plant mass (g)
C. odorata	16.9 (2.5)	71.2 (4.5)	0.19 (0.07)	0.15 (0.01)	0.07 (0.02)	0.41 (0.13)
T. superba	6.5 (1.0)	21.0 (2.0)	0.05 (0.01)	0.02 (0.01)	0.01 (0.00)	0.08 (0.02)

Five plants per species per treatment were randomly sampled and destructively harvested on October 28 and 29, 2004. Leaves and stems (including branches) were separated, and the potting mix washed gently from the roots. Leaf area of each plant at initial and final sampling was measured using leaf area meter (LI-3000A, Li-Cor Inc., Lincoln, NE). Plant components were then oven-dried for at least 48 h at 70°C and cooled in a desiccator for dry mass determination. Total plant mass consisted of leaves, stems and roots. From the final plant harvest, the following variables were derived; leaf mass ratio (LMR, leaf mass per unit plant mass g g^{-1}); stem mass ratio (SMR, stem mass per unit plant mass g g^{-1}); root mass ratio (RMR, root mass per unit plant mass g g^{-1}); leaf area ratio (LAR, leaf area per unit plant mass cm^{2} g^{-1}) and specific leaf area ratio (SLA, leaf area per unit leaf mass cm^{2} g^{-1}).

To determine the relative height growth rate (RHGR), all the seven plants/species/treatment from the two frames were measured for initial and final height using the formula given by Hunt (1978) as follows:

$$\mathrm{RGR} = (\ln H_2 - \ln H_1)\,(t_2 - t_1)^{-1} \tag{1}$$

where H_2 and H_1 represent plant height at the start and end of treatment, respectively; t_2 and t_1 represent time (days) at the start and end of treatment, respectively.

Foliar nutrient analysis was conducted on the oven-dry leaves. The leaves were first milled in a steel mill followed by calcinations at 480°C in a stove and final dissolution of the ash in 0.5 M HCl. Phosphorus (%P g^{-1} dry mass) was determined colorimetrically with the blue ammonium molybdate method, using ascorbic acid as reducing agent (Ricker et al., 2000). Separately, nitrogen (%N g^{-1} dry mass) was determined by acid digestion, according to the micro-Kjeldahl and ammonium molybdate method (Ricker et al., 2000). In order to reduce laboratory cost, leaf samples from each species and treatment were mixed to produce three samples per treatment combination.

5.2.4. Chlorophyll fluorescence measurements

Three healthy seedlings per species without symptoms of leaf damage were selected for measurement from the seven seedlings in each of the treatment combination. Maximal quantum yield of PSII (F_v/F_m) was then measured on two attached and fully developed leaves/ seedlings/ species/ treatment with pulse amplitude modulated fluorimeter (Mini-PAM, Heinz Waltz, Effeltrich, Germany). A miniature 8 V/20 W halogen lamp (Bellaphot, OSRAM) served as internal light source for actinic illumination. A saturation light pulse (SLP) induces maximal fluorescence yield, F_m, and maximal variable fluorescence, F_v, such that also $\Delta F/F_m = F_v/F_m$ is maximal. After 30 min dark-adaptation of the leaves, F_v/F_m was measured at standard measuring intensity of 0.15 µmol m^{-2} s^{-1}; modulation frequency of 0.6 kHz and a 0.8 s SLP over 1800 µmol m^{-2} s^{-1} PAR applied to the leaf. Chlorophyll fluorescence measurements were conducted after sun set on October 3, 2004.

5.2.5. Chlorophyll content estimates using SPAD−502

A rough, non-invasive and rapid estimation of relative chlorophyll content was made on several points of the leaf blade of same leaves used for chlorophyll fluorescence measurements. The mean values of the six leaves from each species and treatment were used for statistical analysis. Hand-held chlorophyll meter, SPAD−502 (Minolta camera Co., Osaka, Japan) was used to estimate Chl content. Measurements were done in the morning (8–10 am) for higher Chl sensitivity as recommended by Guiamet and Martínez (2004). The SPAD-502 estimates Chl content index in 'SPAD units'. It can also provide an objective value of leave greenness compared to a subjective visual rating (SPAD-502 operating manual), and monitoring foliar nitrogen (Ladha et al., 1998; Ommen et al., 1999). Chl content estimation was conducted on the same day of chlorophyll fluorescence measurement.

