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University of Hohenheim  
Crop Waterstress Management in the Tropics and Subtropics  
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**Physiological and growth responses of *Jatropha curcas L.* to water,  
nitrogen and salt stresses**

Dissertation

Submitted in fulfilment of the requirements for the degree  
“Doktor der Agrarwissenschaften”  
(Dr. sc. agr. / Ph.D. in Agricultural Sciences)

to the  
Faculty of Agricultural Sciences

presented by

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2011

This Thesis was accepted as doctoral dissertation in fulfilment of requirements for the degree “Doktor der Agrarwissenschaften” by the Faculty of Agricultural Sciences at University of Hohenheim on February 20, 2012.

**Date of oral examination:** February 20, 2012.

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## Funding

This study is a part of the project «Jatropha Anbau in Madagaskar». We gratefully acknowledge the funding of this work by EnBW-Regenwaldstiftung, Stiftung Energieforschung Baden-Württemberg, project No. 08A26908. All field works were carried out in partnership with Jatrogreen S.A.R.L., Antananarivo, Madagascar.



## **Preface**

This thesis is based upon studies conducted from August 2008 to October 2011 at the Department of Crop Production and Agroecology in the Tropics and Subtropics, Section: Crop Waterstress Management, University of Hohenheim, Germany, and is submitted together with the enclosed three manuscripts in partial fulfilment of the requirements for Ph.D. degree at the Faculty of Agricultural Sciences.

I am really grateful to EnBW-Regenwaldstiftung, Stiftung Energieforschung Baden-Württemberg for their financial support that allowed me to fulfil this work.

I would like to express my sincere gratitude to my leading supervisor Prof. Dr. Folkard Asch who has made a complete readiness to carry to term supervision of this work.

I am thankful to Dr. Holger Brueck who was very kind to share his valuable experience and for all his greatest support throughout the course of my thesis.

I am very grateful to my co-reviewer and examiner, Prof. Dr. Jens Wünsche, and Prof. Dr. Thilo Streck of their kind attention by sitting among the judges of this thesis despite their many responsibilities.

I acknowledge the help from the staff member of the Crop Waterstress Management in the Tropics and Subtropics section.

I would like to thank also the working group of Jatrogreen and particularly, those who were great help for taking care of the field experiment.

I want to thank colleagues and friends, who have supported me.

Finally, I wish to express my greatest thanks to my family without whom the journey would have been tougher.

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Under review of Journal of Arid Environment since July 23, 2011

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Under review of Journal of Agronomy and Crop Science since October 28, 2011.

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Published in: Biomass and Bioenergy, Volume: 35, Issue: 12, December 2011, Page: 4892-4900, <http://dx.doi.org/10.1016/j.biombioe.2011.10.017>

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## Summary

World energy demand is steadily increasing, and since fossil fuel is not renewable and about to be depleted, energy costs and prices of all energy-related market good, including food, have been increasing. Furthermore, projections of future climate indicate manifold risks for human wellbeing as human-induced CO<sub>2</sub> release results in global warming. In view of meeting the world energy demand and respecting commitments that countries have announced under the Copenhagen Accord (UNFCCC, 2010) to reduce their greenhouse gas emissions and limiting the global temperature increase to 2°C, renewable energies are expected to become increasingly important. Among a wide range of plants that can be used for biofuel sources, *Jatropha curcas* L. has been claimed to be cultivable on marginal and degraded land, and as a drought tolerant plant, can be grown profitably on sites with 300-1000 mm of water availability. Establishment of plantations could potentially contribute to ameliorate waste lands, increase poor farmers' income, and avoid competition with food production.

This thesis provides necessary and complementary information for an improved understanding of jatropha growth to guide further research to evaluate the response of jatropha to abiotic stressors and for designing plantations adapted to the plants' requirements. Given the fact that jatropha is claimed to grow on marginal lands, we studied effects of water supply, salt stress, nitrogen and air humidity as major abiotic stressors on gas exchange parameters and biomass production followed by management options for pruning the trees to positively influence biomass productivity and to contribute to optimize resource use.

The effects of water availability (rainfed *versus* irrigated) on growth and gas exchange parameters were investigated for 4-year old jatropha grown in a semi-arid environment at a plantation site in Madagascar in 2010. The results confirmed that 1250 mm water in addition to a 500 mm rainfall did not affect biomass production and instantaneous gas exchange. Nevertheless, leaf light responses of irrigated plants were higher than that of rainfed plants. The similarity of leaf area density of trees did not help clarifying this response.

In dry areas, salinity often is a major abiotic stress factor influencing water use. The study showed to what extent salt stress affected water use, canopy water vapour conductance, leaf growth and Na and K concentrations of leaves of 3-year old and young jatropha plants. 3-year old plants were exposed to seven salt levels (0-300 mmol NaCl L<sup>-1</sup>) during 20 days and young plants to five salt levels (0-200 mmol NaCl L<sup>-1</sup>) during 6 days. In both experiments, plants responded rapidly to salt stress by reducing water loss. The threshold value of responses was

between 0 and 5 dS m<sup>-1</sup>. Leaf area increment of young jatropha had a threshold value of 5 dS m<sup>-1</sup> implying that jatropha is sensitive to external salt application in terms of canopy development, conductance and CO<sub>2</sub> assimilation rate. The rapid decrease of canopy conductance indicated that jatropha is effectively protecting leaves from massive Na import over short time periods. In general, salt stress leads to stomatal closure and a decrease in transpiration rate in salt-sensitive plants. The rapid stomatal closure after first application of salt is an immediate response to salt stress in jatropha. However, subsequent salt application did not induce stomatal closure indicating that plants had adapted to stress. Comparing the response of jatropha over the first 6 days after onset of treatments, both 3-year old and young plants reduced their canopy conductance at external salt application higher than 50 mmol NaCl L<sup>-1</sup>. Transpiration of plants in both experiments was reduced to 55% at EC values between 11 and 12 dS m<sup>-1</sup> as compared to non-stressed plants. The threshold level for leaf expansion rate in this study was about 5 dS m<sup>-1</sup> for young jatropha, corresponding to an external supply of 25 mmol NaCl L<sup>-1</sup>. These findings indicate that jatropha responds sensitive to salt stress in terms of leaf elongation rate and consequently canopy development, and to immediate physiological responses.

On marginal sites, additionally to water availability and/or salinity issues, nutrient availability is often limiting plant growth. The prospect that jatropha would also yield in a low input system render the profitability of this crop more attractive. Leaf gas exchange characteristics of jatropha as affected by nitrogen supply and leaf age were intensively studied, as carbon assimilation is one of the central processes of plant growth and consequently a key process embedded in modelling approaches of plant productivity. This study showed that N supply effects on leaf gas exchange of jatropha leaves were small with only the treatment without nitrogen resulting in lower rates of CO<sub>2</sub> assimilation rate and light saturated CO<sub>2</sub> assimilation rate, nevertheless, effects of N supply on biomass formation were pronounced.

As indicated by the increase of leaf area with increasing N supply, jatropha responded rapidly in terms of leaf expansion. Nevertheless, little variation of SPAD values of plant leaves treated with 2 to 16 mM N indicated that enzyme densities were not affected by N supply once a threshold value of N supply was ensured. Therefore, the SPAD value cannot be used for N management of jatropha plantations, as it would only allow detecting severe N deficiency.

Given the fact that jatropha can adapt to soil drought, and considering that salinity and nutrient availability are limiting factor for biomass production, seasonal changes of atmospheric conditions also can affect productivity via relative air humidity and temperature influencing transpirational water loss and CO<sub>2</sub> assimilation rates. Therefore, instantaneous rates of leaf gas exchange of different leaves subject to variable air humidity (atmospheric vapour pressure deficit

(VPD)) were investigated. This study showed that CO<sub>2</sub> assimilation rate (A) and stomatal conductance (g<sub>s</sub>) were correlated in a hyperbolic fashion, and that g<sub>s</sub> declined with increasing VPD. The invert logistic function reported by Webb (1990) was used hereafter allowing for calculation of a maximal stomatal conductance. These measurements are considered to reflect the response of g<sub>s</sub> to VPD, and indeed, as shown with our data which were measured on many different leaves and VPD conditions, g<sub>s</sub> declined with increasing VPD. However, the results presented in this study indicated that responses of g<sub>s</sub> to VPD differed between the experiments with data of greenhouse experiment on leaf age effect on gas exchange parameters deviating from the general trend obvious in the other experiments indicating that other factors such as signal responses, leaf age, or diurnal dynamics may have had an influence on stomatal conductance independent of VPD... However, applying the inverse logistic function of Webb (1990), excluding the greenhouse experiment on leaf age effects, the maximal stomatal conductance of jatropha was in the range of 382 mmol m<sup>-2</sup> s<sup>-1</sup> and g<sub>s</sub> is predicted to be close to zero at 6 kPa. Effects of VPD, via stomatal conductance, by preventing high transpiration rates, have been demonstrated to be decisive on water use efficiency. Our findings are in this regard relevant for the estimation of water use efficiency of jatropha. The outcome further indicates favourable conditions at which stomatal opening is high and thereby allowing for biomass formation. This information should be considered in approaches which aim at quantifying leaf activity of field-grown bushes which are characterized by spatially highly diverse conditions in terms of microclimatic parameters.

Microclimatic parameters can be modified by the tree structure. The reported field experiment on 4-year old jatropha indicated that the biomass production and canopy size depended mainly on primary branch length. A comparison of plants of different pruning types with regard to trunk height (43 *versus* 29 cm) and total length of primary branches (171 *versus* 310 cm), suggest that higher biomass production and greater leaf area projection was realized by trees with short trunks and long primary branches. Total dry mass formation varied from 489 to 912 g m<sup>-2</sup> and leaf area projection from 3.26 to 7.37. Total aboveground biomass increased from 2.3±0.5 to 4.89±1.4 kg tree<sup>-1</sup> and from 4.6±1.8 to 8.9±1.0 kg tree<sup>-1</sup> for the pruning types with shorter and longer lateral branches, respectively. Growth of twigs and leaves was positively correlated with total length of branches. Relative dry mass allocation to branches, twigs and leaves, length of twigs per cm of branches and specific leaf area (13.57±0.72 m<sup>2</sup> kg<sup>-1</sup>) were not affected by pruning and water supply. Trees with shorter branches had a higher leaf area density.

Pruning of the jatropha trees at a lower height of trunk and allowing for longer primary branches did not change the wood density and resulted in a higher productivity in terms of total

aboveground biomass. As opposed to an allometric relationship between the average diameter of primary branches and total above ground biomass, our data suggest that these traits were not constantly correlated. Our data indicate that the length of newly formed twigs, where the leaves are attached to, can be related to the total length of already established branches. Leaf area density and relative dry mass allocation to leaves were not affected by the two pruning techniques, indicating that pruning differences in leaf area size were proportionally converted to corresponding pruning differences in the canopy volume exploited by plants.

The results reported in this study are relevant for understanding jatropha growth. It helps farmers first for a better plantation management and researchers as well as contribution to future modelling purpose concerning jatropha growth under variable climatic conditions. Additionally, it should complement information for a better set of priorities in research, contribute indirectly to breeding programs and adjust agricultural policies in terms of encountering global change.

## Zusammenfassung

Aufgrund des stetig ansteigenden Weltenergiebedarfs und der absehbaren Erschöpfung fossiler Energiequellen, kam es in der Vergangenheit immer wieder zum Anstieg der Energiepreise und davon abhängiger Wirtschaftsgüter. Durch den fortgesetzten Ausstoß von CO<sub>2</sub> und dem dadurch verursachten Klimawandel wird die Weltagarproduktion weiteren Risiken ausgesetzt sein. Um den Weltenergiebedarf zu decken, und um die, in der Übereinkunft von Kopenhagen (UNFCCC, 2010) festgelegten, Klimaschutzziele einer maximalen Erderwärmung von 2°C und Reduzierung der Emissionen von Treibhausgasen einzuhalten, wird den erneuerbaren Energiequellen eine steigende Bedeutung zugemessen. Unter den verschiedenen Nutzpflanzen, die als Rohstofflieferanten für Biokraftstoffe in Frage kommen, eignet sich *Jatropha curcas* L. angeblich sowohl für den Anbau auf unfruchtbaren und erschöpften Böden, wie auch für den Anbau in Gebieten mit 300-1 000 mm verfügbarem Niederschlag. Ein Jatrophaanbau in Plantagenwirtschaft könnte degradierte Flächen nachhaltig verbessern, die finanzielle Situation einkommensschwacher Landwirte positiv verändern und darüber hinaus die Konkurrenz um Flächen für die Nahrungsmittelproduktion mindern.

Um aber tatsächlich auf marginalen Flächen *Jatropha* erfolgreich anbauen zu können, müssen die Pflanzen unter Bedingungen angebaut werden, die den Mindestanforderungen der Pflanze genügen. Um diese wiederum zu definieren, ist es notwendig grundlegende und weiterführende Erkenntnisse über das Wachstum und das Management von *Jatropha* zu generieren. In dieser Arbeit werden daher zunächst der Einfluss von Wasserversorgung, Salzstress, Stickstoffversorgung und Luftfeuchte als maßgebliche abiotische Stressfaktoren auf Gaswechselfparameter und Photosynthese sowie die Produktion von Biomasse untersucht. Schließlich wird der Effekt von Schnittmaßnahmen auf die Produktivität von *Jatropha* analysiert, um mit potentiellen Managementmaßnahmen Biomasseproduktion und Ressourcennutzung in Plantagen zu beeinflussen bzw. zu optimieren.

Die Arbeit baut auf drei internationalen Publikationen auf, die im Anhang wiedergegeben sind. Zunächst wurde im Jahr 2010 auf einer Plantage in Madagaskar der Einfluss von Wasserverfügbarkeit (Regenfeldbau vs. Bewässerungsfeldbau) auf Wachstum und Gaswechselfparameter von 4-jährigen Jatrophapflanzen in einer semiariden Umwelt untersucht. Die Ergebnisse bestätigten, dass bei einem Niederschlag von 500 mm eine zusätzliche Bewässerungsmenge von 1 250 mm keinen Einfluss auf Biomasseproduktion oder grundlegende Gaswechselfparameter hatte. Bewässerte Pflanzen reagierten allerdings auf Änderungen der Lichtintensität deutlich stärker

positiv als die unbewässerten Pflanzen. Diese Unterschiede konnten nicht über Blattflächendichte erklärt werden, da hier zwischen den Behandlungen nur geringe Unterschiede bestanden.

In Trockengebieten stellt Salzstress einen der wichtigsten abiotischen Stressfaktoren dar. In dieser Studie diskutieren wir den Einfluss von Salzstress auf die Wasseraufnahme, die Wasserleitfähigkeit der Bestandesblattfläche, die Blattentwicklung und Natrium und Kalium Gehalte von Blättern 3-jähriger und junger Jatropha-Pflanzen. Drei-jährige Jatropha-Pflanzen wurden für 20 Tage mit 7 verschiedenen Salzkonzentrationen (0-300 mmol NaCl L<sup>-1</sup>), Jungpflanzen mit 5 verschiedenen Salzkonzentrationen (0-200 mmol NaCl L<sup>-1</sup>) für 6 Tage behandelt. Die Pflanzen reagierten in beiden Versuchen umgehend mit einer verringerten Wasseraufnahme. Der Schwellenwert für die Reaktion lag dabei zwischen 0 und 5 dS m<sup>-1</sup>. Der Schwellenwert für eine Reduzierung des Blattflächenzuwachses lag zwischen 0 und 5 dS m<sup>-1</sup>, was nahe legt, dass Bestandesblattflächenentwicklung, Bestandesleitfähigkeit und CO<sub>2</sub> Assimilationsraten von Jatropha empfindlich auf Salzapplikationen reagieren. Die schnelle Abnahme der Bestandesleitfähigkeit lässt vermuten, dass Jatropha für kurze Zeitabschnitte effektiv den Na-Import in die Blätter unterbinden kann. Generell führt Salzstress in salzempfindlichen Pflanzen zu einer verringerten stomatären Leitfähigkeit und zu einer Abnahme der Transpirationsraten. Bei Jatropha stellt die rapide Abnahme der stomatären Leitfähigkeit eine unmittelbare Reaktion auf Salzstress dar. Eine weitere Salzapplikation führte dagegen nicht zu einer Verringerung der stomatären Leitfähigkeit, was darauf hindeutet, dass die Pflanzen bereits an Salzstress adaptiert waren. Bei einem Vergleich der Reaktionen der Pflanzen in den ersten 6 Tagen nach Beginn der Behandlung fällt auf, dass sowohl die 3-jährigen Pflanzen als auch die Jungpflanzen die Bestandesleitfähigkeit reduzierten, wenn die Salzkonzentration höher als 50 mmol NaCl L<sup>-1</sup> lag. In beiden Versuchen war die Transpiration im Vergleich zu ungestressten Pflanzen auf 55% reduziert. Der Schwellenwert für eine Reduktion der Blattwachstumsrate lag für Jungpflanzen bei 5 dS m<sup>-1</sup>, was einem Salzgehalt der Nährlösung von 25 mmol NaCl L<sup>-1</sup> entspricht. Dieses Ergebnis zeigt, dass Jatropha bezüglich des Blattwachstums und somit auch bezüglich der Bestandesblattflächenentwicklung empfindlich auf Salzstress reagiert.

Neben Wasserverfügbarkeit und Salzstress stellt auf marginalen Standorten die Nährstoffverfügbarkeit einen limitierenden Faktor für pflanzliches Wachstum dar. Die Erwartung, auch in low-input Systemen gute Erträge zu erwirtschaften, macht den Anbau dieser Pflanze noch attraktiver. Die C-Assimilation stellt als zentraler Prozess pflanzlichen Wachstums eine Schlüsselrolle in der Modellierung der Produktivität dar. In einer weiteren Studie wurde der Einfluss der N-Versorgung und des Blattalters auf den Gaswechsel von Jatropha untersucht. Die Untersuchung zeigt, dass die N-Versorgung nur einen geringen Einfluss auf den Gaswechsel von Jatropha hat,

denn nur die N-freie Behandlung induzierte eine verringerte C-Assimilationsrate bzw. eine verringerte lichtgesättigte C-Assimilationsrate, und das obwohl generell Effekte der N-Versorgung auf die Biomasseentwicklung messbar waren.

Die Steigerung der Blattfläche durch verbesserte N-Versorgung zeigte, dass *Jatropha* bezüglich der Blattwachstumsrate eine unmittelbare Reaktion gezeigt hat, während die nahezu konstanten SPAD-Werte der Blätter von Pflanzen, die mit jeweils 2 bis 16 mM N behandelt wurden, nahe legen, dass die Enzymdichte nicht von der N-Versorgung beeinflusst war, wenn ein Mindestwert in der N-Versorgung sichergestellt war. Folglich können SPAD-Messungen nicht für Entscheidungen bezüglich der N-Düngung für *Jatropha* herangezogen werden, da sie ausschließlich einen schweren N-Mangel zeigen würden.

Neben Bodensalzgehalt und Nährstoffverfügbarkeit als wichtigste produktionslimitierende Faktoren sind die Anpassung an saisonale Unterschiede in der Wasserversorgung (sowohl bodenbürtig (Bordentrockenheit) als auch atmosphärisch (geringe Luftfeuchtigkeit)) elementar für eine erfolgreiche *Jatropha*produktion in semi-ariden Gebieten. Um den Einfluss von Luftfeuchtigkeit auf die Produktivität von *Jatropha* zu untersuchen, haben wir unmittelbare Gaswechselraten an Blättern, die einem variablen Dampfdruckdefizit ausgesetzt waren, gemessen. Das Ergebnis der Untersuchung zeigt, dass die CO<sub>2</sub>-Assimilationsrate (A) und die stomatäre Leitfähigkeit (g<sub>s</sub>) in einer hyperbolischen Funktion korreliert waren, und dass g<sub>s</sub> mit zunehmendem Dampfdruckdefizit abfiel. Für die Berechnung der maximalen stomatären Leitfähigkeit wurde die von Webb (1990) beschriebene umgekehrte logistische Funktion genutzt. Wie wir mit unseren an verschiedenen Blättern und unter variablem Dampfdruckdefizit aufgenommenen Daten zeigen konnten, nahm die stomatäre Leitfähigkeit mit zunehmenden Dampfdruckdefizit ab. Dennoch weisen die Ergebnisse dieser Studie darauf, dass die Reaktion von g<sub>s</sub> auf das Dampfdruckdefizit sich zwischen den Experimenten unterschieden, da Daten aus einem Versuch von dem offensichtlichen generellen Trend aus dem anderen Versuch abwichen. Dies weist darauf hin, dass andere Faktoren z. B. Wurzelsignale, Blattalter, oder Tageszeiten unabhängig vom Wasserdampfdruckdefizit der Luft einen Einfluss auf die stomatäre Leitfähigkeit ausgeübt haben. Dennoch war unter Anwendung der umgekehrten logistischen Funktion von Webb (1990) und unter Nichtbeachtung der Gewächshausexperimente zum Blattalter die maximale stomatäre Leitfähigkeit von *Jatropha* im Bereich von 382 mmol m<sup>-2</sup> s<sup>-1</sup> und g<sub>s</sub> wird bei 6 kPa auf null geschätzt. Es wurde gezeigt, dass der Effekt des Dampfdruckdefizits über die stomatäre Leitfähigkeit und die Vermeidung hoher Transpirationsraten entscheidend auf die Wassernutzungseffizienz wirkt. Diesbezüglich sind unsere Ergebnisse relevant für die Abschätzung der Wassernutzungseffizienz von *Jatropha*. Weiterhin erlauben diese Ergebnisse Bedingungen zu definieren, unter denen die

Spaltöffnungsweite groß ist und damit auch eine hohe Biomassebildung erlaubt. Diese Erkenntnisse sollten bei der Quantifizierung der Blattaktivität von Jatrophaanbau im Freiland berücksichtigt werden, da diese in verstärktem Maße Schwankungen im Bestandesmikroklima ausgesetzt sind.

