

LEAF FUNCTION IN TROPICAL RAIN FOREST CANOPY TREES

THE EFFECT OF LIGHT ON LEAF MORPHOLOGY AND
PHYSIOLOGY IN DIFFERENT-SIZED TREES

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THE EFFECT OF LIGHT ON LEAF MORPHOLOGY AND
PHYSIOLOGY IN DIFFERENT-SIZED TREES

TOON RIJKERS

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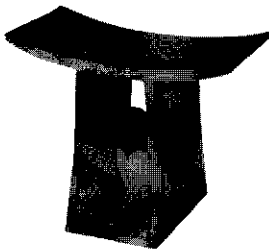
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Saramaca woodcarving, Suriname and French Guiana.

Stool with individual motifs each having a symbolic meaning; e.g. the artistic plaiting without edging means everlasting ties.

Abstract

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In this thesis the effect of constant and fluctuating light availability on several leaf traits was studied for naturally growing trees of different sizes, *i.e.* from sapling to adult canopy tree, of five species in a tropical rain forest in French Guiana. Leaf acclimation responses were examined throughout the life time of leaves in order to evaluate whether leaves can profit from these adjustments in terms of carbon gain. The five species, arranged in order from most shade-tolerant to pioneer, were: *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis*, *Pourouma bicolor* spp. *digitata*, and *Goupia glabra*.

For *Duguetia*, *Vouacapoua*, *Dicorynia* and *Goupia*, it was shown that tree height and light availability had independent effects on photosynthesis and other features of leaf function. Direction and magnitude of the variation in leaf variables tended to be similar among species. The morphological variable leaf mass per unit area seemed to be a key variable as it determined most of the variation in other leaf variables.

The time needed to increase the photosynthetic rate (up to 90% of its capacity) to a sudden increase in light was between 7 to 11 min for shade and sun growing saplings of *Vouacapoua*, *Dicorynia* and *Pourouma*. The readiness to exploit the next lightfleck was substantial in these plants as the induction loss was moderate to low, except in gap saplings of *Dicorynia*. The time needed to reach 75% of the maximum carboxylation efficiency (V_{cmax}) was used to separate the relative importance of biochemical and stomatal limitation during the time course of photosynthetic induction.

The mean leaf life span of different-sized trees for *Vouacapoua* was 61 months (range 27-101) and for *Dicorynia* 32 months (range 17-54). The variation in traits in response to leaf age was low. Photosynthetic capacity and nitrogen concentration were relatively constant with time; leaf mass per unit area increased during the first 18 months. Simulations of the daily carbon gain with a low- and highlight regime showed differences among both sun- and shade-leaves and trees of different sizes. Leaf construction cost was independent of leaf life span. Leaf payback time was relatively short (4 to 40 days); it was constant during a wide range of irradiance, but increased sharply in a narrow range of low light. The rate of net return on carbon investment was slow in long-lived leaves of *Vouacapoua*. Leaf mass per area decreased with increased leaf life span, which could be explained by a light- and height-dependent selection pressure for leaf life span and leaf mass per area.

The integration of the results with those at higher organisation levels, such as branch and tree crown, is briefly discussed, and the applicability in silvicultural systems in which light is manipulated to enhance growth and production of timber species is evaluated.

Key-words: tropical rain forest, leaf morphology, photosynthesis, leaf age, tree height, shade tolerance, leaf construction costs, leaf payback time, ecophysiology.

Voorwoord / Foreword

Laat ik beginnen te vertellen dat mijn in totaal veertien maanden durende verblijf in het tropisch regenwoud een zeer diepe indruk heeft gemaakt. De schoonheid, rust, maar ook het plotselinge omslaan naar complete chaos vanwege een opkomende storm of door een confrontatie tussen twee groepen apen zijn ongekend. De veelvoud aan vormen, kleuren, geuren en geluiden zijn in mijn geheugen gegrift. In de periode tussen mijn eerste veldtrip en het uiteindelijke resultaat dat hier voor u ligt is veel gebeurd. Dit heeft zich natuurlijk nooit tot een goed einde kunnen voltooien zonder de hulp van...

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Chapter 1

Introduction

Lowland rain forests are densely populated and largely evergreen, and generally contain a high plant and animal diversity. Given this richness in plant species, the question arises how these various species manage to coexist. How do they compete for the same, often limited, resources such as light, nutrients and water? Do species differ in their response to these resources? Several systems for categorising tropical tree species have been developed, in order to facilitate our understanding. The majority of these classifications are based upon the relationship between gap dependency and successful regeneration. That is, to what extent do species require forest canopy openings ('gaps'), and hence high light conditions, for seed germination and seedling establishment (e.g. Bazzaz 1979; Denslow 1980; Swaine and Whitmore 1988; Popma et al. 1992). Despite the effort to categorise, today only a few species are regarded as a distinct group sharing a common life history: the pioneer. With few exceptions, these species, which range from small short-lived to large long-lived species, are light-demanding and are dependent on gaps to complete every stage of their life cycle (Swaine and Whitmore 1988). Seeds from the rest of the tree species can germinate under forest shade, and are generally called non-pioneer or climax species (Whitmore 1989). Moreover, they are capable to tolerate shady forest environments in all or some stage(s) of their life cycle beyond germination. During their life span in a tropical rain forest, they are often exposed to several alternate phases of high and low light conditions (e.g. Clark and Clark 1992). These "shade-tolerant" tree species make up the most abundant class of species in the forest (Whitmore 1989; Clark and Clark 1992); and it is this group of shade-tolerant trees that this thesis focuses on. In the forest, trees mainly compete for light, water and soil nutrients. Since light exhibits the greatest variability both in time (e.g. sunflecks) and space (e.g. tree fall) (Kira and Yoda 1989; Chazdon and Fetcher 1984), it is generally assumed that in many rain forests, light has the strongest impact on growth and performance of plants.

A heterogeneous forest light environment: how to exploit it?

In a tropical rain forest the distribution of photosynthetically active radiation (PAR, 400-700nm) is highly variable on both a spatial and temporal scale. On a time scale, light conditions can alter within seconds, minutes up to months and

years (e.g. Chazdon 1988). On a spatial scale, light contrasts occur when gaps are formed or canopies develop (Van der Meer and Bongers 1996) or, on a smaller scale, among leaves within a tree crown (Ellsworth and Reich 1993). These variable light conditions have a strong influence on plant physiological and ecological processes (cf. Chazdon 1988). For example, short sunflecks affect photosynthetic rates and stomatal behaviour, and hence determine the leaf's photosynthetic induction state (Percy et al. 1994), whereas light availability on a scale of months or years may lead to different leaf morphology or tree architecture, respectively (Popma et al. 1992; Ackerly 1996; Sterck 1999).

Similar patterns are observed with respect to the spatial variation in available light. For example, at the leaf level light availability may affect photosynthetic capacity (Boardman 1977), whereas at the whole crown level it may affect self-shading among leaves and hence variation in nitrogen distribution (Pons et al. 1989; Ellsworth and Reich 1993). At the whole-plant level light conditions may influence tree architecture or biomass allocation (Bongers and Sterck 1998) and at the level of plant populations it may affect population growth and recruitment (Zagt and Werger 1998).

Depending on weather, solar path, and spatial and temporal distribution of the vegetation, forest light regimes make up a wide range of different habitats. At the forest floor in the understorey the photon flux density (PFD) is usually less than $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ and often less than 2% of full sunlight, whereas in gaps much higher light levels occur, although the total daily PFD usually does not exceed 50% of that received above the forest canopy (Chazdon and Fetcher 1984, Chazdon 1986). Forest canopies do not only alter the quantity of light but also its spectral composition (e.g. Endler 1993). Although light quality has an important influence on several plant processes (e.g. seed germination and morphogenesis), the scope of this thesis is on the effects of availability and quantity of light on physiological and morphological traits at the leaf level.

In general leaf adjustments to a changing light environment will be directed towards a maintained or enhanced total leaf carbon gain in the changed condition. This may be achieved by a change in the photosynthetic traits as well as by increasing or decreasing the life span in newly formed and possibly also in existing leaves (Chabot and Hicks 1982). Leaf acclimation to changing light environments may involve changes in photosynthesis and respiration (Pons 1977; Björkman 1981; Pons and Percy 1994), anatomy (Bongers and Popma 1988), leaf mass per area (LMA) (Raaijmakers et al. 1995), chlorophyll concentration (Poorter et al. 1995; Pons and Jordi 1996), chlorophyll a to b ratio (Evans and Seemann 1989) and concentration of ribulose biphosphate carboxylase (Rubisco) (Evans and Seemann 1989; Poorter and Evans 1998).

Leaves acclimated to low light usually exhibit a number of traits that distinguishes them from leaves exposed to high light. In low light, leaf adjustments are often directed towards an increase in the capacity of light absorption at the expense of photosynthetic capacity, and little carbon losses through respiration (e.g. Evans 1989; Pons and Pearcy 1994). As a result, shade leaves, when compared to sun leaves, usually are thinner, have a lower mass per unit area, a higher mass-based chlorophyll content and lower area-based rates of photosynthesis and dark respiration (Björkman 1981; Bongers and Popma 1988; Kitajima 1994). Sun leaves, on the other hand, can make more efficient use of the high irradiance for carbon gain via thicker leaves with more chloroplasts per unit area and a greater investment in compounds that determine photosynthetic capacity per unit leaf area (e.g. Rubisco and electron transport components) (Boardman 1977).

A number of arguments have been put forward to explain the adaptive value of variation in leaf life span among and within species (e.g. Chabot and Hicks 1982; Kikuzawa 1995). For plants growing in shaded environmental conditions net carbon gain generally is low. Therefore it is argued that shade plants should extend the life span of their leaves as this will increase the time period for photosynthesis and hence enables them to return or payback the initial construction costs of the leaves (Chabot and Hicks 1982; Williams et al. 1989).

In the forest understorey long periods of low light are often punctuated by sunflecks, when a direct light beam penetrates through openings in the forest canopy. Depending on duration and frequency of these sunflecks, a large fraction (up to 60%) of the daily carbon gain can be contributed to their utilization (Chazdon 1988; Pearcy et al. 1994). Sunfleck utilization for carbon gain depends on the rate of photosynthetic activation, the photosynthetic induction state, the capacity to continue with CO₂ fixation in low light immediately following a sunfleck and the extent of instantaneous photosynthetic rate (Pearcy 1990). Photosynthetic response to a sudden increase in light includes, among other things, light-activation of Rubisco and opening of stomata (Kirschbaum and Pearcy 1988b; Pons et al. 1992). Species may exhibit substantial differences in these photosynthetic induction characteristics. The degree of photosynthetic induction and loss determines the readiness of a leaf to respond to subsequent sunflecks.

It has been argued that species adapted to high-light environments (pioneers) are more flexible in response to variations in light conditions than shade-tolerants which often show little or no plasticity (Bazzaz 1979; Strauss-Debenedetti and Bazzaz 1996; but see Turnbull 1991; Popma et al. 1992 for the reverse). The greater phenotypic plasticity for pioneer species was

suggested to be related to the larger variation in environmental conditions of the gap habitats in which they grow, compared to the forest understorey.

Owing to, often, practical arguments, studies on acclimation responses to light are mainly restricted to seedlings and small saplings. Since shade-tolerant tree species have a great probability to encounter high-light conditions when growing to the forest canopy (Clark and Clark 1992), they need to adapt to these changing conditions. Therefore, one might expect these species to exhibit an increase in phenotypic plasticity with increasing tree height.

From small sapling to large canopy tree

When plants grow, changes in morphology and physiology may result from inherent ontogenetic development rather than from responses to the micro-environment (Poorter and Pothmann 1992; Coleman et al. 1994). An increase in tree height involves greater costs of support structures, such as twigs and branches (Givnish 1986; Bongers and Sterck 1998), roots and xylem vessels (Tyree and Ewers 1996) and stem material (Sterck and Bongers 1998). In addition, with increasing height the ratio of total leaf area to total non-photosynthetic living tissue declines substantially resulting in less foliage relative to total plant mass that has to provide sufficient assimilates for tree growth and maintenance. Consequently, the minimum amount of light necessary to balance carbon gain and loss should increase with height. Thus, in order to realize net growth a decrease in shade tolerance with increasing tree height is expected.

An increase in height in the forest is often accompanied with greater light availability, which has a beneficial effect on leaf net carbon gain. On the other hand, the changes in micro-climate along a vertical gradient in the tropical forest (Kira and Yoda 1989; Koop and Sterck 1994) may also result in sub-optimal conditions for leaf performance: mechanical stress (wind) and drought (wind, light). There is also the possibility of photoinhibition, however this usually causes a problem at extreme high light levels only. As a result, a different investment strategy in leaves might be expected in tall trees compared to small saplings. Strong wind, for example, can induce morphological and anatomical leaf changes, such as thickening of the cuticula and epidermis resulting in a higher leaf mass per area and formation of more and smaller stomata (see review by Jaffe and Forbes 1993).

Outline of thesis

From the foregoing it is apparent that canopy tree species cross a wide range of light conditions when they grow to the forest canopy. As a result growing trees continuously have to adjust their leaf properties to changing light environments in order to maximise carbon gain. The aims of this thesis are: [1] To examine the variation in photosynthetic and morphological responses of leaves to contrasting forest light environments, [2] To determine whether these leaf acclimation responses differ between tree species and between the different phases of one tree species' life cycle, and [3] To examine acclimation responses throughout the life time of leaves in order to evaluate the extent to which leaves can utilize these adjustments to enhance leaf carbon gain. In this thesis, the focus is on shade-tolerant trees as they form the majority of species in a tropical rain forest, and they have been studied in less detail than pioneer species. Naturally growing trees in the forest were selected ranging from saplings of about 1 m to canopy trees up to 55 m in height. The thesis was set up in close connection with an earlier PhD thesis conducted by Sterck (1997) in the Nouragues area. In that study, the effect of light on crown development was investigated in two economically valuable tree species that are also studied in this thesis, i.e. *Vouacapoua americana* and *Dicorynia guianensis*. Hence, I sought to select the same individual trees in the forest that were studied by Sterck (1997).

Chapter 2 deals with the variation in leaf physiology and morphology as dependent on light availability and tree height of four species that differ in shade tolerance. Particularly, I intend to discriminate between light and height effects on leaf traits. In chapter 3, the effects of brief periods of high light ('lightflecks') on photosynthetic carbon gain are analysed. For this, the photosynthetic induction responses to fluctuating light are investigated for saplings of three species, growing in gaps and under a closed forest canopy. Hereby attention is paid to quantification of the removal of biochemical and stomatal limitation during photosynthetic induction, and to evaluate inter- and intra-specific differences herein. In addition, for two species, it is investigated whether leaf age has an effect on the rate of photosynthetic induction. Age-related changes in photosynthetic capacity and leaf morphology, and the benefits of light- and height-oriented leaf adjustments for the daily net carbon gain are addressed in chapter 4. Chapter 5 analyses the variation in leaf life span for two species and examines whether photosynthetic and morphological leaf traits are associated with leaf life span. Furthermore, for trees growing in contrasting light environments it is evaluated whether the rate of return on carbon investment (i.e. leaf payback time) is affected by the leaf's life span.

Finally, in chapter 6, the main goals and results of this thesis are evaluated on the basis how they compare to results obtained from the preceding studies and by others. In addition, results obtained with the two earlier mentioned species *Vouacapoua americana* and *Dicorynia guianensis* at the leaf level (this thesis) as well as at higher organisational level (e.g. branch, crown; Sterck 1997), are briefly discussed with respect to the application in silvicultural systems in which light is manipulated to enhance growth and production of timber species.

Tree species of study and research site

The complete names of the study species and some of their morphological characteristics are shown in Table 1. The species are arranged in order from most shade-tolerant to light demanding. They are all timber species, except *Pourouma bicolor* spp. *digitata*. *Duguetia surinamensis* is locally extracted, while the others are harvested by logging companies. *Vouacapoua americana* and *Dicorynia guianensis* serve as the leitmotif in this thesis. *Duguetia surinamensis* and *Goupia glabra* produce plagiotropic (horizontal) branches from the seedling phase, whereas in the other species plagiotropic branching occurs later in the life cycle. All species have single leaves, except *Vouacapoua americana* and *Dicorynia guianensis*, which have compound leaves (with 5-13 and 5-7 leaflets per leaf, respectively). In all species the repeated growth unit or constructional unit is a metamer (i.e. internode, leaf and bud), except in *Vouacapoua* which forms an extension unit (i.e. a vegetative axis consisting of a sequence of metamers) (see also Sterck 1999).

Table 1. List of species, morphological data and reference to the chapter in which they were studied.

Species	Family	Classification	Height max.(m)	Leaf shape	Shoot growth (seedling stage)	Chapter
<i>Duguetia surinamensis</i> R.E.Fr.	Annonaceae	shade-tolerant	30	simple	plagiotropic	2
<i>Vouacapoua americana</i> Aubl.	Caesalpiniaceae	shade-tolerant	45	compound	orthotropic	2,3,4,5
<i>Dicorynia guianensis</i> Amshoff	Caesalpiniaceae	shade-tolerant	55	compound	orthotropic	2,3,4,5
<i>Pourouma bicolor</i> Mart. spp. <i>digitata</i> Trec.	Cecropiaceae	shade-tolerant	35	simple	orthotropic	3
<i>Goupia glabra</i> Aubl.	Celastraceae	pioneer (long-lived)	55	simple	plagiotropic	2

The study was carried out at the Nouragues field station ($4^{\circ}05'N$, $52^{\circ}40'W$) (Fig.1). The station is named after a group of Amerindians who abandoned the area about two centuries ago. It was established in 1987 by the Centre National de Recherche Scientifique (C.N.R.S.) in France. The mean annual precipitation is about 3,000 mm which is distributed over about 310 days (Grimaldi and Riéra in press). There is a distinct dry period in September and October, although the limits of the dry season may vary between July and December (Fig. 2). The annual average temperature is approximately $26^{\circ}C$.

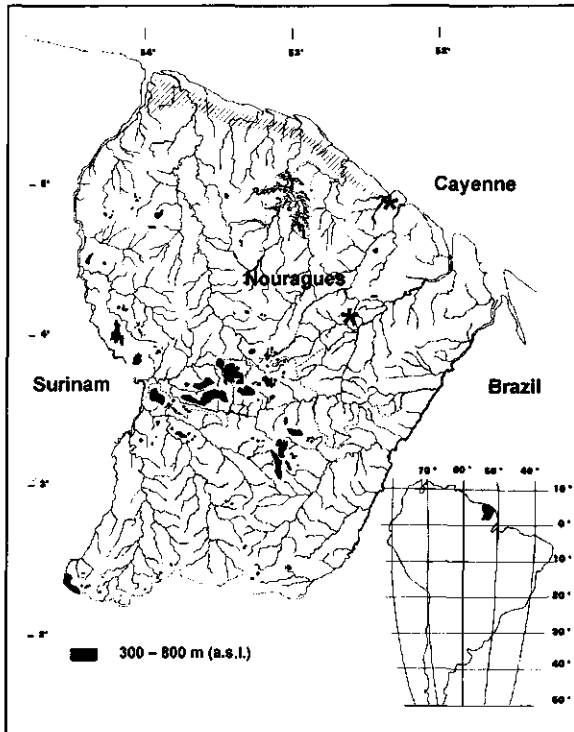


Fig. 1. Map of French Guiana and location of the Nouragues field station.

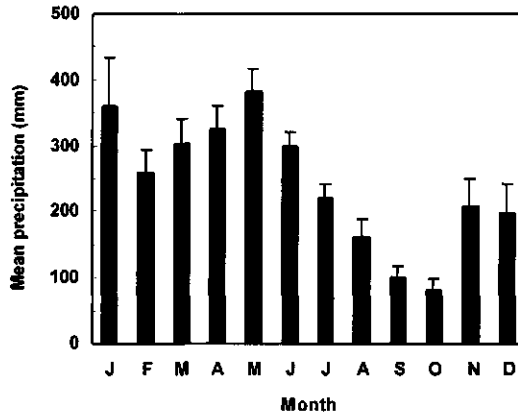


Fig. 2. Mean monthly precipitation (\pm SE) recorded at the Nouragues field station over the period 1987 to 1998. Data is recorded for 104 months out of a total of 132. January and December are the least well documented, as the station is usually unoccupied in these months.

The Nouragues area consists of two permanent forest sample plots that are situated at the base of a granitic inselberg which is about 410 m high. A creek that forms the boundary between two geologically different areas separates the forest plots. The eastern plot (50 ha) consists of metavolcanic rocks of the Paramaca series and is covered with clayey ferralsols. The western plot (12 ha, the study area of this thesis) consists of granite rocks of the series Caraibe which is partly covered with sandy clay ferralsols (Poncy et al. 1998; Grimaldi and Riéra in press). The maximum height of the forest canopy is about 50 m, with some emerg trees of up to 60 m.

Chapter 2

The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance

With Thijs Pons and Frans Bongers

Abstract

Light-saturated rate of photosynthesis (A_{\max}), nitrogen (N), chlorophyll (Chl) content and leaf mass per unit area (LMA) were measured in leaves of trees of different heights along a natural light gradient in a French Guiana rain forest. The following four species, arranged in order from most shade-tolerant to pioneer, were studied: *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis* and *Goupia glabra*. Light availability of trees was estimated using hemispherical photography.

The pioneer species *Goupia* had the lowest LMA and leaf N on both an area and mass basis, whereas *Duguetia* had the highest values. In general, leaf variables of *Vouacapoua* and *Dicorynia* tended to be intermediates. Since A_{\max}/area was similar among species, *Goupia* showed both a much higher light-saturated photosynthetic nitrogen use efficiency (PNUE_{\max}) and A_{\max}/mass . Leaves of *Vouacapoua* demonstrated the greatest plasticity in A_{\max}/area , particularly in small saplings.

A distinction could be made between the effect of tree height and light availability on the structural, i.e. LMA, and photosynthetic leaf characteristics of all four species. The direction and magnitude of the variation in variables were similar among species.

LMA was the key variable which mainly determined variation in the other leaf variables along tree height and light availability gradients, with the exception of changes in chlorophyll concentration. A_{\max}/area , N/area , LMA and stomatal conductance to water vapour (g_s) increased, whereas Chl/mass decreased, with both increasing tree height and canopy openness. A_{\max}/mass , PNUE_{\max} and A_{\max}/Chl increased with increasing openness only. N/mass and Chl/area were independent of tree height and openness, except for small saplings of *Goupia* which had a much lower Chl/area .

Introduction

In tropical forests events such as the formation and closure of canopy openings of different dimensions create a light environment which is highly heterogeneous (Chazdon and Fetcher 1984; Kira and Yoda 1989). Consequently, a tree may experience several alternate phases of high and low light conditions in the course of its life span. Because light is a major determinant for survival and growth, it is expected that plants are able to adjust their leaf and/or whole plant traits to changing light availability.

Leaf responses to different light environments vary widely within and among species. In general, within species, shade-growing leaves are thinner, have lower mass per unit area, and have higher mass-based chlorophyll content than do sun-growing leaves (Bongers and Popma 1988; Kitajima 1994). In addition, shade leaves usually have low area-based rates of photosynthesis and dark respiration and low light saturation points (Boardman 1977). As a result, leaf adjustments to low light increase the capacity of light absorption at the expense of photosynthetic capacity and minimise carbon losses through respiration (e.g. Evans 1989; Pons and Pearcy 1994). In contrast, sun leaves can make more efficient use of the prevailing high light intensities for carbon gain while avoiding a possible reduction in photosynthetic performance as a result of photoinhibition. Therefore, acclimation to a high light condition often results in thicker leaves with more chloroplasts per unit area and in greater investments in leaf compounds related to the carboxylation and electron transport processes needed to increase photosynthetic capacity (e.g. Boardman 1977).

Increasingly more evidence indicates that both light-demanding and shade-tolerant species are capable of phenotypic plasticity, indicating adjustments are not necessarily related to the successional status of species (Turnbull 1991; Popma et al. 1992). However, when comparing species leaves of shade-tolerant species often have both lower rates of photosynthesis (Koike 1988; Raaimakers et al. 1995) and a higher leaf mass per unit area (Kitajima 1994; but see Popma et al. 1992) than do light-demanding species in both shade and high light conditions.

Independent of the light-environment, leaf and/or whole plant traits may also change with plant size (Coleman et al. 1994). For example, Hoflacher and Bauer (1982) found a greater plasticity in leaves of adult plants of *Hedera helix* as in high light these plants were able to develop thicker leaves with higher area-based rates of photosynthesis than did juvenile plants. For several woody species, seedlings showed a decrease in mass-based rates of photosynthesis (Walters et al. 1993a), an increase in photosynthetic saturation irradiance (Bormann 1958) and an increase in leaf mass per unit area (Veneklaas and Poorter 1998) as the plant grew. Studies on mature canopy trees are scarce,

however, and mainly focusing on the distribution patterns of leaf level traits within a crown (e.g. Ellsworth and Reich 1993). However, in temperate forests it was found that an increase in tree height of some conifer species was accompanied by an increase in leaf mass per unit area and a decrease in mass-based leaf nitrogen content (Schoettle 1994; Niinemets and Kull 1995; Niinemets 1997a) and a decrease in mass and area based rates of photosynthesis (Schoettle 1994; Kull and Koppel 1987).

Several factors may explain physiological and morphological alterations in leaf characteristics as tree height increases. For instance, because the ratio of total leaf area to total non-photosynthetic, living tissue decreases with increasing tree height less available foliage has to provide the tree with assimilates for growth and maintenance. Also, increasing tree height necessitates extra carbon investments to construct and maintain support tissues such as twigs and branches (Givnish 1986) or to lessen the risk of windthrow (Telewski 1995). Furthermore, because tall trees are likely to have a steeper water potential gradient from soil to leaf, their leaves must have a sufficiently negative water potential to take water from the soil to the leaf (Friend 1993; Niinemets 1997a).

Beside tree size dependent changes, leaf traits may also change in response to the environment, as an increase in height in the forest is often accompanied by an increase in air temperature, vapour pressure deficit, irradiance and/or wind speed (e.g. Kira and Yoda 1989). Increasing wind speed, for example, may cause mechanical stress for leaves, inducing morphological and anatomical changes in leaves (e.g. Jaffe and Forbes 1993).

To date studies investigating variations in leaf characteristics as related to tree size are scarce. Our objective in this study was to discriminate between tree height and light availability effects on leaf physiology and morphology of four tree species differing in shade tolerance. Arranged in order of decreasing shade tolerance these species were: *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis* and *Goupia glabra*. We selected individuals of different heights growing in a range of natural light conditions in a French Guiana rain forest. Measurements of light-saturated rate of photosynthesis, leaf nitrogen and chlorophyll content, and leaf mass per unit area were performed on exposed leaves in the upper part of the tree crown.

Materials and Methods

Study site and species

The research was carried out in an area of 12 hectares at the biological field station Les Nouragues in a lowland tropical rain forest in French Guiana (4°05'

N; 52°40' W) in the period from June through November during three consecutive years (1995-1997). The area has well-drained, clayey to sandy-clayey ferralitic soils on weathered granite parent material. Mean annual rainfall is about 3,000 mm with dry periods from September to November and February to March. The maximum height of the forest canopy is about 50 m with some emergent trees reaching 60 m in height.

Duguetia surinamensis R.E.Fr. (Annonaceae) is considered to be the most shade-tolerant because it is a sub-canopy species reaching 30 m at maturity. *Goupia glabra* Aubl. (Celastraceae) is the least shade-tolerant. It is a long-lived pioneer species which needs large gaps to germinate and establish, but may survive under the forest canopy (Schulz 1960). The large canopy trees *Dicorynia guianensis* Amshoff and *Vouacapoua americana* Aubl. (both Caesalpiniaceae) are of intermediate shade tolerance, and the latter one is considered the most shade-tolerant of the two (Favrichon 1994). Both species can establish themselves in the forest understorey and in small gaps. For brevity, species will hereafter be referred to by genus only. All species except *Goupia* are common in the area studied. Maximum tree height for *Vouacapoua* is about 45 m, and for *Dicorynia* and *Goupia* about 55 m. *Dicorynia* and *Vouacapoua* have compound leaves with five to 15 leaflets. *Goupia* and *Duguetia* produce plagiotropic branches with simple leaves from the seedling stage. In this study, the leaflets of compound leaves are considered to be functionally similar to simple leaves.

Selection of trees and leaf sampling

The total height of the selected trees ranged from 0.7 to 21 m. Individuals in or nearby gap sites of different sizes and under a closed canopy were chosen. They were selected on the basis of their height at that moment: (1) small saplings (range 0.7-2 m), (2) small juvenile trees (range 4-9 m) and (3) tall juvenile trees (12-21 m). For similar sized trees both shade and light growing individuals were sampled. In the upper, peripheric part of the tree crown, six to 20 young, fully expanded leaves were harvested to determine leaf mass per unit area (LMA). Access to leaves was obtained by using climbing poles or rope techniques. Prior harvesting, three or four leaves from this sample were used to measure the area-based light-saturated rate of photosynthesis ($A_{max}/area$), chlorophyll per unit leaf area (Chl/area) and nitrogen per unit leaf mass (N/mass). All the measurements were averaged per tree. Gas-exchange measurements were made before the leaves were harvested. In addition, for *Duguetia*, *Vouacapoua* and *Dicorynia* mature canopy trees (n=1, 3 and 3, respectively) were sampled, ranging in tree height from 26 m for *Duguetia* and 34-55 m for the other two species. All canopy trees were exposed to full sun, except the *Duguetia* tree. Owing to the small sample of these individuals, they

were not included in the statistical analyses. For *Goupia* no canopy trees were accessible.

Gas-exchange measurements

Light-saturated rate of photosynthesis at ambient CO₂ partial pressure was measured using a portable infra-red gas-exchange system (CIRAS-1, PP-system, Hitchin, UK) with a Parkinson leaf chamber (2.5 cm²). Leaves were brought to photosynthetic light saturation under a quartz halogen bulb (12V, 20 Watt) at a photon flux density (PFD) of 735 μmol m⁻² s⁻¹. Preliminary measurements showed that this light intensity was well above the light-saturated point for these leaves. The time needed for photosynthetic induction was about 15 min. During all measurements mean (± SD) air temperature in the leaf chamber was 28 ± 1°C, CO₂ concentration was 355 ± 4 ppm and relative air humidity was 66 ± 8%. Gas-exchange measurements were always carried out in the morning between 09.00 and 12.00 h. The rate of photosynthesis and stomatal conductance to water vapour (g_s) at light saturation were calculated according to Von Caemmerer and Farquhar (1981).

Leaf morphology, nitrogen and chlorophyll analyses

The circumference of each harvested leaf was first drawn on paper; these drawings were used, in the Netherlands, to calculate leaf area with a video area-meter (DIAS, Delta-T Image Analyse System, Eijkelkamp BV, Giesbeek, the Netherlands). The leaves were then dried in a field oven at 50 °C for 3 days and afterwards dry-stored. In the Netherlands leaves were oven-dried at 70 °C for 48 h before leaf dry weight was measured. From these data LMA was calculated for each leaf. Leaf chlorophyll and nitrogen content were measured for the leaves used for gas-exchange measurements. Chlorophyll was extracted from one leaf disk (1.23 cm²) per fresh leaf with 3 ml N,N-Dimethylformamide (Inskeep and Bloom 1985) and stored in the dark at 4 °C for 2.5 weeks. Chlorophyll content was analysed with a spectrophotometer in the Netherlands. The leaf N-analysis was carried out for a pooled sample of three to four leaves per tree; these leaves were also used for the gas-exchange measurements. In the Netherlands, leaf N was analysed with either an elemental analyser (Carlo Erba, model 1106, Milano, Italy) or, after extraction with hot water, spectrophotometrically with a segmented flow analyser (Skalar San⁺ System, Breda, the Netherlands).