5.2.6. Data analysis

Shade treatment was not replicated due to logistical constraints. For each species, plant size and morphological data at the last sampling and foliar responses were subjected to a two factorial ANOVA analysis, with sunlight and nutrient being the variable factors. One-way ANOVA was done for the sunlight and nutrient treatment combination where a significant effect was detected by the two-factorial ANOVA. Multiple pair-wise comparisons of means were performed with LSD test for mean separation. All statistical tests were performed using SYSTAT® 10.2 (Statistical software Inc., Richmond, CA, USA). Significant differences are reported as $P < 0.05$, $P < 0.01$ and $P < 0.001$.

5.3. Results

5.3.1. Plant size

In *C. odorata* and *T. superba*, nutrient had a much stronger influence on the size parameters (H, LA and total plant mass) than sunlight (Table 2). Hence, in both species, plants from fertilized pots had larger H, LA and total plant mass than plants from non-fertilized pots (control) irrespective of the sunlight treatments (Figs. 1A–C). In the control pots of *C. odorata* and *T. superba*, there was no significant difference in total plant mass between the 100 and 33% sunlight treatment, respectively, (Fig. 1C). However, in the fertilized pots, plant mass of *C. odorata* was significantly greater at 100 than 33% sunlight treatment (Fig. 1C). H and LA response to the treatment combinations in the two species were similar (Figs. 1A and 1B), respectively.

Table 2. Results of a two-way ANOVA applied to final plant size parameters and allocation to organs and morphological traits rate of *C. odorata* and *T. superba*. Degrees of freedom (*df*) and the ANOVA *F*-values representing levels of significance ($P > 0.05$, ns; $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***) are shown.

Source of variation	*df*	H	LA	Total plant mass	Leaf mass	Stem mass	Root mass	RHGR†	LAR	SLA	LMR	SMR	RMR
C. odorata													
Nutrient	1	63.4***	149.5***	259.5***	203.0***	143.8***	77.1***	46.4***	0.8 ns	32.8***	16.6**	6.3*	62.7***
Sunlight	1	26.3***	3.2 ns	34.8***	10.2**	20.5**	27.5***	15.0**	47.0***	88.5***	10.7**	0.4 ns	10.7**
Nutrient X Sunlight	1	0.3 ns	0.3 ns	37.7***	14.2**	20.7***	27.3***	0.2 ns	0.1 ns	0.3 ns	0.7 ns	0.4 ns	3.1 ns
Residual	16												
Total = [(2x2x5)-1]	19												
T. superba													
Nutrient	1	54.82***	34.0***	19.9***	16.6**	28.2***	13.1**	66.9***	0.01 ns	10.5**	0.02 ns	17.1**	9.1**
Sunlight	1	33.20***	1.9 ns	0.3 ns	0.02 ns	0.04 ns	3.4 ns	22.3***	19.7***	100.0***	3.3 ns	9.1**	28.2***
Nutrient X Sunlight	1	0.1 ns	1.0 ns	1.9 ns	1.7 ns	0.5 ns	4.1 ns	3.8 ns	0.1 ns	1.0 ns	0.1 ns	1.3 ns	0.4 ns
Residual	16												
Total = [(2x2x5)-1]	19												

†*n* = 7 plants: (*df* for Nutrient, Sunlight, Nutrient × Sunlight, Residual and Total = 1, 1, 1, 24 and 27, respectively).

Abbreviations used are as follows: Height (H; cm), Leaf area (LA; cm^2), and Relative height growth rate (RHGR; cm cm^{-1} day^{-1}), leaf area ratio (LAR; cm^2 g^{-1}), Specific leaf area (SLA; cm^2 g^{-1}), leaf mass ratio (LMR; g g^{-1}), stem mass ratio (SMR; g g^{-1}) and root mass ratio (RMR; g g^{-1}).

5.3.2. Plant organs and allocation

In C. *odorata*, leaf mass, stem mass and root mass were significantly influenced by nutrient and sunlight treatments, whereas in *T. superba*, the mass of plant organs were influenced only by nutrient treatment (Table 2). Hence, plants from the fertilized pots of both species had larger mean mass of plant organs than plants from the control pots at 100 and 30% sunlight (Figs. 1D–F). In the control pots of *C. odorata* and *T. superba*, the mass of all plant organs were not significantly different between 100 and 33% sunlight treatment. In the fertilized pots of *C. odorata*, the mass of all plant organs from the 100% sunlight-grown seedlings were larger than the mass of all plant organs from the 33% sunlight-grown seedlings. On the other hand, in the fertilized pots of *T. superba*, the mass of all plant organs from the 100 and 33% sunlight were not significantly different (Figs.1D–F).