Mikroklimatische Parameter wiederum können über die Struktur des Baumes beeinflusst werden. Das Feldexperiment mit 4-jährigen Jatrophapflanzen lieferte Hinweise darauf, dass die Biomasseproduktion und die Größe der Bestandesblattfläche hauptsächlich von der Länge der Hauptäste und nicht von der Wasserversorgung abhängen. Ein Vergleich von Pflanzen mit unterschiedlichen Schnittbehandlungen in Bezug auf Stammhöhe (43 vs. 29 cm) und Gesamtlänge der Hauptäste (171 vs. 310 cm) zeigte, dass Bäume mit kurzen Stämmen und langen Hauptästen mehr Biomasse produzieren und eine größere Blattprojektionsfläche bilden. Die Gesamttrockenmasseproduktion variierte zwischen 489 und 912 g m<sup>-2</sup> und die Blattprojektionsfläche zwischen 3.26 und 7.37(m<sup>2</sup> blatt pro m<sup>2</sup> projektion Grundfläche). Die gesamte oberirdische Biomasse stieg von 2.3±0.5 auf 4.89±1.4 kg Baum<sup>-1</sup> für Bäume mit kurzen Sekundärverzweigungen und von 4.6±1.8 auf 8.9±1.0 kg Baum<sup>-1</sup> für Bäume mit langen Sekundärverzweigungen. Das Wachstum von Zweigen war mit der Gesamtlänge der Verzweigungen positiv korreliert. Schnitt und Wasserversorgung hatten keinen Einfluss auf die relative Trockenmassellokation von Ästen, Zweigen und Blättern, die Länge von Zweigen pro cm Ast und die spezifische Blattfläche (13.57±0.72 m<sup>2</sup> kg<sup>-1</sup>). Bäume mit kürzeren Ästen hatten eine höhere Blattflächendichte.

Das Zurückschneiden der Jatrophapflanzen auf einer geringere Stammhöhe und längere Hauptäste hatte keinen Einfluss auf die Dichte des Holzes und führte unabhängig von der Wasserversorgung zu einer höheren Produktivität in Bezug auf die gesamte oberirdische Biomasse. Im Gegensatz zu einer allometrischen Beziehung zwischen dem durchschnittlichem Durchmesser der Hauptäste und der gesamten oberirdischen Biomasse legen unsere Daten nahe, dass diese Merkmale nicht konstant miteinander korreliert waren. Unsere Daten zeigen, dass die Länge der neugebildeten blättertragenden Zweige mit der Gesamtlänge des Astes in Beziehung stehen. Die Schnittvarianten hatten keinen Einfluss auf die Blattflächendichte und die relative Trockenmassellokation. Dies lässt darauf schließen, dass die durch die verschiedenen Schnitttechniken hervorgerufenen Unterschiede bezüglich der Blattfläche durch die Pflanze in Form einer entsprechend vergrößerten Gesamtblattfläche kompensiert wurden.

Die Ergebnisse dieser Arbeit sind relevant für das Verständnis des Wachstums von Jatropha und für die Beurteilung der effektiven Nachhaltigkeit und Profitabilität eines Anbaus auf bestimmten degradierten Flächen. Dies kann in erster Linie Landwirten bei der Entwicklung verbesserter Managementpraktiken dienen, ist aber ebenso eine Hilfe für Wissenschaftler bei der zukünftigen



Entwicklung von Modellierungsansätzen für Wachstum von *Jatropha* unter verschiedenen Klimabedingungen. Zusätzliche liefern die hier dargestellten Ergebnisse weitere Informationen für die Ausrichtung der Forschung, leisten indirekt einen Beitrag für Zuchtprogramme und können bei der Ausrichtung der Agrarpolitik im Hinblick auf die Herausforderungen des Globalen Wandels hilfreich sein, weil sie eine Beurteilung des Potentials potentieller Anbauflächen für *Jatropha* ermöglichen.



## General Introduction

Over the last three decades, energy demand increased by 2% every year and is predicted to step up with the ongoing economic rise of China and India (IEA, 2010). Fossil fuel resources are not renewable and are expected to be more or less exploited within this century such that the demand will, at a certain time, be higher than the daily production potential. This 'peak oil' event is shifting the global economy into a completely new framework as prices are predicted to increase sharply threatening not only the economic growth of industrial societies but more importantly the bottom-line concerns and basic livelihoods of the majority of the world's population. Such trends are already obvious as fuel price rose from 43 US \$ in 2004 to 88 US \$ per barrel in 2011 (IEA, 2011).

Irrespective of global market responses to changing resource availability of oil, effects of CO<sub>2</sub> release by use of fossil fuels on climate change is additionally dominating the public awareness to a great extent. Shafiee and Topal (2009) computed that oil, coal and gas will be depleted approximately by 2042, 2112 and 2144, respectively. Combustion of fossil fuel induces the greenhouse effects by changing the reflectivity of the earth's surface and atmosphere. Carbon dioxide in the atmosphere has increased by 35% during the industrial era (Le Treut et al., 2007) and if atmospheric CO<sub>2</sub> were doubled, the mean global temperature would increase by 2°C (Callendar, 1938). To-date, 250 idealized CO<sub>2</sub> emissions scenarios have been elaborated and these scenarios agree on the fact that the projected 3.67 trillion of CO<sub>2</sub> emitted by human activities (out of it half has been already released) will induce warming of 2 °C above pre-industrial temperature (Allen et al., 2009).

Adding complexity to this, permanent removal of forests constitutes another significant anthropogenic source of CO<sub>2</sub>. More than 80% of the biomass feedstock used for energy are derived from wood and shrubs. The remaining 20% come from the agricultural sector with only 3% from energy crops (Chum et al., 2011). Particularly in Madagascar, the primary source of energy (81%) is wood (directly or as charcoal) used for cooking, followed by fossil fuel (14%). Those 14% correspond to an annual average of 400 000 m<sup>3</sup> of oil during the past 5 years (OMH, 2011).

In view of meeting the world energy demand and respecting commitments that countries have announced under the Copenhagen Accord (UNFCCC, 2010) to reduce their greenhouse gas emissions and limiting the global temperature increase to 2°C, renewable energies are expected to become increasingly important. Among the many options of using renewable energies (solar radiation, wind, geothermal, hydropower, ocean energy) to fulfil these commitments, the idea of

energy production derived from plant biomass to contributing significantly to the worldwide demand in the near future, attracted clean development mechanism projects, private enterprises, and public and political stakeholders. Global biofuel use is predicted to grow more than fourfold from 2008 to 2035 (IEA, 2010). Use of bioenergy is manifold ranging from household-related demands such as heating and cooking to production of electricity and energy demand for transport. E.g., biofuels already meet 8% of global road transport fuel demand (IEA, 2010).

Efforts to include biofuel into national and regional energy panels are growing rapidly and are as diverse as production systems are along the global climatic zones (Chum et al., 2011). A wide range of plants are already used for biofuel sources such as herbaceous and woody lignocellulosic plants (Miscanthus, switchgrass, reed canary grass, poplar, willow, eucalyptus) and first-generation biofuel feedstocks (sugarcane, maize, cassava, rapeseed, soybean, sorghum, palm oil, jatropha).

Bioenergy production systems, though being highly required in the near future, are embedded in already existing land-use patterns dominated by food and fibre production. Consequently, questions concerning fuel versus food and the costs of land use in terms of land and water availability must be given answers to. On productive farm land, "It's 36 percent more efficient to grow grain for food than for fuel," said Ilya Gelfand (MSU News, Apr 19 2010). The FAO (2010) in frame of the Bioenergy and Food Security (BEFS) project proposed both to offer decision tools for policy makers and to 'assess if and how bioenergy developments could be implemented without hindering food security', to increase incomes and employment for smallholders. At least, the conflict between food and fuel would require production systems of bioenergy which are more or less independent of the food production value chain (Asch and Huelsebusch, 2009). In this context, many companies are optimistic about the potential of *Jatropha curcas* L. as a potential fuel substitute (Villena-Denton, 2008; Achten et al., 2008). *Jatropha* has been claimed to be able to replace kerosene and diesel and to substitute fuelwoods (Openshaw, 2000).

## **1. *Jatropha curcas* L.**

### 1.1. Description

The origin of *Jatropha* remains controversial as it can be found over a wide range of countries in Central and South America; in humid forest, tropical dry forest, cactus and thorn scrub, on shrubby slopes, thicket near river banks, dry steep hillsides, in woodland, hillsides with dense shrubs and woods, or coastal thickets (Heller, 1996). Nowadays, it is found in tropical and subtropical countries all over the world (Henning, 2009). Karl von Linne classified the plant in 1753 and gave it the botanical name *Jatropha curcas*. It belongs to the genus *Euphorbiaceae* containing 2 subgenera, 10 sections and 10 subsections with up to 175 species (Dehgan and Webster, 1979). *Jatropha curcas* L. is a perennial tree or shrub with woody succulent. Leaves have 5 to 7 shallow lobes and are arranged in alternate with spiral phyllotaxis. The inflorescence of *Jatropha curcas* L. is composed by a main florescence and a distinct coflorescence. Flowers of *Jatropha curcas* L. are unisexual or occasionally hermaphrodite and are pollinated by moths and bees (Raju and Ezradanam, 2002; Dehgan and Schutzman, 1994). *Jatropha curcas* L. has a trilocular, ellipsoidal, sudrupaceous fruit, the exocarp of which remains fleshy until the seeds are mature, finally separating into 3 cocci. The fruit is 2.5-3.5 cm long to 2-2.5 cm wide (Singh, 1970). Seeds of *Jatropha curcas* L. are black, oblong, 2.5 to 3 cm long and 1 cm thick, more or less spherical or ellipsoidal with very small caruncles. *Jatropha* seeds are toxic for human and animal (Henning, 2009).

### 1.2. Occurrence and uses

*Jatropha* is a multipurpose plant of considerable potential in bioenergy systems (Openshaw, 2000). World *Jatropha* plantations occupied 936.000 ha in 2008 (with 85% in Asia, 13% in Africa and 2 % in Latin America) (GEXSI, 2008). Global plantation area increased dramatically to 4.72 Mio. ha in 2010 and is predicted to reach 12.8 Mio ha in 2012 (GEXSI, 2008). The plant itself has certain functions such as fencing, and tutoring of vanilla and pepper. Annual seed yield of *Jatropha* varies from 100 to 8 000 kg ha<sup>-1</sup>.

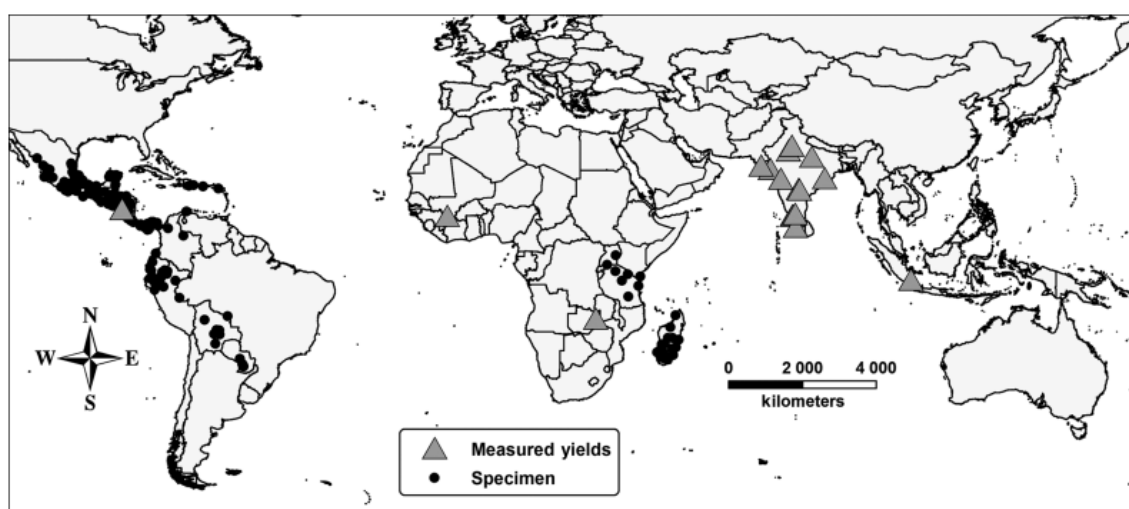
*Jatropha* seed contains on average 34.4% oil on a mass basis (Achten et al., 2008). Depending on the extraction method, 38 to 99% of the oil can be extracted (Achten et al., 2008). This oil can be

used in place of kerosene and diesel, or substitute fuel wood for cooking purposes. Additionally, the oil can be used as insecticide or in soap fabrication. Seed cake can be used as fertilizer (Uellenberg, 2007), and as feedstock for biogas production (Karve, 2005; Visser and Adriaans, 2007).

*Jatropha curcas* L. can be cultivated on marginal and degraded land that is not used for food production (Heller, 1996). *Jatropha* is adapted to arid and semi-arid locations and can be grown profitably on sites with 300-1000 mm of water availability (Dehgan and Schutzman, 1994). Besides being a multipurpose crop and being able to grow on marginal sites and giving the option of energy self sufficiency for cooking and local electricity demand, *jatropha* could provide employment and enhance the quality of rural life (Brittaine and Litaladio, 2010). Properly managed it also has a positive influence on soil fertility by preventing soil erosion and enhancing soil organic carbon content if seed cake is returned to the field site (Jongschaap et al., 2007).

## 2.Rationale

Tigerstedt (1994) stipulated that when established at marginal environment, plant adaptation must be insured prior to yield production. *Jatropha curcas* L. has been identified in area (Fig. 1) with more or less extreme conditions such as arid and semi- arid environment.

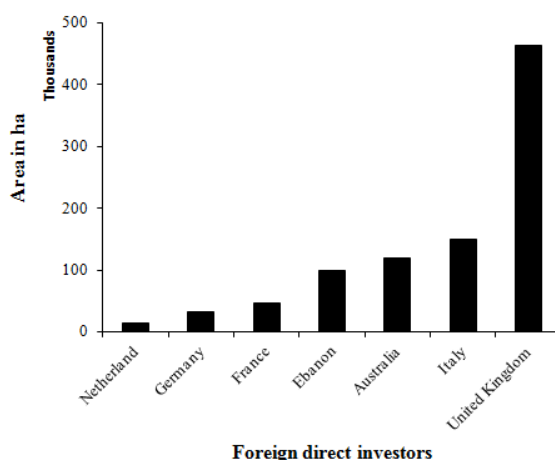


Source: Trabucco et al., 2010

**Figure 1:** Locations of *jatropha* natural occurrence used for model training ('Herbarium Specimen') and locations of on-site yield assessments ('Measured Yields') used for model validation.

It was then obvious that this plant demonstrates a certain adaptation to marginal area. Assessing the carbon budget of jatropha cultivation in terms of the balance between CO<sub>2</sub> source and sink, it was shown that jatropha has advantages compared to food crops like maize, soybean, sugar cane, and oil-palm (Becker and Makkar, 2008). Jatropha was claimed to grow on marginal land implying efficient use of scarce resources such as water and nutrients and to use less carbohydrates to build wood while growing big (Jongschaap et al., 2007). At this point, the interest in jatropha as potential energy source constrain producers to domesticate this plant. However, examples of studies performed on jatropha plantation demonstrated that not all suitable sites will bring profitable yield (Trabucco et al., 2008; Achten et al., 2010) even though this plant adapted to the given conditions.

The particular interest given to jatropha plantation in Madagascar arise in the context that over the past 20 years, Madagascar has lost over 1 million hectare of its forest by conversion to agricultural land in order to meet the growing food demand of the population. At least 10% of the land area has become infertile as a result of slash-and-burn deforestation and/or shifting cultivation. Several regions across Madagascar have favourable conditions for the cultivation of jatropha. Uellenberg (2007) reported 11 actual and potential actors in jatropha production in Madagascar and, in 2008, WWF (GEXSI, 2008) estimated that jatropha plantations in Madagascar cover 20 000 ha. However, they identified project-based areas of totally 37 700 ha in 2009 (Rakotoarivelo, 2008; GEXSI, 2008). If these 37 000 ha would produce a seed yield of 3 000 kg ha<sup>-1</sup> and if these seeds would contain 50% oil content, it were possible to produce enough biodiesel to substitute for 71% of the annual national oil import. In 2009, 926 700 ha of underutilized grass land and other whose land use has not been described has been identified as potential investment targets (Fig. 2).



Source: GTZ/Uellenberg, 2009

**Figure 2:** Estimated land area coverage for jatropha in Madagascar. Study from 2005 to March 2009.

Nevertheless, jatropha is currently still a non-domesticated plant of which basic agronomic properties are not thoroughly understood and the environmental effects have not been investigated yet (Achten et al., 2008). The establishment, management, and productivity of jatropha under various climatic conditions are not fully documented (Openshaw, 2000).

At present, research interest on jatropha focuses on seed oil content and quality (Openshaw, 2000), water use (Holl et al, 2007), ability to grow on marginal sites (Heler, 1996; Jongschaap, 2007) and to convey benefits for socio-economy and ecology (Maes et al., 2009). Physiological studies have been conducted on leaf gas exchange, leaf area development and dry weight partitioning under different environmental conditions but mostly using seedlings (Maes et al., 2009; Behera et al., 2009; Jongschaap et al., 2009; Holl et al., 2007).

With increasing atmospheric CO<sub>2</sub> concentration and concomitant global warming, the proportion of arid areas of the world is likely to increase. Additionally, full potential of jatropha to produce yield at marginal conditions is not complete. Taking poor sites as one of the dominant target environments in which jatropha will be cultivated, an improved understanding of jatropha growth under environmental stressors is required.

Generally, water scarcity is one of the dominant growth constraints in arid and semiarid areas and water availability has been proven to have positive effects on jatropha growth (Wu et al., 2009). High salt content of soils comes second as a major stressor in these climates. Additionally, environmental factors such as light, temperature, humidity may one at a time or by interaction limit growth and cause harvest failure. Furthermore, nutrient availability may affect plant growth in an almost threshold manner. Finally, optimization of resources use efficiency depend also on plant structure.

Therefore, for sustainable production of jatropha, we supply reliable information on relationships between biomass productions, seasonal water use, and environmental conditions are needed.

### **3.Objectives**

In view of improving productivity of jatropha established in marginal areas, this thesis considers some of the essential parameters of jatropha growth in response to abiotic stressors. Water availability and use, salinity, and nitrogen supply are basic abiotic factors influencing productivity of plants via their effects on gas exchange and CO<sub>2</sub> assimilation. Considering in addition the effects of seasonal changes in atmospheric conditions develops an improved



understanding of responses of gas exchange parameters of jatropha plants and thus productivity. Additionally, management-driven practices to optimize resources use for the sake of farmers first and then researchers, urge to an evaluation of effects of pruning on biomass production. The results of this thesis will contribute to future modelling purposes focusing on simulating and predicting jatropha growth and yield. Additionally, it should complement information for a better set of priorities in research, contribute to directing breeding programs, and adjust agricultural policies to global changes.