Estimation of light availability

Hemispherical photography was used to estimate the light availability for a tree. A photograph was taken above the centre of each individual, using a camera (Canon Ti-70) with a fish-eye lens (Canon, 7.5 mm f5.6), except for tall juvenile

trees (12-21 m) where photographs were taken above and close to the position of the harvested leaves. For small saplings (< 2m) the camera was mounted on a tripod, whereas for all other trees the camera was fixed in an aluminium leveller and mounted on a telescopic aluminium pole (maximum range 6 m). For each photograph canopy openness was calculated as the percentage of unobstructed sky, weighted for angle of incidence, according to Ter Steege (1994); we used a radiation distribution for a standard overcast sky. Percentage canopy openness was used as an estimation of the light availability per tree.

In order to verify if canopy openness was a good estimator of light availability, we measured the daily PFD above 16 trees, differing in height, of *Dicorynia* and *Vouacapoua*, which were also used in this study. Five gallium-arsenide phosphide photo-diodes (Model G-1118, Hamamatsu Photonics, Hamamatsu, Japan), calibrated against a Li-Cor quantum sensor under full sunlight, were attached to petioles of leaves at the top of the tree crown, and close to the position where the hemispherical photograph was taken. The light sensors were oriented horizontally. They monitored PFD every 5 s, and 1 min means were stored with a Li-Cor 1000 data logger from 7.30 until 17.00 h, over five to eight consecutive days during the period from September to November in 1996 and 1997. Simultaneously, the daily PFD above the forest canopy was measured at a fixed site in the research area using a Li-Cor quantum sensor. Daily integrated PFD was calculated for each sensor and expressed as a percentage of the daily integrated PFD measured above the forest canopy. Above the forest canopy the PFD ranged from 30 to 45 mol m⁻² day⁻¹.

Statistical analyses

The effects of light availability, tree height and species on leaf variables were analysed with an analysis of covariance (ANCOVA), with species as main factor and tree height and percentage canopy openness as covariables. We tested the assumption of homogeneity of regression slopes of leaf variable on both tree height and canopy openness for all four species; if no significant differences were found then the interaction term was removed from the model (Sokal and Rohlf 1995). Within species scatterplots showed linear relationships between all leaf variables and both tree height and canopy openness, except for Chl/area, as for tree height, no linear relationship was found for trees of *Goupia*. Therefore, Chl/area was excluded from the ANCOVA. The interspecific differences in leaf variables were tested using leaf characteristics for a common tree with an average height and canopy openness; the so-called test for adjusted means. Box plots were used to show the intraspecific variation in leaf variables. For all species pooled, trends of LMA, A_{\max} /area and Chl/area with tree height and canopy openness were analysed by linear regression. Data were log-transformed if variances were not equal among species.

Results

Estimation of light availability

A significant positive relationship was found between direct measurements of PFD and percentage canopy openness above 16 trees of *Dicorynia* and *Vouacapoua* ($r^2=0.60$, $P<0.001$). We therefore concluded that, in this study, canopy openness could be used as an estimator for light availability, and that the hemispherical photographs sufficiently represent the light environment of the leaves on which gas-exchange measurements were made.

For all species the range of canopy openness was moderately low, as almost 95% of the trees had an openness of less than 15% (Fig. 1). This rather

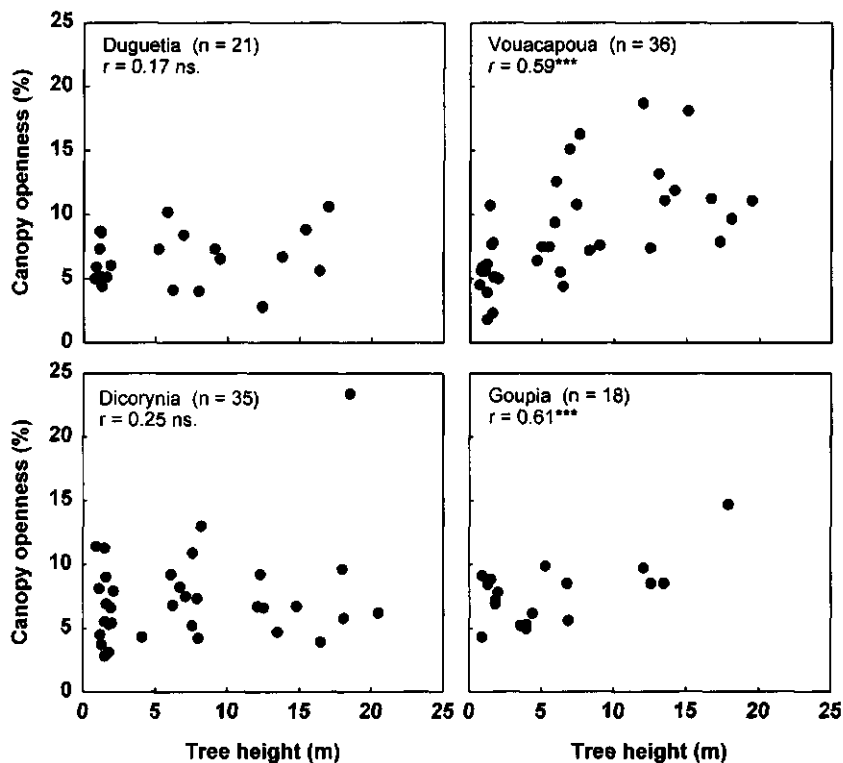


Fig. 1. Light availability, expressed as percentage canopy openness derived from hemispherical photographs, plotted against tree height (m) for *Duguetia*, *Vouacapoua*, *Dicorynia* and *Goupia*. Pearson's correlation coefficient (r), number of individuals (n) and level of significance (ns., $P>0.05$; ***, $P<0.001$) are given.

low range of canopy openness resulted from the lack of large gaps in the research area. There was no correlation between canopy openness and tree height for individuals of *Dicorynia* and *Duguetia* (Fig. 1). This was expected because, for similar sized trees, individuals were selected both in gap sites and under a closed forest. However, trees of *Vouacapoua* and *Goupia* showed a positive correlation between openness and height (Pearson's correlation coefficients of 0.59 and 0.61, respectively). When the canopy openness value of the tallest tree of *Goupia* was excluded, no correlation was found for this species (data not shown). In general, we found a fairly uniform distribution of percentage canopy openness over the different tree heights and species; this is a prerequisite the data analysis.

Table 1. Results of an one-way Ancova with species (n=4) as main factor and tree height (m) and canopy openness (%) as covariables. The following leaf variables are given: light-saturated rate of photosynthesis per unit leaf area ($A_{max}/area$) and per unit leaf dry mass ($A_{max}/mass$); nitrogen content per unit leaf area (N/area) and per unit leaf dry mass (N/mass); chlorophyll content per unit leaf dry mass (Chl/mass); leaf mass per unit leaf area (LMA); light-saturated rate of photosynthesis per unit leaf nitrogen (PNUE_{max}) and per unit chlorophyll (A_{max}/Chl) and stomatal conductance to water vapour (g_s). Units for the variables are given in parentheses. F-values, level of significance (P), intercept, common regression slope of tree height (b1) and canopy openness (b2), coefficient of determination (r^2) and number of individuals (n) per variable are given. $A_{max}/mass$, N/mass, PNUE_{max}, A_{max}/Chl and g_s were log transformed prior to analysis. Significant levels: ns., P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001. F-values for intercepts are not shown (P<0.001). Regression Model: variable = intercept + (b1 x tree height) + (b2 x canopy openness) + species effect + error.

Variable	Species		Intercept	Tree height		Canopy openness			Model		
	F	P		F	P	b1	F	P	b2	r^2	n
$A_{max}/area$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	3	*	3.0	31	***	0.11	37	***	0.21	0.55	112
$A_{max}/mass$ ($\text{nmol g}^{-1} \text{s}^{-1}$)	23	***	1.9	0.3	ns	-0.001	14	***	0.01	0.47	110
N/area (mmol m^{-2})	45	***	67	34	***	1.77	20	***	2.23	0.69	112
N/mass (mmol g^{-1})	19	***	0.2	0.4	ns	-0.001	1	ns	0.002	0.36	112
Chl/mass (mmol mg^{-1})	23	***	13	13	***	-0.14	11	**	-0.22	0.53	105
LMA (g m^{-2})	48	***	42	97	***	1.18	26	***	1.01	0.77	112
PNUE _{max} ($\mu\text{mol mol}^{-1} \text{s}^{-1}$)	40	***	1.7	0.1	ns	0.001	6	*	0.01	0.57	112
A_{max}/Chl ($\mu\text{mol mmol}^{-1} \text{s}^{-1}$)	6	***	0.8	3	ns	0.004	23	***	0.020	0.38	105
g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	0.2	ns	1.9	3	*	0.005	14	***	0.017	0.23	112

Variation in leaf variables among species

Species differed in their expression of leaf variables, and these differences were greatest for LMA, $N/area$ and $PNUE_{max}$ as indicated by their high F-values (Table 1). Interspecific differences in leaf variables, derived from the test for adjusted means, are shown in Fig. 2. Differences in leaf variables were most pronounced between the pioneer species *Goupia* and the most shade-tolerant species *Duguetia*, whereas leaf traits of *Dicorynia* and *Vouacapoua* tended to be intermediates (Fig. 2). *Duguetia* had the highest LMA, followed in decreasing

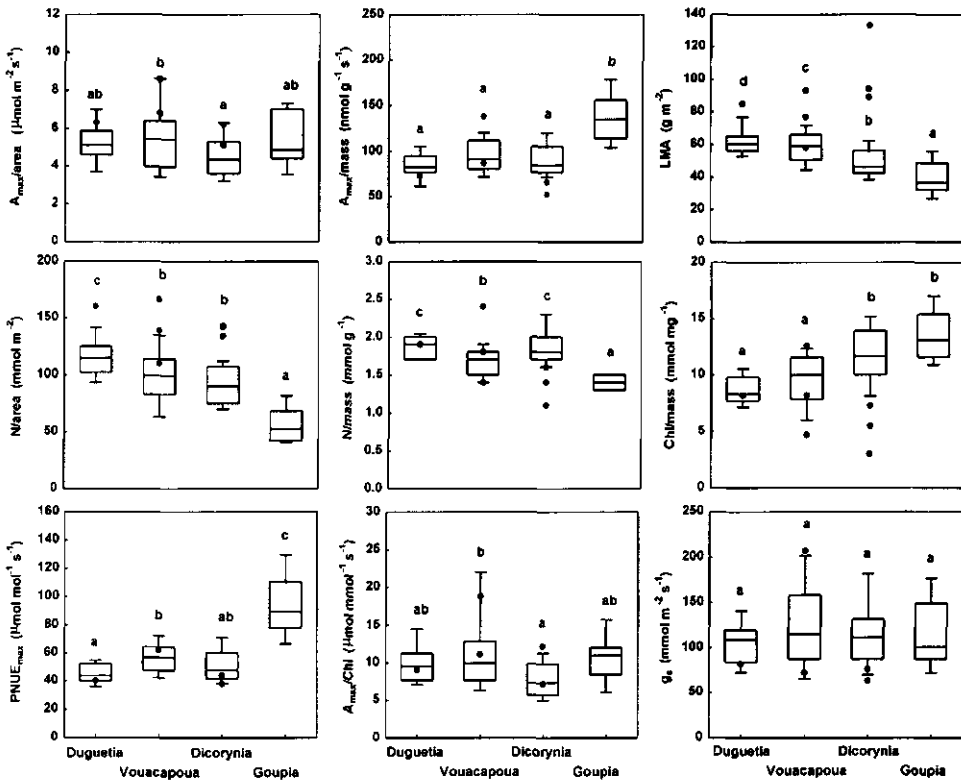


Fig. 2. Box plots of $A_{max}/area$, $A_{max}/mass$, $N/area$, $N/mass$, LMA, $Chl/mass$, $PNUE_{max}$, A_{max}/Chl and g_s for *Duguetia*, *Vouacapoua*, *Dicorynia* and *Goupia* (abbreviations as in Table 1). The upper and lower border of the box are the 75th and 25th percentiles, respectively, the black horizontal line within the box is the median and the error bars are the 10th and 90th percentiles. For each leaf variable species with the same letter were not significantly different ($P > 0.05$). Interspecific differences in leaf variables were analysed using the test for adjusted means. The closed circles represent values for the leaf variables of the large, mature canopy trees of *Duguetia* (n=1), *Vouacapoua* (n=3) and *Dicorynia* (n=3).

order by *Vouacapoua*, *Dicorynia*, with *Goupia* having the lowest LMA. Because *Goupia* had the lowest LMA and N/mass this resulted in a much lower N/area and a much higher A_{\max}/mass as compared to the other species. Among the three shade-tolerant species PNUE_{\max} was significantly higher for leaves of *Vouacapoua* than for *Duguetia*, whereas PNUE_{\max} was almost twice as high for *Goupia* than for the other species (Fig. 2). Both *Duguetia* and *Vouacapoua* had a significantly lower Chl/mass than did the two other species. Although A_{\max}/area

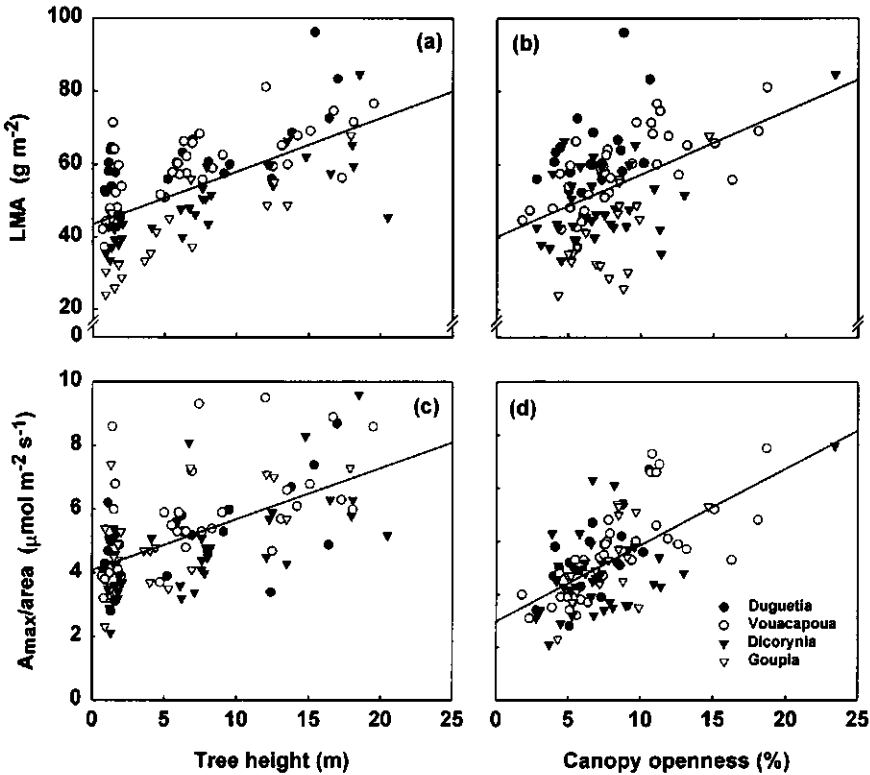


Fig. 3. Leaf mass per unit area (LMA) and light-saturated rate of photosynthesis per unit area (A_{\max}/area) plotted against (a,c) tree height and (b,d) canopy openness for *Duguetia* (closed circle), *Vouacapoua* (open circle), *Dicorynia* (closed triangle) and *Goupia* (open triangle). Solid lines represent linear regression for all species pooled. Regression equations:
 $43.52 + 1.46 \times \text{Height}$ ($r^2 = 0.40$, $P < 0.001$, LMA)
 $40.12 + 1.72 \times \text{Openness}$ ($r^2 = 0.21$, $P < 0.001$, LMA)
 $4.08 + 0.16 \times \text{Height}$ ($r^2 = 0.32$, $P < 0.001$, A_{\max}/area)
 $2.98 + 0.29 \times \text{Openness}$ ($r^2 = 0.39$, $P < 0.001$, A_{\max}/area).

was similar among the four species (Fig. 2), *Vouacapoua* tended to have a greater plasticity in photosynthetic capacity, which is particularly evident in small saplings having a great variation in A_{\max}/area (Fig. 3). A_{\max}/Chl was similar among species, except for *Dicorynia* which had a significantly lower value than had *Vouacapoua*. Stomatal conductance to water vapour (g_s) did not differ among the four species.

Comparison of tree height and light availability effects

The measured leaf variables LMA and A_{\max}/area were used to illustrate the single effect of tree height and canopy openness on leaf traits (Fig. 3); N/mass was not included because it was unaffected by height and openness (Table 1). For all species, both LMA and A_{\max}/area increased significantly with increasing height and openness (Fig. 3).

Table 1 summarises the results of the ANCOVA, which discriminates between tree height and light availability effects on leaf variables. For most of the dependent variables the explained variation of the model was moderate to fairly high (range 23-77%). For A_{\max}/mass , N/mass, A_{\max}/Chl and g_s this variation was less than 50%.

A_{\max}/area , N/area and LMA were strongly positively affected by both tree height and canopy openness as indicated by their high F-values. A_{\max} and N on a mass basis, however, were constant across different heights and openness, except for A_{\max}/mass , which slightly increased with increasing canopy openness. Furthermore, PNUE_{\max} and A_{\max}/Chl were unaffected by tree height, whereas N/mass was unaffected by canopy openness. Chl/mass was the only variable which decreased with both increasing height and openness as indicated by the sign of the regression slopes (b_1 and b_2 , respectively). Stomatal conductance moderately increased with increasing canopy openness and, to a lesser extent, with increasing tree height.

The variable Chl/area was excluded from the ANCOVA (see Statistical Analyses). Because LMA and Chl/mass showed opposite patterns Chl/area was independent of tree height in all species, except for leaves of *Goupia*, which showed a positive logarithmic relationship (Fig. 4). This non-linear relationship for *Goupia* was the result of a much lower leaf chlorophyll content found in small saplings compared to the content in the large trees. Chl/area was independent of canopy openness for all species (data not shown).

Leaf characteristics of the additional mature canopy trees of *Duguetia*, *Vouacapoua* and *Dicorynia* were added in the box plots as closed circles (Fig. 2). It seemed that traits changed when trees reach their maximum height. In all three species, LMA and N/area tended to be higher for canopy trees than for the smaller individuals. Mass-based A_{\max} and leaf N tended to be lower in canopy

trees of *Dicorynia*, resulting from a higher LMA in these large individuals (Fig. 2).

Discussion

Differences in leaf variables among species

Considerable variation in leaf variables existed among the species, especially between the pioneer *Goupia* and the three shade-tolerant species (Fig. 2). Rather low rates of A_{\max}/area were found for light growing individuals of *Goupia*, whereas higher rates were expected for a pioneer species. Higher rates of A_{\max}/area for *Goupia* have been reported, however, for individuals growing in much brighter light conditions (Raaimakers et al. 1995; Reich et al. 1995a; Huc et al. 1994). Moreover, Huc et al. (1994) found a higher A_{\max}/area ($8.9 \mu\text{mol m}^{-2} \text{s}^{-1}$) for trees of *Dicorynia* growing in artificial stands than for similar sized trees in our study. These findings suggest that, in our study, light availability might not be enough for *Goupia* and *Dicorynia* to express their full photosynthetic capacity.

LMA was lowest for the pioneer species *Goupia* and increased with increasing shade tolerance of the other species (Fig. 2), which is consistent with other studies (e.g. Kitajima 1994; Reich et al. 1995a). However, Popma et al. (1992) investigated 68 tropical tree species and found no differences in LMA between obligate gap species and gap-independent species. In general, interspecific variation in LMA is caused by differences in leaf anatomy, morphology (e.g. leaf hairs, thorns) and/or chemical composition (e.g. starch, secondary compounds) (Lambers and Poorter 1992; Van Arendonk and Poorter 1994). A higher LMA, typically found for shade tolerant species, is often owing to extra investment in compounds, e.g. lignin, or in support tissue which reduce the palatability of leaves (Lambers and Poorter 1992).

The greater efficient use of nitrogen in terms of photosynthetic capacity, i.e. PNUE_{\max} , in leaves of *Goupia* was probably owing to its very low N/mass (Fig. 2). In general, high values of mass-based leaf N are observed in pioneer species because they usually occupy resource-rich habitats such as large gaps. Reich et al. (1995a), however, found that only the earliest successional species, e.g. *Cecropia*, had a high N/mass, and that other species along the successional gradient, such as *Goupia*, had low to moderate N/mass. The low PNUE_{\max} found for the three shade-tolerant species, despite their higher N/mass, may have resulted from a large investment of N in leaf components that are not associated with photosynthesis (Lambers and Poorter 1992; Pons et al. 1994). It may also be a consequence of a sub-optimal distribution of N within the photosynthetic apparatus (Pons et al. 1994).

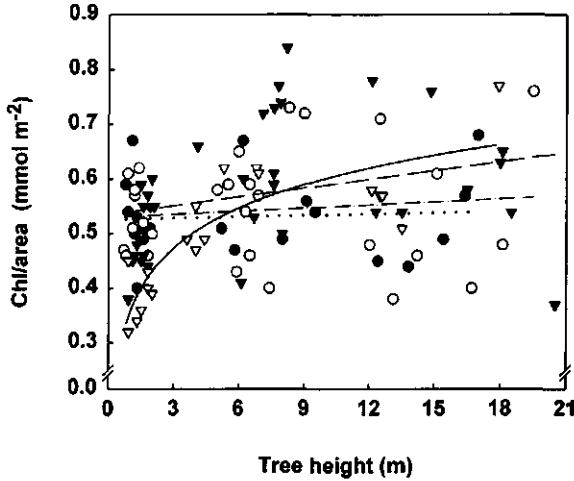


Fig. 4. Chlorophyll content per unit area (Chl/area) plotted against tree height (m) for *Duguetia*, *Vouacapoua*, *Dicorynia* and *Goupia*. Species symbols are the same as in Fig. 3. Mean values of Chl/area for three to four leaves per tree are shown. The solid line and broken lines represent non-linear and linear regression, respectively. Regression equations:

$0.53 + 8.85 \times 10^{-4} \times \text{Height}$	$(r^2 = 0.005, \text{ ns.}, \text{ Chl/area } Duguetia, \text{ dotted line})$
$0.53 + 1.89 \times 10^{-3} \times \text{Height}$	$(r^2 = 0.012, \text{ ns.}, \text{ Chl/area } Vouacapoua, \text{ dash-dot line})$
$0.54 + 5.39 \times 10^{-3} \times \text{Height}$	$(r^2 = 0.074, \text{ ns.}, \text{ Chl/area } Dicorynia, \text{ long-dash line})$
$0.36 + 0.24 \times \log(\text{Height})$	$(r^2 = 0.72, P < 0.001, \text{ Chl/area } Goupia, \text{ solid line}).$

Leaf variables as dependent on tree height and light availability

Both PNUE_{max} and $A_{\text{max}}/\text{mass}$ only marginally increased with increasing light availability, with the exception of changes in chlorophyll content (Table 1), suggesting that the species were hardly capable of physiological light acclimation. Mass-based chlorophyll content increased with both decreasing tree height and canopy openness (Table 1). The increase of Chl/mass with decreasing light often results from enhanced investments of resources, especially nitrogen, in chlorophyll (Chazdon 1992; Poorter et al. 1995; Niinemets 1997b). Furthermore, for all species, $A_{\text{max}}/\text{Chl}$ was only affected by canopy openness (Table 1). The ratio of photosynthetic capacity to chlorophyll content is an important aspect of shade adjustment in leaves. The higher $A_{\text{max}}/\text{Chl}$ at high light availability indicates that in high light conditions species were able to invest more of their resources in carbon-assimilation, rather than in light-harvesting which, in turn, should be more advantageous in low light conditions (Seeman et al. 1987; Evans 1989). Moreover, Chl/mass also decreased with increasing tree height, which may be a consequence of an increased investment in cell-wall material (e.g. Niinemets 1997a). LMA and Chl/mass showed opposite patterns, resulting in Chl/area to be

unaffected by tree height and light availability. This suggests that, within species, leaves of small and/or shade-growing individuals could capture a similar amount of light as do large and/or bright-growing individuals (Poorter et al. 1995; Niinemets 1997b). Small individuals of *Goupia*, however, showed a contrasting pattern, as their leaves had a much lower Chl/area compared to those of saplings of the other three species (Fig. 4).

Significant positive area-based A_{\max} -N relationships ($P < 0.001$), with similar slopes (about 0.052), were found for leaves of the four species (data not shown). When expressed on a mass basis regressions were only significant for *Duguetia* and *Vouacapoua*; however, these slopes were almost horizontally oriented (data not shown). Within species, a stronger A_{\max} -N relationship on an area than on a mass basis is caused by a larger variation in LMA than in N/mass across different environmental gradients, and by a strong correlation between LMA and N/area (Ellsworth and Reich 1993; Reich et al. 1994; Table 1). In our study the variation of A_{\max} /area and N/area could also be traced back to variation in LMA, as this variable was strongly correlated with both tree height and canopy openness (Table 1). Other studies also found positive relationships between LMA and light availability (Walters et al. 1993b; Kitajima 1994; Raaimakers et al. 1995) and between LMA and tree height, independently of light, (Niinemets and Kull 1995; Poorter et al. 1995; Niinemets 1997a), suggesting a consistent trend within tree species.

An increase in LMA with increasing light availability often is the result of the formation of a thicker mesophyll tissue, especially the development of elongated or more layers of palisade parenchyma (Bongers and Popma 1988; Chazdon and Kaufmann 1993). In addition, leaves with thicker mesophyll typically have more chloroplasts per unit area, resulting in a higher A_{\max} /area (Hoflacher and Bauer 1982; Turnbull 1991; Chazdon and Kaufmann 1993). LMA, however, also increased with increasing tree height, independently of light, suggesting the developmental phase constrained plasticity in LMA; that is, small trees were prevented from developing heavy and thick leaves like in large trees (e.g. Hoflacher and Bauer 1982; Knapp and Fahnstock 1990).

When trees grow taller they often experience an increased water limitation, generally owing to changed environmental conditions, such as increasing vapour pressure deficits and wind speed with height, and an increased hydraulic and gravitational resistance to water flow in the xylem, as water has to travel a longer distance from the soil to a leaf (Tyree and Ewers 1991; Friend 1993; Ryan and Yoder 1997). For leaves in a tree crown to be supplied with water they have to reduce their water potential, e.g. by increasing the amounts of solutes, to below that in the soil to overcome the effects of gravity and hydraulic resistance in the trunk and branches. In *Picea abies*, Niinemets (1997a) showed that the higher LMA of needles in tall trees compared to those

in small trees was owing to larger investments of structural (e.g. cell-wall thickening) and non-structural (e.g. starch) carbon per needle dry weight; in that study an increased water stress in taller trees was given as an explanation for the greater LMA.

An adverse effect of higher carbon investments in leaves of tall trees, however, is the dilution of nitrogen and/or other compounds involving photosynthesis, leading to a lower A_{\max}/mass . In light grown trees of *Pinus aristata*, Schoettle (1994) found that needles of tall trees had a higher LMA, a lower N/mass and a lower area and mass based light-saturated photosynthesis than those of small trees. Our results did not support this phenomenon, as N/mass and A_{\max}/mass were unaffected by tree height (Table 1), indicating that allocation of nitrogen to the leaves was similar between tall and small trees.

In light grown trees of two *Pinus* species, Yoder et al. (1994) found from diurnal light-saturated rates of photosynthesis that tall trees had a much lower daily mean A_{\max}/area than small trees, despite similar N/mass and peak net photosynthesis rate between tall and small trees. In that study, the authors suggested that the reduced net photosynthesis rate in tall trees was related to the earlier closure of stomata in the day resulting from an increased hydraulic resistance in these tall trees. Limiting transpirational water loss through stomatal closure in tall trees, which have an increased hydraulic resistance, is a necessity to prevent cavitation and embolism in the xylem, i.e. to avoid air entering in the xylem vessels causing a decrease in water transport (e.g. Tyree and Sperry 1988; Sperry et al. 1993). Fredericksen et al. (1996) also suggested that the effect of increased hydraulic resistance could explain the reduced mean diurnal gas exchange rates in canopy trees of *Prunus serotina* compared to saplings and seedlings. In our study, gas exchange rates were measured between 09.00 and 12.00 h, as we assumed that environmental conditions in the afternoon might reduce the photosynthetic potential. For trees up to 21 m in height, stomatal conductance was almost unaffected by tree height, whereas A_{\max}/area increased with tree height (Table 1). In addition, leaves of the large, mature canopy trees tended to have a higher A_{\max}/area and LMA than those of the smaller trees, despite their somewhat lower stomatal conductance (Fig. 2). Apparently, photosynthetic capacity was uniformly higher in taller trees than in smaller ones for all species. However, midday depression of gas exchange rates, owing to increasing air temperature and light, and decreasing humidity along a vertical gradient in the forest, could diminish the observed difference in photosynthesis rates between tall and small trees.

In summary, the results of this study show that tree height and canopy openness had independent effects on the physiological and structural, i.e. LMA, leaf characteristics of all the four species. Moreover, the direction and magnitude of leaf responses to height and openness were similar among the

species. Overall, leaves of taller trees had higher LMA, N/area and A_{\max}/area , and a lower Chl/mass than those of small trees, whereas canopy openness had an effect on all leaf variables, except on N/mass. LMA seemed to be a key variable, as the variation in photosynthesis rates, nitrogen and chlorophyll content could be traced back to variation in LMA. Our results show that the developmental phase of trees constrained plasticity in leaf variables, especially LMA. Beside environmental effects, differences in leaf variables between different sized trees might also be explained by changing water relations within a tree caused by increased hydraulic limitations in taller trees.

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Chapter 3

Photosynthetic induction in saplings of three shade-tolerant tree species: comparing understorey and gap habitats in a French Guiana rain forest

With Peter Jan de Vries, Thijs Pons and Frans Bongers

Abstract

The photosynthetic induction response under constant and fluctuating light was examined in naturally occurring saplings (about 0.5-2 m in height) of three shade-tolerant tree species, *Pourouma bicolor* spp *digitata*, *Dicorynia guianensis* and *Vouacapoua americana*, growing in bright gaps and in the shaded understorey in a neotropical rain forest. Light availability to saplings was estimated by hemispherical photography. Photosynthetic induction was measured in the morning on leaves that had not yet experienced direct sun light. In *Dicorynia*, the maximum net photosynthesis rate (A_{\max}) was similar between forest environments (ca $4 \mu\text{mol m}^{-2} \text{s}^{-1}$), whereas for the two other species it was twice as high in gaps (ca 7.5) as in the understorey (ca 4.5). However, the time required to reach 90% of A_{\max} did not differ among species, and was short, 7-11 min. Biochemical induction was fast in leaves of *Pourouma*, as about 3 min were needed to reach 75% of maximum carboxylation capacity (V_{cmax}); the two other species needed 4-5 min. When induction continued after reaching 75% of V_{cmax} , stomatal conductance increased in *Pourouma* only (ca 80%), causing a further increase in its net photosynthesis rate. When fully-induced leaves were shaded for 20 min, loss of induction was moderate in all species. However, gap saplings of *Dicorynia* had a rapid induction loss (ca 80%), which was mainly due to biochemical limitation as stomatal conductance decreased only slowly. When leaves were exposed to a series of lightflecks separated by short periods of low light, photosynthetic induction increased substantially and to a similar extent in all species. Although A_{\max} was much lower in old than in young leaves as measured in *Dicorynia* and *Vouacapoua*, variables of the dynamic response of photosynthesis to a change in light tended to be similar between young and old leaves. Old leaves, therefore, might remain important for whole-plant carbon gain, especially in understorey environments. The three shade-tolerant species show that, particularly in low light, they are capable of efficient sunfleck utilization.