In the fertilized pots of *C. odorata*, 33% sunlight-grown plants had greater LMR than 100% sunlight-grown plants, whereas no significant difference in LMR was observed for *T. superba* in all the treatments (Fig. 1G). Plants in the fertilized pots of *T. superba* grown at 33% sunlight had larger SMR than plants in the fertilized pots of *T. superba* grown at 100% sunlight, whereas no significant difference in SMR ratio was observed for *C. odorata* grown at the same treatment combination (Fig. 1H). Plants in the control pots of *T. superba* grown at 100% sunlight had larger RMR ratio than plants in the control pots of *T. superba* grown at 33% sunlight, whereas no significant difference in RMR ratio was observed for control plants of *C. odorata* grown at 100 and 33% sunlight (Fig. 1I). However, with fertilization, RMR of both species were greater at 100 than 33% sunlight.

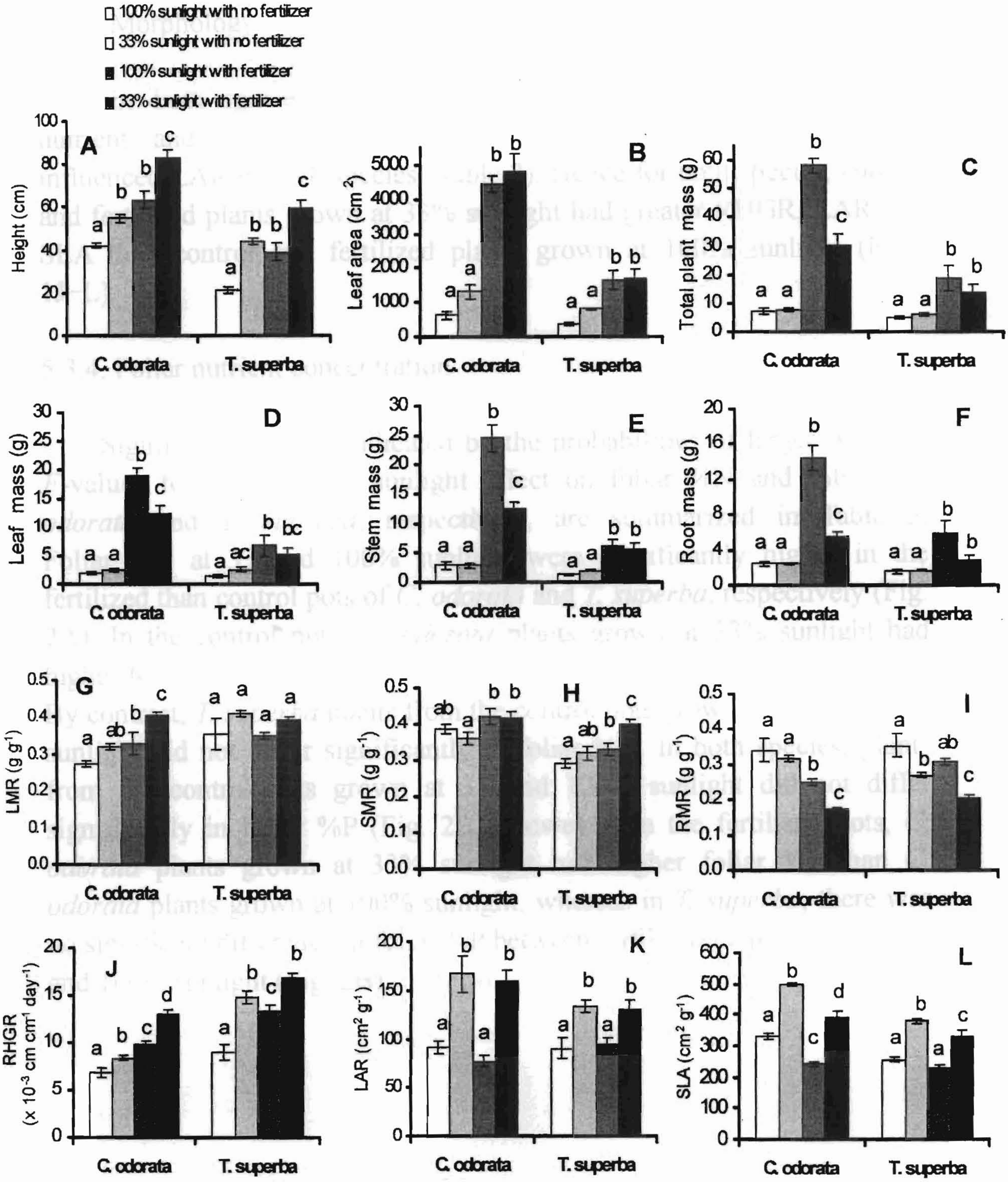

Fig. 1. Plant size, dry mass of plant organs, allocation and morphology of *C. odorata* and *T. superba* grown at 100 and 33% sunlight with or without fertilizer, respectively. Bars with different lettering indicate significant difference among the four treatment combinations for each species, respectively, (ANOVA, $P < 0.05$; $n = 5$).