The specific objectives of this research project were:

- To evaluate the effect of water availability on biomass and gas exchange parameters at a plantation site in Madagascar;
- To assess physiological response of jatropha to salt stress;
- To characterize basic relationships between CO<sub>2</sub> assimilation rate and stomatal conductance in response to N supply and varying vapour pressure deficit of the atmosphere,
- To assess the effect of pruning types on biomass production of jatropha plants at a plantation site in Madagascar.

#### **4. Research hypothesis**

1. As jatropha is supposed to be drought tolerant showing no symptoms of leaf wilting under severe soil water deficit, we speculated that irrigation may improve biomass production but not affect gas exchange parameters.
2. Salt stress is one of the major abiotic stresses and can, particularly in arid and semi-arid regions, severely limit plant growth and yield (Mc William, 1986). But, given the fact that currently jatropha is often seed-propagated from local and not well defined genetic material and assuming that breeding will improve genetic traits in the near future, jatropha may become a crop well-tailored to stressful environments with low water availability and salt stress (Brittaine and Litaladio, 2010). We therefore expected that moderate salt stress has no influence on plant performance concerning leaf expansion and gas exchange.
3. Gas exchange between the leaf and the atmosphere occurs via stomata which are regulated by the plants' internal status and by external conditions. We tested here that

CO<sub>2</sub> assimilation rate and stomatal conductance were influenced by radiation, atmospheric humidity and nitrogen availability.

4. Behera et al. (2009) found that pruning the main branch of 2-years old jatropha at 30–45 cm height was ideal for maximizing growth as indicated by annual increment in plant height. In this thesis, the hypothesis was tested that productivity is positively correlated with trunk basal diameter.

## **5. Overview of experimental studies**

### **5.1. Materials and methods**

For this research a combination of field and greenhouse studies was used.

The experiments were performed in a greenhouse of Hohenheim University, Germany.

The first greenhouse study, described more in detail in Appendix I, concerned effect of salinity on canopy water vapour conductance of young and 3-year old *Jatropha curcas* L. In one of the experiment, 35 jatropha plants of 3-year-old were grown in pots containing peat soil. Plants were subjected to seven different salt treatments of 0, 30, 60, 120, 180, 240 and 300 mmol NaCl L<sup>-1</sup> applied as irrigation water. In the other experiment, a set of 2-month old jatropha plants were grown in modified Hoagland solution (Hoagland and Arnon, 1950) and were subjected to salt application of 0, 25, 50, 75, 100, 150 and 200 mmol NaCl L<sup>-1</sup>.

Electrical conductivity of soil solution and irrigation water (EC) were measured. During the experiment, daily water consumption was measured by recording the weight of pots.

Daily canopy water vapour conductance per plant was calculated from daily water loss corrected for the daily mean VPD. Additionally, average daily water loss during the last three days before harvest and the final leaf area of plants were used for calculating canopy transpiration rates per unit leaf area. These data were complemented by measurements of single leaf gas exchange. Finally, leaf sodium and potassium content were measured using a flame photometer (Jenway, Bibby Scientific Limited, Essex, UK).

The second greenhouse studies concerned mainly the effects of nitrogen supply and leaf age on leaf gas exchange of 2-months old plants grown in nutrient solution and 6-months old plants grown in soil, respectively.

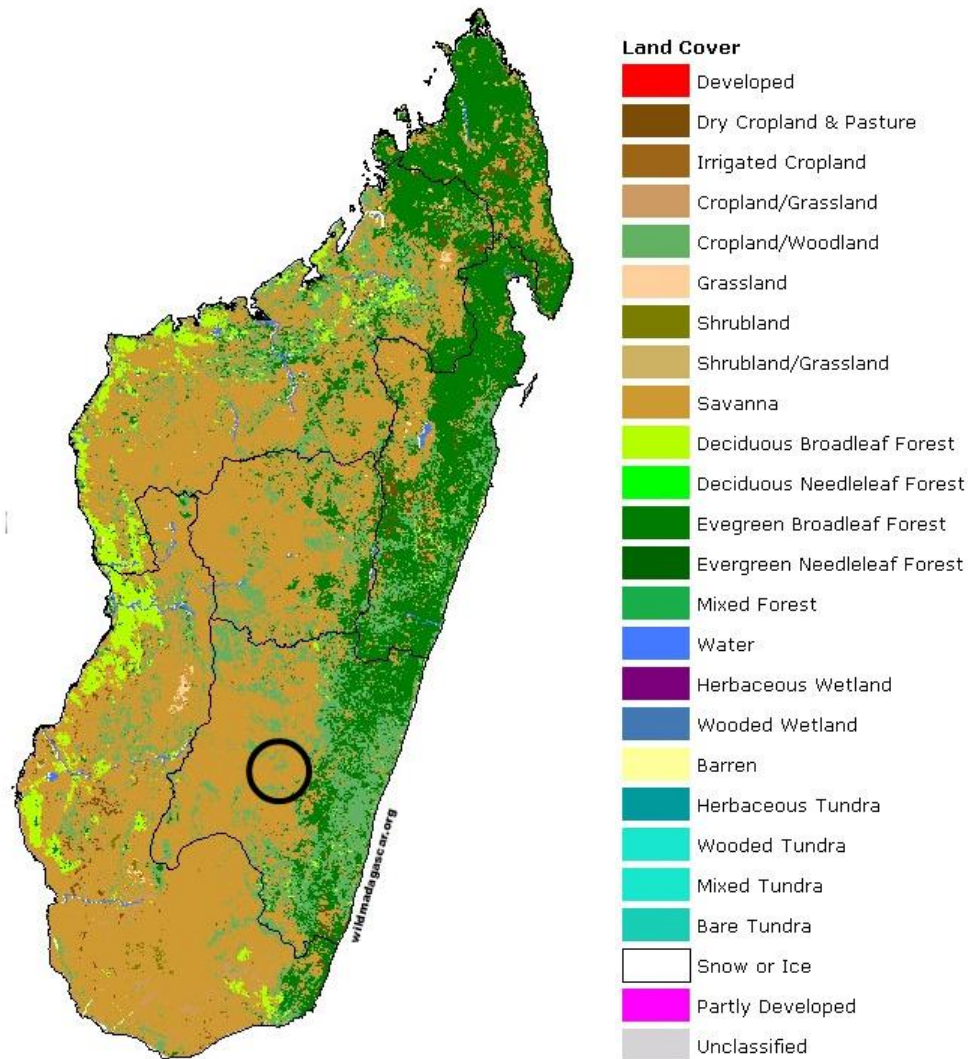
The N treatment was established by applying Hoagland nutrient solution (Hoagland and Arnon, 1950) with a pH of 6.0, and with 0, 2, 4, 8 and 16 mM N. Gas exchange parameters were measured and recorded when stomatal conductance and intercellular CO<sub>2</sub> concentration were stable. Light response curves were also measured. A chlorophyll index value of leaves was recorded prior to gas exchange measurement. At the end of the experimental period, plants were harvested and leaf, stem and root biomass and total leaf area measured.

Effect of leaf age on leaf gas exchange was assessed by monitoring the leaf area development of the 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> oldest leaves of plants from leaf emergence to full leaf expansion. Leaf gas exchange measurements were performed over the experimental period under greenhouse ambient conditions.

Field experiments were performed on a plantation site in the South-Western part of Madagascar (21°43'35.58"S, 46°23'05.63"E, altitude 750 m.a.s.l.) during summer 2009 and 2010. Effects of tree age, water supply, leaf age, and leaf position on gas exchange parameters of jatropha were investigated in 2009. Gas exchange was measured on plants at two sites five km apart from each other. These sites differed in plantation establishment with 1 and 2-year old plants at the sites. At each site, plants of similar size were selected. Gas exchange measurements were done on expanding (less than 3 weeks old) and fully expanded (more than 4 weeks old) leaves. The selected leaves were either inside (shaded leaves) or outside the canopy (sunlit leaves). Noticing during the data analysis that leaf gas exchange was affected by time of the day, data were grouped in three different time groups: early (09:00-10:30), before noon (10:30-12:00), and afternoon (12:00-13:30).

The second experiment performed in the field combined pruning effect on biomass production and gas exchange parameters characteristics of 3-years old jatropha trees in 2010. Plants grown at the same plantation site at similar environment conditions were classified according to two pruning types identified at two sub-area of the plantation sites. Trees were pruned in a way that trees had tall trunk (43 cm) and short total primary branches (171 cm) (Pruning type A), or had short trunk (29 cm) and long total primary branches (310 cm) (Pruning type B). Plants were either rainfed or irrigated. Aboveground dry matter were harvest at 5 harvest dates and effects of water supply and pruning types on aboveground biomass allocation were analyzed. Additionally,

effects of water supply pruning type, leaf age and time of measurement (morning and afternoon) were analyzed. The 5<sup>th</sup> or 6<sup>th</sup> leaf from the apex of branches was selected for gas exchange measurements. Measurements were performed at the last two weeks of April 2010. The leaf age effect on gas exchange was measured on the same leaf at two different times, namely in the expanding stage and the fully expanded stage.



**Source:** FAO Country Profiles and Mapping Information System (The United Nations Food and Agricultural Organization) [http://www.wildmadagascar.org/maps/land\\_cover.html](http://www.wildmadagascar.org/maps/land_cover.html).

**Figure 3:** Land cover and vegetation type in Madagascar. Field experiment site location (black circle): Fenoarivo, District of Ambalavao, Region: Haute Matsiatra, ( $21^{\circ}43'35.58''S$ ,  $46^{\circ}23'05.63''E$ , altitude 750 m.a.s.l.). This region is characterized by tropical climate with an annual minimum and maximum monthly temperature of 17.4 and 25.3 °C, respectively and total rainfall of 597 mm typically occurring from November to April (FAO-climnet, 2010) and is mainly cover by grassland.

## 5.2. Main findings

### 5.2.1. Biomass and leaf gas exchange responses of field- grown 4-year old jatropha.

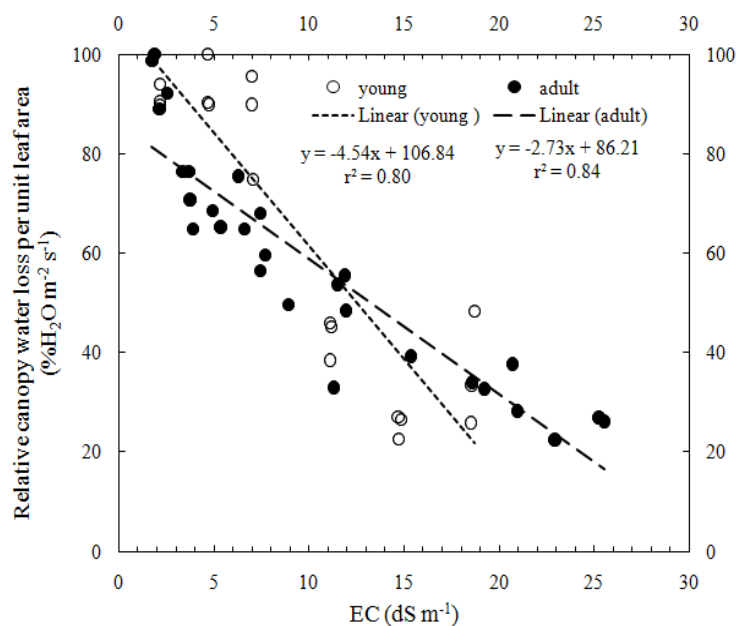
Experiment performed in a field site in Madagascar in 2010 demonstrated that water supply (1250 mm more than the rainfed regime) affected neither biomass production, not the instantaneous gas exchanges parameters, but affected the leaf light responses parameters (Table 1).

**Table 1:** Comparison of leaf light response parameters: light-saturated CO<sub>2</sub> assimilation rate ( $A_{\max}$ ), light compensation point ( $I_C$ ), quantum yield (QY), dark respiration ( $R_d$ ) of jatropha leaves in field experiment on effect of pruning on biomass formation of 4-year old jatropha. Trees were subjected to two different types of pruning, water supply, leaf age, and time of measurement. Average $\pm$ SE. Data presented are pooled over pruning type, leaf age and time of measurements. Differences between water supply are indicated by different letters. Significance of water supply effects are listed at the bottom (For details, see Table 4 in Appendix III)

		$A_{\max}$	$I_C$	QY	$R_d$
Water	Rainfed	10.83 $\pm$ 4.29 b	19 $\pm$ 11 b	0.028 $\pm$ 0.001 b	2.43 $\pm$ 1.69 a
	Irrigated	13.09 $\pm$ 4.39 a	23 $\pm$ 11 a	0.033 $\pm$ 0.001 a	1.27 $\pm$ 1.01 b
Pr>F		0.0001	0.0426	<0.0001	<0.0001

### 5.2.2. Rapid physiological response of young and adult jatropha to salt stress

In our study, salinity induced a decrease of daily transpiration rate of canopies and a reduction of canopy conductance of 3-year old and young plants was observed for concentrations higher than 50 mmol NaCl L<sup>-1</sup> external salt application. The threshold value of the response is in the range of 0 to 5 dS m<sup>-1</sup>. Transpiration of plants in both experiments was reduced to 55% at EC values between 11 and 12 dS m<sup>-1</sup> (Fig. 4) as compared to non-stressed plants.



**Figure 4:** Linear regression between EC of irrigation water and relative canopy water loss (RWL) per unit leaf area of 3-year old (closed circle) and young (open circle) *Jatropha curcas* L. RWL was calculated relative to that of the plant with highest canopy transpiration rate. (Details, see Fig. 6 in Appendix I).

### 5.2.3. CO<sub>2</sub> assimilation rate (A) and stomatal conductance (g<sub>s</sub>) responses of jatropha to nitrogen supply.

Gas exchange measurements performed under fixed environmental conditions on single jatropha leaves indicated that A and g<sub>s</sub> were similar for all treatments supplied with 2 mM N or higher (Table 2). Only the treatment with 0 mM N had significantly lower A, g<sub>s</sub> and ratio of intercellular to external CO<sub>2</sub> concentration (ci/ca). Plants supplied with 16 mM N had the highest A and g<sub>s</sub> and a significantly higher ci/ca compared to plants supplied with 2-8 mM N.

Additionally, the increase of N supply from 0 to 4 mM N resulted in increases of leaf, stem and root dry weights (Table 3), while leaf and total dry weight of plants supplied with 8 and 16 mM N tended to be lower than that of plants supplied with 4 mM N. Leaf area, specific leaf area (SLA) and chlorophyll index (SPAD) values of plants supplied with 0 mM N were lower compared to that of plants supplied with 2 mM N and higher.

**Table 2:** Effects of N supply (mM) on CO<sub>2</sub> assimilation rates (A,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), the ratio of intercellular to external CO<sub>2</sub> concentration (ci/ca), and chlorophyll index values (SPAD) of jatropha leaves measured in Exp. 1. Plants were supplied with 5 different N levels, average  $\pm$  SE. Letters indicate significant differences between N levels. Significance of N effects and least significant differences (LSD) are listed at the bottom; n.s.: not significant. (Details see Table 1 in Appendix II)

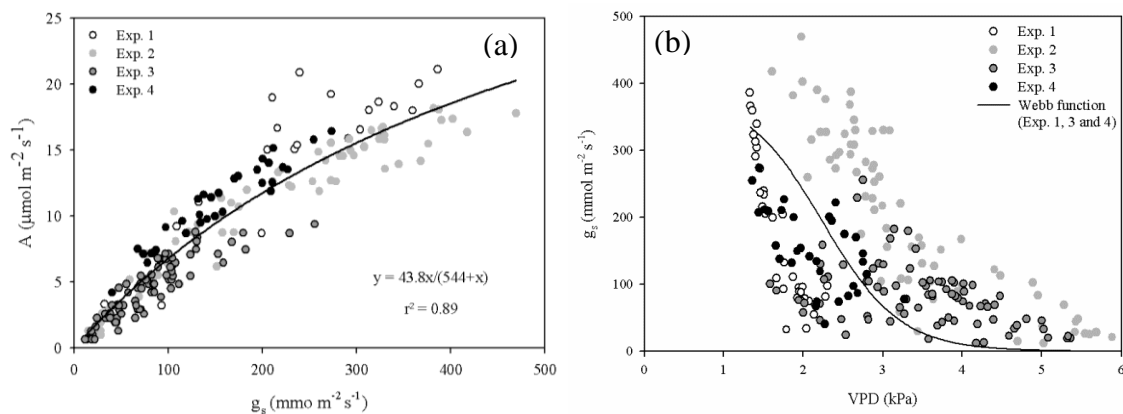
N level	A	$g_s$	ci/ca	SPAD
0	6.12 $\pm$ 1.65 b	108 $\pm$ 34 b	0.61 $\pm$ 0.03 c	26.75 $\pm$ 1.11 c
2	18.77 $\pm$ 0.92 a	288 $\pm$ 44 a	0.70 $\pm$ 0.03 b	43.50 $\pm$ 0.87 b
4	17.77 $\pm$ 1.57 a	261 $\pm$ 36 a	0.70 $\pm$ 0.03 b	46.25 $\pm$ 0.75 ab
8	15.69 $\pm$ 1.70 a	250 $\pm$ 40 a	0.72 $\pm$ 0.03 b	49.25 $\pm$ 1.11 a
16	17.52 $\pm$ 0.72 a	308 $\pm$ 26 a	0.75 $\pm$ 0.02 a	45.75 $\pm$ 1.49 b
P>F	0.0002	0.0015	<0.0001	<0.0001
LSD	4.20	81.91	0.03	3.47

**Table 3:** Effects of N supply (mM) on leaf, stem, root, total biomass (DW, g), leaf area (LA, m<sup>2</sup>), and specific leaf area (SLA, m<sup>2</sup> kg<sup>-1</sup>) of jatropha leaves measured in Exp. 1. Plants were supplied with 5 different N levels, average  $\pm$  SE. Significant differences between N levels are indicated by different letters. Significance of N effects and least significant differences (LSD) are listed at the bottom; n.s.: not significant. (Details, see Table 2 in Appendix II)

N	Leaf DW	Stem DW	Root DW	Total DW	LA	SLA
0	1.6 $\pm$ 0.1 d	3.9 $\pm$ 0.5 b	1.0 $\pm$ 0.1 b	6.5 $\pm$ 0.5 c	0.03 $\pm$ 0.00 b	18.8 $\pm$ 0.5 a
2	15.1 $\pm$ 0.5 c	17.7 $\pm$ 1.9 ab	3.9 $\pm$ 1.4 ab	36.7 $\pm$ 3.0 b	0.31 $\pm$ 0.02 a	21.0 $\pm$ 1.6 a
4	24.0 $\pm$ 2.3 a	30.9 $\pm$ 8.2 a	6.7 $\pm$ 1.2 a	61.7 $\pm$ 11.4 a	0.42 $\pm$ 0.08 a	17.5 $\pm$ 3.1 a
8	16.7 $\pm$ 2.1 bc	30.6 $\pm$ 10.7 a	7.3 $\pm$ 2.3 a	42.4 $\pm$ 7.5 ab	0.43 $\pm$ 0.10 a	19.5 $\pm$ 1.8 a
16	21.1 $\pm$ 2.8 ab	25.0 $\pm$ 4.7 a	6.1 $\pm$ 1.2 a	52.2 $\pm$ 8.6 ab	0.44 $\pm$ 0.06 a	20.8 $\pm$ 0.3 a
P>F	<0.0001	0.0183	0.0172	0.0022	0.0037	n.s.
LSD	3.75	16.49	3.67	3.75	0.18	5.81

#### 5.2.4. $CO_2$ assimilation rate ( $A$ ) and stomatal conductance ( $g_s$ ) responses of jatropha to vapour pressure deficit (VPD)

Measurements of instantaneous rates of leaf gas exchange of different leaves subject to variable VPD in this study illustrated that  $A$  and  $g_s$  were correlated in a hyperbolic fashion (Fig. 5a), and that  $g_s$  declined with increasing VPD (Fig. 5b). However, the response of  $g_s$  to VPD differed between the experiments.



**Figure 5:** Correlation between stomatal conductance ( $g_s$ ,  $mmol\ m^{-2}\ s^{-1}$ ) and  $CO_2$  assimilation rate ( $A$ ,  $\mu mol\ m^{-2}\ s^{-1}$ ) (a), and correlation between of atmospheric vapour pressure deficit (VPD, kPa) and  $g_s$  (b) of jatropha leaves. Regression curve between VPD and  $g_s$  of measurements at Exp. 1, 3 and 4 is given following an inverse logistic function (webb, 1990). Measurements were performed during 4 different experiments in the greenhouse (Exp. 1 and 2) and at a field site in Fenoarivo, Madagascar (Exp. 3 and 4).  $n=217$ . (Details see Fig. 4 in Appendix II).