Introduction

Plants growing in a forest understorey experience extreme fluctuations in light conditions, as long periods of low light alternate with brief, unpredictable periods of high light during sunflecks. These periods of high light can last for a few seconds to several minutes, and can contribute from 10 to 80% of daily photon flux density (PFD) received by a plant (Chazdon 1988). In closed-canopy forests, sunflecks can be isolated, but they usually form clusters separated by relatively long periods of low light (Pearcy et al. 1994). In this unpredictable understorey light environment, plants depend on sunflecks to maintain a positive carbon balance (Chazdon 1988). The photosynthetic apparatus of shade plants is therefore expected to be adapted to increase the utilization of high light in the fluctuating light environment (e.g. a rapid photosynthetic induction response to a light increase and/or slow decrease of photosynthetic induction in low light). Moreover, studies have shown that the growth of a variety of species is strongly correlated with sunfleck availability (Pearcy 1983; Sims and Pearcy 1993; Watling et al. 1997).

Sunfleck utilization for carbon gain depends on the rate of photosynthetic activation, the photosynthetic induction state of the leaf and the capacity to continue with CO₂ fixation in low light immediately following a sunfleck (i.e. post-illumination CO₂ assimilation) (Pearcy 1990). In general, the photosynthetic response to a light increase can be separated into two phases: an initial fast-induction phase which requires 1 to 2 min for completion and involves light-activation of some Calvin-cycle enzymes and build up of metabolic pools, particularly ribulose-1,5-bisphosphate (RuBP) regeneration (Kirschbaum and Pearcy 1988a; Sassanrath-Cole and Pearcy 1992); followed by a slow-induction phase, lasting 5 to 30 min or more, in which ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is activated and stomata open (Kirschbaum and Pearcy 1988b; Pearcy 1990). Approximately the first 10 min in the slow-induction phase are dominated by light-activation of Rubisco (Pons et al. 1992), after which, the intercellular partial pressure of CO₂ increases as stomata continue to open, causing a further increase in assimilation rate (Kirschbaum and Pearcy 1988b). When the leaf is shaded again after high light, the fast-induction component deactivates rapidly, while deactivation of the slow-induction phase is much slower (Pearcy 1990; Pons et al. 1992). These processes together determine the photosynthetic induction state of the leaf and hence its readiness to respond to subsequent sunflecks.

To date, however, most studies on inter-specific differences in photosynthetic induction properties have compared species differing in life form (herbaceous vs woody species) and/or light requirement (pioneer vs climax species) (e.g. Chazdon and Pearcy 1986a; Ögren and Sundin 1996). In tropical forests, most woody

species are characterized as shade-tolerant in all or some stage(s) during their life cycle (Whitmore 1989; Clark and Clark 1992; Popma et al. 1992). At present, there is little information on inter-specific differences in induction properties in this large group of shade-tolerant woody species (but see Kursar and Coley 1993; Lei and Lechowicz 1997; Zipperlen and Press 1997). In particular, data from naturally growing plants in the forest are scarce, as field and laboratory experiments are usually carried out with potted plants.

In this study, we investigated the photosynthetic induction responses to light increases and decreases in leaves of saplings of three neotropical shade-tolerant tree species: *Pourouma bicolor* spp. *digitata*, *Dicorynia guianensis* and *Vouacapoua americana*. In addition, the effect of leaf age on these properties was studied. Specifically, we investigated differences between naturally occurring understorey- and gap-growing forest saplings in (1) photosynthetic induction under constant high light, (2) loss of induction in low light, and (3) induction response to a sequence of simulated sunflecks. We separated the relative importance of biochemical and stomatal limitation during the time course of photosynthetic induction.

Materials and Methods

Study site and species

The field work was carried out at Les Nouragues field station located in an undisturbed lowland tropical rain forest in French Guiana (4° 5'N, 52°42'W). The area has a mean annual rainfall of about 3,000 mm and a distinct dry period from September to November (Poncy et al. 1998). The three species studied are locally abundant, *Pourouma bicolor* Mart. spp. *digitata* Tréc. (Cecropiaceae), *Dicorynia guianensis* Amshoff and *Vouacapoua americana* Aubl. (both Caesalpinaceae). Hereafter they will be referred to by their generic name only. All the species are considered shade tolerant and can survive in the forest shade (Bongers and Sterck 1998; Favrichon 1994). *Pourouma* is a more light dependent species, as it is often found in recently disturbed or secondary forest. Moreover, tall individuals of *Pourouma* (i.e. tree height > 4m) depend on high light to complete their life cycle (F. Bongers, unpublished data). *Vouacapoua* is the most shade-tolerant species of the three; *Dicorynia* has intermediate light requirements (Favrichon 1994). *Pourouma* has simple leaves, and the other two species have compound leaves. In this study, leaflets of compound leaves are considered functionally equal to simple leaves.

Measurements

All field measurements were carried out in September and October 1997; the mean monthly rainfall was about 70 mm in that period. For each species, four to six plants were selected in two different forest light environments: shaded understorey and bright gap sites. Sapling height ranged from 0.5 to 2 m. Above the center of each sapling a hemispherical photograph was taken with a 7.5 mm Canon fish-eye lens and a Canon T70 camera. From these photographs canopy openness was calculated as the percentage of unobstructed sky, weighted for angle of incidence, according to Ter Steege (1994). In addition, mean daily ratios of direct (DSF) and indirect (ISF) site factor, and total daily photon flux density (PFD, $\text{mol m}^{-2} \text{d}^{-1}$) were calculated using 12 days in a year; for this purpose each 21st day of a month was taken to obtain a representative year (Ter Steege 1994). We used a radiation distribution for a standard overcast sky. Site factors indicate the percentage of direct and indirect (or diffuse) light above the forest canopy that is received by the sapling.

For each sapling, photosynthetic measurements were made on the youngest, fully-developed leaf using an open gas-exchange system (CIRAS-1 with PLC-B cuvette, PP-System, Hitchin, UK). For all leaves, the mean (\pm SD) CO_2 concentration of the reference air stream was 354 ± 4 ppm and mean relative air humidity was $70 \pm 4\%$, which was similar to ambient. Mean leaf temperature was $27 \pm 1^\circ\text{C}$; leaf temperature was calculated using the energy balance equation (Parkinson 1985). Calculations of net photosynthetic rate (A), stomatal conductance to water vapour (g_s) and intercellular partial pressure of CO_2 (p_i) were based on the equations of Von Caemmerer and Farquhar (1981).

All measurements were made in the morning between 0900 and 1200 hours. To ensure a leaf was not yet photosynthetically induced that day prior to measurement, a screen was placed around the sapling. In this way leaves were exposed to low diffuse light only ($\leq 20 \mu\text{mol m}^{-2} \text{s}^{-1}$). Light was provided by a quartz halogen bulb (12V, 20 Watt) mounted on the leaf cuvette. Photosynthetic induction was monitored at a PFD of $735 \mu\text{mol m}^{-2} \text{s}^{-1}$; preliminary measurements showed that this light intensity was well above the light-saturated point for all species and did not cause photoinhibition.

The photosynthetic induction time of a leaf was measured by first monitoring A in low PFD ($\leq 5 \mu\text{mol m}^{-2} \text{s}^{-1}$) until a steady state was reached after about 2 min, followed by recording measurements every 7 s for half a minute before and for 15 min after the lamp was switched on until A levelled off. Then, induction loss was measured for the same leaf. The leaf was kept in the cuvette and the lamp was switched off. After 20 min in low light, A was measured 60 s following reillumination.

In all measurements, the flow of air through the cuvette was maintained at 285 ml min^{-1} . At this flow rate, the system response time to a change of 10%

CO₂ concentration, which was determined by injecting a steady stream of CO₂ into the chamber, was about 15 s (for H₂O it was about 35 s). The time course of induction was adjusted for the CO₂ detection time lag by omitting the two photosynthetic measurements recorded immediately after the light was switched on and then shifting the subsequent measurements two time-intervals backwards.

Shapes of the time course of induction were different among species and forest environment (Fig. 1). To obtain the maximum net photosynthetic rate (A_{\max}) a logistic function was fitted to the last 10 minutes of the time course of induction only. This procedure best described the time course of induction for all three species, and estimated A_{\max} accurately. The time required to reach 90% of A_{\max} was taken as a measure for the rate of the induction process. Because maximum stomatal conductance was not yet reached after 15 min in high light in most leaves, the stomatal conductance attained after 15 min (g_{s-15}) was used in comparisons of species. For both non-induced and induced leaves, the induction state at 60 s (IS_{60}) was calculated as the net photosynthetic rate 60 s after reillumination expressed as a percentage of the leaf A_{\max} (cf. Pons et al. 1992).

To measure the induction response to fluctuating light, leaves which were previously restricted to low light were exposed to a sequence of five lightflecks, each 20 s long, separated by 2 min of low light. Lightfleck PFD was 735 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For this experiment, the leaves used for the photosynthetic induction response in constant light were used again but measured on a different day. The photosynthetic induction state was calculated in each lightfleck.

Note that because the response time of the gas-exchange system was 15 s induction loss was actually measured at 45 s instead at 60 s. Similarly, the induction state in each light fleck was measured at 5 s instead at 20 s. For simplicity, we used IS_{60} as notation to quantify induction loss at 45 s.

The effect of leaf aging on photosynthetic induction was measured in saplings of *Vouacapoua* and *Dicorynia* only. The old leaves chosen were about 2-4 years old and were not senescent or covered by epiphylls; they were measured from saplings that were also used in the other experiments. Mean leaf life span of saplings (1-2 m height) of *Dicorynia* and *Vouacapoua* is about 3 and 6 years, respectively (Rijkers et al. chapter 5); for *Pourouma* it is more than 1 year (F. Bongers, unpublished data). No measurements were made on old leaves of the *Pourouma* saplings, as their age was not known.

Modeling the $A-p_i$ relationship of a fully-induced leaf

During the induction response, the demand for CO₂ depends on the rate of biochemical activation, whereas a decrease in stomatal limitation reflects the supply of CO₂. To distinguish between the demand for and the supply of CO₂

during induction, A was plotted as a function of p_i . The photosynthesis model of Farquhar and von Caemmerer (1982) was used to describe the steady-state response of A to p_i at full induction (so-called "demand function"). For this purpose, the maximum carboxylation efficiency (V_{cmax}) was estimated by taking the average of the last five measurements of A and p_i of the induction response (data not shown). The parameter V_{cmax} represents the CO_2 assimilation rate in the CO_2 -limited region of the A - p_i curve, which in turn depends on the amount of active Rubisco. Furthermore, we took the CO_2 -compensation point in the absence of day respiration (Γ^*), because it is not affected by induction state and shows little variation between plant species (e.g. Brooks and Farquhar 1985). A mean leaf night respiration rate of $0.22 \mu\text{mol m}^{-2} \text{s}^{-1}$ was assumed in all species; this value was calculated from steady-state photosynthetic light responses of leaves of saplings of *Dicorynia* and *Vouacapoua* (Rijkers et al. chapter 4). Because Γ^* is temperature dependent, true values of Γ^* were calculated using the equation by Brooks and Farquhar (1985): we took Γ^* at 25°C as 3.7 Pa . Finally, to quantify the removal of the biochemical and stomatal limitation during photosynthetic induction, the time needed to reach 75% of V_{cmax} was calculated (Küppers and Schneider 1993). At 75% of carboxylation efficiency, the time needed to reach this percentage could be determined accurately; that is, the intersection of the imaginary 75% carboxylation efficiency curve with steady state measurements of A versus p_i was most distinct (see Fig. 2).

Statistical analysis

Differences in induction properties and light variables, derived from hemispherical photographs, between saplings of the three species growing in two contrasting forest light environments were analyzed by a two-way ANOVA. Multiple contrasts were analyzed using the Tukey HSD test after a one-way ANOVA. Within species, differences in induction responses to a sequence in lightflecks between saplings growing in gaps and those growing in the understorey were analyzed using a Student t-test. Data were natural log-transformed if they deviated from normality and/or homogeneity of variance.

Results

Forest light environment

From hemispherical photographs, mean DSF and ISF for saplings growing in gaps were estimated to be about four times higher than for those growing in the forest understorey (Table 1). As a result, the mean total daily PFD received by gap saplings was fourfold higher than that received by understorey saplings.

Table 1. Light environment of saplings growing in the forest understorey and in gaps. Mean values (\pm SD) of the canopy openness (CO), total photon flux density (total PFD), direct site factor (DSF) and indirect site factor (ISF). Since a two-way ANOVA showed that for all light variables there were environmental effects only ($P < 0.001$) data of species were pooled in each forest environment. Light variables between environments were significantly different (Student *t*-test, $P < 0.05$). Data were ln-transformed prior to analysis. *n* = number of saplings.

Environment	CO (%)	Total PFD (mol m ⁻² d ⁻¹)	DSF (%)	ISF (%)	<i>n</i>
Understorey	3.1 \pm 0.5	2.5 \pm 0.6	5.4 \pm 1.4	4.7 \pm 0.7	18
Gap	8.6 \pm 2.7	10.2 \pm 3.2	22.3 \pm 6.9	16.5 \pm 5.0	16

Using the data of Van der Meer and Bongers (1996), who measured gap sizes in the same Nouragues forest area, we estimated the average size of our selected forest openings at 400 m², characterizing a medium sized gap. Note, however, that this average gap size is likely to be underestimated, as most of the studied saplings were not growing in the center of the gap.

Photosynthetic induction

When the PFD was increased from 5 to 735 $\mu\text{mol m}^{-2} \text{s}^{-1}$ the time course of photosynthetic induction resembled a hyperbolic response type in all species, with the exception of leaves of *Pourouma* growing in bright light, which showed a two-phase (somewhat sigmoidal) type of induction (Fig. 1b).

Table 2 shows the values of the photosynthetic induction and stomatal opening responses to a light increase for the three studied species. A two-way ANOVA (Table 3) showed a significant interaction effect (species \times environment) only for A_{max} and the time to reach 90% of A_{max} . The interaction effect indicates that species responded differently to forest light environment for these two variables. Furthermore, the ANOVA demonstrated a significant environment effect for all the variables, except for the time to reach 90% of A_{max} , and a significant species effect for A_{max} , IS_{90} and the g_{s-15} . Subsequently, we analyzed the differences in induction properties between both species and environment (Table 2). The A_{max} for *Pourouma* and *Vouacapoua* gap saplings was the same, but it was almost twice as high as that in understorey saplings. Overall, A_{max} values in saplings of *Dicorynia* was equal to those in understorey saplings of the other two species. All leaves required 7 to 11 min to reach 90% of their A_{max} . The initial stomatal conductance ($g_{s\text{-initial}}$) in low light did not differ between forest environment for all species, despite the significant

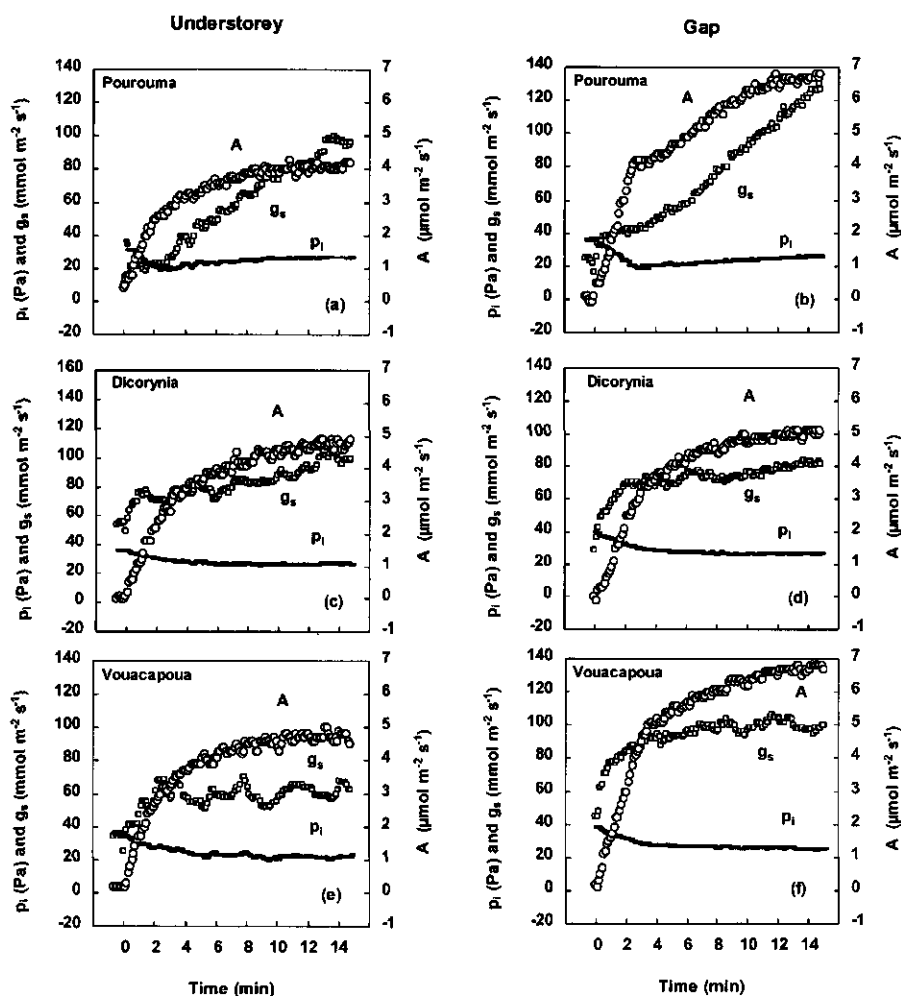


Fig. 1. Time course of the response of net photosynthetic rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) and intercellular CO_2 pressure (p_i , Pa) to an increase in light in a non-induced leaf of a *Pourouma* (a,b), *Dicorynia* (c,d) and *Vouacapoua* (e,f) sapling growing in a gap and in the forest understorey, respectively. Note the difference in the p_i and g_s scale of the understorey growing *Dicorynia* to clear this graph (c).

environmental effect (see Table 3). Overall, however, $g_{s\text{-initial}}$ tended to be higher for gap saplings (Table 2), despite the fact that prior to induction measurements, all saplings experienced uniform-shading conditions from the shade screen above each individual ($\leq 20 \mu\text{mol m}^{-2} \text{s}^{-1}$). Understorey saplings of *Vouacapoua* had the lowest g_{s-15} and gap ones of *Pourouma* had the highest

Table 2. Photosynthetic induction and stomatal opening responses to a light increase in saplings of three species growing in the forest understorey and in gaps. Mean values (\pm SE) of the light-saturated rate of photosynthesis (A_{\max}), time required to reach 90% of A_{\max} , induction state after 60 seconds (IS_{60}), initial stomatal conductance ($g_{s\text{-initial}}$) and stomatal conductance after 15 minutes ($g_{s\text{-15}}$). Means in one column with the same letter (or without a letter) were not significantly different ($P > 0.05$). Multiple contrasts were analysed using the Tukey HSD test after an one-way ANOVA.

Species	Environment	A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Time to 90% of A_{\max} (min)	IS_{60} (%)	$g_{s\text{-initial}}$ ($\text{mmol m}^{-2} \text{s}^{-1}$)	$g_{s\text{-15}}$ ($\text{mmol m}^{-2} \text{s}^{-1}$)
<i>Pourouma</i>	Understorey	4.1 \pm 0.3a	7.4 \pm 0.8	30 \pm 3ab	29 \pm 10	96 \pm 15bc
	Gap	7.5 \pm 0.6b	9.9 \pm 0.8	22 \pm 4a	50 \pm 18	156 \pm 22bd
<i>Dicorynia</i>	Understorey	4.3 \pm 0.2a	10.8 \pm 1.4	25 \pm 1ab	29 \pm 14	73 \pm 13acd
	Gap	5.1 \pm 0.3a	8.2 \pm 1.0	18 \pm 3a	41 \pm 5	69 \pm 10ac
<i>Vouacapoua</i>	Understorey	4.1 \pm 0.2a	8.2 \pm 0.8	40 \pm 6b	20 \pm 4	45 \pm 5a
	Gap	7.2 \pm 0.5b	8.4 \pm 0.6	29 \pm 2ab	42 \pm 12	88 \pm 14bc

Table 3. Results of a two-way ANOVA of the effect of forest light environment on A_{\max} , time required to reach 90% of A_{\max} , IS_{60} , $g_{s\text{-initial}}$ and $g_{s\text{-15}}$ in saplings of three species (abbreviations as in Table 2). F-values are given with their significant levels: * $p < 0.05$, ** $p < 0.01$, *** $P < 0.001$, ns not significant. $g_{s\text{-initial}}$ and $g_{s\text{-15}}$ were ln-transformed prior to analysis.

Effect	A_{\max}	Time to 90% of A_{\max}	IS_{60}	$g_{s\text{-initial}}$	$g_{s\text{-15}}$
Species	4.3*	0.9ns	5.5*	0.1 ns	10.5***
Environment	61.1***	0.0ns	6.7*	4.7 *	8.3**
Species x Environment	6.9**	4.0*	0.1ns	0.02ns	2.7ns

values. Within species the IS_{60} of non-induced leaves tended to be lower in gap than in understorey saplings, suggesting a lower capacity to respond to a light increase for leaves developed in gaps. Gap saplings of *Pourouma* and *Dicorynia* had a significantly lower IS_{60} than understorey saplings of *Vouacapoua*; note, however, that owing to the higher A_{\max} of gap leaves of *Pourouma* induction may take longer.

To distinguish between the relative importance of biochemical and stomatal limitation during photosynthetic induction, A was plotted as a function of p_i ; for

simplicity, only responses of understorey saplings are shown (Fig. 2a-c). In *Pourouma* the plotted measurements showed two phases: first, a gradual decrease in p_i caused by light activated biochemical components of the photosynthetic apparatus and slow opening of stomata; secondly, an increase in p_i due to decreasing stomatal limitation (Fig. 2a). The other two species showed a constant decrease in p_i until A reached the steady-state A- p_i curve, suggesting no difference in time span between removal of stomatal and biochemical limitation (Fig. 2b,c).

The intersection of the 75% $V_{c_{max}}$ curve with the measured A- p_i values was used to quantify removal of biochemical and stomatal limitation during induction. Within species there were no differences between gap and understorey saplings in the time to reach 75% of $V_{c_{max}}$ (Table 4). *Pourouma* saplings growing in the understorey required significantly less time to reach 75% of $V_{c_{max}}$ than those in gaps of *Dicorynia* and *Vouacapoua* (Table 4), indicating a rapid activation of the biochemical components, especially Rubisco, associated with the photosynthetic system. In addition, among understorey saplings, mean time to 75% of $V_{c_{max}}$ tended to be lower in *Pourouma* (ca 2.5 min) than in the other two species (4.5 - 4.9 min). Although g_s at 75% of biochemical induction did not differ significantly between species and forest environment, it tended to be lower in saplings growing in the understorey (data not shown). When induction continued for 5 min after reaching a biochemical induction state of 75%, g_s almost doubled (ca. 80%) in saplings of *Pourouma* in both forest environments, whereas it remained constant or increased slightly in saplings of *Vouacapoua* and *Dicorynia* (Table 4). These results indicate that in *Pourouma* leaves the increase in stomatal opening, following full activation of biochemical factors involved in induction, determined a further rise in the net photosynthetic rate. This corresponds to the two-phase response seen in Fig. 1, particularly in *Pourouma* gap saplings.

The shape of the time course of induction in old leaves of *Dicorynia* and *Vouacapoua* was similar to that in young leaves (data not shown). Although old leaves reached a much lower A_{max} than young ones, times to reach 90% of A_{max} and IS_{60} were similar (Table 2 and 5), suggesting that photosynthetic induction did not change with increasing leaf age. Note, however, that the small sample size of old leaves, especially in *Dicorynia*, hampered an adequate comparison between young and old leaves. Within species, $g_{s-initial}$ and g_{s-15} were the same for old and young leaves for both gap and understorey saplings, except for g_{s-15} in old leaves of understorey saplings of *Dicorynia* which was much lower. In *Vouacapoua*, old leaves of understorey saplings showed a significantly lower A_{max} , $g_{s-initial}$ and g_{s-15} than in gap saplings (Table 5).

Table 4. The response to a light increase in non-induced leaves of three species in the forest understorey and gaps. Mean values (\pm SE) are given of the time required to reach a biochemical induction state of 75% (75% of V_{cmax}) and percentage change in g_s when induction continued for 5 minutes after reaching 75% of V_{cmax} . Means in one column with the same letter were not significantly different ($P > 0.05$). Multiple contrasts were analysed using the Tukey HSD test after an one-way ANOVA. n = number of saplings. Results of two-way ANOVA for the time to 75% of V_{cmax} : effects of species significant ($F = 6.7$, $P < 0.01$); environment and interaction not significant ($P > 0.05$). Results of two-way ANOVA for % change in g_s after 75% of V_{cmax} : effects of species significant ($F = 12.2$, $P < 0.001$); environment and interaction not significant ($P > 0.05$).

Species	Environment	Time to 75% of V_{cmax} (min)	% Change in g_s After 75% of V_{cmax}	n
<i>Pourouma</i>	Understorey	2.5 \pm 0.3a	82 \pm 24b	6
	Gap	3.6 \pm 0.7ab	78 \pm 22b	5
<i>Dicorynia</i>	Understorey	4.7 \pm 0.7ab	21 \pm 13ab	3
	Gap	4.9 \pm 0.4b	-4 \pm 5a	5
<i>Vouacapoua</i>	Understorey	3.5 \pm 0.5ab	6 \pm 5a	5
	Gap	4.5 \pm 0.3b	19 \pm 10ab	5

Table 5. Photosynthetic and stomatal induction responses to a light increase in old leaves of saplings of *Dicorynia guianensis* and *Vouacapoua americana* growing in the forest understorey and in gaps. Mean values (\pm SE) of A_{max} , time to reach 90% of A_{max} , IS_{90} , $g_{s\text{-initial}}$ and $g_{s\text{-15}}$ (abbreviations as in Table 2). For *Vouacapoua*: means in one column with the same letter were not significantly different (Student t-test, $P > 0.05$). For *Dicorynia* no statistical analysis was done because of small sample size. n = number of saplings.

Species	Environment	A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Time to 90% of A_{max} (min)	IS_{90} (%)	$g_{s\text{-initial}}$ ($\text{mmol m}^{-2} \text{s}^{-1}$)	$g_{s\text{-15}}$ ($\text{mmol m}^{-2} \text{s}^{-1}$)
<i>Dicorynia</i>	Understorey	1.8 \pm 0.0	9.5 \pm 4.0	29 \pm 1	22 \pm 5	33 \pm 9
	Gap	3.5 \pm 0.5	10.1 \pm 0.2	23 \pm 0.5	33 \pm 9	75 \pm 1
<i>Vouacapoua</i>	Understorey	2.7 \pm 0.2a	9.8 \pm 2.2a	47 \pm 9a	18 \pm 3a	34 \pm 4a
	Gap	5.0 \pm 0.4b	6.0 \pm 0.6a	39 \pm 5a	37 \pm 5b	72 \pm 8b

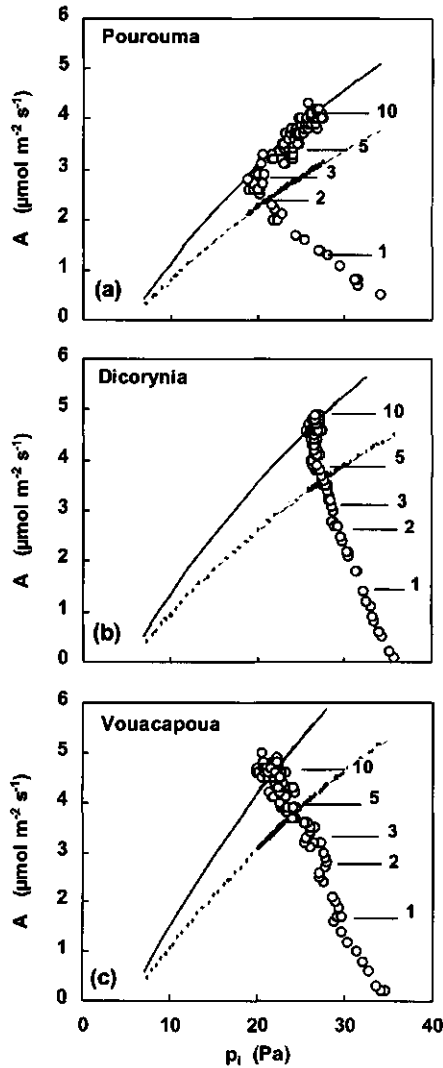


Fig. 2. Net photosynthetic rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) plotted against intercellular CO_2 partial pressure (p_i , Pa) during photosynthetic induction in a leaf of a *Pourouma* (a), *Dicorynia* (b) and *Vouacapoua* (c) sapling growing in the forest understorey. The solid curve is the A - p_i relationship of a fully-induced leaf modeled as Rubisco-limited CO_2 assimilation rates, the dotted line represents the modeled CO_2 assimilation rate at 75% of carboxylation efficiency (V_{cmax}) (see Materials and Methods). The same data are used as shown in Fig.1 (forest understorey saplings). Numbers indicate minutes after exposure to high light.