5.3.3. Morphology

For both species, RHGR and SLA were strongly influenced by nutrient and sunlight treatments, whereas only sunlight treatment influenced LAR of both species (Table 2). Hence for both species, control and fertilized plants grown at 33% sunlight had greater RHGR, LAR and SLA than control and fertilized plants grown at 100% sunlight (Figs. 1J–L).

5.3.4. Foliar nutrient concentration

Significance levels, indicated by the probabilities of larger ANOVA *F*-values for nutrient and sunlight effect on foliar %N and %P of *C. odorata* and *T. superba*, respectively, are summarized in Table 3. Foliar %N at 33 and 100% sunlight were significantly higher in the fertilized than control pots of *C. odorata* and *T. superba*, respectively (Fig. 2A). In the control pots, *C. odorata* plants grown at 33% sunlight had higher foliar %N than *C. odorata* plants grown at 100% sunlight (Fig. 2A). By contrast, *T. superba* plants from the control pots grown at 33 and 100% sunlight did not differ significantly in foliar %N. In both species, plants from the control pots grown at 33 and 100% sunlight did not differ significantly in foliar %P (Fig. 2B). However, in the fertilized pots, *C. odorata* plants grown at 33% sunlight had higher foliar %P than *C. odorata* plants grown at 100% sunlight, whereas in *T. superba,* there was no significant difference in foliar %P between fertilized plants grown at 33 and 100% sunlight (Fig. 2B).

Table 3. Results of a two-way ANOVA applied to foliar nitrogen (%N) and phosphorus (%P) concentration of C. odorata and T. superba. Degrees of freedom (*df*) and the ANOVA F-values representing levels of significance ($P > 0.05$, ns; $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***) are shown.

Source of variation	*df*	%N	%P
C. odorata			
Nutrient	1	57.0***	1.1 ns
Sunlight	1	12.0**	0.9 ns
Nutrient X Sunlight	1	0.1 ns	5.9*
Residual	8		
Total = [(2x2x3)-1]	11		
T. superba			
Nutrient	1	142.5***	11.6**
Sunlight	1	0.8 ns	0.0 ns
Nutrient X Sunlight	1	0.8 ns	0.2 ns
Residual	8		
Total = [(2x2x3)-1]	11		

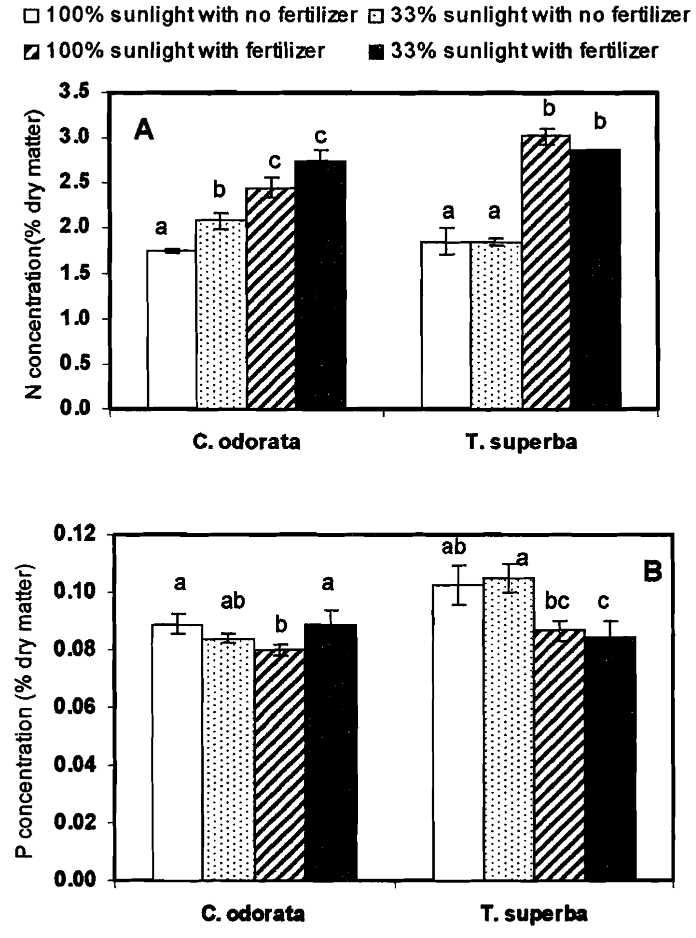

Fig. 2. Foliar nitrogen (N) and phosphorus (P) concentration (% dry mass) of *C. odorata* and *T. superba* grown at 100 and 33% sunlight with or without fertilizer, respectively. Bars with

different lettering indicate significant difference among the four treatment combinations for each species, respectively, (ANOVA, $P < 0.05$; $n = 5$).