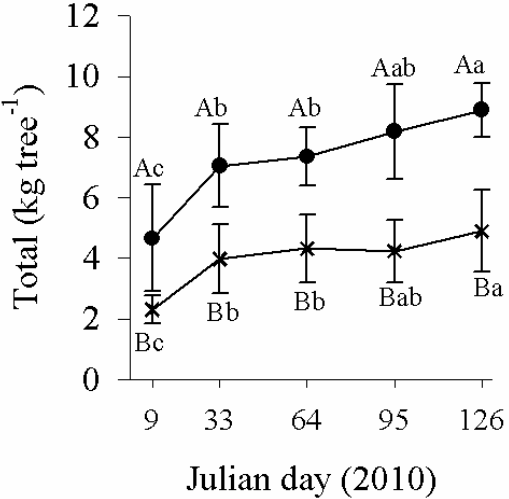
#### 5.2.5. Pruning affects aboveground biomass production

We report here no effects of pruning on leaf gas exchanges but pronounced effects of pruning on biomass formation of adult field grown jatropha that have not been reported in any publications (to the best of our knowledge) and are putatively relevant for modelling of jatropha growth dynamics. This aspects concerns mainly the correlation between branch length and newly formed twigs and leaves, the comparison of leaf area projection (LAP,  $m^2$  of leaves per  $m^2$  of projected ground area) and leaf area density (LAD,  $m^2$  of leaves per  $m^3$  of canopy volume) in the field and formation of twigs per unit length of branches.

Trees that were subjected to one pruning type, that allowed short trunk and long total primary branches (Pruning type B), had a higher total biomass towards the end of the growing period and

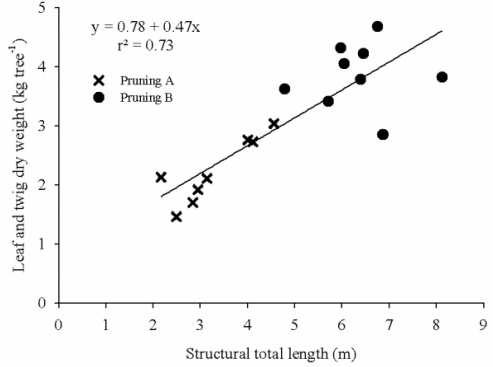


a higher biomass increase over the experimental period, compared to tree pruned at higher trunk and shorter total length of primary branches (Pruning type A) (Fig. 6).



**Figure 6:** Total dry weight of *Jatropha curcas* L. trees under pruning type A (✕) and B (●) at 5 harvest dates. Data are pooled over water treatments. Capital and small letters indicate differences between pruning type and harvest dates, respectively (Pr > F: 0.001) (Details see Fig. 3 in Appendix III).

In an attempt to predict total aboveground biomass from stem diameter using classical allometric relationships, no correlation was found between the two parameters. In this study, the total length of branches appeared as a determinant parameter for biomass increase (Fig 7).



**Figure 7:** Regression between structural total length (sum of branch lengths) and leaf and twig dry weight of *Jatropha curcas* L. at the last harvest date under the two different pruning types (Details see Fig. 6 in Appendix III).

Additionally, leaf area index (LAI) and LAP are key parameters in production ecology and are used for predicting primary production (Xiao et al., 2006). LAP increased over the first 4 harvest dates and decreased towards the end of the growing season. Due to the central function of LAI, and therefore of LAP considering a low density planting, in terms of light interception, the higher LAP of jatropha at pruning type B (5.40 versus 4.07) formed the basis of higher biomass production.

Additionally to LAP the three-dimensional distribution of leaf area is of relevance in canopy analyses of bushes and trees which are not forming a closed canopy. Canopy structure (spatial distribution and individual canopy components) and consequently the LAD can be an indicator of plant growth and productivity (Broadhead et al., 2003) and could be used to define the quantity and pattern of radiation interception. The similarity of LAD values under both pruning types as opposed to the differences in LAP indicate that the larger leaf area under pruning B was proportionally invested in exploiting a larger crown value at the same time. So far, LAD appears as a parameter which, in this case, is not explaining pruning differences in biomass increase.

### 5.3. Discussion

The use of biofuel was presented as an option to face increasing world energy demand and in view of reducing CO<sub>2</sub> release in the atmosphere (IEA, 2010). *Jatropha curcas* L. Has been proposed among many other bioenergy plants because of its ability to grow on marginal and degraded land that is not used for food production (Heller, 1996). Additionally, jatropha is adapted to arid and semi-arid locations and can be grown profitably on sites with 300-1000 mm of water availability (Dehgan and Schutzman, 1994). Jatropha has been identified to adapt to stressed environmental conditions (drought, heat, and low nutrient availability). To what extent jatropha is profitable under those conditions is still debatable.

#### 5.3.1. *Jatropha can adapt to soil drought*

Water stress is the first main factor encountered in marginal areas. It was obvious from description of occurrence of jatropha that it adapted to drought stress. Nevertheless, profitability of jatropha cultivation in these conditions needs to be corroborated.

In South-Africa, despite jatropha being claimed to be drought tolerant, rainfall (less than 800 mm per annum) has been assessed to be determinant for survival and productivity (Holl et al., 2007).

Additionally, a study at a small scale farm jatropha production in India highlighted that jatropha under irrigation perform better than under rainfed conditions but is still not viable for poor farmers (Ariza-Montobbio and Lele, 2010). In Tanzania, poor farmers grow jatropha on marginal land without irrigation and without or with moderate nitrogen supply. It results in low yield ( $2000 \text{ kg ha}^{-1} \text{ year}^{-1}$ ) despite the huge investment in labour work and irrigation. Consequently, those farms run into negative net present value (US \$ -65 per hectare) of the five years investment (Wahl et al., 2009). In our study performed in semi-arid area in South-Western part of Madagascar in 2010, showed that jatropha grown well under 500 mm of rainfall and the simulation of 1250 mm water more provided by rain did not improve neither biomass formation nor instantaneous gas exchange parameters but leaf light responses parameters. As explained later in this study, trees presented similar leaf area density that will exclude light interception issues to explain the higher leaf light responses parameters of irrigated plants compared to rainfed plants. Unfortunately, we were also not able to provide seed yield data to reinforce the fact that jatropha can grow on marginal area and can or not be profitable without irrigation.

### 5.3.2. Responses of jatropha to salt stress

In areas where droughts prevail, high salt content of soils often is a major stressor. In contrast to the projected extension of cultivation, jatropha, although able to survive extended drought spells and to grow during dry seasons, is not particularly efficient in water use and seedlings were classified as only moderately tolerant to salinity (Li, 2002). Furthermore, seed yield and growth of jatropha have been shown to be strongly reduced by salt stress (Silva et al., 2010). Our work describes physiological responses of young and 3-year old *Jatropha curcas* L. to salinity. These responses, particularly in regard to water use, water vapour conductance,  $\text{CO}_2$  assimilation rate, leaf growth, and sodium and potassium concentration in leaves, decide to a large extent on the potential productivity of jatropha. This study illustrate that jatropha could still grow under saline substrate. Nevertheless, 3-year old and young jatropha responded rapidly to salt stress by reducing daily transpiration rate of canopies and canopy conductance for external NaCl concentration of 50 mmol and higher, and a threshold value range of this parameters at  $0\text{-}5 \text{ dS m}^{-1}$ . In a survey across several countries, Prescod (1992) reported EC values of wastewater suited for irrigation between 1.02 and 3.1  $\text{dS m}^{-1}$  following the FAO recommendation of using only slightly to moderately saline wastewater with EC values between 0.7 and 3.0  $\text{dS m}^{-1}$ . Therefore, the rapid responses of jatropha reported in this study indicate that jatropha is sensitive to external

salt application implying that pre-existing salinity issues and/or the use of wastewater should be re-considered if high productivity of plantations is of central economic interest.

### *5.3.3. Responses of jatropha to nitrogen supply*

Even though jatropha can adapt to drought and reconsideration of salinity issue is solved, immediate main abiotic drivers of carbon assimilation are nutrient availability (Evans, 1983), air humidity, and temperature (Sage and Kubien, 2007). As such, N supply is expected to have a major effect on leaf gas exchange, as the built-up of the photosynthetic machinery greatly relies on N availability (Evans, 1983).

Contrarily to the work of Young et al. (2010), this study illustrated that N supply effects on leaf gas exchange of jatropha leaves were small with only the treatment 0 mM N resulting in lower rates of CO<sub>2</sub> assimilation rate and maximum CO<sub>2</sub> assimilation rate at ( $A_{max}$ ), although effects of N supply on biomass formation were pronounced. As indicated by the increase of leaf area with increasing N supply, jatropha responded rapidly to N deficiency with a decrease in leaf expansion, while little variation in SPAD values from 2 to 16 mM N supply indicates that enzyme densities were not affected by N supply once a threshold value of N supply was ensured. This response is typical for a variety of dicotyledonous plant species which regulate leaf expansion in a way to keep enzyme densities fairly constant over a range of external N supply (Vos and Van Der Putten, 1998). Our findings indicate that the SPAD value cannot be used for N management of plantations, as it would only allow detecting severe N deficiency. Additionally, biomass production is affected by amount of nitrogen supply.

### *5.3.4. Gas exchanges parameter responses of jatropha to atmospheric vapour pressure deficit (VPD)*

Given the fact that jatropha can be grown under soil drought, and the results that jatropha is sensitive to salt stress and nitrogen deficiency, environmental factors such as temperature and/or humidity may limit growth and cause harvest failure.

Our study showed that stomatal conductance ( $g_s$ ) and CO<sub>2</sub> assimilation rate ( $A$ ) are correlated in hyperbolic function. This hyperbolic response function can be expected from the increasing substrate saturation of Rubisco with increasing stomatal opening (Jones, 1992), while other

experimental work indicated a strictly linear relationship between  $g_s$  and A (Grantz, 1990). Our findings are similar to that of Tezara et al. (1998) on *Jatropha gossypifolia*, whereas Young et al. (2010) reported a linear relationship between A and  $g_s$  for 3 varieties of *Jatropha curcas* L. Those response pattern between  $g_s$  and A have implications for the instantaneous water use efficiency which is expected to decrease with increasing  $g_s$  (Jones, 1992; Hetherington and Woodward, 2003; Damour et al., 2010).

Additionally, other studies reported a consistent decline of  $g_s$  with increasing VPD in a linear (Aphalo and Jarvis, 1991) or invert logistic function (Webb, 1990) both allow for the calculation of a maximal stomatal conductance. However, the results presented in this study indicated that responses of  $g_s$  to VPD differed between the experiments with data of greenhouse experiment on leaf age effect on gas exchange parameters deviating from the general trend obvious in the other experiments indicating that other factors such as signal responses, leaf age, or diurnal dynamics may have had an influence on stomatal conductance independent of VPD. Effects of VPD, via reduced  $g_s$ , preventing high transpiration rates, have been demonstrated to be decisive on water use efficiency (Sinclair et al., 1983; Chaves and Oliveira, 2004, Lawlor and Tezara, 2009), particularly in this study. The outcome further indicates favourable conditions at which stomatal opening is high and thereby allowing for biomass formation. This information should be considered in approaches which aim at quantifying leaf activity of field-grown bushes which are characterized by spatially high diverse conditions in terms of microclimatic parameters.

#### *5.3.5. Pruning types affects biomass production.*

Microclimatic parameters are (light, temperature, air humidity) are strongly influences by the tree structure. Light interception and variation of temperature and air humidity depend on the canopy density. In a plantation, the establishment of tree structure and biomass production before flowering and fruit setting are mainly depend on management and environmental conditions. Since, plants were grown in a semi-arid area, in a typical low input system with limited nitrogen and phosphate availability, we assume that environmental conditions and resource availability of the greenhouse experiments are more or less similar.

Trees are pruned to optimize the tree architecture by maximizing the number of lateral branches for a given planting density (Behera et al., 2009) and for jatropha, pruning the trunk at 30-45 cm height has been reported optimal for biomass production (Behera et al., 2009). Our results reported that biomass production was higher under pruning of trees that allows shorter trunk and

longer total length of primary branches. Additionally, in an attempt to predict total aboveground biomass from stem diameter using classical allometric relationships (Ghezehei et al., 2009), no correlation was found between the two parameters. In this study, the total length of branches appeared as a determinant parameter for biomass increase (Fig 6)

Manipulating the tree structure by pruning allows predicting biomass formation and our results can be relevant tool for modelling purpose since we found a more or less constant ratio between length of newly formed twigs and branches such that productivity in terms of twig formation is positively related to the length of already existing branches.

#### 5.4. Conclusions

Based on experimental results,

- Results reported in this study are complementary information for both plantation management and growth model of jatropha. It has been demonstrated here that jatropha can adapt to drought stress and is there for suited for soil where food production is not possible anymore.
- Additionally, although the reduction of canopy transpiration did not reduce Na uptake, it allowed Na deposition in the stem tissue thus protecting photosynthetically active leaf tissues from excessive Na concentrations. Nevertheless, jatropha should be considered as salt-sensitive with regard to immediate physiological responses, such as canopy transpiration and conductance, leaf elongation rate, and consequently canopy development, implying that the use of wastewater should be re-considered if high productivity of plantations is of central economic interest.
- This study illustrated that N supply effects on leaf gas exchange of jatropha leaves were small with only the treatment 0 mM N resulting in lower rates of CO<sub>2</sub> assimilation and maximum CO<sub>2</sub> assimilation compared to 2-16 mM N supply, although effects of N supply on biomass formation were pronounced. As indicated by the increase of leaf area with increasing N supply, jatropha responded rapidly in terms of leaf expansion. Biomass, and yield production of jatropha depend on nutrient availability.

- Measurements of instantaneous rates of leaf gas exchange of different leaves subject to variable VPD in this study illustrated that  $A$  and  $g_s$  were correlated in a hyperbolic fashion and that  $g_s$  declined with increasing VPD. However, the response of  $g_s$  to VPD differed between the four experiments. The outcome further indicates favourable conditions at which stomatal opening is high and thereby allowing for biomass formation. This information should be considered in approaches aiming at quantifying leaf activity of field-grown bushes which are characterized by spatially highly diverse conditions in terms of microclimatic parameters.
- The reported field experiment on 4-year old jatropha indicates that the biomass production and canopy size depended mainly on primary branch length but not on water supply. Pruning of the jatropha trees at a lower height of trunk and allowing for longer primary branches resulted in a higher productivity in terms of total aboveground biomass. Classical allometric relationship between average diameter of primary branches and total aboveground biomass was not consistent. Instead, a better correlation was found using the total length of already established branches to predict leaves and newly formed twigs dry weight. Leaf area density and relative dry mass allocation to leaves were not affected by the two pruning techniques, indicating that pruning differences in leaf area size were proportionally converted to corresponding pruning differences in the canopy volume exploited by plants.

*Jatropha curcas* L. was introduced as potential bioenergy source and a plant that is able to grow on marginal land and adapt to a given environmental condition. Our studies demonstrate that jatropha can grow on marginal lands predominated with soil drought, but biomass productivity is reduced by salt stress, nitrogen deficiency, and external vapour pressure deficit by affecting leaf gas exchange parameters. Initiation of large scale plantation targeting at profitable yield requires proper management accounting for tree structure and fertilizer application since these management processes decide to a large extent on the potential productivity of jatropha, particularly when grown under marginal conditions. Additionally, the results presented in this work are relevant for research-oriented activity, especially for crop growth modeling.

## 5.5. Perspectives

Biomass formation and plant development at various plant-environment systems has been studied in quantitative and qualitative way. In contrast of the interests given to rice (Kropff, 1994) or maize (Yang, 2004) for example, for their food values, modelling of jatropha growth has not been established so far. Nevertheless, published studies already introduced yield assessment of jatropha depending on specific agro-climatic and physiological conditions (Abou Kheira and Atta, 2009; Trabucco et al., 2010; Young et al., 2010) that accounts for subsequent step to fully understand the plant's behavior under specific conditions.

**If high biomass production under manageable optimal conditions would be a prerequisite for high yield potential in jatropha plants, similar to soybeans (Salvagiotti et al., 2008) or to oil palm tree (Van Kraalingen et al., 1989) to refer to biofuel crop, there is no choice but improving breeding strategies and developing models from the data presented here and other data in further research oriented in plant growth in terms of morphological and physiological features.**

A simplified relational flow diagram of physiological responses of jatropha as affected by abiotic environmental factors and allocation of assimilates to different parts of the plant can already be summarized as shown in Fig. 8.

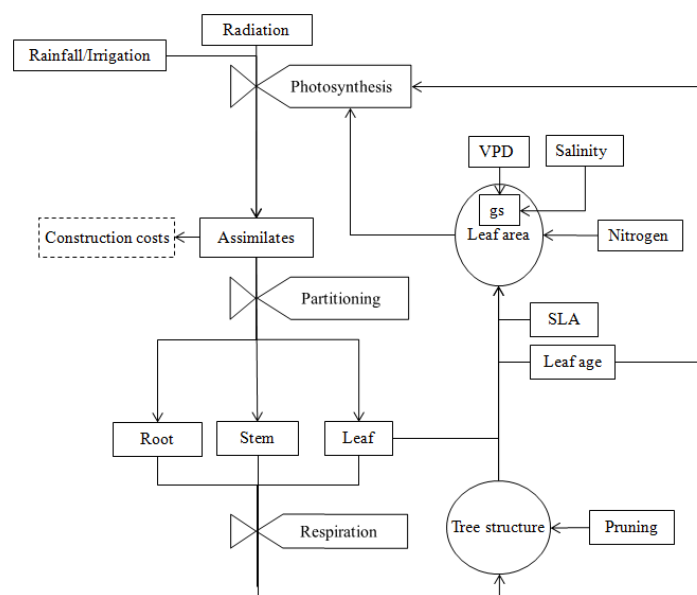


Figure 8: Biomass production and partitioning diagram affected by plant internal status and environmental factors. ( $g_s$  = stomatal conductance; SLA = specific leaf area; VPD = vapour pressure deficit)



Therefore, aiming at high profitability of jatropha plantation and providing efficient management tool for producers and accurate prediction process for policy makers, further relevant information need to be discussed. Within the framework of plant physiology, further studies should focus on the following aspects:

- How to optimize light interception, growth and profitability considering branch length and number, canopy volume and shape?
- Which factors influence leaf appearance, flowering, and seed production?
- Does the allometric relationship established in the present study hold true for other plantation sites?
- Do other jatropha eco-types result in constant leaf area density when leaf area projection vary?

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## Appendix I

Effect of salinity on canopy water vapour conductance of young and 3-year old

*Jatropha curcas* L.

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2011

Under review of Journal of Arid Environment since July 23, 2011

## Abstract

With increasing demand for biofuels, *Jatropha curcas* L. is considered as one of the biofuel crops, which is suitable for growth under adverse conditions such as salinity. We report in this study to what extent salt stress affected water use, canopy water vapor conductance, leaf growth and Na and K concentrations of leaves of 3-year old and young plants. 3-year old plants (grown on peat substrate) were exposed to seven salt levels (0-300 mmol NaCl L<sup>-1</sup>) during 20 days and young plants (in a nutrient solution experiment) to five levels (0-200 mmol NaCl L<sup>-1</sup>) during 6 days.

In both experiments, plants responded rapidly to salt stress by reducing water loss. The threshold value of responses was between 0 and 5 dS m<sup>-1</sup>. Leaf area increment of young *Jatropha* had a threshold value of 5 dS m<sup>-1</sup> implying that *Jatropha* is sensitive to external salt application in term of canopy development, conductance and CO<sub>2</sub> assimilation rate. The rapid decrease of canopy conductance indicates that *Jatropha* is effectively protecting leaves from massive Na import into leaves over short time periods. Our data indicate that use of wastewater should be carefully considered if high productivity is the key of plantation management.

Keywords: biofuel, conductivity, Physic nut, salt stress, transpiration, water use.