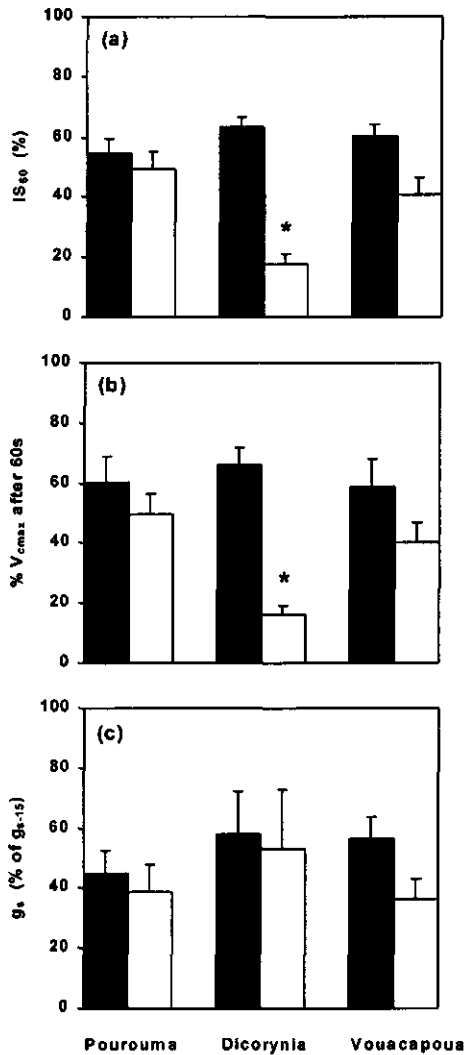


Fig. 3. Mean values (\pm SE) of the photosynthetic induction (IS_{60}) (a), the percentage of maximum carboxylation capacity (V_{cmax}) (b) and the decrease in stomatal conductance (g_s) as percentage of g_{s-15} (c) for fully-induced leaves after 60 seconds of exposure to high light following a low-light period of 20 min. Data are shown for saplings of the three species growing in the understorey (filled bars) and in gaps (open bars). Bars with no asterisk were not significantly different ($P > 0.05$). Multiple contrasts were analyzed using the Tukey HSD test after one-way ANOVA. A two-way ANOVA for IS_{60} showed that there were effects of species ($F = 7.4$, $df = 2$, $P < 0.01$), environment ($F = 38.4$, $df = 1$, $P < 0.001$), and their interaction ($F = 12.5$, $df = 2$, $P < 0.001$). Results of two-way ANOVA for the biochemical induction state at 60s showed effects of species ($F = 4.9$, $df = 2$, $P < 0.05$), environment ($F = 26.5$, $df = 1$, $P < 0.001$), and their interaction ($F = 8.1$, $df = 2$, $P < 0.01$). For percentage decrease in g_s , no significant differences were found.

Induction loss

After fully-induced leaves were exposed to a 20 min low-light period the induction state decreased by 50-60% for all species in both environments, except for gap saplings of *Dicorynia* which showed a rapid loss of induction (Fig. 3a). As the percentage biochemical induction 60 s after reillumination was also very low, about 20%, in leaves of *Dicorynia* gap saplings (Fig. 3b), this suggests a rapid decline in the activity of biochemical factors associated with induction. After 20 min of low light, stomatal conductance decreased, on average 50%, in relation to g_{s-15} in all species. However, *Pourouma* and gap saplings of *Vouacapoua* tended to have a greater decrease in g_s (ca 60%) than the other saplings (ca 40%) (Fig. 3c).

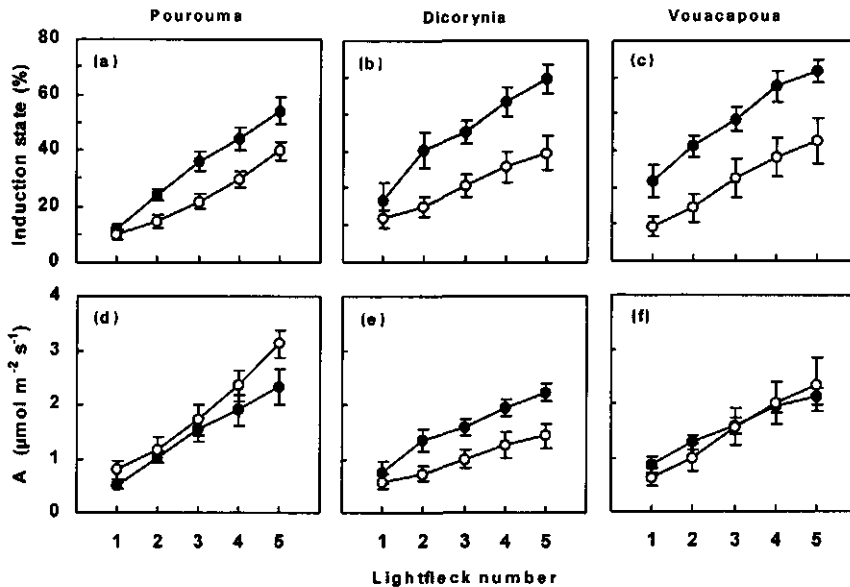


Fig. 4. Mean values (\pm se) of the induction state (%) (a-c) and net photosynthetic rate (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$) (d-f) for leaves of three to five saplings of *Pourouma*, *Dicorynia* and *Vouacapoua* during a sequence of five lightflecks, each 20s long, separated by 2 min of low light. Lightfleck PFD was $735 \mu\text{mol m}^{-2} \text{s}^{-1}$. In each plot filled dots are understorey saplings and open dots are gap saplings.

Response to a sequence of lightflecks

Photosynthetic induction increased during a sequence of five lightflecks, for all species (Fig. 4a-c). Within species, induction state at the end of the first lightfleck was significantly higher in forest understorey saplings of *Vouacapoua* (Fig. 4c) (Student t-test, $P < 0.01$); in *Pourouma* and *Dicorynia* induction state did not differ between the two light environments (Fig. 4a,b). After the fifth lightfleck, leaves in the forest understorey had reached about 50% of A_{max} , whereas those in gaps of *Dicorynia* and *Vouacapoua* had reached about 30% and those of *Pourouma* about 40% of A_{max} (Fig. 4a-c). Within species, induction state at the end of the last lightfleck was significantly higher in forest understorey saplings of *Dicorynia* and *Vouacapoua* than in gap saplings (Fig. 4b,c) (in both species, Student t-test, $P < 0.01$). In *Pourouma*, it tended to be higher in understorey than in gap saplings; however, this was not significant (Student t-test, $P > 0.05$). Leaf induction state at the end of each intermediate lightfleck (i.e. number 2, 3 and 4) was significantly higher in understorey than in gap saplings in all species (Student t-test, $P < 0.05$). In *Pourouma* and *Vouacapoua*, the A reached after each specific lightfleck was similar between gap and understorey saplings ($P > 0.05$; Fig. 4d,f). In *Dicorynia*, however, A tended to be consistently higher in understorey than in gap saplings (Fig. 4e), although this was only significant after lightflecks 2 and 5 (Student t-test, $P < 0.05$). During the sequence of lightflecks, g_s remained rather stable, ranging from 25 to 60 $\text{mmol m}^{-2} \text{s}^{-1}$, in all species, except for gap saplings of *Dicorynia* in which g_s decreased slightly (data not shown). In *Vouacapoua*, gap saplings tended to have a higher g_s than those in the understorey (data not shown).

Discussion

Overall, the mean time to reach 90% of A_{max} was short, 7 to 11 min, which is consistent with induction times found in other field growing shade-tolerant species (e.g. Kursar and Coley 1993; Valladares et al. 1997; Naumburg and Ellsworth 2000). However, induction studies are accumulating indicating that fast induction times (< 10 min) are not restricted to shade-tolerant species but can also be found in light-demanding ones (Poorter and Oberbauer 1993; Roden and Pearcy 1993; Yanhong et al. 1994; Valladares et al. 1997). Additionally, slow induction, lasting up to 1 h or more, is also found in a variety of species, regardless of life form and/or light requirement for growth (Chazdon and Pearcy 1986a; Poorter and Oberbauer 1993; Pons et al. 1992; Ögren and Sundin 1996; Zipperlen and Press 1997). Note, however, that photosynthetic induction times in field growing plants may differ from those measured in potted or greenhouse grown plants. For example, Kursar and Coley (1993) found that

for two shrubs, the induction time was much faster in naturally occurring than in potted plants. Moreover, rates of induction can also differ between seasons (wet vs dry), or times of day (morning vs afternoon). Poorter and Oberbauer (1993), for example, found slower induction times in the afternoon for two tropical tree species. Allen and Pearcy (2000a,b), observed that induction times for four tropical shrubs were fastest during wet season mornings. They found that season and time of day affected stomatal behavior; i.e., induction responses were limited by stomata, except on wet season mornings, owing to a low $g_{s\text{-initial}}$ and/or maximum g_s .

We found no differences between gap- and shade-growing plants in the time needed to reach 90% of A_{max} (Table 2), indicating a lack of plasticity in the rate of photosynthetic induction. In some other species, induction time was also not affected by light environment (tropical: Chazdon and Pearcy 1986a; Kursar and Coley 1993; Zipperlen and Press 1997; temperate: Roden and Pearcy 1993). When comparing seedlings of *Shorea leprosula*, a light-demanding dipterocarp species, growing in three contrasting forest light environments, Zipperlen and Press (1997) found that those in medium light (DSF 15.1%) induced much faster than those in low and high light (DSF 5.5 and 39.4%, respectively) with a similar induction rate, suggesting a light optimum for maximizing the rate of induction in this species. However, other studies did find that plants grown in low light induced faster than those in high light (Küppers and Schneider 1993; Yanhong et al. 1994).

Because the time course of photosynthetic induction after 1 or 2 min is predominantly determined by g_s and Rubisco activation (Kirschbaum and Pearcy 1988b; Pons et al. 1992), inter- and intra-specific differences in induction times are likely caused by differences in the dynamic responses of these slow-inducing components. Among species, leaves of understory saplings of *Pourouma* required the least time to reach 75% of biochemical induction (Table 4) suggesting a more rapid activation of Rubisco in these leaves. In several studies, however, Rubisco activity did not vary among and within species (Seemann and Kobza 1988; Tinoco-Ojanguren and Pearcy 1993). Non-uniform stomatal opening (i.e. stomatal patchiness) in response to a sudden increase in light, however, might also cause the apparent difference in biochemical activation (e.g. Tinoco-Ojanguren and Pearcy 1993; Kirschbaum and Pearcy 1988b; but see Küppers et al. 1999; Allen and Pearcy 2000b).

In general, time courses of photosynthetic induction are either hyperbolic or sigmoidal, the latter being attributed to a low initial g_s causing a relatively slow increase in A during induction. A hyperbolic induction type is observed when g_s is initially high, photosynthetic induction being mainly determined by removal of the biochemical limitation (e.g. Kirschbaum and Pearcy 1988b). Although *Pourouma* gap saplings showed a weak sigmoidal type of induction (Fig. 1b),

$g_{s\text{-initial}}$ was quite high and similar to the values measured in the other two species. Therefore, the delay in the rate of photosynthesis observed in gap saplings of *Pourouma* was likely caused by much higher attainable stomatal conductances compared to the other species (Table 2). Indeed, we found that, unlike *Dicorynia* and *Vouacapoua*, g_s in leaves of *Pourouma* continued to increase substantially after reaching 75% of the biochemical induction (Table 4). Moreover, the rate of biochemical induction seemed to be faster in *Pourouma* leaves, rendering stomatal conductance an important factor in the further increase in net photosynthesis rate.

Although times to 90% of A_{max} were similar among species, within species A_{max} differed between light environments, except in *Dicorynia* (Table 2). Gap saplings of *Pourouma* and *Vouacapoua* showed much higher photosynthetic capacities than saplings in the understorey, indicating greater photosynthetic plasticity compared to *Dicorynia* saplings (see also Rijkers et al. 2000). A decrease in A_{max} with increasing leaf age was found in both *Dicorynia* and *Vouacapoua* (Table 5). However, the time to 90% of A_{max} , IS_{60} , $g_{s\text{-initial}}$, and g_{s-15} tended to be similar to values observed in young leaves. Apparently, old leaves of *Dicorynia* and *Vouacapoua* still have a similar capacity to respond to light increases. Therefore, they might continue to make an important contribution to whole-plant carbon gain, especially in shaded understorey environments where sunflecks are abundant. By contrast, comparing old and young leaves of fast-growing *Salix* sp. and slow-growing *Pinus sylvestris* plants, Ögren and Sundrin (1996) found that induction rates were slower in old leaves, probably due to increased closure of stomata in low light and retarded opening of stomata in high light.

When fully-induced leaves are shaded, they lose induction, limiting their ability to respond to subsequent sunflecks. We found that the IS_{60} after 20 min low light remained fairly high, except in gap saplings of *Dicorynia* (Fig. 3a); a result comparable with other studies showing similar slow rates of induction loss, even for light demanding species (e.g. Roden and Pearcy 1993; Valladares et al. 1997). A slow loss of induction is mainly due to slow decreases of g_s and Rubisco activity (Pons et al. 1992; Tinoco-Ojanguren and Pearcy 1993). This seemed to occur in all the understorey saplings as g_s was still quite high after 20 min of low light (Fig. 3c). The rapid loss of induction in *Dicorynia* gap saplings appeared to be mainly due to biochemical limitation, because g_s remained relatively high (Fig. 3). A stronger decrease in Rubisco activity compared to g_s during the 20-min low-light period could explain the observed limitation in photosynthetic induction in *Dicorynia*. In *Vouacapoua*, induction loss also tended to be higher in gap than in understorey saplings, which in turn might be due to the somewhat greater relative decrease in g_s (Fig. 3c). In addition to decreasing g_s and Rubisco activity in long periods of low light, a high

loss of induction has also been attributed to a limitation in RuBP regeneration (Sassenrath-Cole and Pearcy 1992). Allen and Pearcy (2000a) found that induction loss after 20 min low light in shade-tolerant shrub species was greater in the afternoon (IS_{90} about 55%) than in the morning (about 75%) owing to a lower maximum A and g_s in the afternoon; induction loss was less affected by season. Rapid induction losses have been reported in other studies as well, though in light demanding species only (Poorter and Oberbauer 1993; Tinoco-Ojanguren and Pearcy 1993; Valladares et al. 1997; Zipperlen and Press 1997). As far as we know, only Zipperlen and Press (1997) and Naumburg and Ellsworth (2000) have also observed a fast induction loss in shade-tolerant tree species.

Overall, photosynthetic induction increased rapidly after exposure to a series of lightflecks (Fig. 4), a result found in many other studies (e.g. Chazdon and Pearcy 1986a; Poorter and Oberbauer 1993). For leaves exposed to short and frequent lightflecks, the induction gain is greater than for those exposed to short, infrequent ones (Roden and Pearcy 1993; Tinoco-Ojanguren and Pearcy 1993; Yanhong et al. 1994), owing to stomatal and Rubisco activity remaining high; photosynthesis is then limited by the relatively fast loss of RuBP regeneration capacity (Sassenrath-Cole and Pearcy 1992). In the Nouragues forest, daily direct PFD measurements above saplings of *Vouacapoua* and *Dicorynia* showed that understorey plants experience very low light levels, i.e., below $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD during 80% daytime, while gap ones are more frequently exposed to high PFD, receiving more than $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD during 25% daytime (Sterck et al. in press). For *Pourouma* saplings, the same light conditions are expected because in the seedling and sapling phase all three species are similarly distributed through different forest light environments (Bongers and Sterck 1998).

What is the relative importance of the rate of induction gain and loss in relation to carbon gain in the three studied species? In our study, the data recording of the gas exchange system was too slow to calculate total carbon gain during a lightfleck. Data logging at very short time intervals, for example at 1 s, is required to monitor accurately an increase in photosynthetic rate and post-illumination CO_2 fixation during, and shortly after, a lightfleck (e.g. Pearcy 1990). Using this approach, true carbon gain can then be calculated via integration of the response curve; and, in addition, lightfleck use efficiency can be determined (cf. Chazdon and Pearcy 1986b). In the current study, the photosynthetic induction state increased gradually during a sequence of lightflecks that followed a long period of low light, indicating an increase in net carbon gain. Despite similar A_{max} among *Dicorynia* saplings, after each lightfleck, A tended to be higher in understorey than in gap saplings, suggesting a lack of acclimation response to lightflecks in gap-growing saplings (Fig. 4b,e). Because

$I_{S_{60}}$ after 20 min of low light was still high and similar for all saplings (except *Dicorynia*), a short time after a lightfleck induction loss might be expected to be marginal, resulting in a net carbon gain quite similar to that of a fully induced leaf. Within species, gap saplings of *Pourouma* reached a higher net photosynthetic rate 60 s after reillumination than did understorey saplings (3.9 and 2.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ respectively) (Student-t-test $P < 0.05$). In *Vouacapoua* no differences in photosynthetic rate were found between gap and understorey saplings (2.9 and 2.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ respectively, $P > 0.05$). Apparently, gap saplings of *Pourouma* are able to retain a high photosynthetic capacity after a long period of low light. Simulating daytime carbon gain of two tropical shrub species taking into account their seasonal (wet, dry) and diurnal (morning, afternoon) induction gain and loss properties Allen and Percy (2000a) showed that it was most limited by the induction responses during short and frequent sunflecks and during dry season afternoons. Within a species, however, total carbon gain for a given total PFD was rather unaffected by season or time of day. Overall, the results of our study show that among shade-tolerant tree species, photosynthetic induction responses to light differ significantly. The three species studied can utilize sunflecks efficiently, particularly in low-light conditions. This may contribute to a positive whole-plant carbon balance, though some attention has to be paid to the effect of diurnal and seasonal changes in induction properties for sunfleck utilization and total carbon gain.

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Chapter 4

Photosynthetic carbon gain in two tropical species: leaf age, tree size and canopy openness effects

With Thijs Pons and Frans Bongers

Abstract

We studied the effects of leaf age on light-saturated rate of photosynthesis (A_{\max}), leaf nitrogen concentration (N) and leaf mass per unit area (LMA) for trees of different heights growing under a closed forest canopy (shade leaves) and in gaps (sun leaves) of two shade tolerant tropical canopy species: *Dicorynia guianensis* and *Vouacapoua americana*. Both species have long-lived leaves (*Dicorynia*, mean 2.5 years; *Vouacapoua*, 5 years). Measurements were made at three time points during the first 30 months of the leaf's life. Furthermore, the effect of light- and height-orientated leaf adjustments for daily leaf carbon gain was evaluated by means of photosynthetic light response curves that were measured for six months old shade and sun leaves; these curves were used to calculate the carbon gain from diurnal measurements of photon flux density in a gap and closed-canopy site. For both species, the morphological and physiological variation in response to leaf age was moderate to low. Area and mass based A_{\max} stayed relatively constant with time in all trees, while LMA increased until leaves were 18 mo old. Variation in mass based leaf N and A_{\max} /leaf N stayed relatively constant with leaf age. This variation in leaf morphology and physiology with leaf age was independent of light. Variation in parameters of the light response curves were similar for both species. The quantum yield and convexity values were similar among heights, light and species. Dark respiration rates tended to be higher in sun leaves and tall trees, although this was not always clear. In a low-light regime, carbon gain was lowest for sun leaves of tall trees owing to the higher dark respiration rates in these leaves. In a high-light regime, there was an effect of tree height rather than of sun or shade acclimation in the variation in leaf carbon gain. That is, carbon gain for both sun and shade leaves was significantly lower in saplings than in other trees, except for sun leaves of *Vouacapoua* saplings of which A_{\max} was similar to other trees.

Introduction

Plants are expected to adjust their leaf and/or whole-plant traits to the environment in such a way that they maximize a net carbon gain (Givnish 1988). The necessity of photosynthetic carbon gain is reflected in the fact that, depending on species and environmental conditions, about half of the assimilates produced per day are respired during the same time period (e.g. Van der Werf et al. 1994). Moreover, a higher net carbon gain increases the availability of carbon for future growth. Increasing plant carbon gain can be achieved either by increasing the intensity of resource acquisition (e.g. enhancing the photosynthetic capacity or increasing the total leaf area), and by increasing the longevity of the foraging structures involved (e.g. leaves or branches).

Since a leaf is the carbon gaining organ of the plant, it is essential to the plant that its carbon gain exceeds its costs of construction and maintenance. In general, a growing leaf is using more assimilates than that it is producing, but when fully expanded it produces a surplus of assimilates. This surplus is exported to other parts of the plant (branch, trunk, roots). From the start of leaf senescence, carbon fixation rapidly declines owing to the breakdown of components involved in the photosynthetic apparatus (e.g. chlorophyll, enzymes) and reallocation of nutrients (e.g. nitrogen) to other parts of the plant (Pons and Jordi 1998). Data on aging effects of photosynthetic activity is important when estimating lifetime carbon balances for single leaves or even whole canopies.

In tropical rain forests, studies concerning the variation in photosynthetic activity, and hence carbon economy, over the full life span of leaves are relatively scarce (e.g. Miyaji et al. 1997; Kitajima et al. 1997) in comparison to those found in temperate regions (e.g. Küppers 1984; Reich et al. 1991a; Witkowski et al. 1992; Schoettle 1994; Diemer and Körner 1996; Morecroft and Roberts 1999). The lack of literature concerning the effect of age on leaf traits in tropical species is presumably owing to very long leaf lifespan many plants have in these habitats (e.g. Reich et al. 1991b) making long-term studies rather difficult.

For species with continuous leaf production, such as early successional species, maximum photosynthetic rates after full leaf expansion show an almost linear decrease with time (Šesták 1981; Witkowski et al. 1992; Ackerly and Bazzaz 1995). In contrast, for species with long-lived leaves (> 1 year) and discontinuous leaf production, such as found in many shade-tolerant species, photosynthetic activity usually remains rather constant with time until it declines rapidly during the relatively short senescent phase (Witkowski et al. 1992; Miyaji et al. 1997).

With respect to the daily leaf carbon balance, plants can have a negative carbon balance for short periods as they may live on their stored reserves, but in the long term a positive balance is inevitable for survival and growth. Daily leaf carbon balance has been constructed for many plant species using different approaches. The most straightforward method is to measure daily courses of photosynthesis rates; this approach, however, is rather time-consuming (e.g. Zotz and Winter 1996). Photosynthetic light-response curves (simple steady-state model) can be applied to calculate carbon gain from daily courses of photon flux density (PFD). This approach, however, neglects transient photosynthetic responses to sudden increases or decreases in PFD resulting in an often overestimation of the daily carbon gain, particularly in shade growing plants (Percy et al. 1994). Other, more sophisticated, models consider these dynamic photosynthetic responses to fluctuating light conditions (e.g. Percy et al. 1994; Stegemann et al. 1999); as a result, these complex models require more input parameters. In this paper, we calculate the potential daily carbon gain from photosynthetic light-response curves and the daily course of PFD; we think that this can be useful tool for examining comparative differences in carbon gain between different-sized trees and different light conditions.

Nowadays, there is a large body of knowledge about photosynthetic acclimation responses of plants to different light conditions (see reviews by Björkman 1981; Chazdon et al. 1996). However, as trees grow taller they probably also adjust to the higher wind velocities and air temperatures, and lower air humidity in the forest canopy. Beside these environmental factors, inherent ontogenetic changes might also affect leaf and whole-plant characteristics when trees grow (Coleman et al. 1994; Rijkers et al. 2000).

In the this paper, we investigated different-sized trees of two tropical shade tolerant canopy tree species that have long-lived leaves: *Dicorynia guianensis* and *Vouacapoua americana*. The following two questions were addressed. (1) What is the effect of leaf age on the photosynthetic capacity and leaf morphology? (2) To what extent do light- and height-orientated adjustments affect the daily net carbon gain of the leaf? These questions were examined by analysing measurements of the light-response of photosynthesis, leaf mass per unit area and nitrogen concentrations made at several time points during the leaf's life for leaves from different-sized trees growing in two contrasting forest light environments in a tropical rain forest in French Guiana.

Materials and Methods

Study site and tree species

The study was carried out at the research station Nouragues in an undisturbed lowland tropical rain forest in French Guiana (40°5' N; 52°40' W). Mean annual rainfall is about 3,000 mm and there is a distinct dry period from September to November (Grimaldi and Riera in press). Both *Vouacapoua americana* and *Dicorynia guianensis* are shade tolerant tree species, but *Vouacapoua* is considered more shade tolerant than *Dicorynia* (Favrichon 1994); they both belong to the family Caesalpiniaceae. The maximum tree height of *Dicorynia guianensis* is about 55m and that of *Vouacapoua americana* is about 45m. They both have compound leaves with 5 to 13 leaflets as dependent on tree size. In this study leaflets are considered functionally equal to simple leaves. For brevity, the species will be referred to by their generic names only.

Tree- and leaf sampling

In a 12-ha plot, five to eight individual trees in a specific height class were selected for each species. Selection was based on the tree height at that moment: about 1m (saplings), 7m (small trees) and 16m (medium-sized trees). The saplings did not have sufficient leaves of the same age cohort per plant, and therefore more individuals were selected, 11 and 13 for *Dicorynia* and *Vouacapoua*, respectively. Trees were selected in gaps, that is growing beneath a forest canopy opening (sun leaves) and under a closed forest canopy (shade leaves) using the crown position index (CPI). This index is based on a visual estimation of the vegetation cover above and around an individual tree, and consists of 5 categories: 1, no direct light; 2, crown lit only from the side; 3, partial vertical illumination; 4, full vertical illumination and 5, crown fully exposed to vertical and lateral light (Dawkins and Field 1978). Category number 2 was subdivided into low (1.5), medium (2) and high (2.5) crown lit from the side (cf., Clark and Clark 1992). In this study, trees with a CPI ≤ 2 were considered closed-canopy individuals and those with a CPI ≥ 2.5 represented gap individuals. All trees had produced new foliage in June and July of 1995, and for each tree, newly produced leaves were tagged and coded to follow their development over time. The studied leaves were positioned in the upper part of the tree crown without being shaded by leaves from the same crown. Access to a tree crown was done by using climbing poles and a single rope technique.

At three time points during the leaf life span, i.e. in the period from September through December from 1995 to 1997, light-saturated rate of photosynthesis (A_{max}), leaf mass per unit area (LMA) and leaf nitrogen concentration (N) were measured for leaflets of the same age cohort.

Photosynthetic light response curves were obtained in six months old leaves only. Table 1 shows the leaf sampling procedure per tree based on the selection of leaves for which gas-exchange measurements were made. Per tree, six leaves were selected for gas-exchange measurements of which three leaves were used to measure the light response of photosynthesis and the other three leaves to measure the A_{\max} only. Finally, this resulted in six leaves for which the A_{\max} was obtained. In each census year, leaves for which only the A_{\max} was measured were harvested together with three additional leaves of the same cohort. For saplings, however, a different leaf sampling scheme was used. That is, individuals were divided in two groups: one for which leaves were used to obtain the light response curve, and the other group for which the leaves were used to measure the A_{\max} only. Leaves of the latter group of saplings were harvested after measuring photosynthesis, hereby no additional leaves were harvested as was done in the taller trees.

During the course of the experiment, a number of the tagged leaves and/or individual trees were lost owing to branch and/or tree fall, hence this reduced the number of measurements in 1997. Leaf loss particularly occurred in individuals of *Dicorynia*.

Table 1. General leaf sampling scheme for *Vouacapoua* and *Dicorynia* trees based on gas-exchange measurements. For saplings leaf sampling is different (see Materials and Methods).

Census year	Age (y)	Number of leaves	Measurement	Result
1995	0.5	3	light-response curve	3 curves
		3	A_{\max} + harvest	6 A_{\max}
		3	harvest	6 harvest
1996	1.5	3	A_{\max}	6 A_{\max}
		3	A_{\max} + harvest	6 harvest
		3	harvest	
1997	2.5	3	A_{\max}	6 A_{\max}
		3	A_{\max} + harvest	6 harvest
		-	harvest	

Measurements

Diurnal courses of photon flux density (PFD) at the leaf level were monitored for one small tree growing in a gap and for one growing under a closed forest

canopy (both *Vouacapoua*) using gallium-arsenide phosphide light sensors (model G-1118, Hamamatsu Photonics, Hamamatsu, Japan). These light measurements were then used to calculate the daily carbon gain for leaves of all studied trees (see Section Calculations). Five light sensors were used which were attached on petioles of leaves located in the upper, periphery part of the tree crown. They were calibrated against a LiCor LI 190SA (Lincoln, NE, USA) in full-sun light. Sensors were placed in a horizontal position and light was measured from 7.30 to 17.00 h, over four consecutive days during the dry season in October 1996 and September 1997. This was done for the closed-canopy and gap individual, respectively. The PFD was recorded every five seconds and one-minute means were stored with a LiCor LI 1000 data logger (Lincoln, NE, USA). Simultaneously, the PFD above the forest canopy was measured at a fixed place in the study area, that is from a permanent platform installed in an emergent tree, using a LiCor LI 190SA sensor. All measurements were carried out under predominately clear-sky conditions; mean daily PFD above the forest canopy ranged from 30-45 mol m⁻².

Circumferences of all harvested leaves were drawn on paper. These drawings were used to calculate leaf area with a video area-meter (Dias, Delta-T Image Analyse System, Eijkelkamp BV, Giesbeek, the Netherlands). Leaves were then dried in a field oven at 50°C for three days and afterwards dry-stored. In the Netherlands, leaf dry weight was measured after they were oven-dried at 70°C for 48 hours. These data were used to calculate the leaf mass per unit area (LMA) for each leaf.

Net photosynthetic rate of a leaf at ambient CO₂ partial pressure was measured using a portable infra-red gas exchange system (CIRAS-1, PP-System, Hitchin, UK) with a Parkinson leaf chamber (2.5 cm²). Photosynthetic light response curves were developed using eight PFD's, from 0 to 1420 μmol m⁻² s⁻¹. The PFD was decreased in a step-wise fashion using a quartz halogen bulb (12V, 20 Watt) which was mounted on the leaf chamber in combination with different neutral light filters. Successively, dark respiration rate (R_d) was measured for the same leaf; hereby the lamp was switched off and the leaf chamber was covered with a black cloth. Preliminary measurements showed that a PFD of 1420 μmol m⁻² s⁻¹ did not cause a decline in photosynthetic rate over a time-period of 30 minutes. In leaves for which the A_{max} was measured only, the time needed for photosynthetic induction was about 15 minutes. Measurements were done under ambient CO₂ concentrations, air temperature and relative humidity. Leaf temperature was calculated using the energy balance equation (Parkinson 1985). All gas-exchange measurements were done in the morning. Gas-exchange parameters were calculated according to the equations of Von Caemmerer and Farquhar (1981).

Per tree, leaves that were harvested and for which the A_{\max} was measured were pooled to determine total nitrogen concentration (N). Total leaf N was measured with an elemental analyser (Carlo Erba, model 1106, Milano, Italy) on oven-dried leaves.

Calculations

The light-saturated rate of photosynthesis per unit leaf nitrogen ($A_{\max}/\text{leaf N}$) was calculated using the mean A_{\max} per tree.

In order to evaluate if light- and height-orientated leaf adjustments result in a different carbon gain, we predicted the daily carbon gain for leaves from trees growing in a gap (sun leaves) and under a closed forest canopy (shade leaves) using the diurnal incident PFD's. Photosynthetic light response curves were fitted using a nonrectangular hyperbola:

$$P_{\text{net}} = \frac{\phi I + P_{\max} - \sqrt{(\phi I + P_{\max})^2 - 4\theta\phi I P_{\max}}}{2\theta} - R_d \quad (\text{eq. 1})$$

(e.g. Johnson and Thornley 1984), where P_{net} is the net photosynthetic rate at a given PFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$), ϕ is the quantum yield (the initial slope of the light response curve, $\text{mol CO}_2 \text{ mol}^{-1} \text{ photon}$), I is the irradiance (PFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$), θ is the curvature factor ($0 \leq \theta \leq 1$, dimensionless), P_{\max} is the asymptotic light-saturated gross photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and R_d is the respiration rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$).

Using the light response curve of a given leaf, the daily net carbon gain (daytime only) of that leaf was calculated from the 1-minute PFD measurements of each light sensor. In this way the potential maximum net carbon gain is calculated because we assumed instantaneous responses of the rate of photosynthesis to changes in PFD and we did not take into account the transient photosynthetic responses to sudden increases or decreases in PFD (e.g. Pearcy et al. 1994) and/or the variability in photosynthetic rates owing to time of day (e.g. Zotz and Winter 1996).