5.3.5. Foliar Chl estimate and chlorophyll fluorescence

Significance levels, indicated by the probabilities of larger ANOVA F-values for nutrient and sunlight effect on Chl content and F_v/F_m ratio of *C. odorata* and *T. superba*, respectively, are summarized in Table 4. At 100 and 33% sunlight treatments, fertilized plants of both species had higher Chl content than control plants, respectively (Fig. 3A). In the control pots of both species, only *C. odorata* showed significantly higher Chl content at 33 than 100% sunlight (Fig. 4A).

Plants from fertilized pots of *C. odorata* had higher F_v/F_m ratio than plants from control pots at 100 and 33% sunlight treatments, respectively (Fig. 3B). On the other hand, in *T. superba*, plants from fertilized pots had significantly higher F_v/F_m ratio than plants from control pots at only 33% sunlight treatment.

Table 4. Results of a two-way ANOVA applied to relative Chl content and F_v / F_m ratio of *C. odorata* and *T. superba*. Degrees of freedom (*df*) and the ANOVA F-values representing levels of significance ($P > 0.05$, ns; $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, ***) are shown.

Source of variation	*df*	Chl Content	F_v/F_m ratio
C. odorata			
Nutrient	1	77.99***	7.99*
Sunlight	1	11.11**	20.10***
Nutrient X Sunlight	1	13.48**	0.48 ns
Residual	20		
Total = [(2x2x6)-1]	23		
T. superba			
Nutrient	1	10.64 **	51.46***
Sunlight	1	0.02 ns	0.01 ns
Nutrient X Sunlight	1	0.66 ns	0.30 ns
Residual	20		
Total = [(2x2x6)-1]	23		

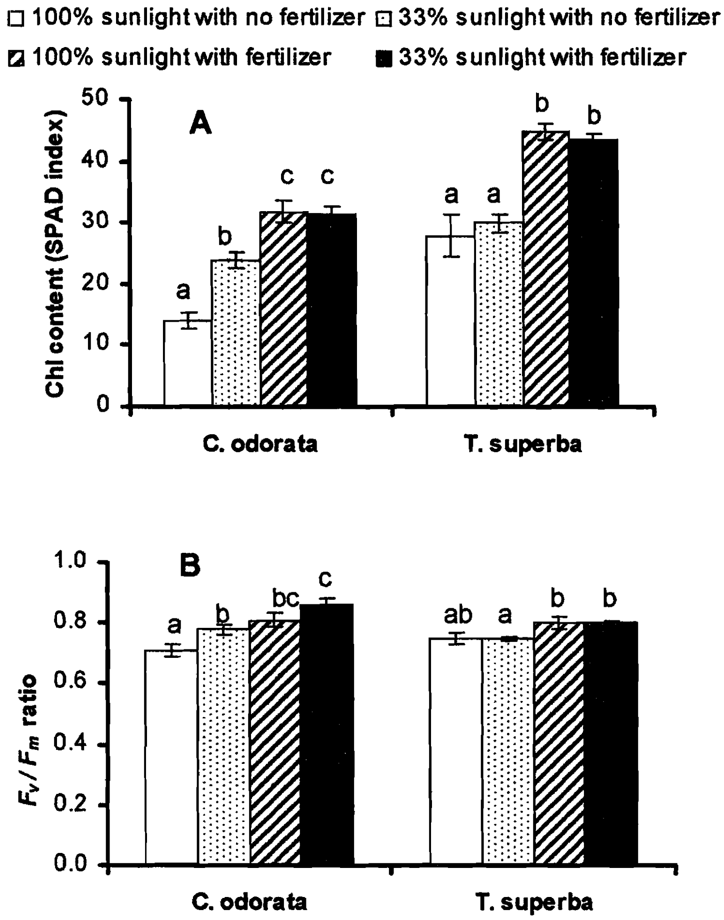

Fig. 3. Relative Chl content and F_v / F_m ratio of *C. odorata* and *T. superba* grown at 100 and 33% sunlight with or without fertilizer, respectively. Bars with different lettering indicate significant difference among the four treatment combinations for each species, respectively, (ANOVA, $P < 0.05$; $n = 5$).