## Introduction

Salinity affects about 6% of the world's land (Munns and Tester, 2008) and salt stress as one of the major abiotic stresses can, particularly in arid and semi-arid regions, severely limit plant growth and yield (Mc William, 1986). *Jatropha curcas* L. is increasingly grown as biofuel and has been claimed to be drought tolerant and suitable for cultivation on marginal land (Openshaw et al., 2000; Achten et al., 2008). This makes *Jatropha* an obvious choice for biofuel production as it potentially does not compete with food production for land and water, which constitutes a major threat to food security particularly in rural areas (Asch and Huelsebusch, 2009). Globally, the area of *Jatropha* cultivation was estimated to have reached 900.000 ha in 2008 and projected 12.8 Mio. hectares by 2015 (PTI, 2010). Given the fact that currently *Jatropha* is often seed-propagated from local and not well defined genetic material and assuming that breeding will improve plant traits in the near future, *Jatropha* may become a crop well-tailored to stressful environments with low water availability and salt stress (Brittaine and Lutaladio, 2010). Furthermore, waste water will be increasingly used for irrigation (UNESCO, 2009; Qadir et al. 2007) resulting in exposure of plants to external salt load from these grey water sources.

In contrast to the projected extension of cultivation, *Jatropha*, although able to survive extended drought spells and grow during dry seasons, is not particularly efficient in water use and seedlings were classified as only moderately tolerant to salinity (Li, 2002). Furthermore, seed yield and growth of *Jatropha* have been shown to be strongly reduced by salt stress (Silva et al., 2010).

Salinity effects on plants can be observed on different scales, ranging from the whole-plant, down to physiological traits, biochemical processes, and molecular expression pattern (Parida and Das, 2005). Studies on *Jatropha curcas* L. considering whole-plant aspects in response to salt stress so far focused on biomass reduction and changes in allocation patterns, e.g., increased allocation to root at the expense of leaf growth (Silva et al., 2009). Effects of salt stress on primary processes of photosynthesis and transpiration have been investigated on the single leaf level by assessing stomatal responses, photosynthesis rates (Silva et al., 2011) and the damage to the photosynthetic apparatus as indicated by chlorophyll fluorescence (Silva et al., 2009).

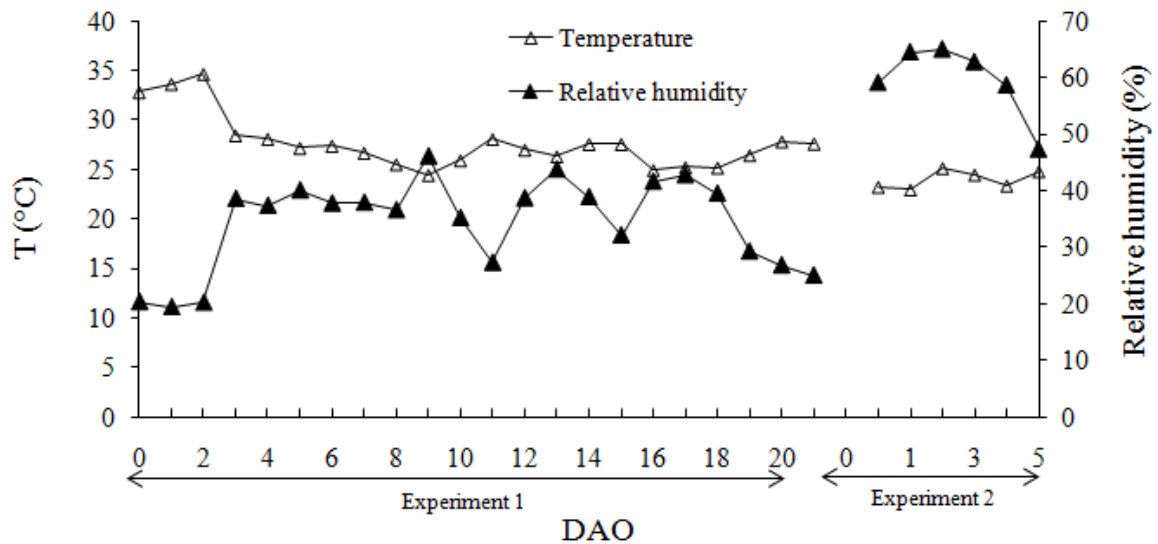
However, there is no information as to how these single leaf responses translate into effects on the entire organism; in particular, the effects of salt stress on canopy transpiration and conductance have not yet been investigated in *Jatropha*. Here we report on the responses of entire canopies to salt stress in young plants and 3-year old *Jatropha* trees. Whereas the study on young plants covered a relatively short time (6 days) of exposure to salinity, 3-year old *Jatropha* was

exposed to salinity conditions for 22 days in order to take into account time scale and plant size effects on the responses of plants exposed to salinity (Munns, 2002). This approach allows strengthening model-based approaches aiming at assessing potential physiological responses of *Jatropha* to salinity, which this plant is expected to be exposed to when cultivated on marginal land in order to avoid competition with food production.

## **Materials and methods**

### *Plant materials:*

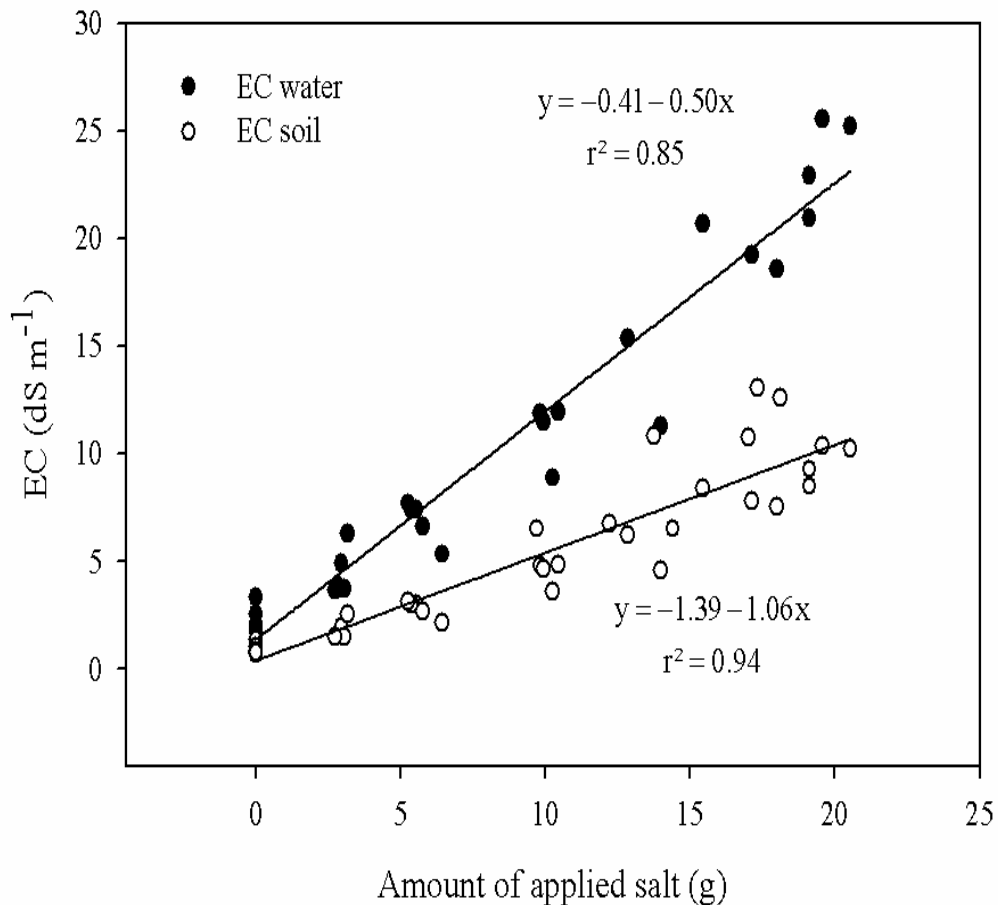
For the first experiment (Exp. 1), 3-year-old *Jatropha* plants were grown in a greenhouse of the University of Hohenheim, Germany, from April 27 to May 19 2010. Average temperature and relative humidity (RH) in the greenhouse were recorded with Tinytag data loggers (Type TGP-4500, Gemini data loggers, Chichester, UK). Average air temperature in the greenhouse was 25.5 °C and RH 38% with considerable variation over the experimental period (Fig. 1). Vapor pressure deficit (VPD) varied between 1.8-3.1 kPa. 35 plants were selected for the experiment and grouped in 5 blocks according to initial height (minimum and maximum initial heights were 40 and 69 cm, respectively). They were grown in pots of 18 cm height and 16 cm diameter containing commercially available, fertilized peat substrate. Fertilizer was applied weekly as 0.2% (vol/vol) universal fertilizer (Wuxal<sup>®</sup>, MANNA, Ammerbuch-Pfäffigen, Germany). Plants were subjected to seven different salt treatments of 0, 30, 60, 120, 180, 240 and 300 mmol NaCl L<sup>-1</sup> applied as irrigation water on April 28, May 2 and May 8. The daily water consumption varied between plants and application days, thus, in order to supply the same amount of salt to all plants within a treatment, salt solution was additionally supplied at April 29 and May 9 to those pots that did not receive the full amount of salt the day before. Electrical conductivity of soil solution and irrigation water (EC) were measured with an EC-meter (Cole-Parmer Instr., Chicago, Illinois), varied from 0.94 to 9.59 and 1.26 to 25.97 dS m<sup>-1</sup>, respectively, and was linearly correlated to the total amount of salt applied (Fig. 2). Prior to the onset of treatments the water holding capacity (WHC) of each pot was determined. During the experiment, daily water consumption was measured at 9:00 a.m. by recording the weight of pots and tap water was supplied to 90% of WHC.



**Figure 1:** Average temperature and relative humidity in the greenhouse during the two experiments. DAO, Days after onset of treatment.

For the second experiment (Exp. 2) *Jatropha* seeds were germinated in sand on November 26 2010 in a growth chamber at 20°C air temperature. On December 10, when seedlings were in 1<sup>st</sup>-leaf stage, plants were transferred to the greenhouse and fixed on styropor plates floating on quarter-strength Hoagland nutrient solution (Hoagland and Arnon, 1950) of 23°C and pH of 6.0. Nutrient solution was changed every other day. On December 18, seedlings (in the 2<sup>nd</sup>-leaf stage) were transferred into pots (3 seedlings per 5 L pot) containing half-strength nutrient solution. Full-strength nutrient solution was supplied from December 24. Nutrient solutions were changed every 4 days and plants were thinned to one plant per pot on January 4, 2011. Salt treatments were established on January 7 for 6 days by supplying nutrient solution of 0, 25, 50, 75, 100, 150 and 200 mmol NaCl L<sup>-1</sup>. EC of the nutrient solution varied from 2.15 -18.58 dS m<sup>-1</sup> and was linearly correlated with the amount of salt applied (Fig. 2). Transpirational water loss was recorded daily at 9:00 a.m by weighing the individual pot. The nutrient solution was renewed at January 10. Mean air temperatures and RH were 22°C and 50%, respectively, and VPD varied from 1.1-1.8 kPa.

In both experiments, a maximum photosynthetic photon flux density (PPFD) of approximately 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was supplied for 14h (Exp.1) and 12h (Exp. 2) with lamp type: PL SON-K-400 (DHLicht GmbH, Wülfrath, Germany).



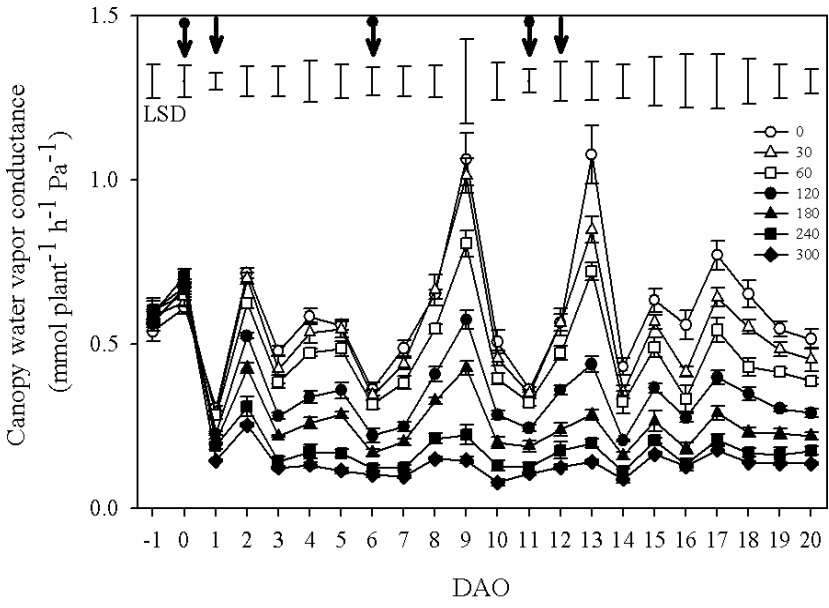
**Figure 2:** EC values of nutrient solution and soil solution as a function of the total amount of applied salt (g NaCl).

*Measurements:*

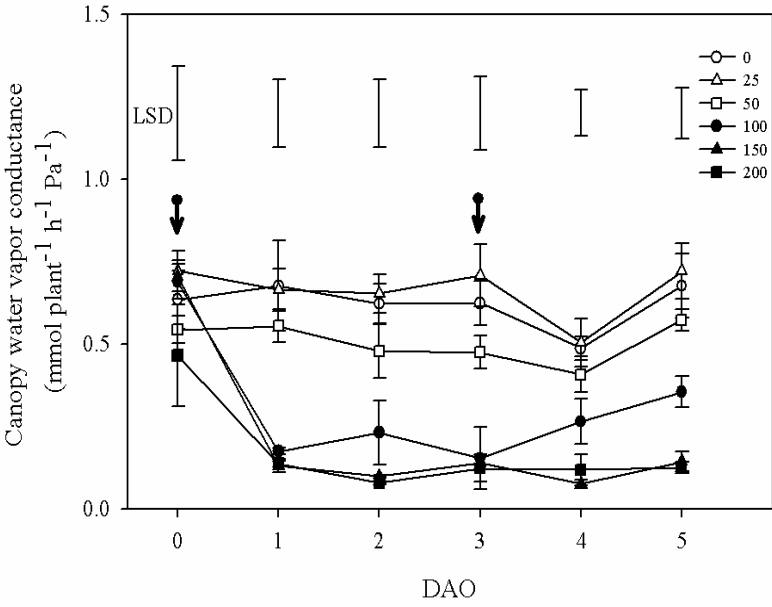
### Leaf and canopy water vapor conductance and transpiration

Daily canopy water vapor conductance per plant (Fig. 3, 4) was calculated from daily water loss corrected for the daily mean VPD. Additionally, average daily water loss during the last three days before harvest and the final leaf area of plants were used for calculating canopy transpiration rates per unit leaf area. These data were complemented by measurements of single leaf gas exchange. Leaves ~ 20 cm below the shoot apex of the 3-year old *Jatropha* and the 2<sup>nd</sup> youngest leaf of young *Jatropha* were selected and inserted in a 4 cm<sup>2</sup> leaf chamber of the GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany) and exposed to a PPFD of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and a CO<sub>2</sub> concentration of 380 ppm. Stability of readings was reached after 15-30 minutes. Three

plants of each of the treatments 0, 60 and 120 mmol NaCl from Exp. 1 were compared at 5, 9 and 14 days after onset of treatments (DAO) and three plants of each of the treatments 0, 25 and 100 mmol NaCl from Exp. 2 were compared at 4 and 5 DAO.



**Figure 3:** Canopy water vapor conductance (average±SE) of 3-year old *Jatropha curcas* L. over 21 days. Arrows indicate time of salt application. n=5, LSD at 0.05.



**Figure 4:** Canopy water vapor conductance (average±SE) of young *Jatropha curcas* L. over 5 days. Arrows indicate time of salt application. n=3, LSD at 0.05.

### Leaf area determination

Effects of salt application on leaf area increment were quantified in Exp. 2. Digital images of single leaves were taken against a millimeter background the day before onset of treatments. The leaf area was determined using the image analyzing software Mesurim Pro (Vers. 3.3) (Académie d'Amiens, Amiens, France). Total leaf area after destructive harvesting at the end of the experiment was measured with the  $\Delta T$  area meter (Burwell, Cambridge, England).

### Determination of plant sodium and potassium concentrations

Oven-dried leaves and stems were finely ground in a ball mill (PAT COSHH Ltd, Kent, UK). 10 mL distilled water (Millipore S.A. Molsheim, France) was added to 1 g of plant material and autoclaved for 1 h at 100°C (Wolf SANOclav, Bad Überkingen-Hausen, Germany). This extract was filtered (LLG filter paper folded,  $\phi$  150 mm, Lab Logistics group GmbH, Meckenheim, Germany) into volumetric flasks and filled up to 100 ml by adding distilled water. Leaf sodium and potassium content were measured using a flame photometer (Jenway, Bibby Scientific Limited, Essex, UK). The flame photometer was calibrated with Flame photometry standards of 0, 12.5, 25, 50 and 100 ppm Na and K (Jenway, Essex, UK).

### *Experimental design*

Exp. 1 with 3-year old *Jatropha* plants was arranged in a randomized complete block designs with 5 replicates. Exp. 2 with *Jatropha* seedlings was arranged in a fully randomized design with 3 replicates. Data were analyzed with the statistical software package SAS 9.2 (SAS Institute Inc., North Caroline, USA). ANOVA of the 1-factorial experiments were performed with PROC GLM and multiple comparisons of means with the LSD-range tests at 0.05 level of confidence. Test of normality of residuals was performed with PROC UNIVARIATE.



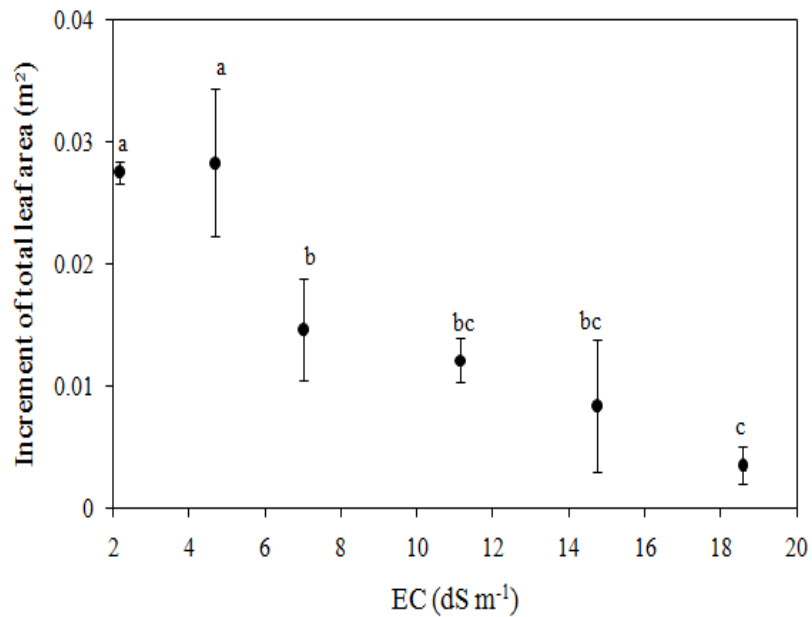
## Results

### *Canopy water vapor conductance*

The amount of water transpired by plants depends on leaf area size and transpiration rate which is modified by environmental conditions (temperature, relative humidity) which were highly variable during the experiment (Fig. 1). Values of hourly canopy water vapor conductance ( $g_{\text{Canopy}}$ ) were affected by salinity and varied from 0.08 to 1.8 mmol H<sub>2</sub>O plant<sup>-1</sup> h<sup>-1</sup> Pa<sup>-1</sup> for 3-year old *Jatropha* (Fig. 3). Plants treated with 30 mmol NaCl had similar  $g_{\text{Canopy}}$  compared to control plants and  $g_{\text{Canopy}}$  decreased with any further increase in salt load. The response to the first salt application was fast as indicated by the decrease in  $g_{\text{Canopy}}$  already at the first day after salt application. However, the second and third salt application did not induce a further decrease of  $g_{\text{Canopy}}$ . The  $g_{\text{Canopy}}$  of plants treated with 240 and 300 mmol NaCl were similar and the lowest among all treatments. In Exp. 2,  $g_{\text{Canopy}}$  of young plants ranged from 0.07 to 0.72 mmol plant<sup>-1</sup> h<sup>-1</sup> Pa<sup>-1</sup> throughout the experiment (Fig. 4). Control plants and those treated with 25 and 50 mmol NaCl had similar values of  $g_{\text{Canopy}}$ . Salt concentrations of 100 mmol NaCl and above induced decreases in  $g_{\text{Canopy}}$ , and rapid responses of plants to external salt application were again observed at the first day after salt application.

### *Leaf area increment*

The daily leaf area increment of young *Jatropha* plants in the control treatment over the 6 days experimental period was 0.028 m<sup>2</sup>, and increases in leaf area were substantially reduced when the EC of the nutrient solution was higher than 5 dS m<sup>-1</sup> (Fig. 5).