Per leaf, the carbon gain was averaged over 4 days in which the PFD was measured. For each tree height class (i.e. sapling, small- and medium-sized tree) in a forest growth environment (i.e. gap and closed canopy), individual leaves were pooled and used for data analysis.

Statistical analyses

Data were analyzed using the SPSS statistical package. The change in chosen leaf traits after each year was analysed by an one-sample t-test. A two-way Anova was used to analyze the effect of tree height and forest light

environment on daily carbon gain in sun and shade leaves. Multiple contrasts were analyzed using the Tukey HSD test after a one-way Anova. Data were ln-transformed if they deviated from normality and/or homogeneity of variance.

Table 2. Leaf age related changes in light-saturated rate of photosynthesis ($A_{max}/area$), leaf mass per unit leaf area (LMA) and leaf nitrogen content (N/area) of different-sized trees of *Dicorynia* and *Vouacapoua*. Since there were no significant differences between sun and shade leaves in the degree of change with leaf age in any of the traits the data were pooled. Asterisks indicate that the change in leaf trait is significantly different from zero. Mean values of $A_{max}/area$, LMA and N/area are given in each age category. Sample size is the number of trees used (per tree 3-6 leaves were measured). - = no statistical analysis was done for the change in traits from 18 to 30 mo old leaves in 16m tall individuals of *Dicorynia* owing to small tree sample size. Note that the significance of change in traits between 18 and 30 months old leaves was based on the leaf sample size which were 30 months old. Significant levels: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

	Height (m)	Sample size (mo)		Leaf age (mo)			Significance of change in leaf trait		
		6, 18	30	6	18	18 ⁽¹⁾	30	6-18 mo	18-30 mo
<i>Dicorynia</i>									
$A_{max}/area$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1	11	11	2.9	3.4		2.6	*	***
	7	5	5	4.7	4.5		2.9	ns	**
	16	5	2	6.2	6.2	5.7	6.3	ns	-
LMA (g m^{-2})	1	8	7	32	40	40	40	***	ns
	7	6	4	41	47	46	48	*	ns
	16	5	2	61	70	62	56	***	-
N/area (mmol m^{-2})	1	7	7	67	87		72	**	*
	7	6	4	89	85	86	84	ns	ns
	16	5	2	127	117	105	100	ns	-
<i>Vouacapoua</i>									
$A_{max}/area$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1	13	13	3.7	4.8		4.3	***	*
	7	6	6	5.4	5.5		5.5	ns	ns
	16	6	3	6.3	6.8	5.4	5.4	ns	ns
LMA (g m^{-2})	1	8	8	40	45		51	***	***
	7	6	6	53	59		60	***	ns
	16	6	4	62	69	66	65	***	ns
N/area (mmol m^{-2})	1	6	6	71	61		74	***	**
	7	6	6	103	111		96	ns	ns
	16	5	3	108	109	103	91	ns	ns

⁽¹⁾ = mean value for 18 months old leaves based on the sample size of leaves which are 30 months old

Results

Leaf age, morphology, nitrogen concentration and photosynthesis

Because the effect of leaf age on chosen traits was similar between gap and closed-canopy individuals, the measured traits were pooled in each tree height class per year (data not shown). In saplings, A_{\max}/area significantly increased during the first 18 months (for *Dicorynia* about 17%, *Vouacapoua* about 31%) (Table 2), whereas it remained rather constant in the other trees (Table 2). In saplings and small trees of *Dicorynia*, the A_{\max}/area significantly decreased when leaves were 30 mo old, which might be an indication that these leaves entered the phase of senescence. In trees of *Vouacapoua*, there were no significant differences in A_{\max}/area between 18 mo and 30 mo old leaves, except in saplings. In all trees of both species, 18 mo old leaves had a significantly higher LMA than 6 mo old leaves (Table 2). Moreover, LMA continued to increase in 30 mo old leaves of saplings of *Vouacapoua*, whereas it remained constant in all other trees.

The mean (\pm SE) life span of leaves of *Dicorynia* is about 32 ± 1 months (range 17-54), and for *Vouacapoua* it is about 61 ± 3 months (range 27-101) (Rijkers et al., chapter 5). In the present study, the leaf life span of *Vouacapoua* exceeded the time period substantially (i.e. 30 mo) for which traits were measured, whereas for leaves of *Dicorynia* the maximum life span was almost approached. Therefore, in *Vouacapoua* only, for some saplings ($n=4-13$) and

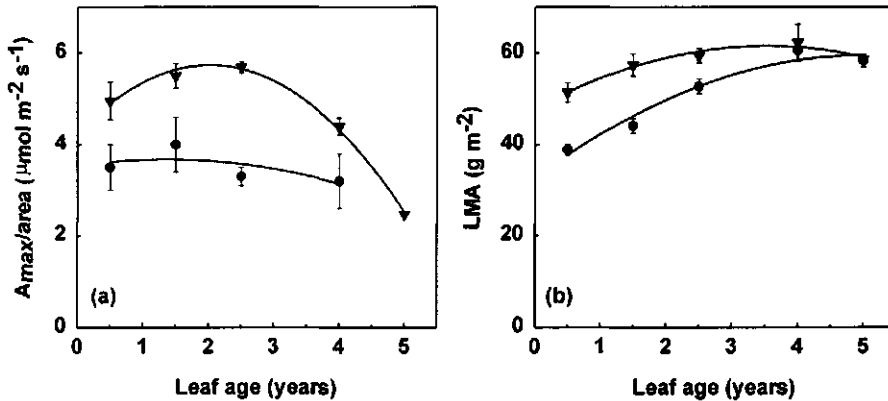


Fig. 1. Light-saturated rate of photosynthesis (A_{\max}/area) (a) and leaf mass per unit area (LMA) (b) in relation to leaf age (years) for saplings (filled dots) and small trees (filled triangles) of *Vouacapoua*. Mean (\pm SE) values are given; a polynomial curve is fitted through the data. Note that for the small trees only 1 tree was used in the leaf age category 5 years.

small trees ($n=4$) the A_{\max}/area and LMA were measured for leaves that were positioned below the leaves that were used in the present study. Based on their position, these leaves were estimated to be between 4 and 5 years old. For medium-sized trees no such data were taken. In saplings, A_{\max}/area was independent of leaf age, whereas it strongly decreased with leaf age in small trees (Fig. 1a). LMA increased with age in saplings, and it remained rather constant in small trees (Fig. 1b).

Since in each census year mass-based A_{\max} and leaf N, and $A_{\max}/\text{leaf N}$ were independent on tree height (see also Rijkers et al. 2000), data for different-sized trees were pooled for a given leaf age. Photosynthetic capacity expressed on a mass basis decreased with leaf age, although only significant for *Dicorynia* ($P<0.05$, from 6 to 30 mo old leaves) (Fig. 2a). Leaf N/mass significantly decreased with leaf age in both species ($P<0.001$) (Fig. 2b); that is, in both species 30 mo old leaves had about 15% less N/mass than 6 mo old leaves. The $A_{\max}/\text{leaf N}$ was independent of leaf age in *Dicorynia*, whereas it slightly increased with leaf age in *Vouacapoua* ($P<0.05$) (Fig. 2c).

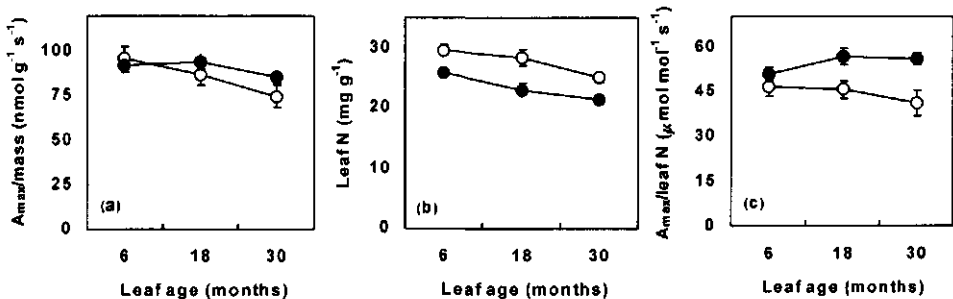


Fig. 2. Mass based light-saturated rate of photosynthesis (A_{\max}/mass) (a), nitrogen concentration (N/mass) (b) and light saturated rate of photosynthesis per unit leaf nitrogen ($A_{\max}/\text{leaf N}$) (c) in relation to leaf age (months) for trees of *Dicorynia* (open dots) and *Vouacapoua* (filled dots). Since these leaf variables were independent of tree height they were pooled in each leaf age category. For *Dicorynia* tree sample size was 11 to 13 and for *Vouacapoua* 14 to 16.

Estimation of daily leaf carbon gain

The resulting parameter estimates of the photosynthetic light response curve for all trees of both species are shown in Table 3. For both species, the quantum yield (ϕ) and curvature factor (θ) appeared to be insensitive to tree height and/or growth environment (i.e. shade and sun leaves). Fitted values of maximum rates of photosynthesis (P_{\max}) were always highest in trees growing in

Table 3. Photosynthetic light response curve parameters of leaves from different-sized trees of *Dicorynia* and *Vouacapoua* growing in a gap (sun leaves) or under a closed forest canopy (shade leaves). Mean values (\pm SE) of the curvature factor (θ , dimensionless), asymptotic light-saturated rate of photosynthesis (P_{\max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), quantum yield (ϕ , $\text{mol CO}_2 \text{ mol}^{-1} \text{ photons}$), respiration rate (R_d , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and light compensation point ($R_d \phi^{-1}$, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). In each species means in one column with the same letter were not significantly different ($P > 0.05$). Multiple contrasts were analysed using the Tukey HSD test after one-way Anova. n = number of leaves; number of trees are given in parentheses.

Species	Height (m)	Leaf	θ	P_{\max}	ϕ	R_d	$R_d \phi^{-1}$	n
<i>Dicorynia</i>	1	Shade	0.727 \pm 0.080 ab	3.1 \pm 0.1 a	0.047 \pm 0.005 a	0.19 \pm 0.01 a	4.1 \pm 0.4 ab	5 (2)
		Sun	0.843 \pm 0.037 ab	3.5 \pm 0.2 ab	0.049 \pm 0.003 a	0.16 \pm 0.03 a	3.3 \pm 0.6 a	6 (2)
	7	Shade	0.886 \pm 0.020 b	5.3 \pm 0.3 bc	0.044 \pm 0.003 a	0.18 \pm 0.03 a	4.1 \pm 0.6 a	10 (4)
		Sun	0.842 \pm 0.026 ab	7.4 \pm 0.7 de	0.041 \pm 0.002 a	0.15 \pm 0.06 a	3.6 \pm 1.2 a	6 (2)
	16	Shade	0.805 \pm 0.024 ab	5.9 \pm 0.2 cd	0.043 \pm 0.001 a	0.26 \pm 0.03 a	6.1 \pm 0.7 ab	12 (4)
		Sun	0.715 \pm 0.070 a	7.7 \pm 0.8 e	0.040 \pm 0.002 a	0.34 \pm 0.06 a	8.8 \pm 1.7 b	9 (3)
<i>Vouacapoua</i>	1	Shade	0.810 \pm 0.021 a	3.7 \pm 0.1 a	0.047 \pm 0.002 bc	0.14 \pm 0.02 a	3.0 \pm 0.5 a	12 (4)
		Sun	0.826 \pm 0.022 a	5.3 \pm 0.3 b	0.054 \pm 0.001 c	0.37 \pm 0.05 b	6.9 \pm 1.0 bc	6 (2)
	7	Shade	0.826 \pm 0.014 a	5.5 \pm 0.4 b	0.046 \pm 0.002 abc	0.16 \pm 0.04 a	3.4 \pm 0.8 a	9 (3)
		Sun	0.806 \pm 0.024 a	6.6 \pm 0.2 bc	0.046 \pm 0.002 ab	0.17 \pm 0.03 a	3.6 \pm 0.6 a	9 (3)
	16	Shade	0.818 \pm 0.024 a	5.8 \pm 0.3 bc	0.049 \pm 0.001 bc	0.25 \pm 0.03 ab	5.1 \pm 0.6 ab	5 (2)
		Sun	0.875 \pm 0.009 a	7.1 \pm 0.4 c	0.040 \pm 0.001 a	0.38 \pm 0.04 b	9.3 \pm 0.8 c	11 (4)

gaps; moreover rates increased with increasing tree height. For *Vouacapoua*, fitted values of R_d tended to be higher for trees growing in gaps. In both species, the light compensation point (i.e. the amount of light at which the net rate of photosynthesis is zero and that was estimated by $R_d \phi^{-1}$) was low, i.e. $< 10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, which is normal for shade leaves but unusual for sun leaves. For *Vouacapoua*, it tended to be higher for trees growing in gaps.

The daily courses of PFD, that were measured at the leaf level for a small *Vouacapoua* tree growing in a gap (high light) and in a closed-canopy site (low light), were used as uniform light measurements to calculate the net carbon gain of either sun- or shade leaves of all studied trees. The daily total PFD varied almost 10-fold, from 0.8 mol m^{-2} under a closed canopy to 7.8 mol m^{-2} in a gap (Fig. 3). Almost 90% of the daily 1-minute PFD measurements in the closed-canopy site were less than $20 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereas in the gap site this was 6% (Fig. 3). In the gap site, the majority of PFD measurements ranged between 20 and $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, although PFD measurements over $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ were not rare, about 13% of the light measurements (Fig. 3). In the closed-canopy light environment, the percentage of daily total PFD that was

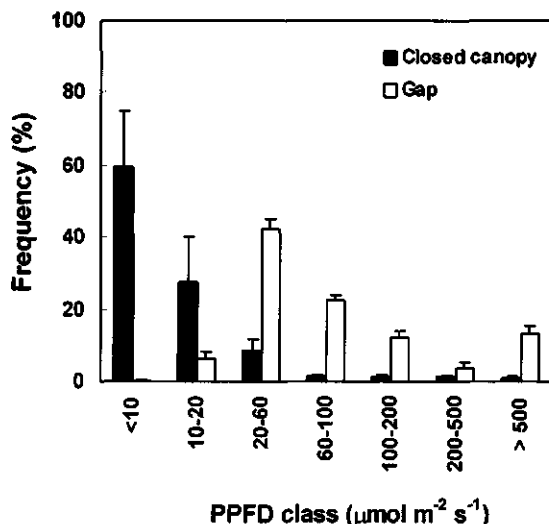


Fig. 3. Frequency histogram of 1-minute means (\pm SD) of photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) measured in the upper peripheral part of the crown of one small *Vouacapoua* tree growing in a gap and under a closed forest canopy, respectively. Light was monitored between 07.30 and 17.00 h using five light sensors during four days in October 1996 and September 1997. The daily total PFD was 0.8 and 7.8 mol m^{-2} , the daily mean PFD was 23 and 228 $\text{mmol m}^{-2} \text{s}^{-1}$, and the percentage PFD of full sunlight was 1.9 and 18.5 for the forest canopy and gap environment, respectively.

contributed by periods of high light (arbitrarily set at $\text{PPFD} > 60 \mu\text{mol m}^{-2} \text{s}^{-1}$) ranged from 24–63%, and the mean daily duration of these high-light periods ranged from 15 to 35 minutes (data not shown).

Table 4. Results of a two-way Anova of the effect of growth environment (sun and shade leaves) on the daily carbon gain of leaves of different-sized trees of *Dicorynia* and *Vouacapoua* using a closed-canopy (low photon flux density, PFD) and a gap light environment (high PFD). F-values are given with their significant levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns not significant. The predicted carbon gain of *Dicorynia* leaves in the high PFD environment was ln-transformed prior to analysis.

Effect	Daily carbon gain			
	Low PFD		High PFD	
	<i>Dicorynia</i>	<i>Vouacapoua</i>	<i>Dicorynia</i>	<i>Vouacapoua</i>
Tree height	6.8 **	8.7 ***	46.4 ***	9.8 ***
Growth environment	0.2 ns	10.2 **	16.1 ***	9.4 **
Height x growth environment	2.2 ns	3.8 *	3.6 *	4.5 *

Figure 4 shows the mean daily carbon gain of sun and shade leaves in both low- and high-light environments for each species separately. A two-way Anova (Table 4) showed a significant interaction effect (height \times growth environment) for the net daily carbon gain calculated in a high-light regime (high PFD) in both species, and in a low-light regime (low PFD) in *Vouacapoua* only. The interaction effect indicates that, for trees of different heights, sun and shade leaves responded differently in their carbon gain when exposed to the same

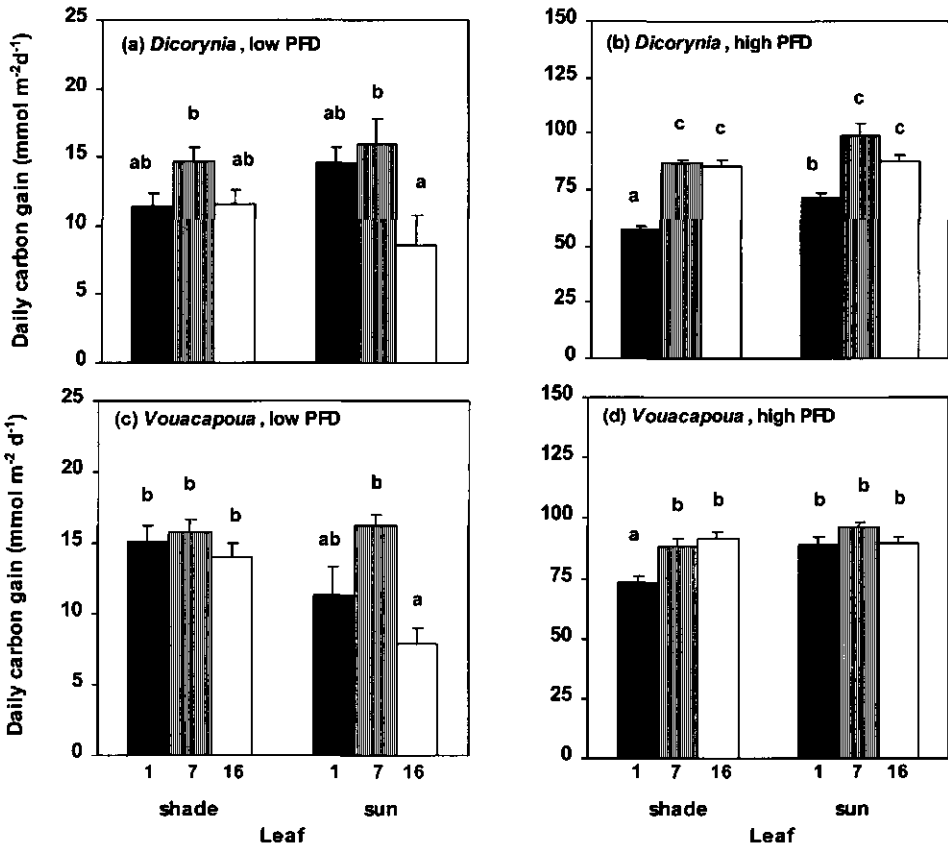


Fig. 4. Predicted daily carbon gain of leaves from different-sized trees of *Dicorynia* (a,b) and *Vouacapoua* (c,d) growing in a gap (sun leaves) or under a closed forest canopy (shade leaves). For both sun and shade leaves the daily net carbon gain was calculated for both a low and high PFD (closed canopy and gap light environment, respectively). Bars represent mean values (\pm SE). Filled bars are saplings, hatched bars are small trees and open bars are medium-sized trees. Within a species and light environment means with the same letter were not significantly different ($P > 0.05$). Multiple contrasts were analysed using the Tukey HSD test after an one-way Anova. Leaf (and tree) sample size per species is the same as in Table 3.

light regime. Furthermore, Table 4 showed a significant tree height effect in all predicted values of daily carbon gain; this was also true for the growth habitat effect except for *Dicorynia* in a closed-canopy light environment. Subsequently, we analyzed the differences in predicted daily carbon gain for sun and shade leaves of different-sized trees exposed to both low and high PFD conditions (Fig. 4). Obviously, for both sun and shade leaves the mean daily carbon gain was much higher when they were exposed to a high than to a low PFD (about 85 mmol m⁻² and 13 mmol m⁻², respectively). In general, there were no significant differences between sun and shade leaves when they were exposed to the same light regime. However, in tall trees of *Vouacapoua*, sun leaves exposed to a low-light regime had only 56 % of the daily carbon gain for shade leaves (Fig. 4c). Furthermore, in saplings of both species, sun leaves had a significantly higher carbon gain than shade leaves when exposed to a high PFD (Fig. 4b,d). The effect of tree height on carbon gain was most profound for leaves exposed to high PFD; that is, leaves of saplings had a lower carbon gain than those in other trees, except in sun leaves of *Vouacapoua* (Fig. 4b,d).

Discussion

Age related changes in leaf traits

The rather constant leaf traits throughout the life span of leaves found for *Vouacapoua* and *Dicorynia* is consistent with other studies in which leaf aging effects on photosynthesis were investigated (Reich et al. 1991b; Witkowski et al. 1992; Miyazawa et al. 1998; Morecroft and Roberts 1999). After attaining the maximum rate, however, photosynthesis can also decrease immediately as leaf aging proceeds. A linear decline of photosynthetic capacity with time is typically found in species with short-lived leaves and/or continuous leaf production, and which have a high photosynthetic capacity, such as fast growing pioneer and agricultural crop species (e.g. Šesták 1981; Chabot and Hicks 1982; Ackely and Bazzaz 1995; Miyaji et al. 1997; Ishida et al. 1999). A rapid decline in photosynthesis has been associated not only with leaf age as such, but also with a reduction in irradiance as existing leaves are shaded by newly formed leaves or by faster growing neighbouring plants (Ackerly and Bazzaz 1995; Ackerly 1999; but see Ishida 1999). Partial shading often leads to redistribution of resources such as nitrogen from leaves in order to improve carbon gain at the whole-plant level. That is, resources present in shaded lower located leaves are more efficiently utilised in new leaves exposed to more favourable light conditions (Field 1983; Pons and Pearcy 1994; Kikuzawa 1995). Consequently, redistribution of N from shaded leaves results in an accelerated senescence (Woledge 1986; Pons and Pearcy 1994; Pons and Jordi

1998). A decrease of carbon gain in shaded leaves might, however, also be caused by redistribution of N from components that determine photosynthetic capacity to those that determine light harvesting (e.g. Pons and Pearcy 1994; Ishida et al. 1999). We did not observe differences in leaf aging effects on area and mass based A_{\max} between sun and shade leaves. By contrast, some studies showed that maximum rates of area-based photosynthesis for sun leaves decreased rapidly while those for shade leaves declined more gradually (Miyaji et al. 1997; Ishida et al. 1999).

In the present study, many individuals of both species formed new leaves above the monitored leaves. Overshading of monitored leaves occurred in about 85 and 85% of the saplings, and in 30 and 65% of the small and in 65 and 0% of the medium-sized trees of *Dicorynia* and *Vouacapoua*, respectively. Apparently, self-shading did occur in the two species, especially in saplings, but the likely reduced light levels did not cause a decline in photosynthetic capacity. Despite the increase in LMA over the first 18 months of the leaf life span, it can be concluded that both *Dicorynia* and *Vouacapoua* exhibit a low morphological and physiological variability in response to age and self-shading. This might not come as a surprise, because owing to the fact that slow growing woody plant species generally have long-lived leaves, and that rapid changes in the light environment at the leaf level do not commonly occur in these species, it is likely that leaf adjustments to light are retained on a more long-term basis. Since leaf N concentration and photosynthetic capacity were relatively constant with time, A_{\max} /leaf N also remained rather unchanged, indicating that old leaves of both species kept their functional efficiency with leaf age. These results are consistent with other studies (e.g. Field et al. 1983; Witkowski et al. 1992), although in some other species a decline in A_{\max} /leaf N with leaf age was observed which, in turn, was caused by a decrease in leaf nitrogen concentration, photosynthetic capacity or both with time (e.g. Sobrado 1994; Kitajima et al. 1997; Ishida et al. 1999; see also Witkowski et al. 1992).

In both species, N/mass decreased with about 15% after about 30 months. This decline, however, could be a dilution effect as LMA increased simultaneously (Table 2). Nevertheless, for *Dicorynia* a somewhat greater percentage of N resorption might be expected as leaves exhibit yellowing (i.e. loss of chlorophyll) just before they are shed (T Rijkers, personal observation). This yellowing is an indication of leaf senescence. For leaves of *Vouacapoua*, on the other hand, a low N resorption is expected as leaves stay green throughout their life span (personal observation). Beside loss of chlorophyll (yellowing), resorption also includes loss of compounds such as protein, lipids and RNA. The following additional field observation supports this hypothesis of a low N resorption in *Vouacapoua*. In 1997, several leaves of a medium-sized

tree were measured two weeks prior to leaf abscission. When these data were compared with those from the previous year, A_{\max} /area declined sharply with about 95% (from 8.6 to 0.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$), whereas N/mass was reduced by 24% only. Apparently, these leaves were senescing. Nitrogen resorption from leaves can range from 0 to 55% in tropical tree species growing in different environmental conditions (Reich et al. 1995a; Raaimakers 1994). In a rain forest in Guyana in which nitrogen was not a limiting factor for growth, Raaimakers found for three species an N resorption of 0%, and for another species a resorption of 33%. Since N/mass values for leaves in the present study were high compared with those found in other tropical forests (e.g. Reich et al. 1995a; Schulze et al. 1994), this suggests that plant growth in the forest at our research site is not N-limited either, and hence low N-resorption efficiencies are not disadvantageous for the plant.

Photosynthetic carbon gain in sun and shade leaves

The variation in parameters of the light response of photosynthesis was rather similar for both species (Table 3). The fitted area-based maximum rate of photosynthesis (P_m) and dark respiration rate (R_d) increased with light availability and tree height, although the R_d was more affected by height than by light.

The quantum yield (ϕ) was not affected by either light or tree height, and it did not differ much between the two species. In many other species, it has also been found that there are no marked differences in ϕ between plants growing in contrasting light conditions (review by Veneklaas and Poorter 1998; Björkman 1981; Ramos and Grace 1990; Lewis et al. 2000). The overall range of light compensation point values, which was estimated as the ratio $R_d \phi^{-1}$, was rather low, 3 to 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$, regardless of tree height and light condition. These low ϕ values, however, reflect the often very low light conditions many plants are exposed to in tropical forests. From diurnal measurements of PFD, taken at different heights in the forest, it appeared that trees of *Dicorynia* and *Vouacapoua* growing under a closed forest canopy were exposed to very low light during most of the day (Fig. 3; Sterck et al. in press). For example, saplings in the understorey received less than 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ nearly 80% of the time; in medium-sized trees, almost 20% of PFD was less than 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but they were more frequently exposed to PFD above 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Sterck et al. in press). In contrast, trees growing in gaps were frequently exposed to high PFD ($> 100 \mu\text{mol m}^{-2} \text{s}^{-1}$) and hence received a greater total daily PFD.

In the present paper, daily carbon gain for sun and shade leaves was calculated using the steady-state responses of photosynthesis to light. The steady-state model is generally inadequate when carbon gain is to be predicted with much precision as it ignores the induction responses of photosynthesis to fluctuating light. It usually leads to an overestimation of carbon gain (see review

Pearcy et al. 1994), particularly in shaded understorey environments in which lightflecks are very common. However, steady-state models may also underestimate carbon gain as they do not take into account the effect of post-illumination CO₂ fixation, i.e. after a lightfleck photosynthesis often continues for a brief period (Pearcy 1990). Dynamic photosynthetic models which consider these induction responses have shown that the overestimation of carbon gain may range from 12-50% in the understorey plant *Alocasia macrorrhiza* (Pearcy et al. 1997) to about 85% in the late successional species *Billia colombiana* (Stegemann et al. 1999). In high light environments, however, the differences between the modelled carbon gains were much lower (<15%). Induction responses for shade-growing saplings of both *Dicorynia* and *Vouacapoua* were moderate to fast (Rijkers et al. in press), suggesting that, in the present study, carbon gain in saplings might be overestimated. On the other hand, along a vertical gradient in the forest, the enhanced irradiance levels, which are usually accompanied with lower vapor pressure deficits and higher air temperatures may cause stomata to closure earlier during the day resulting in reduced rates of photosynthesis (Ryan and Yoder 1997). In this case, a decline in daily carbon gain may also occur, contrary to what is predicted when using steady state models, resulting in an overestimation of carbon gain.

The steady-state modelling approach was applied because it is a useful tool in a comparative study, such as this one, analysing differences in leaf carbon gain of trees of different heights and in contrasting light environments. Regardless of leaf light acclimation or tree height, predicted daily carbon was always higher in the high-light than in the low-light regime owing to the much higher light levels in the gap site. In general, within each forest light environment carbon balances tended to be very similar for all leaves, although there were a few exceptions (Fig. 4). This suggests that the variation in light- and height-orientated leaf adjustments had only a minor impact on daily carbon gain in these two species. The low carbon gain for sun-acclimated leaves of tall trees in closed-canopy light environments is likely caused by the high respiration rates in these leaves. In the high-light regime, the variation in leaf carbon gain showed a tree height effect rather than a leaf light acclimation effect. That is, carbon gain for both sun and shade leaves was significantly lower in saplings than in leaves of the other trees. This was due to a lower A_{\max} /area in saplings.

Conclusions

Leaves of both species exhibited a low variability in morphology and physiology in response to leaf age because for both *Dicorynia* and *Vouacapoua*, leaf traits

stayed relatively constant during the first 30 months of the leaf life span. In the case of *Vouacapoua*, for which the mean leaf life span was much longer than the studied time period, it is expected that these traits will remain unchanged prior to leaf fall (see Fig. 1). In general, the differences in predicted leaf daily carbon gains, when leaves were 6 months old, between species, height and leaf light acclimation were small. In a high-light regime, carbon gain was significantly lower for both sun- and shade-acclimated leaves of saplings, whereas in a low-light regime sun-acclimated leaves of medium-sized trees exhibited reduced carbon gains.

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Chapter 5

Leaf life span related variation in morphological and physiological leaf traits of different-sized trees in a tropical rain forest

With Thijs Pons, Frank Sterck and Frans Bongers

Abstract

Intra-specific variation in leaf life spans was investigated in two shade-tolerant tree species, *Dicorynia guianensis* and *Vouacapoua americana*, in a tropical rain forest in French Guiana. For different-sized trees, leaf life span related variation in photosynthesis, nitrogen concentration, leaf mass per unit area and construction costs were determined. Leaves of *Vouacapoua* lived longer (mean 61 months, range 27-101) than those of *Dicorynia* (mean 32, range 17-54). Life span was inversely related to tree height and light availability in *Vouacapoua*, whereas in *Dicorynia* such a correlation was lacking. Leaf life span related variation in leaf traits was most pronounced in *Vouacapoua*. Intraspecific variation in leaf traits with leaf life span generally did not follow interspecific variation. Only for the variable $A_{max}/area$ (*Vouacapoua*) the direction of the leaf life span related variation was similar to the one generally found among species. For LMA, on the other hand, the life span related variation within a species was opposite to the variation found among species. For *Vouacapoua*, this could be explained by a light- and tree height-dependent selection pressure for leaf life span and LMA. For *Dicorynia* no such selection pressure for leaf life span occurred. Long-lived leaves showed lower light-saturated rates of photosynthesis ($A_{max}/area$) and leaf mass per unit area (LMA) than short-lived ones; mass based leaf nitrogen concentration (N/mass) was not correlated with life span. Both mass based leaf construction costs (CC/mass) and light-saturated net photosynthesis per unit leaf nitrogen ($A_{max}/leaf\ N$) were independent of life span in both species. This suggested a lack of trade-off between investment of resources to high photosynthetic capacity and to extended leaf life span in terms of reduced palatability of leaves. For both species, the time needed to repay the leaf construction costs (payback time) was very short, ranging from 4 to 40 days. Payback time remained rather constant during a wide range of photon flux densities (PFD's), but it increased sharply in a narrow range of low PFD. Only in *Vouacapoua*, payback time tended to be positively correlated with leaf life span, suggesting that long-lived leaves required relatively more time to repay their initial construction costs.