5.4. Discussion

5.4.1. Seedling growth traits as influenced by light and nutrient availability

Greater biomass production as a result of nutrient enhancement was clearly demonstrated by *C.odorata* and *T. superba* at 100 and 33% sunlight. This reflected in the greater seedling height, leaf area and dry mass of plant organs of the fertilized plants in both species when compared to the control plants (Fig. 1). Contrary to our expectation, greater biomass in *T. superba*, a late seral pioneer species, was not evident when compared to C. *odorata* at 100% sunlight. At the initial sampling before treatment (i.e., transplant stage), mean total plant mass of *C. odorata* was greater than *T. superba* by a 5-fold (Table 1). However, at the last sampling (i.e., final harvest), when mean total plant mass of both species are compared in the control pots, *C. odorata* was again greater than *T. superba* by a 1.3-fold (Fig. 1C). This suggests that, at 100% sunlight with no fertilization, growth of the plants, especially the pioneer species (*T. superba*), was suppressed. This may be attributable to severe environmental conditions such as high temperatures and strong heat from direct solar radiations, which affected the species growth. Another probable reason was that the plants were in the exposed site for a relatively short period and thus, they had not fully acclimated to 100% sunlight. However, in both species, fertilized plants grown at 100% sunlight showed a trend of larger biomass production than fertilized plants grown at 33% sunlight, though not statistically significant for *T. superba* ($P = 0.35$; Fig. 1C). Alternatively, without fertilization, 33% sunlight may provide favorable light environment for the nursery seedlings' growth. Studies with some tropical tree seedlings have shown that under intermediate light conditions (25–50% sunlight), many species, including pioneers, attained their highest values of biomass and stem length (Poorter, 1999). Height and leaf area of plants in the control pots of both species exhibited superior growth at the expense of total biomass in the 33 than 100% sunlight treatment (Figs. 1A and 1B). This finding is consistent with other studies, which showed that, in shade conditions, photosynthate allocation patterns favor shoot elongation and chlorophyll production and hence, increase light-harvesting capabilities (Walters et al., 1993).

Plant responses to light in biomass partitioning followed the trends described for sun and shade plants (Osunkoya et al., 1994; Poorter, 1999). Under shade conditions, light interception per unit plant biomass for light-demanding species, is increased by an augmented biomass allocation to leaf material. Hence, LMR of *C. odorata* grown at 33% sunlight with fertilization was significantly higher than 100% sunlight-grown with or without fertilizer (Fig. 1G). Superior SMR was observed for *T. superba* at 33% sunlight with fertilization, whereas *C. odorata*, an intermediate successional species, displayed superior SMR at both 100 and 33% sunlight with fertilization (Fig. 1H). RMR responses followed patterns generally characteristic of plants growing in sun and shade conditions, and was influenced by nutrient availability depending on the species. For instance, *T. superba*, a pioneer species, had higher biomass allocation to root at 100% sunlight compared to 33% sunlight irrespective of fertilization (Fig. 1I). In contrast, *C. odorata* showed higher RMR at 100 than 33% sunlight only in the fertilized pots (Fig. 1I). Generally, higher plasticity in LMR is commonly associated with pioneers (Campbell and Grime, 1989). However, the mean LMR and RMR values suggest that trends of plasticity of both species were similar by nutrient addition.

The two species showed superior relative height growth rate (RHGR) at 33% sunlight with fertilization. However, *T. superba* displayed a trend of higher mean RHGR than *C. odorata* at 100 and 33% sunlight, especially with fertilization (Fig. 1J). Reductions in SLA in response to exposure to full sun have been observed in pioneers and intermediate successional species of the tropics (Poorter, 1999; Maruyama et al., 2005). *C. odorata* and *T. superba* showed significant reductions in SLA at 100% sunlight irrespective of nutrient addition. High SLA resulting from the production of thin leaves which was observed in foliage grown at 33% sunlight, may allow seedlings to harvest light more effectively at lower irradiances (Loach, 1970). At 100% sunlight, *T. superba* had lower mean SLA than *C. odorata* probably due to the observed thicker leaves of the former species. Leaf area ratio (LAR) is known to be the result of parallel patterns in LMR and SLA (Veneklaas and Poorter, 1998). Hence, light interception per unit plant biomass may be increased through high LMR and SLA and conversely, reductions in SLA and / or LMR may lead to a reduction in LAR.