**Figure 5:** Leaf area increment (difference between final and initial total canopy size, average $\pm$ SE) of young *Jatropha curcas* L. as affected by EC value of the nutrient solution. Letters indicate significant differences between salt treatments at  $P < 0.05$ .

#### *Leaf and stem Na and K content*

Leaf Na concentration of 3-year old *Jatropha* increased with salt application from 124 to 477 mmol Na kg<sup>-1</sup> (Table 1). Contrarily, leaf Na concentration of young *Jatropha* reached a plateau value at external NaCl concentrations of 50 to 150 mmol NaCl and decreased to rather low values at the highest external salt level (Table 2). Leaf Na concentration of 3-year old *Jatropha* was substantially higher than in young plants across all salt levels. Leaf K concentration of 3-year old *Jatropha* was highest at high level of external salt concentration (300 mmol NaCl), and lowest in the control and 30 mmol NaCl treatments. Leaf K concentration in young plants was similar in all NaCl treatments. Across all salt levels, leaf K concentration was lower in 3-year old than in young *Jatropha*. A similar trend was observed for the leaf K/Na ratio of young and 3-year old *Jatropha*. It was highest for the control plants in both experiments, followed by the leaf K/Na ratio of plants in the highest salt treatments (200 and 300mmol NaCl for young and 3-year old *Jatropha*, respectively). All intermediate salt concentration resulted in similar K/Na ratios in both experiments.

**Table 1:** Sodium and Potassium contents and K/Na ratio per unit leaf dry weights (mmol kg<sup>-1</sup>) of 3-year old *Jatropha curcas* (average±SE) at different salinity levels (mmol NaCl L<sup>-1</sup>). Letters indicate differences between salt treatments at P<0.05.

NaCl mmol l <sup>-1</sup>	Na ---mmol Na kg <sup>-1</sup> DW---	K	K/Na
0	124±5 d	213±16 bc	1.74±0.17 a
30	271±7 c	194±8 c	0.72±0.04 c
60	305±14 c	228±19 bc	0.76±0.10 bc
120	339±24 bc	235±11 bc	0.71±0.07 c
180	422±37 ab	261±19 b	0.64±0.07 c
240	417±36 ab	261±7 b	0.65±0.07 c
300	477±75 a	262±31 a	1.05±0.12 b

**Table 2:** Sodium and Potassium concentrations, and K/Na ratios of leaves and stems (mmol kg<sup>-1</sup> DW) of young *Jatropha curcas* (average±SE) at different salinity levels (mmol NaCl L<sup>-1</sup>). Letters indicate differences between salt treatments at P<0.05.

NaCl mmol l <sup>-1</sup>	Leaves			Stem		
	Na ---mmol Na kg <sup>-1</sup> DW---	K	K/Na	Na ---mmol Na kg <sup>-1</sup> DW---	K	K/Na
0	10±1 c	417±79 a	46.14±0.38 a	10±2 d	405±11 ab	35.85±0.26 a
25	116±9 b	347±12 a	3.01±0.14 c	58±2 cd	453±14 ab	7.88±0.32 b
50	228±8 a	338±19 a	1.48±0.04 d	83±4 c	366±19 b	4.47±0.44 c
100	255±54 a	378±32 a	1.63±0.39 d	152±31 b	435±16 ab	3.01±0.37 d
150	220±28 a	472±82 a	2.22±0.45 cd	301±16 a	516±5 a	1.71±0.12 e
200	73±16 bc	467±8 a	7.17±0.00 b	344±33 a	489±6 ab	1.43±0.15 e

Salt stress also induced changes in stem Na and K concentrations of young *Jatropha* plants contrasting with that of leaves (Table 2). Na content increased from low to high salt application while K content was more or less constant resulting in a decrease of the K/Na ratio with increasing salt level. Whereas the K concentrations of stems were higher than that of leaves at all NaCl treatments, Na concentrations of leaves were higher than that of the stems at low external salt levels (25, 50 and 100 mmol NaCl L<sup>-1</sup>) and the opposite was observed at high salt levels (150 and 200 mmol NaCl L<sup>-1</sup>). Stem K/Na ratio remained higher than that of the leaves at all NaCl treatments, except for the control plants.

#### *Daily transpiration rates*

For 3-year old *Jatropha*, canopy transpiration decreased with increasing salt levels from 1.20 to 0.34 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> (Table 3). Contrarily, canopy transpiration of young *Jatropha* was high and unaffected by salt application for 0, 25, and 50 mmol NaCl and was reduced by more than 50% with salt levels exceeding 50 mmol NaCl . In all treatments, canopy transpiration of young *Jatropha* was higher than that of 3-year old *Jatropha*. This salt effect on canopy transpiration rates is supported by results from porometer measurements performed for 3 salt levels (Table 4). Transpiration rates decreased with increasing salt application and were numerically similar to those of canopy transpiration rates except for the control treatment in Exp. 2.

**Table 3:** Canopy transpiration ( $\text{mmol m}^{-2} \text{ leaf s}^{-1}$ ) (average $\pm$ SE) of young and 3-year old *Jatropha curcas* at different salt concentrations ( $\text{mmol NaCl}$ ). Letters indicate significant differences between salt treatments at  $P < 0.05$ .

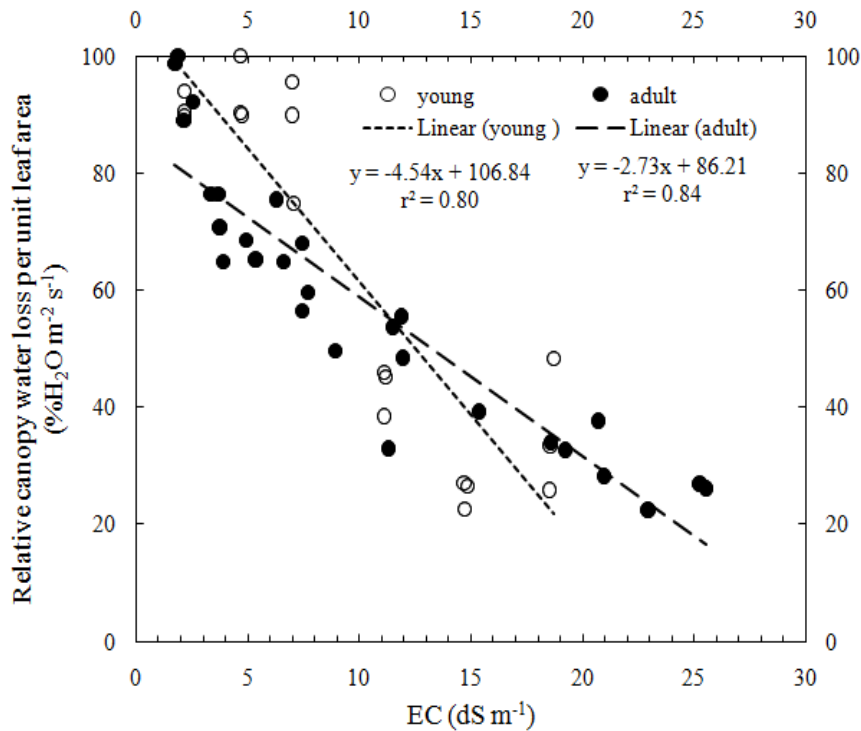
NaCl	Canopy transpiration
3-year old <i>Jatropha</i>	
0	1.20 $\pm$ 0.06 a
30	0.94 $\pm$ 0.03 b
60	0.83 $\pm$ 0.03 c
120	0.66 $\pm$ 0.03 d
180	0.49 $\pm$ 0.02 e
240	0.41 $\pm$ 0.04 ef
300	0.34 $\pm$ 0.02 f
Young <i>Jatropha</i>	
0	2.58 $\pm$ 0.04 a
25	2.63 $\pm$ 0.10 a
50	2.44 $\pm$ 0.17 a
100	1.21 $\pm$ 0.07 b
150	0.72 $\pm$ 0.04 c
200	1.01 $\pm$ 0.19 bc

**Table 4:** Porometer-based gas exchange parameter (average±SE) of 3-year old *Jatropha curcas* at 0, 60 and 120 mmol NaCl and young *Jatropha* at 0, 25 and 100 mmol NaCl: transpiration rate (E), stomatal conductance ( $g_s$ ), CO<sub>2</sub> assimilation rate (A) and ratio between atmospheric and intercellular carbon dioxide concentration ( $c_i/c_a$ ). Letters indicate significant differences between salt treatments at P<0.05.

NaCl	E	$g_s$	A	$c_i/c_a$
Mmol l <sup>-1</sup>	-----	mmol m <sup>-2</sup> s <sup>-1</sup>	-----	
3-year old <i>Jatropha</i>				
0	1.71±0.31 a	71±14 a	6.64± 1.16 a	0.57±0.02 a
60	1.29±0.31 ab	52±13 ab	5.48±1.03 ab	0.51±0.02 ab
120	0.68±0.14 b	26±6 b	3.17±0.69 b	0.47±0.03 b
Young <i>Jatropha</i>				
0	1.42±0.20 b	60±7 b	5.79±2.12 ab	0.73±0.02 ab
25	2.64±0.09 a	117±13 a	9.56±1.53 a	0.65±0.08 b
100	1.02±0.08 b	40±5 b	1.03±0.59 b	0.87±0.05 a

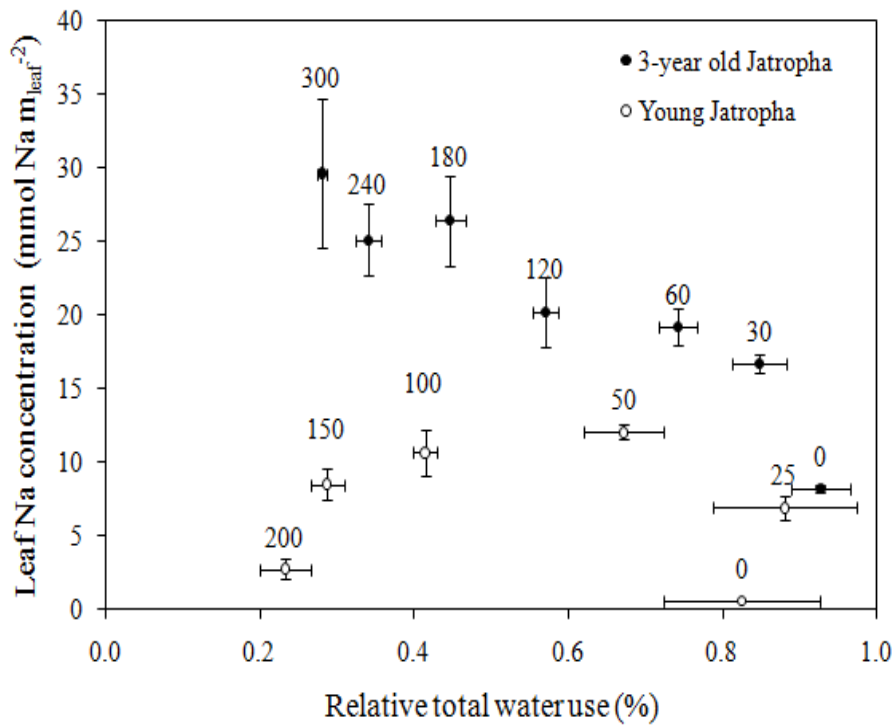
The ratio between atmospheric and intercellular CO<sub>2</sub> concentration ( $c_i/c_a$ ) of 3-year old *Jatropha* was highest for the control treatment and lowest for the 120 mmol NaCl treatment. For young *Jatropha*, salinity decreased  $c_i/c_a$  at concentrations of 25 mmol NaCl. Subjecting the plants to 100 mmol NaCl increased the  $c_i/c_a$  to values higher than that of control plants indicating a severe impairment of photosynthesis.

Following the graphical approach of crop tolerance to salinity (Kenneth and Neeltje, 2002), relative daily water loss per unit leaf area was plotted against EC values (Fig. 6). Regression analyses showed a linear relationship between the two parameters with no indication of saturation or threshold values. The slope and y-axis intercept of the regression were higher for young than for 3-year old *Jatropha* plants.



**Figure 6:** Linear regression between EC of irrigation water and relative canopy water loss (RWL) per unit leaf area of 3-year old (closed circle) and young (open circle) *Jatropha curcas* L. RWL was calculated relative to that of the plant with highest canopy transpiration rate.

In Exp. 1, increasing salt levels resulted in a consistent decrease of total water use associated with an increase of Na concentration in leaves (Fig. 7). In Exp. 2, total water consumption of plants treated with 25 mmol NaCl was higher than that of control plants and then decreased at higher NaCl levels until 100 mmol NaCl. Total water consumption at high NaCl treatments (100, 150 and 200 mmol NaCl) were lowest and of similar magnitude. Consequently, increasing the external salt application from 25 to 50 mmol NaCl was associated with a decrease of total water consumption and an increase of Na concentration in leaves. Further salt application resulted in less water consumption but a similar leaf Na concentration as in the previous treatment (50 mmol NaCl), except for plants treated with 200 mmol NaCl that had Na concentrations similar to that of plants treated with 0 and 25 mmol NaCl.



**Figure 7:** Leaf Sodium concentration (average $\pm$ SE) as a function of relative total water use per unit leaf area (average $\pm$ SE). Relative total water use was calculated relative to that of the plant with the highest total water consumption during the experiment. Numbers above each point indicate the NaCl concentration (mmol L<sup>-1</sup>) of the irrigation water and the nutrient solution.

## Discussion

The assessment of susceptibility of plants to salt stress is usually based on analyses of yield depression in response to increasing EC values in the rooting medium. This stress response is due to osmotic and ionic effects operating on different time (from immediate to long-term), plant organ (e.g., root and leaves), and spatial (tissue, cells, organelles) scales (Pasternak, 1987; Katerji et al., 2003; Parida and Das, 2005). Salt stress affects a multitude of biophysical and – chemical processes which in turn cause several physiological and morphological adaptations (Munns and Tester, 2008; Volkmar et al., 2006). Thus, supplementary to the characterization of species' and varieties' tolerance against salt stress in terms of biomass and yield formation, analyses of early response patterns to salt stress are highly relevant. Cell division and expansion and the control of the leaf water status by stomatal conductance are considered processes which rapidly respond to salt stress (Fricke et al., 2004).



In general, salt stress leads to stomatal closure and a decrease in transpiration rate in salt-sensitive plants. This response is considered an early protective measure of the plant to control the internal water status (Touchette et al., 2009). Work of Silva et al. (2010) illustrated this response in *Jatropha*, as leaf water content was not affected when plants were exposed to 50 mmol NaCl for eight days but stomatal conductance was substantially reduced. This response pattern to salt stress is usually assessed by measuring leaf gas exchange of single leaves over relatively short time intervals. In this experiment, daily transpiration rates of canopies were measured to gain insight into the whole-plant response to salt stress over a longer time period. The rapid stomatal closure after first application of salt (Fig. 3 and 4) is an immediate response to salt stress in *Jatropha*. However, subsequent salt application did not induce stomatal closure indicating that plants had adapted to stress. Comparing the response of *Jatropha* over the first 6 DAO, both 3-year old and young plants reduced their canopy conductance for external salt application higher than 50 mmol NaCl L<sup>-1</sup>. As, due to the differences in cultivation methods (nutrient solution versus compost), this irrigation water salt concentration corresponds to different EC values of the substrate (Fig. 2), the threshold value of the response is probably in the range of 0 to 5 dS m<sup>-1</sup>. Transpiration of plants in both experiments was reduced to 55% at EC values between 11 and 12 dS m<sup>-1</sup> (Fig. 6) as compared to non-stressed plants. In a survey across several countries, Prescod (1992) reported EC values of wastewater suited for irrigation between 1.02 and 3.1 dS m<sup>-1</sup> following the FAO recommendation of using only slightly to moderately saline wastewater with EC values between 0.7 and 3.0 dS m<sup>-1</sup>. Additionally to the increase of EC values due to water loss by evapotranspiration, the leaching fraction of the soil should be considered when using wastewater for irrigation. A case study of *Jatropha* cultivation using treated sewage effluent in Morocco showed that a long term supply of saline wastewater with EC value of 1.4 dS m<sup>-1</sup> into a soil with a leaching fraction of 0.05 may lead to an increase of EC value of 4.2 dS m<sup>-1</sup> and 9.7 dS m<sup>-1</sup> at 60 and 90 cm depth, respectively (Sutterer, 2010). Considering that evapotranspiration and biomass production are highly correlated (Ben-Gal et al., 2003), this finding indicates that *Jatropha* is sensitive to external salt application implying that the use of wastewater should be re-considered if high productivity of plantations is of central economic interest.

Under stress conditions, plants reduce leaf expansion rates and close stomata to minimize water loss (Munns, 2002). The threshold level for leaf expansion rate in this study was about 5 dS m<sup>-1</sup> in Exp. 2, corresponding to an external supply of 25 mmol NaCl L<sup>-1</sup> (Fig. 5). This threshold value is lower than the maximum threshold value of 40 mmol NaCl reported by Munns and Tester (2008). According to Kenneth and Neeltje (2002), moderately tolerant (wheat, barley, maize) and sensitive (rice, beans, sugar cane, apple) crops can withstand salt concentrations of irrigation

water of around 7 and 4.5 dS m<sup>-1</sup>, respectively. These findings indicate that *Jatropha* responds sensitive to salt stress in terms of leaf elongation rate and consequently canopy development.

Salt stress had an immediate and long-lasting effect on canopy transpiration rate and affected the Na concentrations in the leaf tissues. The relationship between transpirational water loss and transpiration-driven import of Na into leaf tissue is highly relevant for salinity resistance strategies in plants as high concentrations of sodium in leaf tissue impair the ionic balance and physiological functioning within the cytosol (Munns and Tester, 2008). Leaf Na concentration increased with external salt load. However, the reduction of canopy transpiration did effectively protect the leaves from excessively high Na concentrations in young *Jatropha* plants (Fig. 7), where values of > 200 mmol Na kg<sup>-1</sup> leaf dry mass were observed at external concentrations of 50 mmol NaCl L<sup>-1</sup> but leaf Na concentrations were lower at higher external salt levels (Table 2). A similar effect was observed by Patel et al. (2010) when applying salt stress up to 10 dS m<sup>-1</sup>, corresponding to 120 mmol external salt application to young *Jatropha* over 9 months. In young *Jatropha* plants, stem Na concentrations were lower than that of the leaves for treatments below 150 mmol NaCl whereas the opposite was observed for higher external salt levels, reflecting a change in Na distribution among organs with increasing salt level. As indicated by the increasingly high salt load in the stem tissue, reduction of canopy transpiration did not reduce Na uptake but allowed Na deposition in the stem tissue thus protecting photosynthetically active leaf tissues from excessive sodium concentrations. Due to the fact that 3-year old *Jatropha* was used for a regrowth study after salt application, there is no information available on the Na concentration in stem tissues of these plants. The results reported here provide a possible explanation for effects of salinity on *Jatropha* observed by Dagar et al. (2006) and Patil et al. (2010) who reported increasing Na concentrations in *Jatropha* stem tissue combined with less accumulation of Na in leaf tissues but they did not relate these observations to transpiration.

Leaf K/Na ratio is considered a reliable indicator of yield loss (Asch et al., 2000) taking into account that Na uptake may induce a reduction of internal K availability. Silva et al. (2009) reported that due to severe ionic imbalances, toxic symptoms (interveinal leaf necrosis) in young *Jatropha* only occurred if the K/Na ratio was below 0.5. In the experiments reported here K concentration of leaves was lower in the experiment with 3-year old *Jatropha* than with young *Jatropha* plants. This indicates that differences in substrate and plant age render interpretation of the importance of K/Na ratios for physiological responses such as leaf elongation rate and canopy transpiration in *Jatropha* difficult. Despite this constraint, our study and those of Silva et al. (2009, 2010) suggest that *Jatropha* should be considered as salt-sensitive with regard to immediate physiological responses. Since these processes decide to a large extent on the potential

productivity of *Jatropha*, particularly when grown under marginal conditions or when irrigated with saline waste water, some serious doubts on the suitability of *Jatropha* for such conditions need to be raised.

## Appendices

A. Canopy water vapor conductance (average $\pm$ SE) of 3-year old *Jatropha curcas* over 21 days. n=5, LSD at 0.05. NaCl effects differences at each day are indicated by different letters. DAO (Days after onset of treatment).