Introduction

Plant species can differ considerably in leaf life span. Among tropical tree species, it can vary from some months up to several years (Coley 1988; Reich et al. 1999). Plants with short-lived leaves usually occur in resource-rich sites, such as fertile soils and bright canopy gaps; those with long-lived leaves predominate low-resource habitats like poor soils or the shady conditions in a forest understorey. There are several arguments that may explain the adaptive value of variation in leaf life span among plants. First, extending the leaf life span increases the residence time of (limited) nutrients, particularly nitrogen, reducing the expenses for re-supplying them via root up-take (Chapin 1980; Chabot and Hicks 1982; Berendse and Aerts 1987). As in many tropical rain forests light, and not nitrogen, is the most important factor limiting plant growth, the nutrient residence time is likely a matter of secondary concern in these habitats. Second, fast growing plants generally have short-lived leaves because resources present in shaded lower located leaves (as a result of self-shading or shading by faster growing neighbour plants) are more efficiently utilised in new leaves exposed to more favourable light conditions (Field 1983; Hirose and Werger 1987; Pons et al. 1989). Third, owing to their low photosynthetic rates, plants in resource limited sites need to extend the lifetime of their leaves in order to repay the leaf's construction costs (Chabot and Hicks 1982). Finally, to deter herbivores plants invest in well-protected lignified leaves (Coley 1988). This, in turn, can dilute compounds involved in photosynthesis reducing the mass based rate of carbon gain and hence necessitates an extending leaf lifetime (Chabot and Hicks 1982; Williams et al. 1989). Although it is predicted that, on a mass basis, long-lived leaves are more expensive to produce than short-lived ones (cf. Kikuzawa 1995), this is still questionable owing to lack of supporting data (Merino et al. 1982; Williams et al. 1989).

It has been proposed that between species leaf life span correlates with a number of other leaf traits (Koike 1988; Reich et al. 1991b, 1992; Diemer 1998). For example, long-lived leaves tend to have low area- and mass-based rates of photosynthesis, mass-based nitrogen concentration, and a high leaf mass per unit area, while the opposite is true for short-lived ones. These relationships, however, are not observed if the range of leaf lifetimes among the species studied is too small (Diemer et al. 1992). Generally, the average lifetime is taken of either sun or shade leaves from plants at a specific developmental phase or age (e.g. seedlings or saplings) leaving out the variability in life span as dependent on plant growth conditions and/or ontogenetic development. This intraspecific variability in leaf life span may not be ignored, particularly if conclusions about life span related leaf traits are generalised over the entire plant population. Therefore, the main objectives of this study was to investigate

whether a relationship exists between leaf life span and physiological and morphological leaf traits within a tree species; if so, to examine if these relationships are similar to those generally found among species.

In woody species, interspecific variations in leaf life span and the combined changes in leaf properties are generally studied for plants differing in light requirements, for example, shade-tolerants vs. light demanders (see Reich et al. 1991b, 1992; Lusk and Contreras 1999). However, within a species comparable patterns might occur, i.e. plants growing in bright (shade) conditions could also have short (long) leaf life spans with comparable changes in traits, like observed in pioneer (late successional) species (Bongers and Popma 1990; Reich et al. 1995b). Variation in leaf life span within a species can also be due to variation in other environmental conditions, for example nutrient availability (Shaver 1983). Beside these environmental causes, life span can also be affected by tree size, as shown by Seiwa (1999) who found that, independent of light, leaf life span was shorter in seedlings than in taller trees of the deciduous species *Acer mono*.

Based on the listed arguments, which explain differences in leaf life span among plants, the following questions were addressed. (1) Is there a variation in leaf life span between different-sized individuals of two shade tolerant tree species? (2) Is the variation in leaf life span affected by increasing light availability? (3) Are physiological, morphological and chemical leaf traits associated with leaf life span? (4) If so, are these relationships similar to those generally found among species? (5) Is the rate of return on carbon investment (expressed on a mass basis), or the leaf payback time, affected by the life span of the leaf?

To answer these questions, the following two canopy trees were studied: *Dicorynia guianensis* and *Vouacapoua americana*. We examined the questions by analysing measurements of photosynthetic capacity, nitrogen concentration, leaf mass per unit area and leaf construction costs (i.e. the amount of glucose needed to construct one gram of leaf biomass) for leaves of different-sized trees growing in contrasting light environments in a tropical rain forest in French Guiana.

Materials and Methods

Study site and species

The study was carried out at the research station Nouragues in an undisturbed lowland tropical rain forest in French Guiana (40°5' N; 52°40' W). A description of the forest site, soil and climate is given in Poncy et al. (1998) and Grimaldi and Riera (in press). *Dicorynia guianensis* Amshoff and

Vouacapoua americana Aubl. (both Caesalpiniaceae) are both shade tolerant; they are common species at the study site. *Vouacapoua americana* is considered more shade tolerant than *Dicorynia guianensis* (Favrichon 1994). Maximum tree height for *Vouacapoua americana* is about 45m and for *Dicorynia guianensis* about 55m. Both species have compound leaves with 5-13 leaflets as dependent on tree size. In this study, leaflets are considered functionally equal to simple leaves. A detailed description of growth characteristics of the two species is given in Sterck (1999). For brevity, species will be referred to by their generic names only.

Tree- and leaf sampling

For each species, individual trees were selected on the basis of their height: (1) saplings (range 0.7-2m), (2) small trees (range 4-9m) and (3) medium-sized trees (13-20m). For similar sized trees, individuals were selected growing both in gaps and under a closed canopy. Climbing poles or a single rope technique were used to access the crown of small and medium-sized trees. All measurements were made on leaves that were about 1.5 years old; leaves were positioned in the upper part of the tree crown in exposed conditions. For each tree, six leaves were sampled in the period from September through November 1996. For saplings, however, three leaves were sampled because of a lack of sufficient leaves. All measurements were averaged per tree. In order to estimate the life span of the leaves, we used data from a long-term study in which the vegetative growth of different-sized trees of the two species was monitored at the Nouragues site for five consecutive years (see Sterck 1997). In that study, the production and loss of leaves of different-sized trees growing across a broad range of forest light environments were monitored annually; light availability on trees was estimated by hemispherical photography (see Measurements). For individuals < 5m all branches and leaves were tagged and coded, whereas for those > 5m a selection of branches positioned in the upper part of the crown was used. In the present study several tree individuals were selected that were also used in this long-term study.

Measurements

Light availability on trees was estimated using hemispherical photographs which were taken with a Canon T70 camera and a 7.5-mm Canon fish-eye lens under a standard overcast sky. From these photographs, canopy openness was calculated as the percentage of unobscured sky (Ter Steege 1994), which was used as an estimation of light availability.

Circumferences of all harvested leaves were first drawn on paper; these drawings were used to calculate leaf area with a video area-meter (DIAS, Delta-T Image Analyse System, Eijkelkamp BV, Giesbeek, the Netherlands). The

leaves were then dried in a field oven at 50°C for three days and afterwards dry-stored. In the Netherlands leaves were oven-dried at 70°C for 48h before leaf dry weight was measured. From these data leaf mass per unit leaf area (LMA) was calculated for each leaf. Prior to harvesting, three out of six leaves were used to measure the light-saturated rate of photosynthesis (A_{\max}). Photosynthetic measurements were done under ambient CO_2 concentrations, air temperature and relative humidity using a portable infra-red gas exchange system (CIRAS-1, PP-System, Hitchin, UK) with a Parkinson leaf chamber (2.5 cm^2). A halogen light unit, mounted on the chamber, was used to set the maximum light intensity at 1420 $\mu\text{mol m}^{-2} \text{s}^{-1}$; preliminary measurements showed that this light intensity did not cause a decline photosynthetic rate over a period of about 30 minutes. All measurements were done in the morning. The rate of photosynthesis and stomatal conductance were calculated according to the equations of Von Caemmerer and Farquhar (1981). For each tree, leaves for which the A_{\max} was measured were pooled to determine nitrogen (N), carbon (C) and ash concentration; these data were then used to calculate mean leaf construction costs per tree (see Section Calculations). In order to calculate the light-saturated rate of photosynthesis per unit leaf nitrogen ($A_{\max}/\text{leaf N}$) the mean A_{\max} per tree was taken. Total N and C content were measured with an elemental analyser (Carlo Erba, model 1106, Milano, Italy) on oven-dried leaves. Ash content was measured after combustion of leaf material in a muffle furnace at 550 °C for five hours.

The payback time of leaves (i.e. the time needed for a leaf to repay its construction costs) was determined for trees for which the leaf construction costs were calculated, and for which the daily photon flux density (PFD) at the leaf level as well as the corresponding photosynthetic light-response curves were measured. In a companion study (see Rijkers et al. chapter 4), the light response of photosynthesis was measured by decreasing the light intensity in a step-wise fashion. Furthermore, GaAsP light sensors (model G-1118, Hamamatsu Photonics, Hamamatsu, Japan) attached to leaves for which the light response of photosynthesis was measured were used to monitor PFD from 7.30 to 17.00 h, for four consecutive cloudless days. The sensors were placed in a horizontal position. PFD was recorded every five seconds and one-minute means were stored with a LiCor LI 1000 data logger (Lincoln, NE, USA).

Calculations

Using the long-term data set of Sterck (1997), the mean leaf life span (months) for the crown was calculated using the following formula (King 1994):

$$\text{leaf life span} = \left\{ \frac{n_a}{0.5 \times (n_p + n_s)} \right\} \times 12 \quad (\text{eq. 1})$$

where n_s is the mean number of leaves alive each year, and n_p and n_s are the mean number of new leaves produced and number of leaves shed per year, respectively. In the present study, for trees for which the leaf life span could not be calculated using equation 1 (for *Dicorynia* 16 out of 22 individuals, and for *Vouacapoua* 12 out of 19; in both species all saplings were included) leaf life span was sought via a multiple regression model describing the relationship between life span and both tree height and canopy openness for each species.

Leaf construction cost, i.e. the amount of glucose needed to construct one gram of leaf biomass, was calculated using the following formula:

$$CC = (-1.041 + 5.077 \times C_{om}) \times (1 - M) + (5.325 \times N) \quad (\text{eq. 2})$$

(Poorter 1994), where CC is the construction cost (g g^{-1}), C_{om} is the carbon content of the organic matter (g g^{-1}), M and N are the mineral and nitrogen concentration respectively (g g^{-1}). The mineral concentration was calculated multiplying the ash concentration by 0.67 (cf. Vetregt and Penning de Vries 1987). Although Poorter (1994) used the organic nitrogen concentration (i.e. total N minus nitrate) to calculate the leaf construction costs, we made no correction for the nitrate content as we assumed it to be a negligible factor in the studied leaves. The approach of Poorter (1994) assumes that nitrate (NO_3^-) instead of ammonium (NH_4^+) is the major nitrogen source in the soil. The carbon costs involved in the conversion of inorganic N to organic N are greater if nitrate is the N source. Since there are no data available whether nitrate or ammonium is the major N source in the forest soil of the Nouragues area, the calculated leaf construction costs represent maximum values in this study.

Leaf payback time, i.e. the time needed for a leaf to repay its construction costs, was determined for a selection of trees only ($n = 5$ for *Dicorynia*, $n = 6$ for *Vouacapoua*). Leaf payback time was calculated as:

$$PT = \frac{\ln(2) \times CC}{A_{net} - LR_m} \quad (\text{eq. 3})$$

(Poorter 1994), where PT is the payback time (days), CC are the leaf construction costs, A_{net} is the daytime net rate of photosynthesis (g glucose g^{-1} dry weight day^{-1}) and LR_m is the nighttime maintenance respiration of the leaf (g glucose g^{-1} dry weight day^{-1}). The payback time is multiplied by $\ln(2)$ to avoid overestimation of the payback time (cf. Poorter 1994); that is, assimilates produced by a leaf could be used to construct another leaf, which in turn may contribute to the payback time of the first leaf (cf. Harper 1989). The photosynthetic light response curves, which were described by a non-

rectangular hyperbola (see Rijkers et al. chapter 4), were used to calculate the net daily carbon gain (A_{net}) from the diurnal measurements of PFD. Note that LR_m was applied as nocturnal maintenance respiration. The LR_m was calculated using the simple approach that maintenance requirements are approximately proportional to the leaf dry weight:

$$LR_m = r \times M_L \quad (\text{eq. 4})$$

where r is the maintenance coefficient ($\text{g g}^{-1} \text{ dry weight day}^{-1}$) and M_L the leaf mass (g). A standard value of 0.03 for r was used (cf. Spitters et al. 1989).

Statistical analyses

Data were analysed statistically using SPSS. Linear regression was used to describe the relationship between the various leaf traits and life span. A multiple regression model, which described the relationship between leaf life span and tree height and canopy openness, was used to predict the life span of leaves of the studied trees.

Results

The calculated mean leaf life span (eq. 1) was plotted against tree height and canopy openness for each species (Fig. 1). Because in *Vouacapoua* leaf life span was dependent on both tree height and light availability (Fig. 1), we used a multiple regression model to predict the life span of leaves that could not be calculated using eq. 1. The following model was fitted through the data in Fig. 1: leaf life span = $78.8 - (1.6 \times \text{Height}) - (1.4 \times \text{Openness})$; ($r^2=0.64$, $P<0.001$; significance of regression coefficient for Height, $P = 0.004$; for Openness, $P = 0.019$). For *Dicorynia*, however, leaf life span was independent of height and openness (Fig.1). Therefore, in this species, for each tree height class, a mean leaf life span was calculated from the overall leaf lifetime distribution.

The mean leaf life span (\pm SE) for *Dicorynia* was 32 ± 1 months (range 17-54) and for *Vouacapoua* 61 ± 3 months (range 27-101) (Fig. 1). Although leaf sampling was restricted to the upper branches in trees > 5 m tall, variation in leaf lifetime between upper and lower branches was quite small (data not shown). Within a species leaf life span varied greatly. In *Vouacapoua*, it

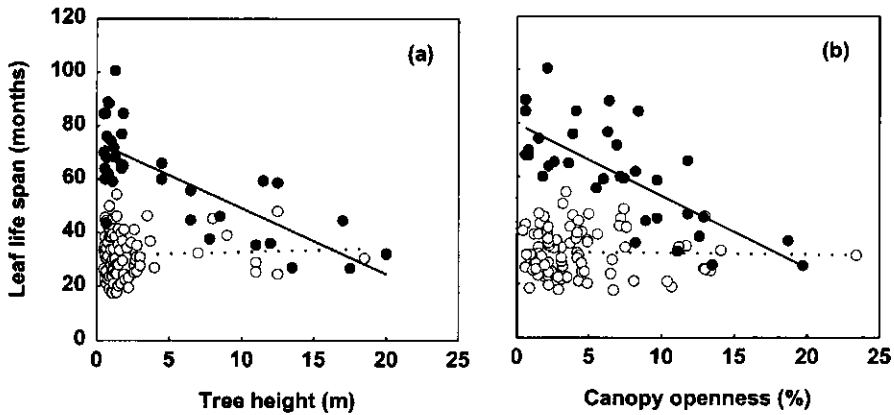


Fig. 1. Leaf life span (months) in relation to tree height (m) (a) and percentage canopy openness (b) for individuals of *Dicorynia guianensis* (open dots, $n = 92$) and *Vouacapoua americana* (filled dots, $n = 35$). Data was derived from the long-term study of Sterck (1997) which investigated growth dynamics of the two species. Regression lines are calculated for each species and they are drawn continuous if significant and broken if not significant. For *Vouacapoua*, $r^2=0.58$, $P<0.001$ for regression with height; $r^2=0.54$, $P<0.001$ for regression with openness.

Table 1. Relationships between various leaf traits (dependent variable) and leaf life span (months, independent variable) of different-sized trees of *Dicorynia* ($n=22$) and *Vouacapoua* ($n=19$). The following leaf traits are shown: light-saturated rate of photosynthesis (A_{\max}/area , A_{\max}/mass), leaf mass per area (LMA), nitrogen concentration (N/area, N/mass), light-saturated net photosynthesis per unit leaf N ($A_{\max}/\text{leaf N}$) and construction costs (CC/area, CC/mass). Regression intercept, slope, coefficient of determination (r^2) and level of significance (P) are given. ns = not significant.

Dependent variable	<i>Dicorynia</i>				<i>Vouacapoua</i>			
	Intercept	Slope	r^2	P	Intercept	Slope	r^2	P
A_{\max}/area ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	5.2	-0.03	0.01	ns	10.7	-0.09	0.64	<0.001
A_{\max}/mass ($\text{nmol g}^{-1} \text{s}^{-1}$)	49.5	1.19	0.13	ns	114	-0.32	0.10	ns
LMA (g m^{-2})	83.1	-1.03	0.10	ns	99.4	-0.75	0.77	<0.001
N/area (mmol m^{-2})	130	-1.2	0.06	ns	174	-1.4	0.54	<0.001
N/mass (mg g^{-1})	22.1	0.13	0.02	ns	27.0	-0.07	0.06	ns
$A_{\max}/\text{leaf N}$ ($\mu\text{mol mol N}^{-1} \text{s}^{-1}$)	39.1	0.3	0.02	ns	61.0	-0.03	0.001	ns
CC/area (g glucose m^{-2})	192	-3.42	0.21	0.037	166	-1.21	0.73	<0.001
CC/mass (g glucose g^{-1})	1.82	-0.005	0.25	0.020	1.66	0.001	0.06	ns

decreased significantly ($P < 0.05$) with both increasing canopy openness and tree height (see above); for *Dicorynia* there were no such relationships observed ($P > 0.05$, Fig. 1a,b).

Significant leaf life span related changes in leaf traits were mainly observed in *Vouacapoua*, whereas for *Dicorynia* life span tended to be of minor relevance in the expression of leaf properties (Table 1). Area-based light-saturated photosynthesis ($A_{max}/area$) decreased significantly ($P < 0.05$) with increasing leaf life span in *Vouacapoua* (Fig. 2a and Table 1). Mass-based net photosynthesis was independent of leaf lifetime (Table 1). The investment of dry-mass per unit

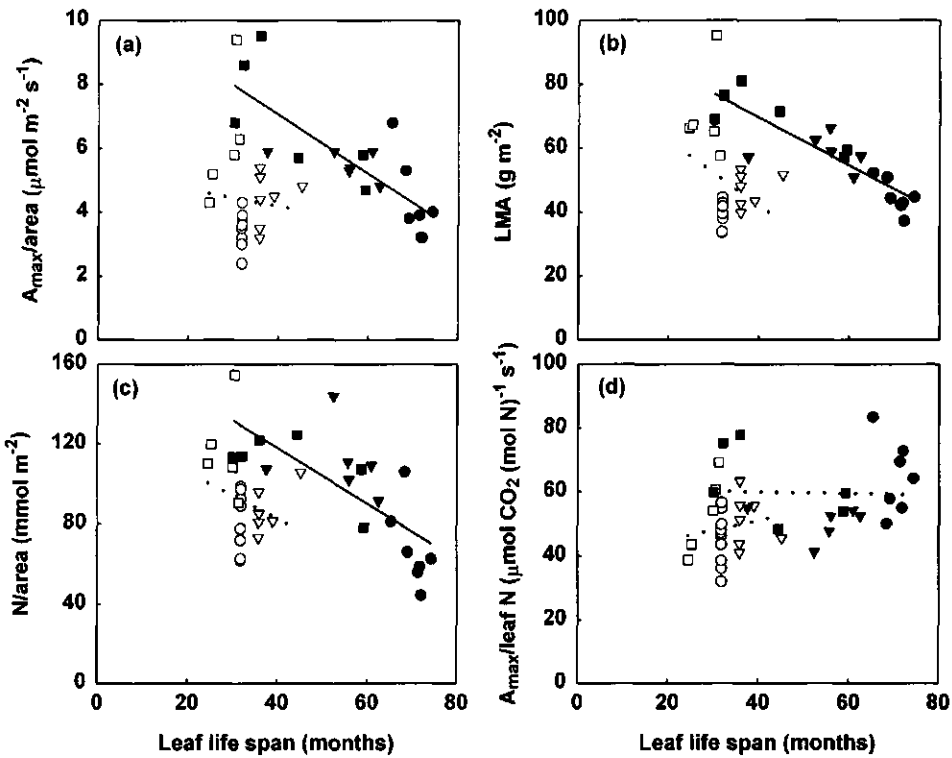


Fig. 2. Area based light-saturated rate of net photosynthesis ($A_{max}/area$)(a), leaf mass per unit area (LMA) (b), area-based nitrogen concentration ($N/area$) (c) and light-saturated net photosynthesis per unit leaf nitrogen ($A_{max}/\text{leaf N}$)(d) in relation to leaf life span for different-sized trees of *Dicorynia* and *Vouacapoua*. Filled symbols are trees of *Vouacapoua* ($n=19$) and open symbols are trees of *Dicorynia* ($n=22$). Dots are saplings (height range 0.7-2 m), triangles small trees (range 4-9 m) and squares medium-sized trees (range 13-20 m). Regression lines are calculated for each species and they are drawn continuous if significant and broken if not significant.

leaf area (LMA) decreased significantly with increasing leaf life span in *Vouacapoua* only ($P < 0.05$, Fig. 2b and Table 1). There was no relationship between N/mass and lifetime (Table 1). Thus, trees with short-lived leaves did not have more nitrogen per unit leaf mass than did trees with long-lived leaves. When expressed on an area basis, nitrogen concentration decreased with increasing leaf life span in *Vouacapoua* (Fig. 2c). Although in *Dicorynia* N/area tended to decrease with leaf life span, it was not significant ($P > 0.05$). When all trees of *Dicorynia*, for which the leaf life span could not be calculated, were omitted from analysis (that is 16 out of 22 trees), only a significant correlation between N/mass and leaf life span was found ($N/mass = 0.23 \text{ leaf span} + 17.2$, $r^2 = 0.70$, $P = 0.04$, $n = 6$). In both species, there was no relationship between $A_{max}/\text{leaf N}$ and leaf life span ($P > 0.05$, Fig. 2d and Table 1), suggesting a lack of trade-off between the investment in CO_2 acquisition and in leaf life span.

Overall, mass based leaf construction costs (CC/mass) of *Vouacapoua* were significantly higher than those of *Dicorynia* (Student t-test, $P < 0.001$, see also Fig. 3b). CC/mass decreased significantly with leaf life span in *Dicorynia* only ($P < 0.05$, Fig. 3b and Table 1), although differences were very small. When expressed on an area basis, construction costs decreased significantly with leaf life span in both species ($P < 0.05$, Fig. 3a and Table 1) which was owing to a similar decrease in LMA (Fig. 2b, 3a). Thus, expenditures for constructing one unit of leaf area are greatest in short-lived leaves.

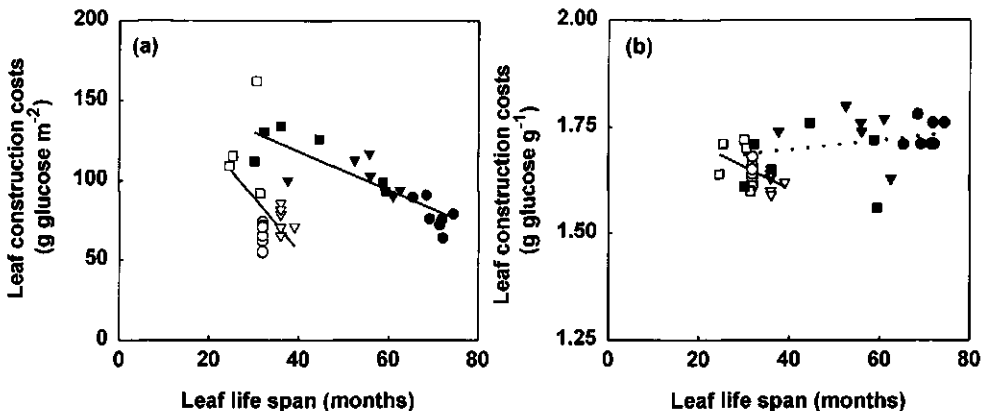


Fig. 3. Leaf construction costs expressed on an area basis (a) and on a mass basis (b) in relation to leaf life span for different-sized trees of *Dicorynia* and *Vouacapoua*. Further information is given in the legend of Fig. 2.

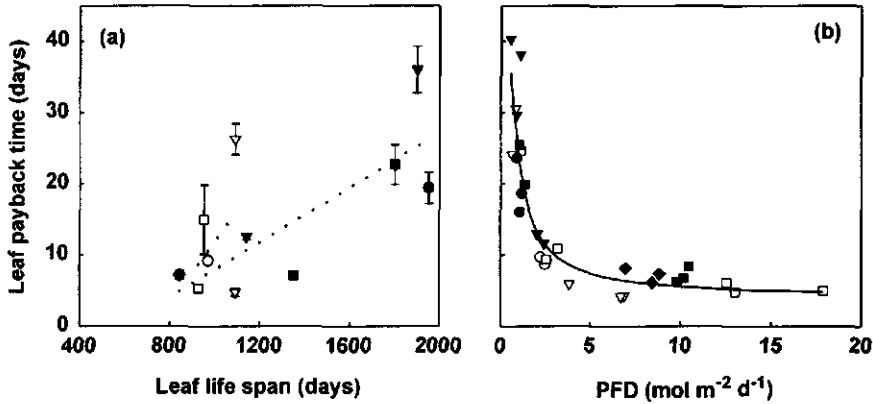


Fig. 4. Leaf payback time in relation to leaf life span (a) and photon flux density (PFD) (b) for different-sized trees of *Dicorynia* and *Vouacapoua*. Filled symbols are trees of *Vouacapoua* ($n=6$) and open symbols are trees of *Dicorynia* ($n=5$). Dots are saplings (height range 0.7-2 m), triangles small trees (range 4-9 m) and squares medium-sized trees (range 13-20 m). Note that for *Vouacapoua* results of a tall canopy tree (about 35m) are added (filled diamond). In the left panel, a symbol represents a mean (\pm SE) for a given tree. In the right panel, a symbol represents a value for a leaf (see Materials and Methods). Regression lines are calculated for each species and they are drawn continuous if significant and broken if not significant. Non-linear relationship between leaf payback time and PFD for all leaves of both species pooled, $r^2 = 0.84$, $P < 0.0001$.

In *Vouacapoua*, long-lived leaves required more time to repay their initial construction costs than did short-lived leaves ($r^2 = 0.63$, $P = 0.06$), while in *Dicorynia* such a relationship was not found (Fig. 4a). Overall, payback times were relatively short, i.e. about 0.4 - 2.4 % of the leaf life span. In both species, the leaf payback time increased strongly with decreasing light availability at low light levels. At higher light levels it remained rather constant (Fig. 4b).

Discussion

Leaf life span

In another site in French Guiana, Sterck (1999) found leaf life spans for small trees of *Dicorynia* and *Vouacapoua* that were comparable to those found in the present study (range 11-36 and 19-50 months, respectively). In the present study, mean leaf lifetime for *Vouacapoua* saplings was high (about 72 mo) compared to maximum life spans reported for other tropical shade-tolerant tree

species (e.g. Coley 1988: 35 months; Reich et al. 1999: 51 months); but it resembled the life span often observed in coniferous species (Reich et al. 1995b, 1999). For *Vouacapoua*, leaf life span was negatively correlated with light availability (Fig. 1), lending support to the hypothesis that leaf lifetime varies among light environments (see also Williams et al. 1989; Bongers and Popma 1990; Reich et al. 1995b; Poorter 1998). The observed variation in leaf lifetimes in *Dicorynia* was not related to light availability and could result from other factors, either on a genetic basis or on an environmental basis, such as water and nutrient availability. The latter one, however, seems unlikely as both species were exposed to similar forest growth conditions. Leaves of saplings of *Dicorynia* showed more incidence of insect herbivory than those of *Vouacapoua*. This might be an indication that a lower fraction of resources is allocated to anti-herbivore defences, such as lignin or other chemical compounds, resulting in a lower potential leaf life span in *Dicorynia* (e.g. Coley 1988; Reich et al. 1991b).

Leaf physiology versus leaf life span

Overall, life span related variation in leaf structure and function were most pronounced in *Vouacapoua* (Table 1). The A_{\max} declined when leaves lived longer; a pattern that can also be found when pioneer species with short-lived leaves are compared with climax species with long-lived leaves (e.g. Reich et al. 1991b, 1992). On the other hand, mass-based leaf nitrogen concentration was independent on life span, while, among species of contrasting ecology, those with short-lived leaves generally have higher nitrogen concentrations. The interspecific relationships between leaf life span and traits postulated by Reich et al. (1991b, 1992) are largely determined by species with very low leaf lifetimes (< 12 months). When, however, these very short-lived leaves are omitted from the analysis, there is no relationship between A_{\max} and N/mass and leaf lifetimes. Lusk and Contreras (1999) also found no relationship between N/mass and leaf life span among several shade-tolerant tree species ranging in leaf life span from 13 to 54 months.

Because in *Vouacapoua* leaf life span was inversely related to tree height, independently of light availability (see Results and Fig. 1b), the observed patterns in this species could also be traced back to differences in tree height: medium-sized trees tended to have higher A_{\max} than small ones (Fig. 2a). For *Dicorynia* a similar trend could be observed, despite that leaf life span and tree height were not related. In a companion study, it was shown that for both species various leaf traits were affected by tree height and light availability independently (Rijkers et al. 2000).