5.4.2. Foliar responses: physiological significance for acclimation to sun and shade conditions

At leaf level, nitrogen is needed for the synthesis of chlorophyll. However, at the whole plant level, nitrogen is also needed to promote growth and nutrient uptake (Bauer and Bazzaz, 2000). As expected, fertilization significantly enhanced foliar %N of *C. odorata* and *T. superba*, respectively (Fig. 2A). However, without fertilization, foliage of *C. odorata* from the shade treatment had 19% higher N concentration than foliage from 100% sunlight. This may be attributed to the fact that generally, shade and/ or shade-grown tropical plants have larger total N-contents in their biomass (Lüttge, 1997). Foliar %P responses of two species to the treatments were different. *C. odorata* and *T. superba* showed maximal foliar %P at 33% sunlight with fertilization and 33% sunlight without fertilization, respectively (Fig. 2B). There is a wide diversity in the ability of tropical tree species to (presumably) acquire and use P under the same edaphic and climate conditions (Webb et al., 2000).

Generally, shade plants have higher chlorophyll content in their leaves (Lüttge, 1997), which enhance the efficiency of the light harvesting complex of the photosystems. Chl content of *T. superba* was higher than *C. odorata*, probably due to the naturally thicker leaves (low SLA) of the former. At the 100% sunlight, Chl content was 97% greater in foliage of *T. superba* than *C. odorata* in the control pots (Fig. 3A). However, with nutrient additions at this high light, *C. odorata* gained more Chl and the difference in Chl content between the two species was reduced to 40% (Fig. 3B). Hence, at 100% sunlight with fertilization, acclimation of *C. odorata* to this light condition was improved and subsequently, growth of the species was enhanced.

Plants subjected to high-irradiance stress, typically have lower F_v/F_m values than non-stressed plants (Björkman and Demmig, 1987) and N concentration decreases plant susceptibility to photoinhibition (Naidu and DeLucia, 1997). In this study, N fertilization significantly enhanced F_v/F_m of *C. odorata* at 100% sunlight (Fig. 4B) and therefore reduced the susceptibility of *C. odorata* to photoinhibition at high irradiance. At 100% sunlight, control plants compared to fertilized plants of *C. odorata*, displayed symptoms associated with high irradiance stress such as leaf bleaching and this might have contributed to the lower F_v/F_m values recorded in this light level. By contrast, in *T. superba*, there was no statistical difference in F_v/F_m between the control and fertilized plants at 100% sunlight. A possible reason may be that physiologically, *T. superba* acclimated to full solar irradiance faster than *C. odorata* and thus, the expected effect of nitrogen fertilization enhancing F_v/F_m was not well expressed in the species. Peterson et al. (1999) reported similar findings for sweet gum seedlings, which showed similar F_v/F_m values in low and high fertilizer treatments when grown at full sunlight. Higher F_v/F_m of the shade-grown foliage of *C. odorata* is an indication of higher quantum yield, which allow for efficient energy transfer from chlorophyll to photosystem II (Groninger et al., 1996).

5.5. Conclusion and implications for reforestation efforts in African tropics

Variations in the effect of light and/ or nutrient additions on growth traits of *C. odorata* and *T. superba* seedlings were evident. The apparent smaller total plant mass of seedlings, especially, in *T. superba* at 100% sunlight without nutrient additions may be due to strong light and heat stress at this light level. However, at 100 and 33% sunlight, RHGR differed between the two species, with the pioneer species (*T. superba*) showing superior seedling RHGR at both light treatments, especially in the fertilized pots. Allocation to leaf and root followed patterns typical of plants growing in sun or shade conditions; and that mean root mass ratio was greater in fertilized plants of *T. superba* than fertilized plants of *C. odorata* at 100% sunlight.

At 100% sunlight, fertilization enhanced F_v/F_m of *C. odorata* and thus, reduced its susceptibility to photoinhibition. In addition, N fertilization enhanced relative Chl content of both species at the two light treatments, and at 100% sunlight, Chl content of *C. odorata* was significantly reduced in the control pots. Whole-plant growth and leaf Chl synthesis competed for available nutrients and this was probably, influenced by the specie's nutrient uptake ability. Foliar N concentration of both species was higher in the fertilized than control pots, whereas P concentration response to the light-nutrient treatments was species-specific.