NaCl (mmol L <sup>-1</sup> )	DAO																					
	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0	a	c	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
30	a	bc	a	ab	ab	ab	a	a	ab	ab	ab	ab	a	a	b	ab	ab	b	ab	a	a	ab
60	a	bc	a	b	b	b	a	a	b	b	b	b	a	a	b	b	bc	b	b	b	b	b
120	a	ab	b	c	c	c	b	b	c	c	c	c	b	b	c	c	cd	bc	c	b	c	c
180	a	ab	b	d	c	ca	b	bc	c	d	c	cd	b	c	cd	cd	de	cd	cd	c	cd	cd
240	a	a	b	e	d	de	c	c	d	e	d	de	c	cd	d	d	e	cd	d	c	d	d
300	a	ab	c	e	d	e	c	c	d	f	d	e	c	d	d	d	e	d	d	c	d	d

B. Canopy water vapor conductance (average $\pm$ SE) of young *Jatropha curcas* over 5 days. n=3, LSD at 0.05. NaCl effects differences at each day are indicated by different letters.

NaCl (mmol L <sup>-1</sup> )	DAO					
	0	1	2	3	4	5
0	a	a	a	a	a	a
25	a	a	a	a	a	a
50	a	a	ab	ab	ab	a
100	a	b	bc	bc	bc	b
150	a	b	c	bc	c	c
200	a	b	c	c	c	c

## **Acknowledgement:**

This study is a part of the project 327 «Jatropha Anbau in Madagaskar». We gratefully acknowledge the funding of this work by EnBW-Regenwaldstiftung, Stiftung Energieforschung Baden-Württemberg, project No. 08A26908.

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## Appendix II

Leaf gas exchange characteristics of jatropha as affected by nitrogen and water supply, leaf age, and atmospheric vapour pressure deficit.

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2011

Under review of Journal of Agronomy and Crop Science since October 28, 2011.



## Abstract

Facing a steadily increasing world energy demand, jatropha, among other energy crops, has been reported to potentially contribute to bio-fuel production. A basic characterisation of plant responses to abiotic environmental factors is important for assessing the model-assisted potential of this plant in view of the many agro-ecological zones in which jatropha is presently cultivated. Two pot experiments and two field studies were used to record gas exchange parameters in response to light, nitrogen supply, atmospheric vapour pressure deficit (VPD), leaf age and time of measurements.

Variation of N supply from 0 to 16 mM resulted in lower rates of photosynthesis ( $A$ ) and stomatal conductance ( $g_s$ ) of treatment 0 mM N compared to other N levels, whereas the light compensation point ( $I_C$ ), quantum yield (QY), and dark respiration rates ( $R_d$ ) were similar in all treatments. In the field, diurnal effects were evident with higher  $A_{max}$  and QY and lower  $I_C$  and  $R_d$  in the morning than in the afternoon. Considering leaf age effects, fully expanded leaves had a lower  $A_{max}$  compared to expanding leaves and this variation in leaf gas exchange was not related to changes in the chlorophyll index value (SPAD) which steadily increased with leaf age. QY of field and greenhouse plants varied from 0.023 to 0.037 and was substantially lower than in C3 plants.  $A$  was positively correlated with  $g_s$  in a hyperbolic function.  $A$  varied from 0.64 to 21.13  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and  $g_s$  varied from 12 to 469  $\text{mmol m}^{-2} \text{s}^{-1}$ . With increasing VPD  $g_s$  decreased, but this response differed between the field experiments and the two pot experiments which contrasted each other distinctively. Applying the inverse logistic function of Webb (1990), the maximal stomatal conductance of jatropha was in the range of 382  $\text{mmol m}^{-2} \text{s}^{-1}$  and  $g_s$  is predicted to be close to zero at 6 kPa. These data altogether indicate that light absorption characteristics of single leaves and carbohydrate status parameters should be investigated further in order to explain the low QY and the pronounced diurnal variation.

Key words: air humidity, Biofuel,  $\text{CO}_2$  assimilation rate, *Jatropha curcas* L., light response curve, Madagascar, stomatal conductance.

## Introduction

Photosynthesis is one of the central processes of the plants' carbon balance and is highly responsive to changes in the abiotic environment. The photosynthetic performance depends on nutrient availability and on the ability of the plant to regulate and adapt to environmental factors. Responses of plants to these abiotic factors are widely used to predict growth under variable climatic conditions. Among the abiotic factors, the responses to radiation, atmospheric humidity and nitrogen availability are central, as these factors vary widely even within one day (radiation and humidity) or are key drivers of the process of carbon gain (nitrogen). Gas exchange between the leaf and the atmosphere occurs via stomata which are regulated by the plants' internal status (e.g., rubisco and electron transport capacity) which is defined by the nitrogen supply (Evans, 1983; Sage and Kubien, 2007) and by external conditions such as light (Marshall and Biscoe, 1980), and humidity (Monteith, 1995). Additionally to abiotic factors, leaf age is known to affect leaf gas exchange due to alterations of the sink-source status of leaves during ontogeny.

Taking marginal sites as one of the dominant target environments in which *Jatropha curcas* L. will be cultivated, an improved understanding of leaf photosynthesis is, therefore, required for any approach aiming at model-assisted productivity estimation. This information combined with canopy size and tree structure will help predicting biomass production. Further, the relationship between carbon assimilation and conductance is frequently used to estimate carbon gain relative to water loss and by this the plant's intrinsic water use efficiency (Field and Mooney, 1983). This intrinsic water use efficiency is affected by atmospheric humidity (Rawson et al., 1977) and nitrogen supply (Brueck, 2008).

Gas exchange of jatropha has rarely been investigated and effects of fertilizer application and leaf age (Young et al., 2010) and drought stress (Maes et al., 2009) have only been considered for seedlings. In order to gain information about the relationship between CO<sub>2</sub> assimilation rate and stomatal conductance under variable climatic conditions, extensive measurements on plants either grown in a greenhouse or in the fields were combined in this study.

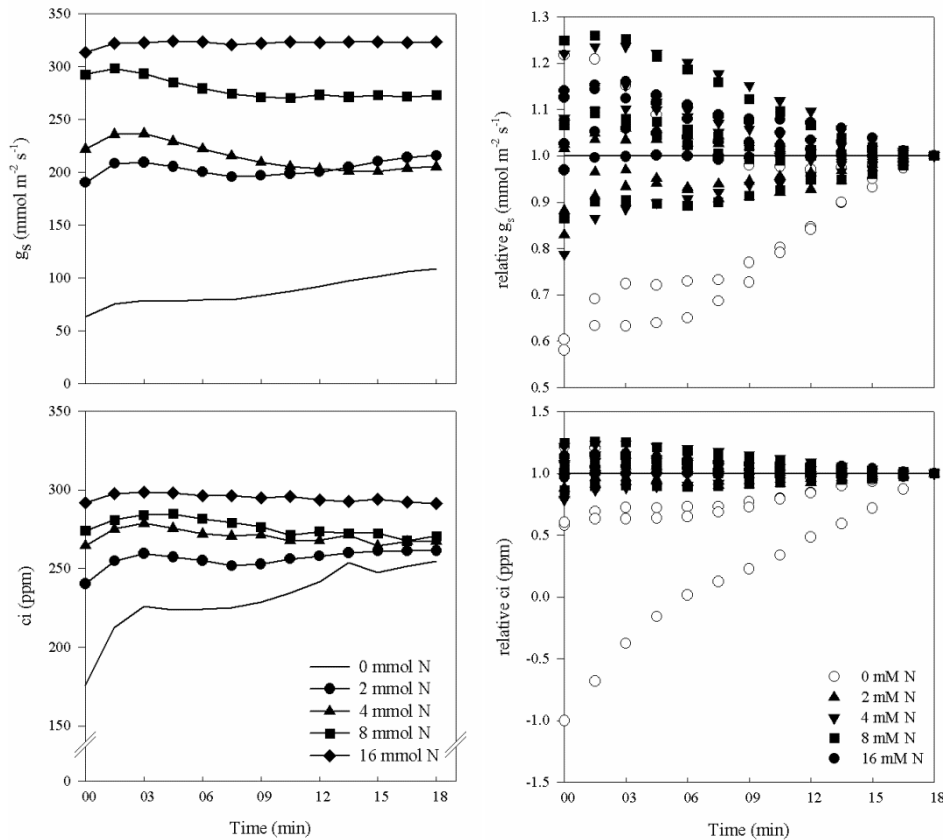
## Materials and methods

### *Greenhouse experiments*

Studies on the effects of nitrogen supply (Experiment 1) and leaf age (Experiment 2) on leaf gas exchange were conducted in a greenhouse of the Hohenheim University, Germany.

Experiment 1 (Exp. 1) was conducted from April 11 to June 24 of 2011. The experiment focused on the effects of N supply on leaf gas exchange parameters of 2-months old jatropha plants in a nutrient solution experiment. Jatropha seeds were germinated in sand on April 11 2011 in a growth chamber at 25°C air temperature. On May 2, when seedlings were in the 1<sup>st</sup>-leaf stage, plants were transferred to the greenhouse and transplanted into pots (1 seedling per 5 L pot) containing water. The N treatment was established on May 20 for 20 days by applying a modified Hoagland nutrient solution (Hoagland and Arnon, 1950) with a pH of 6.0, and with 0, 2, 4, 8, and 16 mM N. The nutrient solution was changed every 4 days and water added 2 days after changing the nutrient solution in order to compensate for transpirational water loss. The pH was adjusted every other day to 6.0. Average day and night air temperatures in the greenhouse were 30 and 23°C, respectively. Daily relative humidity varied from 28 to 60 % during the experimental period. Photosynthetic photon flux density (PPFD) varied from 200-500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  with artificial light for 12h per day using high pressure sodium lamps (PL SON-K-400, DHLicht GmbH, Wülfrath, Germany).

Gas exchange parameters were measured on June 2, 3, 20, 22, and 23 of 2011 using the GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany) photosynthesis system. Leaves were inserted into a 4 cm<sup>2</sup> chamber and exposed to a PPFD of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , a CO<sub>2</sub> concentration of 380 ppm, 50% of relative humidity and at leaf temperature of 25°C. Gas exchange parameters were recorded when stomatal conductance and intercellular CO<sub>2</sub> concentration were stable (between 15 and 20 minutes after leaf insertion) (Fig. 1). The ratio of VPD<sub>chamber</sub> to VPD<sub>air</sub> reached 0.86±0.02 during all measurements. Light response curves were measured by recording the steady-state gas exchange at a light intensity of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PPFD. The light intensity was then increased to 1500 and 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and thereafter decreased stepwise (800, 600, 400, 200, 150, 100, 50, and 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Waiting time between changes of light intensity were 2 minutes in order to minimize stomatal responses to varying light intensity.



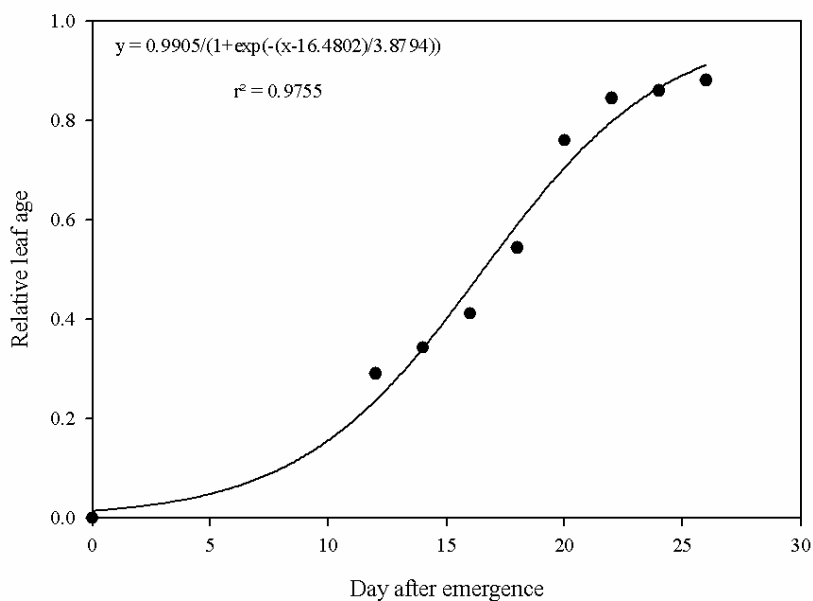
**Figure 1:** Examples of temporal dynamics of stomatal conductance ( $g_s$ ) and intercellular  $\text{CO}_2$  concentration ( $c_i$ ) (absolute value\_left, relative value\_right) of leaves of 2 months-old *Jatropha curcas* L. Plants were grown in a nutrient solution experiment with 5 N levels (0-16 mM N) supplied. Gas exchange parameters were measured at  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, 380 ppm  $\text{CO}_2$  concentration, 50% relative humidity and a leaf temperature of  $25^\circ\text{C}$ . Data were logged every 90 sec after insertion of leaves in the chamber (time 00).

A chlorophyll index value of leaves was recorded prior to gas exchange measurement using the SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan). At the end of the experimental period, plants were harvested and leaf, stem and root biomass and total leaf area measured. Total leaf area of fresh leaves was measured with the  $\Delta T$  area meter (Burwell, Cambridge, England). The different parts of the plants were thereafter oven-dried (72 hours at  $65^\circ\text{C}$ ) to constant weight.

Experiment 2 (Exp. 2) was conducted from June 9 to December 23 of 2008 to assess the effect of leaf age on leaf gas exchange parameters of 6-months old *Jatropha* plants. 16 plants were grown in 4 L pots containing peat soil. The maximum soil water holding capacity was 26 Vol.% and was determined by adding water stepwise to pots filled with dried peat until water was leaking

out at the bottom of the pots. Pots were irrigated every other day with tap water to 70% water holding capacity (WHC) over the experimental period. Average day and night air temperature in the greenhouse was 27 and 24°C, respectively, and relative humidity was 30% during the experimental period. Plants were grown under high-pressure sodium lamps (PL SON-K-400, DHLicht GmbH, Wülfrath, Germany) with 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  on an average during 12 h per day.

Leaf area development of the 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> oldest leaves of plants was regularly monitored from leaf emergence to full leaf expansion. Images of single leaves were taken with a 12 Megapixel camera and millimeter paper as a background below the leaf. Images were analyzed with Mesurim Pro software, version 3.3 (Académie d'Amiens, Amiens, France). Data of leaf area development were fitted by sigmodial growth curves using SigmaPlot version 10.0 (Systat Software, Inc., California, USA) with time as the independent parameter and leaf area development expressed in units relative to full leaf expansion with values from 0-1 (Fig. 2). Four relative leaf age classes were defined: 0.00-0.25 (young leaves); 0.25-0.50 and 0.50-0.75 expanding leaves; 0.75-1.00 (fully expanded leaves).



**Figure 2:** Curve-fitted estimation of relative leaf age using single leaf area measured at different days after leaf emergence. Example of one young leaf of 6-months old *Jatropha* plants irrigated at 70% of soil water holding capacity. Leaf area was measured between 12 and 26 days after emergence.

Leaf gas exchange measurements were performed every two days over the experimental period under greenhouse ambient conditions using the LCi portable photosynthesis system (ADC Bio-Scientific Ltd., Hoddesdon, UK). Measurements were taken under the following conditions: CO<sub>2</sub> concentration: 428±39 ppm, chamber temperature: 36±2°C, relative humidity in the chamber: 14±2%, PPFD: 921±219 μmol m<sup>-2</sup> sec<sup>-1</sup>).

### *Field experiments*

Experiment 3 (Exp. 3) and experiment 4 (Exp. 4) were carried out from February 10 to May 5 of 2009 and from December 2009 to May of 2010, respectively, on the plantation of JatroGreen S.A.R.L, Antananarivo, Madagascar located in the South-Western part of Madagascar (21°43'35.58"S, 46°23'05.63"E, altitude 750 m.a.s.l.). Minimum, maximum and average temperatures and relative humidity were measured with Tinytag data loggers (Type TGP-4500, Gemini data loggers, Chichester, UK) installed at the experimental sites. Rainfall data were not available in the first half of 2009 but were recorded from October 2009 to May 2010 by collecting the rainfall in a rain gauge. In both experiments, leaf gas exchange was measured 10-15 minutes after inserting the leaf in the chamber.

In Exp. 3 the effects of tree age, water supply, leaf age, and leaf position on gas exchange parameters of jatropha were investigated. Gas exchange was measured on plants at two sites five km apart from each other. These sites differed in plantation establishment with 1 and 2-year old trees at the sites, respectively. At each site, plants of similar size were selected. Aluminum trench walls were installed in rectangle of 4x8 m (with 2 trees in each experimental unit) in order to avoid run-on and run-off. At each site, eight replications of rainfed and irrigated plots were established. From February 15 to May 10, every four days 40 L of water were supplied to plots of the irrigation treatment. This simulated rainfall events of 1 mm every 4<sup>th</sup> day.

Gas exchange measurements were done with the LCi portable photosynthesis system (ADC Bio-Scientific Ltd., Hoddesdon, UK). On March 5 of 2009, newly appearing leaves were marked and gas exchange measured when leaves were expanding (less than 3 weeks old) and fully expanded (more than 4 weeks old). The selected leaves were either inside (shaded leaves) or outside the canopy (sunlit leaves). Noticing during the data analysis that leaf gas exchange was affected by time of the day, data were grouped in three different time groups: early (09:00-10:30), before noon (10:30-12:00), and afternoon (12:00-13:30).

In Exp. 4, the effects of water supply (irrigated *versus* rainfed), pruning type, leaf age and time of measurement (morning and afternoon) were analyzed. These data were collected within the experiment described in Rajaona et al. (2011 in press). The 5<sup>th</sup> or 6<sup>th</sup> leaf from the apex of branches was selected for gas exchange measurements. Measurements were performed at the last two weeks of April 2010. The leaf age effect on gas exchange was measured on the same leaf at two different times, namely in the expanding stage and the fully expanded stage. Measurements were performed with leaves receiving 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD using the LCpro portable photosynthesis system (ADC BioScientific Ltd., Hoddesdon, UK).

### *Statistical analysis*

The influence of treatment factors were analyzed with the statistical software package SAS version 9.00 (SAS Institute Inc., Cary, NC, USA). ANOVA of plant traits were performed with a one-factorial for Exp. 1 (N supply), two-factorial for Exp. 2 (leaf age and water supply), five-factorial for Exp. 3 (tree age, water supply, leaf age, leaf position, time of measurement), and four-factorial for Exp. 4 (pruning type, water supply, leaf age, time of measurement) with PROC GLM (type III). Multiple comparisons of means were performed with the LSD test. Test of normality was performed with PROC UNIVARIAT. Average  $\pm$  standard error are reported except indicated otherwise. Analysis of traits was based on 3 (ambient gas exchange) and 4 (light response curves) replications in Exp. 1, and 8, 8, and 6 replications in Exp. 2, 3, and 4, respectively. Mainly due to the uncontrollable leaf chamber temperature, an appreciable amount of data had to be excluded when chamber temperature exceeded 38 °C. Additionally, extremes were excluded based on the criteria if their studentized residual value was  $> \pm 3$ .

## **Results**

### *Effects of N supply on leaf gas exchange parameters*

Gas exchange measurements performed under fixed environmental conditions on single jatropha leaves indicated that CO<sub>2</sub> assimilation rate (A) and stomatal conductance (g<sub>s</sub>) were similar for all treatments supplied with 2 mM N or higher (Table 1). Only the treatment with 0 mM N had significantly lower A, g<sub>s</sub> and ratio of intercellular to external CO<sub>2</sub> concentration (ci/ca). Plants sup-

plied with 16 mM N had the highest A and  $g_s$  and a significantly higher  $ci/ca$  compared to plants supplied with 2-8 mM N.

**Table 1:** Effects of N supply (mM) on CO<sub>2</sub> assimilation rates (A,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), the ratio of intercellular to external CO<sub>2</sub> concentration ( $ci/ca$ ), and chlorophyll index values (SPAD) of jatropha leaves measured in Exp. 1. Plants were supplied with 5 different N levels, average  $\pm$  SE. Letters indicate significant differences between N levels. Significance of N effects and least significant differences (LSD) are listed at the bottom; n.s.: not significant.