It is argued that species with long leaf life spans have thick leaves (i.e. high LMA) to deter herbivores from consuming them. These leaves typically are more

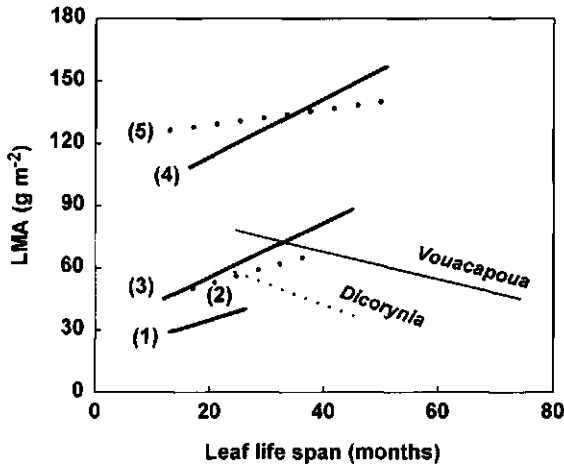


Fig. 5. Leaf mass per unit area (LMA) in relation to leaf life span for various species from a range of studies with interspecific comparisons. Data of *Dicorynia* and *Vouacapoua* are added as intraspecific comparisons. Regression lines are calculated for each data set and they are drawn continuous if significant and broken if not significant. Legend for the different lines: 1. shrubs (n=4): *Williams et al. 1989*; 2. trees (n=5): *Poorter 1998*; 3. trees (n=10): *King 1994*; 4. trees (n=14): *Reich et al. 1999*; 5. trees (n=11): *Lusk and Contreras 1999*. All plant species are from tropical regions except those from Lusk and Contreras who studied temperate rain forest trees. For the interspecific comparisons, always shade growing plants were used, except in Reich et al. who studied leaves in high light. In each study, we omitted species which had a leaf life span <12 mo. Thick lines are interspecific comparisons and thin lines represent intraspecific comparisons. (n) is number of species.

tough and fibrous, and often have greater chemical defence than short-lived leaves (Chabot and Hicks 1982; Coley 1988; Koike 1988; Reich et al. 1991b). Leaf mass per unit area (LMA) can differ considerably among tree species which have inherently long leaf life spans. In both our species, LMA decreased with increasing leaf life span (Fig. 2b). Figure 5 gives a compilation of several studies showing interspecific comparisons between LMA and leaf life spans (intraspecific results from the present study are included). The data in this figure suggest that the leaf life span related variation in LMA among species is opposite to the variation found within a species. Thus, species with inherently long-lived leaves have a higher LMA than those with short-lived leaves, whereas individuals with long-lived leaves have a lower LMA than those with short-lived leaves. The observed negative correlation between LMA and leaf life span within a species can be explained by a selection pressure for these two variables which is light dependent. That is, in low light conditions, there is selection for long-lived leaves as a result of inherent long payback times in the

shade (Fig. 4b), and also selection for leaves with low LMA as a result of reduced leaf investments for the purpose of photosynthetic capacity. For trees of *Vouacapoua* this is true as both leaf life span and LMA are correlated with light availability (Fig. 1b; Rijkers et al. 2000). Additionally, in *Vouacapoua*, there is also an ontogenetically-induced selection pressure for LMA and life span; that is, saplings have longer-lived leaves (Fig. 1a) and a lower LMA (Rijkers et al. 2000) compared to juvenile-adult trees. For trees of *Dicorynia*, on the other hand, leaf life span appeared to be not associated with light availability and tree height (Fig. 1a,b). However, for this species, and also *Vouacapoua*, there may have been a selection for leaf life span, which is dependent on the degree of mechanical damage (e.g. by wind). That is, with increasing windspeed higher in the forest canopy, and hence a greater mechanical stress, there may be selection for shorter leaf life spans, just as there is also selection for leaves with a higher LMA with increasing wind (Fig. 2b; Rijkers et al. 2000; Jaffe and Forbes 1993).

Leaf construction costs

The overall range of mass based leaf construction costs (1.59 - 1.72 and 1.56 - 1.80 g glucose g⁻¹ for *Dicorynia* and *Vouacapoua*, respectively) is within the range generally found among woody species (Poorter and Villar 1997). For leaves of both *Dicorynia* and *Vouacapoua*, CC/mass was not related to leaf life span; this is in agreement with results generally found among species (Merino et al. 1982; Williams et al. 1989). A proposed mechanism that explains the lack of relationship between CC/mass and leaf lifetime among species is that resources are either allocated to improve the photosynthetic apparatus (e.g. investment in enzymes, chlorophyll) or to protective structures that ensure a longer leaf life span (e.g. lignin, phenolics) (Williams et al. 1989; Reich et al. 1991b). Hereby, in both cases, the specific compounds are costly to produce. This allocation-based trade-off among plants is largely determined by the specific growth conditions plants are experiencing. For example, leaves in high light generally will allocate more nitrogen to the photosynthetic apparatus to increase their carbon gain, but they generally do not invest in compounds that also increases their life span. A reduced leaf lifetime in high light can be beneficial for fast growing plants as self-shading among leaves generally has a negative effect on the whole-plant carbon balance (Kikuzawa 1995; Ackerly 1999).

In *Dicorynia* and *Vouacapoua*, light availability had no effect on leaf construction costs, CC/mass (data not shown). Although this is in agreement with other studies (Williams et al. 1989; Sims and Pearcy 1991), it has been found that construction costs of leaves are greater in high than in low light (Williams et al. 1989; Poorter and Villar 1997). In addition, with greater

photosynthetic capacity in high light non-structural carbohydrates might accumulate counter acting the high expenditures for photosynthetic compounds on a mass basis as they are relatively cheap and can form a great part of the total leaf dry-weight. When expressed on an area basis leaf costs increased with increasing light (data not shown).

Photosynthetic carbon gain and leaf payback time

The $A_{\max}/\text{leaf N}$ was unaffected by leaf life span in both species, suggesting that within species, leaves with a different life span have comparable efficiencies of carbon acquisition per unit leaf nitrogen. When comparing species, however, it was found that species with short-lived leaves had higher $A_{\max}/\text{leaf N}$ than those with long-lived leaves, which in turn was explained by the lower LMA and higher N/mass in the short-lived leaves (Reich et al. 1991b). Williams et al. (1989) suggested the payback time (the ratio of carbon costs to carbon gain) as a determinant of leaf life span (see also Poorter 1994). Using this ratio, the authors found that among seven *Piper* species (all shrubs) those with long-lived leaves needed much more time to repay their initial leaf costs than did those with short-lived leaves. The payback times of *Vouacapoua* and *Dicorynia* leaves were short (< 40 days) and likely underestimated. This was owed to an overestimation of the leaf daily carbon gain, because for the calculation of payback times only cloudless days were used and midday depression of photosynthetic rates were disregarded. Nevertheless, for *Vouacapoua* the ratio of construction costs to carbon gain tended to be greater in long-lived than in short-lived leaves (Fig. 4a). Moreover, in both species, payback times strongly increased when the daily irradiance was very low (Fig. 4b) because leaf construction costs were not affected by light whereas daily carbon gain was. This suggests that for plants growing in low-light habitats, the leaf construction costs have a relatively higher impact on the net return on carbon investment than for those in high-light.

Conclusions

In conclusion, in both species leaf life span was remarkably high, and the intraspecific variation in life span was also high, particularly in *Vouacapoua*. Within *Vouacapoua*, leaf life span was negatively associated with increasing light availability. Of all measured variables, only for A_{\max}/area (*Vouacapoua*) the direction of the leaf life span related variation was similar to the one generally found among species. For LMA, it appeared that the life span related variation within a species (*Vouacapoua*, but not significant in *Dicorynia*) was opposite to the variation often found among species. For *Vouacapoua* this could be

explained by the existence of a selection pressure for leaf life span and LMA which is light- and tree height dependent. For *Dicorynia* no such selection pressure for leaf life span was observed. Owing to the fact that leaf payback times were relatively short compared to their life span, it might be expected that the effect of this parameter on leaf lifetime carbon gain is marginal, particularly in high-light conditions. On the other hand, in low-light environments payback times strongly increased, thus illustrating the importance of leaf life span in these conditions.

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Chapter 6

General discussion

In the early stages of life, i.e. as seedlings, pioneer tree species and non-pioneer or shade-tolerant tree species experience highly variable conditions in their respective tropical rain forest environments. That is, pioneer species typically occupy open patches in the forest (gaps) where there is ample light, whereas shade-tolerant species commonly occur under a closed forest canopy in rather light-limited conditions. This in turn, affects their performance, which is reflected in features such as the rate of height growth and leaf turnover (cf. Swaine and Whitmore 1988). However, during the development from seedling to adult individual, trees of pioneer species are not restricted to gaps: they can also be found in more shady environments, just as trees of shade-tolerant species can occur in open forest sites (chapter 2, Clark and Clark 1992). Especially trees of shade-tolerant species are often subject to alternate phases of high and low forest light conditions during their development from seedling to adult tree. At the tree level, various physiological and architectural characteristics are subject to these changing forest light environments. Depending on the scale of light variation, adjustments can be related to different organisational levels such as crown, branch or leaf level. With respect to leaves, responses to changing forest light conditions are species specific and are influenced by the ontogenetic phase of the tree.

Variation in leaf traits: height and light effects

With respect to light conditions, the vast majority of studies on leaf acclimation responses are focussed on seedlings and small saplings. In many cases light-saturated rates of photosynthesis on area ($A_{max}/area$), mass ($A_{max}/mass$) and leaf nitrogen ($A_{max}/leaf\ N$) bases are higher, and leaf mass per unit area (LMA) is lower in pioneer than in shade-tolerant species (e.g. Kitajima 1994, Raaimakers et al. 1995, Reich et al. 1995a). The morphological and physiological leaf traits, demonstrated in this thesis, are consistent with those generally found between pioneers and shade-tolerants (chapter 2). This was also true for the leaf life span: shade-tolerant species have long-lived leaves (more than 1 year) (chapters 5, Coley 1988, Reich et al. 1991b), whereas pioneers such as *Goupia*, have leaves that live less than 1 year (Reich et al. 1999, Rose 2000).

Goupia exhibited a low A_{\max}/area that was unexpected as higher rates of photosynthesis are often found in this species (Huc et al. 1994, Raaimakers et al. 1995, Reich et al. 1995a). The growth irradiance for *Goupia* in the Nouragues area was rather low, however: individuals were found in a lower range of canopy openness compared with those in the studies mentioned above. Owing to this lower light availability shade-acclimated leaves with a lower LMA, leaf nitrogen content (N/area) and A_{\max}/area were formed. *Goupia* might persist in forest canopy shade as it is able to stretch its plagiotropic branches, that have relatively "cheap" leaves (low LMA and fast net return on carbon investment), into forest patches of greater irradiance and hence capture light for photosynthesis. Although *Duguetia* also exhibits plagiotropic shoot growth, with many branches and deep crowns, crown development in this shade-tolerant species is less flexible suggesting a persistence strategy towards low light. The high N/mass values for leaves in all species (chapter 2) compared with those found in other tropical forests (Reich et al. 1995a, Schulze et al. 1994) suggest that plant growth in the Nouragues forest is not N-limited. The increase in chlorophyll concentration (Chl/mass) with decreasing order of shade-tolerance (*Duguetia* < *Vouacapoua* < *Dicorynia* < *Goupia*) was associated with a coincident decrease in LMA such that chlorophyll per unit area (Chl/mass), and hence light absorbance, was similar.

Light acclimation responses of leaf photosynthetic and morphological traits are important for maximising the net carbon gain of a plant (Givnish 1988). Thus, for plants growing in shade, efficient light capture is more important than maximising CO_2 acquisition. Indeed, leaves of shade plants usually have lower area based A_{\max} and respiration rates (R_d), light compensation points, LMA, A_{\max}/Chl and higher Chl/mass compared with those of the same species growing in high light (chapters 2 and 3). It has been argued that phenotypic plasticity in response to light environment is generally greater in pioneer than in shade-tolerant species (Bazzaz and Carlson 1982, Strauss-Debenedetti and Bazzaz 1996). The shade-tolerant species studied in this thesis, however, showed substantial photosynthetic plasticity across a wide range of forest light regimes (chapters 2 and 3). *Vouacapoua* exhibited the greatest ability of photosynthetic plasticity especially in the sapling phase.

Depending on forest canopy structure pronounced vertical gradients in environmental conditions usually are present (e.g. Kira and Yoda 1989). In a vertical transect in a closed canopy forest in the Nouragues area, for example, incident photon flux density (PFD) slowly increased up to 30 m followed by a strong increase in PFD thereafter (Koop and Sterck 1994). Leaf acclimation along a vertical gradient in the forest may therefore follow similar patterns like sun-shade acclimation responses generally found in seedlings and small saplings (chapter 2). However, the variation in acclimation responses might be less with

increasing height in the forest because the range of contrasting light regimes may become narrower with increasing height. On the other hand, effects on leaf function can also be due to changes in tree size. That is, during overall growth, total biomass in plant parts such as leaves, branches, trunk and roots, increases. This, in turn, involves enhanced costs such as construction and maintenance of supporting tissues (Givnish 1986), minimising chances of wind-throw (Sterck and Bongers 1998) and investment in vascular tissue in order to supply leaves with water and nutrients (Friend 1993). These costs act as a sink for the carbohydrates produced by the leaves. Additionally, when a tree grows the leaf area ratio (total leaf area divided by total plant mass) decreases as shown by Veneklaas and Poorter (1998) for seedlings and saplings, and it will continue to decrease dramatically when trees grow taller (Körner 1994). Since this is for part due to the decline in leaf mass relative to whole plant mass, less foliage has to provide sufficient carbohydrates to overcome the increasing sink demands. An increase in photosynthetic rate can be achieved by investment in extra mesophyll tissue, which will increase LMA. As N/mass was independent of light and height, a greater LMA is accompanied with an increase in N/area resulting in a higher A_{\max}/area (chapter 2). Several studies have shown that in canopies of different plant species leaf N is optimally distributed to maximise carbon gain (e.g. Hirose and Werger 1987, Pons et al. 1989).

Variation in LMA, however, can also be brought about by materials not associated with photosynthesis, such as protection against herbivores, structural requirements and storage (e.g. Lambers and Poorter 1992). Furthermore, a greater LMA in taller trees has been associated with increasing water limitations with increasing tree height which is a reflexion of the necessity for osmotic leaf adjustment (Friend 1993, Niinemets 1997a). Consequently, an increase in LMA may cause a decline in N/mass, as a result of a dilution effect, (Leuning et al. 1991) and hence a lower A_{\max}/mass in tall trees (Schoettle 1994). In *Goupia*, *Dicorynia*, *Vouacapoua* and *Duguetia*, however, A_{\max}/mass remained constant across different tree heights as N/mass was unaffected by height (chapter 2). Owing to the high leaf N/mass, which is nutritionally attractive for herbivores, together with a long leaf life span the shade-tolerant species likely invest in compounds which reduce the palatability of their leaves. Overall it appears that for trees in the Nouragues area, LMA is a key variable as changes in this variable regulate the changes in other leaf traits such as N/area and A_{\max}/area .

Photosynthetic utilization of fluctuating light. At the forest floor under a closed forest canopy plants usually experience very low light regimes which can be less than 2% of that of full sunlight (chapters 3 and 4, see review by Chazdon 1988). During most of the day PFD is less than $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, but it

is much higher in sunflecks (chapter 4). A substantial part of the total daily PFD (10-80%) is contributed by sunflecks (chapter 4, Chazdon 1988). Nowadays it is well established that utilisation of these temporary high light intensities is important for, and enhances, daily carbon gain in understory plants, and hence it enables plants to survive and persist in the shaded understory (e.g. Pearcy et al. 1994). To utilise a sunfleck efficiently, a leaf should have a rapid photosynthetic induction response to a light increase, and also a slow decrease of photosynthetic induction in low light in order to stay relatively "standby" to respond to a sequence of sunflecks (chapter 3). During sunflecks, the slowest processes of induction are the biochemical reactions (activation of ribulose-bisphosphate carboxylase, Rubisco) and stomatal responses (Pons et al. 1992). Differences in induction responses among species can often be accounted for by differences in stomatal behaviour (e.g. Tinoco-Ojanguren and Pearcy 1993). Although the time course of induction was similar between the studied species, they differed in the removal of biochemical and stomatal limitation (chapter 3). For both *Vouacapoua* and *Dicorynia* these two limiting processes of induction could not be separated in time, whereas for *Pourouma* the increase in stomata opening, after removal of biochemical limitation, determined further rise in net photosynthetic rate in both gap and closed-canopy growing plants. Since the distribution of sunflecks in the forest understory is often clustered and these clusters are separated by long periods of low light, it is important for plants to enhance the photosynthetic rate during a sequence of sunflecks. Hereby, the loss of photosynthetic induction in periods of low light between successive sunflecks should be minimised (chapter 3). Since light is highly heterogeneous at the forest floor, knowledge of photosynthetic responses to sunflecks in order to calculate daily carbon gain in small plants is evidently significant (Chazdon et al. 1996). The intensity of diffuse PFD (i.e. when there is no sunfleck) is important in maintaining a photosynthetic induction state between sunflecks (Kirschbaum and Pearcy 1988a). That is, at a high diffuse irradiance the relative photosynthetic efficiency of sunfleck utilisation is higher. As a result, it might be arguable whether predictions of carbon gain for taller trees necessitates knowledge of the dynamic responses of photosynthesis to variable light as leaves of tall trees generally experience somewhat higher levels of diffuse irradiance (chapter 4, Sterck et al. in press). Thus, most likely the photosynthetic apparatus of leaves in tall trees is induced relatively stronger for a large fraction during the day (e.g. Roden and Pearcy 1993).

Life span of leaves

The leaf life span can be defined as the period between the emergence and fall of a leaf. Variation in leaf life span is mainly determined by genotypic differences among species (e.g. deciduous vs. evergreen or pioneer vs. non-pioneer) whereas variation within species is mainly determined by acclimation responses to the environment. The leaf's life however, can also be terminated abruptly by severe environmental stress such as mechanical damage (branch fall), water stress or herbivory. Extending the leaf life-time may have an adaptive value in terms of nutrient conservation, carbon balance and nutrient-use efficiency (cf. Chabot and Hicks 1982). In the tropical rain forest leaves of pioneer species typically live less than 1 year (Bongers and Popma 1990, Ackerly 1996) whereas those of shade-tolerants are longer lived (Coley 1988, Reich et al. 1991b, chapter 5: mean for *Dicorynia* and *Vouacapoua* was about 2.5 and 5 years, respectively). These differences in leaf life span generally reflect the forest growth habitats of the species. That is, pioneers usually occur in sites with ample light and nutrients whereby their inherent fast growth rate and hence high leaf turnover, is crucial to out-compete neighbouring plants. Shade-tolerants, on the other hand, usually regenerate in light-limited habitats in which slow growth, characterised by low physiological activity and extended leaf life span, is preferable in terms of reducing carbon loss.

Within a species, leaf life span is usually inversely related to light availability (chapter 5: *Vouacapoua*, Bongers and Popma 1990, Reich et al. 1995b): A reduced leaf life span is likely associated with a greater photosynthetic capacity at higher irradiance. In low light conditions a leaf has to extend its life time in order to acquire sufficient assimilates with which it can return its construction costs. This is illustrated by a longer leaf payback time at low irradiance (chapter 5). In addition, as the mass based leaf construction costs were not affected by light in both *Vouacapoua* and *Dicorynia*, construction costs likely have a relatively stronger impact on the net return on carbon investment in low- than in high-light growing plants. Since survival and persistence in low-light habitats is more important than maximising growth, leaves with a low LMA, A_{max}/area (and per mass), respiration rate and a long life span likely have a greater priority in these habitats (chapters 3, 4 and 5). In *Dicorynia*, leaves were not capable to adjust their life span to the light environment (chapter 5). A lack of flexibility in leaf life span was also found when understory individuals were compared with trees in relatively large gaps (15-30% canopy openness) (Sterck 1999). Beside the likely selection for long-lived leaves in low light, *Vouacapoua* also exhibited an ontogenetically-induced selection for long-lived leaves, as leaves of small and medium-sized trees never reached the life span of those in small saplings (chapter 5). In *Dicorynia*, leaf life span was independent of tree size. The range

in leaf life span that was found among individuals of *Dicorynia* (17-54 months), and which was smaller than that of *Vouacapoua* (27-101), could not be explained by light or height. There are other factors that might account for the observed variation in leaf life span in *Dicorynia*. First, the description of light availability via percentage canopy openness may not have been accurate enough, although the same is true for *Vouacapoua*. Second, the occurrence of locally shallow and not well drained soils, as a result of rock formations in the Nouragues area, may cause drought stress and hence earlier shedding of leaves. Finally, genetic differences among trees might exist.

In order to gain a better understanding of long-term carbon balance for leaves and/or whole-canopy, it is necessary to investigate the influence of leaf aging on leaf morphology and physiology. For species with short-lived leaves it has been hypothesised that the decline in photosynthetic capacity is caused by a reduction in irradiance due to self-shading by leaves and/or neighbouring plants rather than by leaf aging as such (Ackerly and Bazzaz 1995, Ackerly 1999). As a result, in fast-growing species with high leaf turnover rates, the maximum photosynthetic rate generally declines rapidly after full leaf expansion. Leaf nutrients, especially nitrogen, are withdrawn from old leaves as they are more efficiently used in newly formed leaves, which are exposed to higher irradiance. For slow-growing species, which typically have long-lived leaves, the rate of photosynthesis is expected to decline slowly with age (Mooney and Gulmon 1982, Witkowski et al. 1992). Since leaf turnover rates are generally much lower in these species, plants will seek to maximise leaf net carbon gain presumably via a prolonged maximum photosynthetic capacity. In both *Vouacapoua* and *Dicorynia*, the vast majority of leaves are distributed at the periphery of the tree crown. This might indicate that there is relatively low degree of self-shading among leaves within a canopy (Sterck 1999, Sterck and Bongers in press). Formation of new leaves, however, will likely result in a reduction of irradiance at lower positioned leaves, but this was not examined for the species in this thesis. In both species, leaf morphology and physiology remained rather constant throughout the leaf's life, in spite of the formation of new leaves in some trees, especially in small saplings (chapter 4).

From small sapling to large tree

The production of new constructional units, that is leaves with their internodes, is, among other things such as nutrients and water, dependent on carbon availability. Consequently, tree development and the degree of resource acquisition (here CO₂) must be closely linked and typically depend on the environmental growth conditions. For example, in low light photosynthetic

carbon gain is reduced (chapters 2 and 4) and hence it is more advantageous, with respect to survival, to allocate the available carbon to leaves to enhance (future) light interception rather than to long leaf-supporting woody tissue such as internodes or petioles. Although exposure to high irradiance increases net carbon gain of leaves (chapters 2 and 5), too much light also strongly promotes branching, which results in the formation of wide and deep tree crowns and slows down height growth (e.g. Sterck and Bongers in press).

As mentioned in the Introduction, *Vouacapoua americana* and *Dicorynia guianensis*, formed the leitmotif in this thesis. Light and ontogenetic effects on crown development have been studied intensively for trees of these species, up to 25 m in height, over a period of five years (Sterck 1997, 1999; Bongers and Sterck 1998, Sterck and Bongers 1998, in press). Briefly, some of the results showed that when these trees grow taller and/or are exposed to higher irradiance their crowns were found to have greater total branch extension. This, in turn, was due to greater meristem activity (both apical and axillary) and longer constructional growth units. Tree height, however, was not correlated with metamer (*Dicorynia*) and extension unit (*Vouacapoua*) length, but in *Vouacapoua* the number of leaves per unit of extension increased with increasing tree height. Furthermore at high light levels, trees exhibited elongated crowns because the main orthotropic stem ('leader branch') grew much faster than the other branches. With decreasing irradiance crowns became wider in *Vouacapoua*, whereas in *Dicorynia* no such response was observed. The formation of a relatively flat crown with a few leaf layers can be advantageous in a low light environment as it reduces self-shading among leaves. Overall, it appeared that the amount and spatial arrangement of leaf area changed with increasing height and light availability in trees, but the mechanisms to establish this were different between the two species.

Both *Vouacapoua* and *Dicorynia* are valuable timber species in French Guyana. In tropical rain forests, silvicultural systems that are based on natural regeneration are characterised by selective felling of economically desirable trees. That is, only a few individuals at a time are removed in the forest and natural regeneration is allowed to fill the gaps (Gómez-Pompa and Burley 1991). Some of these systems are designed to stimulate growth of trees by means of forest interventions, such as refinement and liberation thinning, which then result in a greater light availability around the trees concerned (e.g. de Graaf 1986). Light manipulations in tropical forests are not species-specific, but usually directed towards many different species (Bongers 1998). Growth responses may differ between species, however.

When light manipulations in the forest are geared towards optimising both resource acquisition (i.e. producing sufficient carbon, 'source') and tree

development (i.e. directing meristem activity, 'sinks'), taking into account the tree's developmental stage, height growth might be steered and enhanced. That is, integrating the results of this thesis with those of Sterck, via for instance cost-benefit analyses, can gain insight into the mechanisms that regulate branch growth and abscission or self-pruning in these two species (e.g. Witowski 1997). Abscission of (often low positioned) branches in the crown for example, takes place when there is a negative carbon balance. Thus, there is no adequate carbon return for the costs of nutrient and water supply a branch receives. This supply of nutrients and water might then be available for other more productive branches located in the upper crown, and hence may improve height growth.

Conclusion

The acclimation responses to light and/or other environmental factors in seedlings and small saplings are important in understanding the growth performance of tree species in question. However, when a tree grows taller it is subject to often strong changes in forest micro-climate, but also to its inherent ontogenetic alterations. Consequently, it was expected that this would bring differences in leaf function and structure. It was demonstrated that tree height and canopy openness had independent effects on several leaf traits such as photosynthetic capacity, chlorophyll concentration, leaf mass per unit area (LMA) and leaf life span. LMA appeared to be the most important determinant of other features of leaf function, regardless of forest light environment or tree height. Mean leaf life span was very long in the shade-tolerant species *Vouacapoua* and *Dicorynia*, and throughout the life span these leaves exhibited a rather low variability in morphology and physiology. Thus, leaves of these two species were capable of retaining photosynthetic capacity across a large part of their lifetime. Moreover, relative to their leaf life span, the rate of net return on carbon investment (leaf payback time) was very fast (< 40 days), suggesting that the effect of this parameter on leaf lifetime carbon gain is likely marginal, particularly in high-light conditions.

Although the shade-tolerant species exhibited a substantial plasticity for the studied leaf traits (e.g. photosynthetic capacity and sunfleck utilisation), the results also suggested that acclimation responses in seedlings and saplings do not necessarily predict the responses of larger trees. In *Dicorynia* the responses of a number of leaf traits to changes in light availability and tree height were less plastic compared to those in *Vouacapoua*. This also appeared to be true for traits at higher organisation levels (e.g. branch and crown) (Bongers and Sterck 1998). This thesis was restricted to only five species, of which two species

were studied in more detail. Nevertheless, its results suggest that when assessing the effectiveness of forest light manipulations to enhance tree growth of timber species species-specific differences in responses to light together with those depending on tree size should be taken into account.

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Summary

Tropical rain forests are characterised by a highly heterogeneous light environment. Consequently, trees are often exposed to several alternate phases of high and low light conditions throughout their life span. As light is commonly considered to be the major determinant for plant survival and growth in the forest, it is expected that plants are able to adjust to these changing light conditions.

In this thesis, the effect of steady-state and dynamic light conditions on photosynthesis and other features of leaf function was studied in five tree species in the Nouragues tropical rain forest in French Guiana. The five species are: *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis*, *Pourouma bicolor* spp. *digitata*, and *Goupia glabra*. The research was especially focused on the variation in leaf traits among trees in different ontogenetic stages: from small sapling to tall canopy tree. Leaf acclimation responses were examined throughout the life time of leaves in order to evaluate whether they can profit from these adjustments in terms of leaf carbon gain.

To date, there is much knowledge about light acclimation responses at the scale of seedlings and small saplings, but how traits alter when a tree grows taller has received only little attention. Besides being exposed to changes in micro-climate, a growing tree is also subject to inherent ontogenetically-induced changes in plant traits, which may include among other things, physiological and morphological changes in leaf function.

In chapter 2, the effects of irradiance and tree height on photosynthetic leaf traits were studied in four species, arranged in order from most shade-tolerant to pioneer: *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis* and *Goupia glabra*. It was demonstrated that tree height and irradiance had independent effects on photosynthesis and other features of leaf function. The direction and magnitude of the variation in leaf variables tended to be similar among species. Overall, leaf mass per unit area, which is a leaf morphological trait, determined most of the variation in the other leaf variables. Leaves in small trees and in low light conditions had a lower photosynthetic capacity, nitrogen content and leaf mass per unit leaf area. The chlorophyll concentration (on a mass basis) however was higher, indicating that shade leaves and leaves of small plants were more efficient in light capture. Photosynthetic capacity on a mass basis and per unit leaf nitrogen was almost independent of irradiance. Leaf nitrogen concentration (on a mass basis) was independent of height and light. Moreover, leaf nitrogen values were high in all species suggesting that

plant growth was not nitrogen-limited. Among species, *Goupia* had a low leaf mass per area and a high photosynthetic capacity per unit leaf nitrogen, which is typically found in pioneer species, whereas *Duguetia* exhibited opposite values characteristic for shade-tolerant species. Values for *Vouacapoua* and *Dicorynia* tended to be intermediate. All four species exhibited substantial plasticity in leaf traits both along a gradient of forest light environments and during their development from sapling to adult tree.

Since the light environment in the forest understorey was highly dynamic, photosynthetic induction responses under constant and fluctuating light were investigated for three species in chapter 3. Induction responses include the time needed to increase the photosynthetic rate to a sudden increase in irradiance, and the ability to hold the photosynthetic apparatus induced under low light conditions, which determines the readiness to exploit the next sunfleck. The selected species differed in their ability to tolerate shady forest environments. Saplings growing in gaps and in the understorey of *Vouacapoua americana*, *Dicorynia guianensis* and *Pourouma bicolor* spp *digitata* were selected. In general, the photosynthetic response to a light increase can be separated into two phases: an initial fast-induction phase which requires 1 to 2 min for completion; followed by a slow-induction phase, lasting 5 to 30 min, in which Rubisco (a key enzyme in the photosynthetic process) is activated and stomata open. Approximately the first 10 min in the slow-induction phase are dominated by activation of Rubisco, after which the stomata continue to open, causing a further increase in photosynthetic rate. The time needed to reach 75% of the maximum carboxylation efficiency (V_{cmax}) was used to separate the relative importance of biochemical and stomatal limitation during the time course of photosynthetic induction. The photosynthetic induction time (i.e. the time required to reach 90% of the photosynthetic capacity) did not differ among species, and was short (7 to 11 min). Thus, in these plants the photosynthetic apparatus was induced quickly. Biochemical induction was fast in leaves of *Pourouma*, as about 3 min were needed to reach 75% of maximum carboxylation capacity; the two other species needed 4-5 min. When induction continued after reaching 75% of maximum carboxylation capacity, stomatal conductance increased in *Pourouma* only (ca 80%), causing a further increase in its net photosynthesis rate. The induction loss in low light determines whether a plant is able to better exploit subsequent sunflecks. When fully-induced leaves were shaded for 20 min, loss of induction was moderate in all species. However, gap saplings of *Dicorynia* had a rapid induction loss (ca 80%), which was mainly due to biochemical limitation as stomatal conductance decreased only slowly. Leaves were very efficient at utilizing fluctuating light environments as they increased the photosynthetic rate when exposed to a sequence of lightflecks separated by

short periods of low light. The three shade-tolerant species exhibited, particularly in low light, a capability to efficiently utilize sunflecks. This may contribute to a positive whole-plant carbon balance, though it is argued that attention has to be paid to the effect of diurnal and seasonal changes in induction properties for sunfleck utilization and total carbon gain.