Evaluating morphological and physiological traits of nursery-grown seedlings under varied light and nutrient availability is important in screening tropical tree species for reforestation programs. This study suggests that while F_v/F_m and relative Chl content at the leaf level may detect early onset of environmental stresses in plants, growth traits may provide more information on seedling suitability for planting in the fields where site resources (i.e., light and nutrient supply) are not uniform. Superior biomass production in fertilized plants grown at 100% sunlight suggests that *C. odorata* and *T. superba* may be suitable for planting in large clearings and/ or exposed sites. However, enhancing acclimation of the transplants through longer hardening periods plus nutrient additions in an exposed nursery conditions may stimulate their initial growth when planted in the field.

CHAPTER 6.

General conclusion and recommendations for forest management in Ghana

Tropical forests are an important economic and environmental resource that are diminishing world-wide due to unsustainable exploitation of economically important tree species, encroachment of agriculture and other land-use pressures. As a consequence, there is increasing concern to conserve remnant natural forests and to develop reforestation programs for the production of forest products, including commercial timbers on previously cleared and/or degraded lands. Hence, experimental research that examines environmental influences on the establishment and growth of seedlings of tropical tree species are extremely necessary for successful reforestation efforts. Controlled experiments in this thesis have provided further information on the eco-physiological characteristics of several economically important tree species, which are native or exotic to Ghana. Thus, this study was an attempt to fill information gaps, which are vital in screening the selected tree species for reforestation efforts in Ghana.

In examining the effects of soil water availability on dry matter production and allocation of two African mahogany (*Khaya*) species, it was found that that seedlings of *K. anthotheca* have considerable potential for use in plantation forestry in the humid tropics of Africa, which has a wide rainfall distribution range and distinct drought periods (4–5 months). Seedlings of *K. ivorensis* on the other hand, are more suited for planting on moist site (low-lying areas with 1500 – 1750 mm of annual precipitation) as demonstrated by the species ability to grow best in wet condition. A further study on responses of three other species to drought conditions revealed that drought tolerance and drought-avoiding adaptations often vary for different species. *C. siamea* and *K. senegalensis*, which show xerophytic traits, are more drought resistant than *C. odorata*, a mesophytic species. This assertion was reflected in the higher sensitivity of *C. siamea* and *K. senegalensis* to the transpiration and chlorophyll fluorescence parameters, and the root-shoot growth characteristics under the drought conditions. The ability of *C. siamea* and *K. senegalensis* to maintain relatively higher mass of foliage and/ or root growth under drought conditions confers greater advantage to them as plantation forestry species in semi-dry environments. In contrast, *C. odorata* followed a drought-avoiding strategy (greater leaf shedding) and was more conservative in its water use and hence displayed restricted growth under drought conditions. Thus, the mesic species, *C. odorata*, may not be a suitable choice for plantation in dry sites.

In examining early growth and photosynthetic responses to light in seedlings of *C. odorata*, *M. altissima* and *T. superba*, this study indicated that light availability, species successional status, biomass allocation and physiological characteristics (i.e., light-saturated maximum photosynthetic rates, net assimilation rate, photochemical efficiency, transpiration and water-use efficiency) play a critical role in determining the success of seedlings in enrichment planting or plantation. Conclusion drawn from this study was that *T. superba* may have greater potential in plantation forestry at exposed sites; *C. odorata* has broad ecological amplitude making it suitable for planting in medium gaps and exposed sites; *M. altissima* may be suitable for enrichment planting in smaller gaps in partially logged forests.

Furthermore, in determining growth responses to fertilization in seedlings of *Cedrela odorata* and *Terminalia superba* grown under varied irradiance, the following conclusions were drawn: (1) Growth traits, such as biomass production and allocation, species level of plasticity and foliar responses may provide more information on seedling suitability for planting in the fields where site resources (i.e., light and nutrient supply) are not uniform. (2) Adequate supply of nutrients to seedlings in a nursery and/or during establishment stage in field planting may enhance production of quality seedling for reforestation success.

C. odorata and *T. superba* are recommendable for planting in large clearings and/ or exposed sites of Ghana, where forest have been classified as "mostly degraded" or ear-marked for plantation development. However, enhancing acclimation of the transplants through longer hardening periods plus nutrient additions in an exposed nursery conditions may stimulate their initial growth when planted in the field.

This work may serve to fill information gaps, which are needed for screening the studied species for successful reforestation schemes in Ghana. However, further research work in growth, survival and physiological traits of the experimental species needs to be done in degraded forest and exposed sites earmarked for reforestation schemes in Ghana. This will allow testing experimental results from controlled conditions against actual field situation for more conclusive results to be drawn on the experimental species for reforestation purposes.

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