In chapter 4 the influence of leaf aging on photosynthetic carbon gain was studied for saplings and small and medium-sized trees growing under a closed forest canopy (shade leaves) and in gaps (sun leaves). The species of study were *Vouacapoua americana* and *Dicorynia guianensis*. Measurements were made at three time points during the first 30 months of the leaf's life. The effect of light- and height-orientated leaf adjustments for daily leaf carbon gain was evaluated by means of photosynthetic light response curves that were developed for six months old shade and sun leaves. With these curves the daily carbon gain was calculated from diurnal measurements of photon flux density in a low and high forest light environment. No differences were found in the variation in leaf morphology and physiology with leaf age between sun and shade leaves. Photosynthetic capacity stayed relatively constant with time in all trees, whereas leaf mass per unit area increased until leaves were 18 months old. Leaf nitrogen concentration (on a mass basis) was rather independent of leaf age. The photosynthetic response curves revealed that the quantum yield was almost similar in all leaves. Dark respiration rates tended to be higher in both sun leaves and tall trees. In a low-light regime, daily carbon gain was lowest for sun leaves of tall trees owing to the higher dark respiration rates. In a high-light regime, daily carbon gain for both sun and shade leaves was significantly lower in saplings than in small and medium-sized trees, except for sun leaves of *Vouacapoua* saplings. In these plants the photosynthetic capacity was equal to the one found in small and medium-sized trees. Overall, for both species the morphological and physiological variation in response to leaf age was moderate to low.

Earlier studies have shown that among species leaf life span is highly correlated with a number of morphological and physiological leaf traits. In chapter 5, it was examined whether similar patterns could be found when focusing on the variation in leaf traits within a species. For *Vouacapoua americana* and *Dicorynia guianensis*, the intra-specific variation in leaf life spans was investigated. For different-sized trees, leaf life span related variation in photosynthesis, nitrogen concentration, leaf mass per unit area and leaf construction costs were determined. The life span of leaves of *Vouacapoua* were longer (mean 61 months, range 27-101) than those of *Dicorynia* (mean 32, range 17-54). Life span was inversely related to tree height and canopy

openness in *Vouacapoua*, whereas in *Dicorynia* no relation was found. *Vouacapoua* exhibited the most leaf life span related variation in leaf traits. Intraspecific variation in leaf traits with leaf life span generally did not follow interspecific variation. For photosynthetic capacity (on area basis) (*Vouacapoua*), the direction of the leaf life span related variation was similar to the one generally found among species. For leaf mass per area however, the opposite pattern was observed. Regarding *Vouacapoua*, this could be explained by a light- and height-dependent selection pressure for leaf life span and leaf mass per area. For *Dicorynia* no such selection pressure for leaf life span occurred. Long-lived leaves showed lower photosynthetic capacity and leaf mass per area than short-lived ones. Both leaf construction costs (i.e. the amount of glucose needed to construct one gram of leaf biomass) and photosynthetic capacity per unit leaf nitrogen were independent of life span. This suggested a lack of trade-off between investment of resources to high photosynthetic capacity and to extended leaf life span in terms of a reduction in the palatability of leaves. For both species, the time needed to repay the leaf construction costs (i.e. payback time) was very short, ranging from 4 to 40 days. Payback time remained rather constant during a wide range of irradiance, but it increased sharply in a narrow range of low light. Only in *Vouacapoua* it was observed that payback time was positively correlated with leaf life span, suggesting that in long-lived leaves the rate of net return on carbon investment is slow.

Finally, in the general discussion (chapter 6) the results of this thesis are compared with those from other studies. For the two shade-tolerant species *Vouacapoua americana* and *Dicorynia guianensis* the integration of the results at the leaf level (this thesis) with those at higher organisation levels, such as branch and tree crown, are briefly discussed. This in the context of the applicability of these results in silvicultural systems in which light is manipulated to enhance growth and production of timber species.

Résumé

Les forêts tropicales humides se caractérisent par un régime lumineux très hétérogènes. Par conséquent, les arbres sont souvent exposés, durant leur vie, à des plusieurs alternances de conditions lumineuses fortes et faibles. Si l'on considère que la lumière est généralement considérée comme le facteur déterminant de la survie et de la croissance des plantes en forêt, on peut s'attendre à ce que les plantes soient capables de s'ajuster à des conditions lumineuses variables.

Nous avons étudié l'effet d'un régime lumineux transitoire ou en steady-state sur la photosynthèse et d'autres processus foliaires chez cinq espèces d'arbres de la forêt tropicale humide de Nouragues en Guyane française. Les cinq espèces choisies étaient *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis*, *Pourouma bicolor* spp. *digitata*, et *Goupia glabra*. Nos recherches ont porté plus particulièrement sur les variations entre arbres des caractéristiques foliaires, à différents stades ontogénétiques, du petit semis à l'arbre de la canopée. Les réponses des feuilles en terme d'acclimatation ont été examinées sur l'ensemble de leur durée de vie afin de mettre en évidence un éventuel bénéfice en terme de gain de carbone résultant de ces ajustements.

Les connaissances actuelles sur l'acclimatation à la lumière sont nombreuses à l'échelle des semis et des gaulis, mais très peu d'attention a été portée sur la manière dont les caractéristiques sont altérées lorsqu'un arbre grandit. Un arbre en phase de croissance est soumis non seulement à des modifications de son environnement micro-climatique, mais il est aussi soumis à des modifications internes ontogénétiquement induites de ces caractéristiques, avec notamment, entre autres, des modifications physiologiques et morphologiques des fonctions foliaires.

Dans le chapitre 2, nous avons étudié les effets de la lumière et de la hauteur de l'arbre sur les caractéristiques photosynthétiques foliaires de quatre espèces, classées de la plus tolérante à l'ombrage jusqu'aux espèces pionnières intolérantes : *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis* et *Goupia glabra*. Nous avons démontré que la lumière et la hauteur agissent de manière indépendante sur la photosynthèse et les autres processus foliaires étudiés. Le sens et l'intensité de variation des variables foliaires ont tendance à être similaires entre espèces. Globalement, la biomasse foliaire par unité de surface, qui est un critère morphologique foliaire, explique la plus grande part de la variabilité observée chez les autres variables foliaires. Les feuilles des petits arbres et celles situées en faibles conditions d'illumination ont

une capacité photosynthétique, un contenu en azote et une masse surfacique plus faibles. Cependant, leur teneur en chlorophylle (par unité de masse) est plus grande, ce qui indique que les feuilles d'ombre et celles portées par de petites plantes interceptent la lumière de manière plus efficace. La capacité photosynthétique par unité de masse et par unité de surface sont pratiquement indépendantes de l'intensité lumineuse. La concentration en azote (par unité de masse) est indépendante de la hauteur de l'arbre et de la lumière. D'autre part, les teneurs en azote observées sont fortes chez toutes les espèces ce qui suggère une absence de limitation azotée de la croissance. Entre espèces, on peut voir que *Goupia* possède une faible masse surfacique et une forte capacité photosynthétique par unité d'azote foliaire ce qui est typique des espèces pionnières, alors que *Duguetia* présente les caractéristiques inverses, typiques des espèces tolérantes à l'ombrage. *Vouacapoua* et *Dicorynia* présentent des valeurs intermédiaires. Les quatre espèces ont fait montre d'une plasticité considérable de leurs caractéristiques foliaires, le long d'un gradient d'environnement lumineux ainsi que pendant leur développement du stade gaulis au stade adulte.

Comme l'environnement lumineux en sous-bois est fortement transitoire, nous avons étudié, dans le chapitre 3, les réponses photosynthétiques de trois espèces, induites par une illumination constante ou fluctuante. Cela inclut le temps requis pour que la photosynthèse augmente en réponse à une augmentation soudaine de l'illumination et la capacité à maintenir l'appareillage photosynthétique actif sous faibles conditions d'illumination, ce qui indique si la plante est prête à exploiter les tâches de soleil ultérieures. Les espèces choisies diffèrent dans leur capacité de tolérance à l'ombrage. Des gaulis de *Vouacapoua americana*, *Dicorynia guianensis* et *Pourouma bicolor* spp *digitata* ont été échantillonnés dans des trouées et en sous-bois. En général, la réponse de la photosynthèse à une augmentation d'intensité lumineuse peut être séparée en deux phases : une phase initiale d'activation rapide qui dure 1 à 2 min, suivie d'une phase d'activation lente, qui dure 5 à 30 min, durant laquelle la Rubisco (une enzyme clé de la photosynthèse) est activée et les stomates ouverts. Les 10 premières minutes, environ, de la phase d'activation lente correspondent essentiellement à l'activation de la Rubisco, puis les stomates continuent de s'ouvrir, provoquant une augmentation supplémentaire de la photosynthèse. Le temps requis pour atteindre 75 % du taux maximum de carboxylation (V_{cmax}) a été utilisé pour déterminer l'importance relative des limitations biochimiques et stomatiques dans la courbe de réponse de la photosynthèse. Le temps d'activation de la photosynthèse (i.e. le temps requis pour atteindre 90 % de la capacité photosynthétique) ne diffère pas entre espèces et est de courte durée (7 à 11 min). Ainsi, chez ces plantes, l'appareil photosynthétique est activé

rapidement. L'activation biochimique est rapide chez les feuilles de *Pourouma*, où environ 3 min sont nécessaires pour atteindre 75 % du taux maximum de carboxylation ; 4-5 min sont nécessaires pour les deux autres espèces. Lorsque l'activation se poursuit après avoir atteint 75 % du taux maximum de carboxylation, la conductance stomatique augmente chez *Pourouma* seulement (ca. 80 %), ce qui résulte en une augmentation supplémentaire de son taux de photosynthèse nette. La baisse d'activité à faible lumière détermine la capacité d'une plante à pouvoir tirer profit des tâches lumineuses postérieures. Sur des feuilles totalement actives placées à l'ombre pendant 20 min, nous avons observé des chutes d'activité modérées quelle que soit l'espèce. Cependant, les gaules de *Dicorynia* situés en trouées montrent une perte rapide d'activité (ca. 80%), principalement liée à une limitation biochimique, puisque que la conductance stomatique décroît lentement. Ses feuilles sont très efficaces dans un environnement lumineux transitoire car elles augmentent leur taux de photosynthèse lorsqu'elles sont exposées à une succession de tâches lumineuses séparées par de courtes périodes de lumière de faible intensité. Les trois espèces tolérantes possèdent la capacité d'utiliser efficacement les tâches de soleil, plus particulièrement de faible intensité. Ceci peut apporter une contribution positive au bilan de carbone de la plante, bien que dans l'étude de l'utilisation des tâches de soleil et du gain total de carbone, il est souligné qu'il faut porter une attention particulière à l'effet des variations journalières et saisonnières sur l'activation de ces propriétés.

Dans le chapitre 4, nous avons étudié l'influence de l'âge de la feuille sur le gain de carbone par photosynthèse, pour des gaules et des arbres de petite taille ou taille moyenne, situés sous une canopée fermée (feuilles d'ombre) ou dans des trouées (feuilles de lumière). Les espèces étudiées sont *Vouacapoua americana* et *Dicorynia guianensis*. Trois mesures ont été faites durant les 30 premiers mois de la vie des feuilles. Les effets des ajustements foliaires liés à la lumière ou à la hauteur de l'arbre sur le gain quotidien de carbone foliaire ont été évalués par l'intermédiaire de courbes de réponse de la photosynthèse à la lumière, obtenues pour des feuilles d'ombre et de lumière âgées de six mois. A l'aide de ces courbes, le gain de carbone journalier a été calculé à partir de mesures diurnes de la densité de flux de photons dans un environnement forestier sous faible et forte luminosité. Il n'apparaît pas de différence entre feuilles d'ombre et de lumière dans les modifications morphologiques et physiologiques de la feuille avec l'âge. La capacité photosynthétique reste relativement constante dans le temps chez tous les arbres, alors que la masse surfacique augmente avec l'âge des feuilles, jusqu'à 18 mois. La teneur en azote (par unité de masse) est plutôt indépendant de l'âge des feuilles. Les courbes de réponse de la photosynthèse montrent que l'efficacité lumineuse est

pratiquement similaire chez toutes les feuilles. Le taux de respiration dans le noir ("dark respiration") a tendance à être plus élevé chez les feuilles de lumière et les grands arbres. Sous faibles conditions lumineuses, le plus faible gain de carbone est obtenu pour les feuilles de lumière des grands arbres, en raison d'un taux de respiration plus élevé. En conditions lumineuses fortes, le gain de carbone journalier des feuilles d'ombre et des feuilles de lumière est significativement plus faible pour les gaulis que pour les petits arbres et les arbres moyens, à l'exception des feuilles de lumière des gaulis de *Vouacapoua*. Chez ces derniers, la capacité photosynthétique est égale à celle trouvée sur les arbres petits et moyens. Globalement, pour ces deux espèces, les variations morphologiques et physiologiques des feuilles avec l'âge sont moyennes à faibles.

Des études précédentes ont montré qu'entre espèces, la durée de vie des feuilles est fortement corrélée à certaines des caractéristiques morphologiques et physiologiques des feuilles. Dans le chapitre 5, nous avons cherché à voir si un résultat similaire pouvait être mis en évidence en regardant les variations des caractéristiques foliaires au sein de chaque espèce. Nous avons caractérisé la variabilité intra-spécifique de la durée de vie des feuilles de *Vouacapoua americana* et de *Dicorynia guianensis*. Les variations de photosynthèse, de teneur en azote, de masse surfacique et des coûts de construction foliaires liées à la durée de vie des feuilles ont été déterminées. La durée de vie des feuilles de *Vouacapoua* (moyenne 61 mois, gamme 27-101) est supérieure à celle des feuilles de *Dicorynia* (moyenne 32, gamme 17-54). Elle est négativement reliée à la hauteur de l'arbre et au degré d'ouverture de la canopée pour *Vouacapoua*, tandis que chez *Dicorynia*, il n'y a aucune relation. *Vouacapoua* présente un plus grand nombre de variations des caractères foliaires liés à la durée de vie des feuilles. Les variations intra-spécifiques des caractères foliaires en fonction de la durée de vie ne correspondent généralement pas aux variations inter-spécifiques. Chez *Vouacapoua*, la capacité photosynthétique (par unité de surface) évolue avec la durée de vie foliaire de manière similaire à l'évolution généralement observée entre espèces. Cependant, la masse surfacique évolue dans le sens opposé. En ce qui concerne *Vouacapoua*, cela pourrait s'expliquer par une pression de sélection, sur les variables durée de vie et masse surfacique, résultant de la lumière et de la hauteur de l'arbre. Chez *Dicorynia*, une telle pression de sélection n'a pas été mise en évidence. Les feuilles à longue durée de vie présentent une capacité photosynthétique et une masse surfacique plus faibles que les feuilles à courte durée de vie. Les coûts de construction foliaire (i.e. la quantité de glucose nécessaire pour construire un gramme de biomasse foliaire) et la capacité photosynthétique par unité d'azote ne dépendent pas de la durée de vie des feuilles. Ceci suggérerait qu'il n'y a pas

de compensation, en ce qui concerne l'investissement des ressources, entre l'obtention d'une capacité photosynthétique plus importante et l'accroissement de la durée de vie des feuilles. Le temps requis pour recouvrir les coûts de construction foliaire (i.e. durée de recouvrement) est très court chez les deux espèces, compris entre 4 et 40 jours. Cette durée de recouvrement reste quasiment constante sous une large gamme de conditions lumineuses, mais elle décroît brusquement dans une petite gamme de faibles valeurs. Le temps de recouvrement est corrélé positivement avec la durée de vie des feuilles uniquement chez *Vouacapoua*, ce qui suggère que les feuilles à longue durée de vie ont un taux lent de recouvrement du carbone investi.

Enfin, nous avons comparé, dans la discussion générale (chapitre 6), les résultats de ce travail de thèse avec ceux d'autres études. Pour les deux espèces tolérantes à l'ombrage que sont *Vouacapoua americana* et *Dicorynia guianensis*, nous discutons rapidement de l'intégration des résultats obtenus ici à l'échelle foliaire à d'autres niveaux d'organisation plus élevés, comme la branche ou la couronne, ceci dans le contexte de l'applicabilité de ces résultats à des systèmes sylvicoles où la lumière est manipulée afin d'accroître la croissance et la production des espèces commerciales.

Samenvatting

Tropische regenbossen worden gekarakteriseerd door een sterk heterogeen lichtklimaat. Hierdoor komen bomen gedurende hun gehele levenscyclus vaak bloot te staan aan afwisselend hoge- en lage lichtcondities. Omdat licht in het algemeen wordt beschouwd als de belangrijkste factor voor het overleven en de groei van planten, kan verwacht worden dat planten in staat zijn zich aan te kunnen passen aan deze constante veranderingen in lichtklimaat.

In dit proefschrift is voor vijf verschillende boomsoorten onderzocht het effect van stabiel- en fluctuerend licht op fotosynthese karakteristieken en ander bladeigenschappen. De studie is uitgevoerd in het onderzoeksstation Nouragues; gelegen in het tropisch regenbos van Frans Guyana. De onderzochte soorten zijn: *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis*, *Pourouma bicolor* spp. *digitata*, en *Goupia glabra*. In het onderzoek heeft de variatie in bladkenmerken tussen bomen in verschillende ontwikkelingsfasen (van zaailing tot kronendakboom) speciale aandacht gekregen. Om te kunnen evalueren of bomen kunnen profiteren van de bladaanpassingen aan een specifiek lichtklimaat zijn de aanpassingen bekeken gedurende de gehele levensduur van de bladeren.

Tot nu toe is er veel studie gedaan naar lichtaanpassingen bij kiemplanten en zaailingen. Er is echter relatief weinig bekend over hoe eigenschappen van planten veranderen naarmate planten ouder of groter worden. Een groeiende boom ervaart niet alleen een verandering in microklimaat maar hij is ook onderworpen aan inherent ontogenetische veranderingen in eigenschappen zoals o.a. fysiologische en morfologische veranderingen in bladkenmerken.

In hoofdstuk 2 zijn de effecten van zowel lichtbeschikbaarheid als boomhoogte op fotosynthese karakteristieken onderzocht. Hiervoor zijn de volgende vier boomsoorten bekeken, gerangschikt van het meest schaduwtolerant tot pionier: *Duguetia surinamensis*, *Vouacapoua americana*, *Dicorynia guianensis* and *Goupia glabra*. Er is aangetoond dat boomhoogte en lichtbeschikbaarheid onafhankelijk van elkaar effect hadden op de onderzochte bladkenmerken. Tussen de soorten bleek richting- en mate van variatie in bladkenmerken hetzelfde te zijn. De hoeveelheid bladgewicht per eenheid van bladoppervlak, een morfologische blad variabele, was dé sleutel variabele die in hoge mate de variatie in ander bladkenmerken bepaalde. De bladeren van kleine bomen groeiende in lage lichtbeschikbaarheid hadden een lage fotosynthese-capaciteit, stikstofgehalte en bladgewicht per eenheid bladoppervlak. De chlorofylconcentratie (per eenheid bladgewicht) daarentegen was hoger. Dit

houdt in dat schaduwbladeren en bladeren van kleine bomen efficiënter zijn in het opvangen van licht. De hoogte van de fotosynthese-capaciteit per eenheid bladgewicht en per eenheid bladstikstof, was vrijwel onafhankelijk van de lichtbeschikbaarheid. De hoeveelheid stikstof per eenheid bladgewicht was onafhankelijk van zowel licht als boomhoogte. Het stikstofgehalte van de bladeren was voor alle soorten hoog. Dit duidt op het feit dat de groei van planten in het onderzoeksgebied waarschijnlijk niet stikstof-limiterend is. De soort *Goupia* liet een lage bladgewicht per eenheid bladoppervlak en een hoge fotosynthese-capaciteit per eenheid stikstof zien, wat vaak gevonden wordt in pioniersoorten. De schaduwtolerante soort *Duguetia* daarentegen liet tegenovergestelde waarden zien. De waarden van de bladkenmerken voor de soorten *Vouacapoua* and *Dicorynia* lagen in het midden van de twee andere soorten. Alle vier de boomsoorten lieten een behoorlijke plasticiteit in de bladkenmerken zien langs een lichtgradiënt in het bos en gedurende hun ontwikkeling van zaailing naar volwassen boom.

Het lichtklimaat in het onderbos is erg dynamisch. Daarom is in hoofdstuk drie onderzocht de fotosynthese-inductiereacties onder constante- en fluctuerende lichtcondities van drie verschillende boomsoorten. Met de inductie reactie van de fotosynthese wordt o.a. bedoeld de tijd die nodig is om de snelheid van de fotosynthese te laten toenemen nadat het blad bloot is gesteld aan een plotselinge lichttoename, en de mogelijkheid om het fotosynthese apparaat geïnduceerd te houden nadat het blad weer bloot wordt gesteld aan lage lichtcondities. Dit laatste bepaalt de mate van gereedheid van het fotosynthese apparaat om een volgende lichtvlek (hoge lichtintensiteit) efficiënt te benutten. De onderzochte boomsoorten verschillen in de capaciteit om donkere boscondities te tolereren. Kleine planten (ca. 1 m) van de soorten *Vouacapoua americana*, *Dicorynia guianensis* and *Pourouma bicolor* spp *digitata* werden geselecteerd in bosopeningen ('gaps') en onder een gesloten kronendak. In het algemeen kunnen tijdens de fotosynthese respons na een toename in lichtintensiteit twee fasen onderscheiden worden. Ten eerste is er een snelle inductiefase, die gewoonlijk één-twee minuten duurt. Deze wordt gevolgd door een langzame inductiefase die ongeveer 5 tot 30 minuten in beslag kan nemen en waarin Rubisco (een belangrijk enzym in het fotosynthese proces) wordt geactiveerd en huidmondjes zich openen. De eerste 10 minuten worden gedomineerd door het activeren van het enzym Rubisco, het daarna verder openen van de huidmondjes bepaalt de verdere toename in fotosynthese snelheid. De tijd die nodig is om 75% van de maximale carboxylatie efficiency (V_{cmax}) te bereiken is gebruikt als relatieve maat om het biochemische proces te onderscheiden van het openen van de huidmondjes gedurende het verloop van de fotosynthese inductie in de tijd. De fotosynthese inductietijd, dat is de tijd

die nodig is om 90% van de maximale fotosynthese snelheid te bereiken, was erg kort, ongeveer 7 tot 11 minuten, en verschillende niet tussen de boomsoorten. Dus deze planten laten een snelle inductie zien van het fotosynthese apparaat nadat ze bloot worden gesteld aan hoog licht. De biochemische inductie was snel in bladeren van *Pourouma*: ongeveer 3 minuten waren nodig om 75% van de maximale carboxylatie capaciteit te bereiken; voor de twee ander soorten was hier vier tot vijf minuten voor nodig. Nadat 75% van de V_{cmax} was bereikt nam de stomataire geleidbaarheid (opening huidmondjes) in bladeren van alleen *Pourouma* nog toe (met ca. 80%). Dit veroorzaakte vervolgens een verdere stijging van de fotosynthesesnelheid in deze planten. De mate van fotosynthese inductieverlies in laag lichtcondities bepaald of een plant in staat is toekomstige zonnevlekken ('korte periodes van hoog licht') efficiënt te benutten. Nadat planten 20 minuten waren blootgesteld aan laaglicht was het inductieverlies van volledig geïnduceerde bladeren redelijk laag. Planten van *Dicorynia* planten in bosopeningen ('gaps') lieten echter een hoge mate van inductieverlies zien (ca 80%). Dit werd voornamelijk veroorzaakt door de biochemische limitering en niet door het sluiten van de huidmondjes. Alle bladeren waren goed in staat fluctuerend licht efficiënt te gebruiken. Dat wil zeggen, wanneer ze werden blootgesteld aan een reeks van lichtvlekken die onderbroken werden door korte periodes van laag licht werd de fotosynthesesnelheid in stappen verhoogd. In het algemeen is gebleken dat de drie schaduwtolerante boomsoorten in staat zijn lichtvlekken efficiënt te benutten, met name in schaduwrijke omstandigheden onder een gesloten kronendak. Dit kan uiteindelijk een bijdrage leveren aan een positieve koolstof balans voor de gehele plant. Echter bij het bepalen van fotosynthese inductie karakteristieken, en het uiteindelijke belang hiervan voor de totale koolstof opbrengst, zou rekening gehouden moeten worden met mogelijke optredende variatie gedurende de dag ('ochtend versus middag metingen') en met seizoensveranderingen ('droge en natte periode').

In hoofdstuk 4 is de rol van de bladleeftijd op de koolstofopbrengst onderzocht voor bomen van verschillende hoogte onder een gesloten kronendak (de schaduw bladeren) en in gaps (de zon bladeren). De geselecteerde soorten waren: *Vouacapoua americana* and *Dicorynia guianensis*. Metingen werden verricht tijdens drie verschillende tijdsintervallen gedurende de eerste 30 maanden van de levensduur van het blad. Het effect van licht- en hoogte- georiënteerde bladaanpassingen op de dagelijkse koolstof opbrengst werd geëvalueerd met behulp van fotosynthese lichtrespons curven. Deze werden gemeten in bladeren die zes maanden oud waren. De dagelijkse koolstofopbrengst werd berekend met behulp van de responscurven die gecombineerd werden met metingen van het dagelijkse verloop van de lichtintensiteit in zowel een laag als een hoog bos

lichtklimaat. De variatie in bladmorphologie en -fysiologie was hetzelfde voor zonen schaduwbladeren met dezelfde leeftijd. De maximale fotosynthesesnelheid veranderde relatief weinig tijdens de gehele levensduur van de bladeren. Er was een toename in het bladgewicht per eenheid bladoppervlak gedurende de eerste 18 maanden van de bladleeftijd. De hoeveelheid stikstof per bladgewicht was onafhankelijk van bladleeftijd. De donkerrespiratie leek hoger te zijn in zowel zonbladeren als in bladeren van grote bomen. Wanneer de dagelijkse koolstofopbrengst werd gesimuleerd met behulp van een laag lichtklimaat bleek dat zonbladeren van grote bomen de laagste koolstofopbrengst hadden. Dit werd veroorzaakt door de hogere donkerrespiratie in deze bladeren. Indien een hoog lichtklimaat werd gebruikt bleek dat de koolstofopbrengst in zaailingen lager was dan in kleine- en middelgrote bomen. Dit gold voor zowel zon- als schaduwbladeren, met uitzondering van zonbladeren van *Vouacapoua* zaailingen. Voor deze planten was de maximale fotosynthesesnelheid gelijk aan die van kleine- en middelgrote bomen. In het algemeen hadden beide boomsoorten een lage variatie in morfologische en fysiologische bladkenmerken gedurende de levensduur van hun bladeren.

Eerdere studies hebben laten zien dat tussen plantensoorten de bladlevensduur sterk gecorreleerd is met een aantal morfologische en fysiologische bladkenmerken. In hoofdstuk 5 is bekeken of deze relaties ook gevonden konden worden indien de variatie in bladkenmerken binnen een soort werden bekeken. Hiervoor zijn de volgende twee boomsoorten onderzocht: *Vouacapoua americana* en *Dicorynia guianensis*. Voor bomen van verschillende hoogte is bepaald of de maximale fotosynthesesnelheid, stikstofconcentratie, bladgewicht per eenheid bladoppervlak en blad constructiekosten gerelateerd waren aan de bladlevensduur. De gemiddelde levensduur van *Vouacapoua* bladeren was langer (61 maanden, range 27-101) dan die van *Dicorynia* (32, range 17-54). In *Vouacapoua* was de bladlevensduur negatief gecorreleerd met boomhoogte en lichtbeschikbaarheid; terwijl in *Dicorynia* geen relatie was gevonden. Veel bladkenmerken van *Vouacapoua* waren afhankelijk van de bladlevensduur. De gevonden relaties tussen bladkenmerken en -levensduur binnen een soort waren meestal niet vergelijkbaar met de relaties die gevonden zijn wanneer soorten onderling met elkaar vergeleken worden. Voor de maximale fotosynthesesnelheid per eenheid bladoppervlak (alleen in *Vouacapoua*) was de richting van de levensduur gerelateerde variatie gelijk aan die normaliter gevonden wordt tussen soorten (i.e. een negatieve richtingscoëfficiënt). Echter voor de variabele bladgewicht per eenheid bladoppervlak werd een tegenovergestelde relatie gevonden. Met betrekking tot *Vouacapoua*, kan dit verklaard worden door een licht- en boomhoogte afhankelijke selectiedruk voor bladlevensduur en bladgewicht per eenheid oppervlak. Binnen *Dicorynia* kon

zo'n selectiedruk voor bladlevensduur niet gevonden worden. Langlevende bladeren hadden een lagere maximale fotosynthesesnelheid en bladgewicht per eenheid oppervlak dan kortlevende bladeren. Zowel de blad constructiekosten ('de hoeveelheid glucose die nodig is om een gram bladmateriaal te maken') als de maximale fotosynthese snelheid per eenheid bladstikstof waren niet gecorreleerd met de bladlevensduur. Dit kan betekenen dat er geen trade-off bestaat tussen de investering in een hoge fotosynthese capaciteit en in een langere bladlevensduur (in termen van een verminderde smakelijkheid van het blad). Voor beide soorten gold dat de tijd die nodig is om de constructiekosten van bladeren terug te betalen ('payback time') erg kort was (van 4 tot 40 dagen). De 'payback time' bleef relatief constant gedurende een brede range van lichtintensiteiten, maar het nam sterk toe in een smalle range van lage lichtintensiteit. Alleen in *Vouacapoua* nam de 'payback time' toe met toenemende bladlevensduur. Dit suggereert dat voor langlevende bladeren de teruggave van de geïnvesteerde koolstof in het blad langzaam is.

Tenslotte worden in hoofdstuk 6, de algemene discussie, de resultaten van dit proefschrift vergeleken met die uit andere studies. Voor de twee schaduwtolerante soorten *Vouacapoua americana* en *Dicorynia guianensis* wordt de integratie van de resultaten op bladniveau (dit proefschrift) met die op een hoger organisatie niveau (zoals op tak en boomkroon) kort bediscussieerd. Dit in de context van de toepasbaarheid van deze resultaten in bosteeltkundige systemen waarin de factor licht wordt gemanipuleerd om de groei en productie van commerciële houtsoorten te verhogen.

List of publications

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- Rijkers T, Pons TL, Sterck FJ and Bongers F. Leaf life span related variation in morphological and physiological leaf traits of different-sized trees in a tropical rain forest. *Submitted for publication*.
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Curriculum Vitae

Toon Rijkers was born on November 30th, 1964 in Uden, the Netherlands. He passed the secondary school in 1985. From 1985 until 1986 he fulfilled his military obligations. In 1986 he started the study Biology at the Wageningen University. For his Master's degree he carried out several studies. At the Department of Forestry, he studied the effect of light availability on the morphological and architectural development of three tropical rain forest tree species (*Vouacapoua americana*, *Dicorynia guianensis* and *Goupia glabra*). At the Department of Phytopathology, the relationship between ash-wilting disease (*Fraxinus excelsior*) and the pathogen *Verticillium dahliae* in the Netherlands was studied. At Indiana University in Bloomington, Indiana USA, a study of mayapple photosynthesis was conducted (*Podophyllum peltatum*, a clonal plant). He obtained his Master's degree in 1992.

From 1993 until 1994 he was an extraneous research scientist at the former Department of Plant Ecology and Soil Biology, Wageningen University. During this period the translocation of ¹⁴C-labelled assimilate during vegetative growth in bindweed (*Calystegia sepium*) was studied.

In 1994 he started his PhD study, in which the results are included in this thesis, in the Silviculture and Forest Ecology Group at the Department of Environmental Sciences. This work was carried out in collaboration with the Department of Plant Ecophysiology, Utrecht University. In the framework of the PhD. work he stayed at the Nouragues field station which is located in the rain forest of French Guiana, for a total of 14 months.

Since October 2000 he has a temporary appointment as teacher in the Silviculture and Forest Ecology Group, Wageningen University.