N level	A	$g_s$	$ci/ca$	SPAD
0	6.12 $\pm$ 1.65 b	108 $\pm$ 34 b	0.61 $\pm$ 0.03 c	26.75 $\pm$ 1.11 c
2	18.77 $\pm$ 0.92 a	288 $\pm$ 44 a	0.70 $\pm$ 0.03 b	43.50 $\pm$ 0.87 b
4	17.77 $\pm$ 1.57 a	261 $\pm$ 36 a	0.70 $\pm$ 0.03 b	46.25 $\pm$ 0.75 ab
8	15.69 $\pm$ 1.70 a	250 $\pm$ 40 a	0.72 $\pm$ 0.03 b	49.25 $\pm$ 1.11 a
16	17.52 $\pm$ 0.72 a	308 $\pm$ 26 a	0.75 $\pm$ 0.02 a	45.75 $\pm$ 1.49 b
P>F	0.0002	0.0015	<0.0001	<0.0001
LSD	4.20	81.91	0.03	3.47

The increase of N supply from 0 to 4 mM N resulted in increases of leaf, stem and root dry weights (Table 2), while leaf and total dry weight of plants supplied with 8 and 16 mM N tended to be lower than that of plants supplied with 4 mM N. Leaf area, specific leaf area (SLA) and chlorophyll index (SPAD) values of plants supplied with 0 mM N were lower compared to that of plants supplied with 2 mM N and higher.



**Table 2:** Effects of N supply (mM) on leaf, stem, root, total biomass (DW, g), leaf area (LA, m<sup>2</sup>), and specific leaf area (SLA, m<sup>2</sup> kg<sup>-1</sup>) of jatropha leaves measured in Exp. 1. Plants were supplied with 5 different N levels, average  $\pm$  SE. Significant differences between N levels are indicated by different letters. Significance of N effects and least significant differences (LSD) are listed at the bottom; n.s.: not significant.

N	Leaf DW	Stem DW	Root DW	Total DW	LA	SLA
0	1.6 $\pm$ 0.1 d	3.9 $\pm$ 0.5 b	1.0 $\pm$ 0.1 b	6.5 $\pm$ 0.5 c	0.03 $\pm$ 0.00 b	18.8 $\pm$ 0.5 a
2	15.1 $\pm$ 0.5 c	17.7 $\pm$ 1.9 ab	3.9 $\pm$ 1.4 ab	36.7 $\pm$ 3.0 b	0.31 $\pm$ 0.02 a	21.0 $\pm$ 1.6 a
4	24.0 $\pm$ 2.3 a	30.9 $\pm$ 8.2 a	6.7 $\pm$ 1.2 a	61.7 $\pm$ 11.4 a	0.42 $\pm$ 0.08 a	17.5 $\pm$ 3.1 a
8	16.7 $\pm$ 2.1 bc	30.6 $\pm$ 10.7 a	7.3 $\pm$ 2.3 a	42.4 $\pm$ 7.5 ab	0.43 $\pm$ 0.10 a	19.5 $\pm$ 1.8 a
16	21.1 $\pm$ 2.8 ab	25.0 $\pm$ 4.7 a	6.1 $\pm$ 1.2 a	52.2 $\pm$ 8.6 ab	0.44 $\pm$ 0.06 a	20.8 $\pm$ 0.3 a
P>F	<0.0001	0.0183	0.0172	0.0022	0.0037	n.s.
LSD	3.75	16.49	3.67	3.75	0.18	5.81

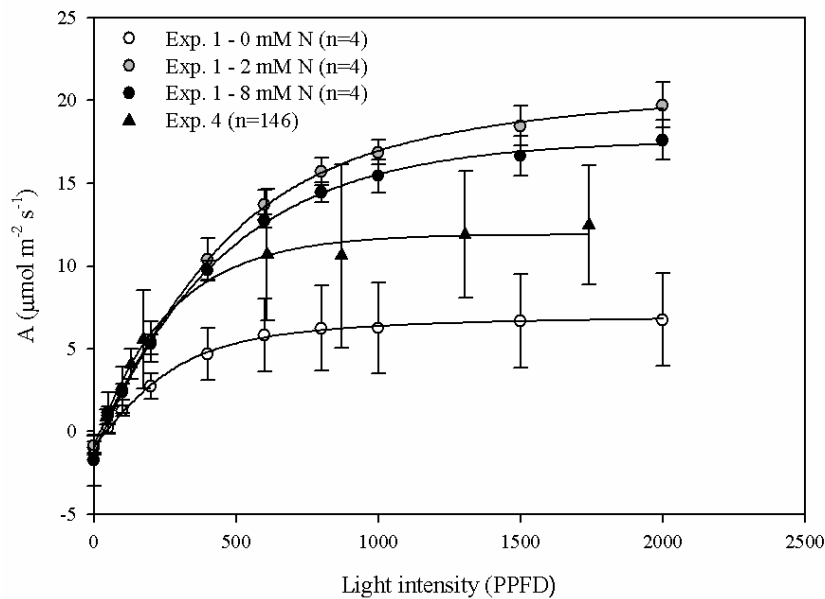
### *Light response curves of jatropha*

Light response curves were measured at three N supply levels (0, 2 and 8 mM N) (Fig. 3) of Exp 1. Light saturated rates of photosynthesis ( $A_{max}$ ) of treatment 0 mM N were significantly lower than that of N levels 2 and 8 mM N (Table 2). Light compensation point ( $I_C$ ), quantum yield (QY), and dark respiration ( $R_d$ ) were similar for all treatments.

Additionally to the data collected under the semi-controlled conditions in the greenhouse (Exp. 1), light response curves were also measured on jatropha leaves at the plantation site in 2010 (Exp. 4) (for further details, see Table 3). Pooling these light response curves over all treatments, it is evident that light response parameters of field-grown plants were lower than that of plants from the greenhouse study supplied with 2 and 8 mM (Fig. 3)

**Table 3:** Comparison of leaf light response parameters: Maximum CO<sub>2</sub> assimilation rates ( $A_{\max}$ ), light compensation point ( $I_C$ ), quantum yield (QY), dark respiration ( $R_d$ ) of jatropha leaves at 0, 2 and 8 mM N were measured in Exp. 1. Average  $\pm$  SE. ANOVA was performed with a one-factorial with PROC GLM (type III). Differences between N levels are indicated by different letters. Significances of N effects are listed at the bottom; n.s.: not significant.

N	$A_{\max}$	$I_C$	QY	$R_d$
0	7.11 $\pm$ 1.51 b	41 $\pm$ 7 a	0.023 $\pm$ 0.001 a	0.96 $\pm$ 0.19 a
2	21.79 $\pm$ 1.21 a	38 $\pm$ 4 a	0.037 $\pm$ 0.005 a	0.84 $\pm$ 0.37 a
8	20.45 $\pm$ 2.33 a	35 $\pm$ 5 a	0.033 $\pm$ 0.003 a	0.94 $\pm$ 0.13 a
P>F	0.003	n.s.	n.s.	n.s.



**Figure 3:** Light response curves of Jatropha supplied with 0, 2 and 8 mM N (Exp. 1) and of jatropha grown in the field (Exp. 4).

Field measurements (Exp. 4) illustrated that plants of the two pruning types did not differ in  $A_{\max}$ ,  $I_C$ , and QY. Nevertheless,  $R_d$  of plants subjected to pruning type B was higher than that of plants subjected to pruning type A. Rainfed plants had a lower  $A_{\max}$ ,  $I_C$  and QY but a higher  $R_d$  than irrigated plants. Finally, the same leaf measured in the morning had higher  $A_{\max}$  and QY and lower  $I_C$  and  $R_d$  than in the afternoon.

*Leaf age effects on leaf gas exchange parameters*

In field measurements of Exp. 4, fully expanded leaves had a lower  $A_{\max}$  compared to expanding leaves (Table 4). Similarly, results from Exp. 2 (greenhouse study) in which leaf age was monitored in a high time resolution, indicated that leaf gas exchange parameters were affected by leaf age (Table 5).  $A$  and  $g_s$  increased from young to expanding leaves of age class 3 and decreased when leaves were fully expanded (leaf age class 4). The  $ci/ca$  was similar for all leaf age classes.

**Table 4:** Comparison of leaf light response parameters light-saturated  $CO_2$  assimilation rate ( $A_{\max}$ ), light compensation point ( $I_c$ ), quantum yield (QY), dark respiration ( $R_d$ ) of jatropha leaves in Exp. 4. Trees were subjected to two different types of pruning, water supply, leaf age, and time of measurement. Average $\pm$ SE. Differences between treatments are indicated by different letters. Significance of pruning, water, leaf age and time of measurement effects are listed at the bottom; n.s.: not significant.

		$A_{\max}$	$I_c$	QY	$R_d$
Pruning	A	11.94 $\pm$ 4.05 a	22 $\pm$ 10 a	0.031 $\pm$ 0.001 a	1.59 $\pm$ 1.50 b
	B	12.13 $\pm$ 4.95 a	21 $\pm$ 12 a	0.031 $\pm$ 0.001 a	2.07 $\pm$ 1.43 a
Water	Rainfed	10.83 $\pm$ 4.29 b	19 $\pm$ 11 b	0.028 $\pm$ 0.001 b	2.43 $\pm$ 1.69 a
	Irrigated	13.09 $\pm$ 4.39 a	23 $\pm$ 11 a	0.033 $\pm$ 0.001 a	1.27 $\pm$ 1.01 b
Leaf age	Expanding leaf	13.09 $\pm$ 4.39 a	19 $\pm$ 11 b	0.033 $\pm$ 0.001 a	1.59 $\pm$ 1.36 a
	Fully expanded leaf	11.10 $\pm$ 4.37 b	23 $\pm$ 11 a	0.029 $\pm$ 0.001 a	1.72 $\pm$ 1.47 a
Time	Morning	14.01 $\pm$ 3.75 a	19 $\pm$ 9 b	0.034 $\pm$ 0.001 a	1.59 $\pm$ 1.36 b
	Afternoon	9.18 $\pm$ 3.88 b	25 $\pm$ 13 a	0.026 $\pm$ 0.001 b	2.14 $\pm$ 1.60 a
P>F					
Pruning		n.s.	n.s.	n.s.	0.03
Water		0.0001	0.0426	<0.0001	<0.0001
Leaf age		0.007	0.0254	0.0014	n.s.
Time		<0.0001	0.0016	<0.0001	0.0053

**Table 5:** Effects of leaf age (1: young leaves, 2 and 3: expanding leaves (lower and upper part of the sigmoid curve), 4: fully expanded leaves) on CO<sub>2</sub> assimilation rate (A,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), the ratio of intercellular to external CO<sub>2</sub> concentration (ci/ca) and chlorophyll index value (SPAD) of jatropha leaves in Exp. 2 (average $\pm$ SE). Significant differences between leaf age classes are indicated by different letters. Significance of leaf age effects are listed at the bottom; n.s.: not significant.

Leaf age	A	$g_s$	ci/ca	SPAD
1	8.04 $\pm$ 3.95 b	162 $\pm$ 76 b	0.68 $\pm$ 0.04 a	30 $\pm$ 2 c
2	11.87 $\pm$ 3.26 ab	224 $\pm$ 85 ab	0.66 $\pm$ 0.04 a	33 $\pm$ 2 c
3	14.56 $\pm$ 2.47 a	291 $\pm$ 84 a	0.65 $\pm$ 0.02 a	38 $\pm$ 1 b
4	8.55 $\pm$ 5.77 b	147 $\pm$ 118 b	0.64 $\pm$ 0.06 a	42 $\pm$ 1 a
P>F	<0.0001	<0.0001	n.s.	<0.0001

#### *Time of measurement*

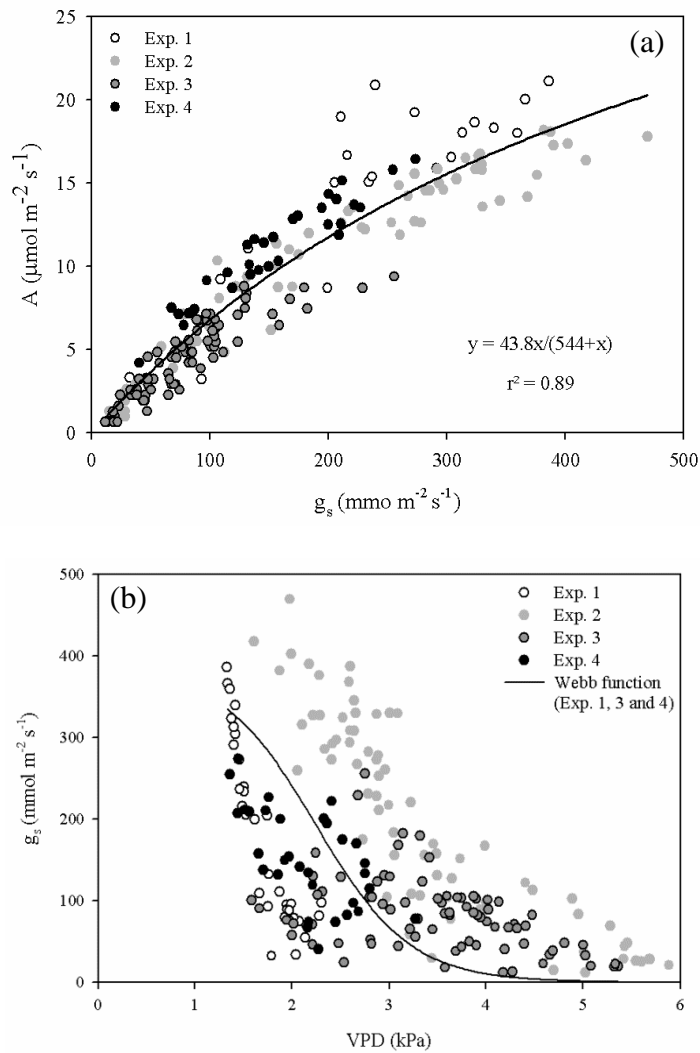
In field measurements of Exp. 4,  $A_{\text{max}}$  was higher in the morning than in the afternoon (Table 4). The ANOVA of data collected in the field in 2009 (Exp. 3) revealed significant (Pr>F: 0.0053 for A, 0.0022 for ci/ca) effects of time of measurement on gas exchange parameters with higher A and lower ci/ca in the morning compared to measurements performed later during the day (Table 6).

**Table 6:** Effect of time of measurement (1: early (09:00-10:30); 2: before noon (10:30-12:00); 3: afternoon (12:00-13:30)) on CO<sub>2</sub> assimilation rates (A,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), and the ratio of intercellular and external CO<sub>2</sub> concentration ( $c_i/c_a$ ) of *Jatropha* leaves measured at ambient field conditions on April and May 2009 (Exp. 3) (average $\pm$ SE). Data are pooled over tree age, water regimen, leaf age, and leaf position. Letters indicate significant differences between time of measurements. Significance of leaf age effects are listed at the bottom; n.s.: not significant.

Time	A	$g_s$	$c_i/c_a$
1	5.21 $\pm$ 0.31 a	87 $\pm$ 07 a	0.64 $\pm$ 0.01 b
2	3.83 $\pm$ 0.45 b	77 $\pm$ 10 a	0.69 $\pm$ 0.01 a
3	3.72 $\pm$ 0.76 b	76 $\pm$ 12 a	0.7 $\pm$ 0.03 a
P>F	0.0053	n.s.	0.0022

#### *Relationship between CO<sub>2</sub> assimilation rate and stomatal conductance*

Pooling all data of Exp. 1 to 4, A was positively correlated with  $g_s$ . Across all experiments, A varied from 0.64 to 21.13  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and  $g_s$  varied from 12 to 469  $\text{mmol m}^{-2} \text{s}^{-1}$  (Fig. 4a). Evident are differences between the two field experiments with lower values of leaf gas exchange in 2009 (Exp. 3) compared to Exp. 4. Field and pot experiments showed a high degree of similarity in the relationship between A and  $g_s$ .



**Figure 4:** Correlation between stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) and  $\text{CO}_2$  assimilation rate ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) (a), and correlation between of atmospheric vapour pressure deficit (VPD, kPa) and  $g_s$  (b) of jatropha leaves. Regression curve between VPD and  $g_s$  of measurements at Exp. 1, 3 and 4 is given following an inverse logistic function (Webb, 1990). Measurements were performed during 4 different experiments in the greenhouse (Exp. 1 and 2) and at a field site in Fenoarivo, Madagascar (Exp. 3 and 4).  $n=217$ .

However, when analyzing the relationship between  $g_s$  and VPD of these four experiments, it became evident that in all experiments  $g_s$  decreased with increasing VPD but that this response differed between the field experiments and the two pot experiments which contrasted each other distinctively (Fig. 4b).

## Discussion

Carbon assimilation is one of the central processes of plant growth and consequently a key process embedded in modelling approaches of plant productivity. A myriad of research results considering the response of carbon assimilation to abiotic factors have been reported for almost all terrestrial plants. Based on this wealth of information, photosynthesis models have been developed which either use mechanistic or empirical approaches to predict plant performance in varying (micro)-climatic environments.

Immediate main abiotic drivers of carbon assimilation are light intensity (Gates, 1980; Amthor, 2010), soil water status (Cowan, 1982; Gollan et al., 1986), nutrient availability (Evans, 1983), and air humidity and temperature (Sage and Kubien, 2007). Contrary to the analysis of carbon assimilation of crops from high-input agricultural systems (e.g., rice, wheat) where abiotic stresses are minimized, many of the tropical and sub-tropical production systems are subject to a variety of stressors of which water and nutrients are more dominating than temperature constraints. Additionally to abiotic factor exerting control over leaf gas exchange, internal plant status parameters are as well known to affect carbon assimilation such as N availability (LeBauer and Treseder, 2008), leaf age (Kitajima et al., 2002) and diurnal variation in photosynthesis (Hari et al., 1999). Parameters such as leaf age and diurnal variation in leaf gas exchange render prediction of leaf gas exchange as this variation is less easily to predict than those induced by e.g., light availability.

Jatropha is considered as one of the plants which may contribute to substituting fossil fuel by bio-energy production (Achten et al., 2008). As this plant is proposed for cultivation on marginal sites, thus not competing with food production; effects of the main abiotic drivers (water, VPD, N availability) on leaf gas exchange need thorough assessment. However, data from field experiments predominantly cover aspects of agronomic practices to enhance plant growth (Behera et al., 2009) and seed yield (Abou Kheira and Atta, 2009) of jatropha. To a certain extent allometric parameters have been investigated recently (Ghezehei et al., 2009; Rajaona et al., 2011 in press) while leaf gas exchange of jatropha was exclusively recorded in greenhouse studies and at seedling stage (Maes et al., 2009; Young et al., 2010; da Silva et al., 2009, 2010). In order to reduce this knowledge gap, the present study considered N supply and VPD as the central abiotic drivers and leaf age, leaf position, and time of measurement as plant status parameters to assess variability in response to these factors.

N supply is expected to have a major effect on leaf gas exchange, as the built-up of the photosyn-

thetic machinery greatly rely on N availability (Evans, 1983). Enzyme densities are positively correlated with biophysical and chemical rates of light and dark reaction of photosynthesis. Contrarily to the work of Young et al. (2010), this study illustrated that N supply effects on leaf gas exchange of jatropha leaves were small with only the treatment N0 resulting in lower rates of A and  $A_{\max}$ , although effects of N supply on biomass formation were pronounced. The leaf SPAD value is a reliable indicator of both tissue nitrate and total N concentration in both monocotyledonous and dicotyledonous plants (Westerveld et al., 2003). As indicated by the increase of leaf area with increasing N supply, jatropha responded rapidly with a decrease in leaf expansion (Table 2), while the constancy of SPAD values from 2 to 16 mM N indicates that enzyme densities were not affected by N supply once a threshold value of N supply was ensured. This response is typical for a variety of dicotyledonous plant species which regulate leaf expansion in a way to keep enzyme densities fairly constant over a range of external N supply (Vos and Van Der Putten, 1998). Our findings indicate that the SPAD value cannot be used for N management of plantations, as it would only indicate severe N deficiency.

As jatropha leaves are, due to the planting density and the arrangement of leaves in the bulky canopy, partially exposed to low light conditions (e.g.,  $<300 \mu\text{mol m}^{-2} \text{s}^{-1}$  of PPFD), the quantum yield is a central parameter for predicting  $\text{CO}_2$  assimilation rate under such low light conditions. The quantum yield (QY) is considered to be fairly stable within the group of  $\text{C}_3$  plants with an average value of 0.052 (Skillman, 2008). For some  $\text{C}_4$  species belonging to the same family as jatropha (Euphorbiaceae) this value varied between 0.058 and 0.065 (Ehleringer and Pearcy, 1983), whereas the QY of CAM plants varied from 0.033 to 0.065; Barrel cactus (Nobel, 1977); *Agave deserti* (Nobel and Hartsock, 1978), *Sedum praealtum* (Spalding et al., 1980); *Heuchera sanguine* (Skillman, 2008). The QY of jatropha plants measured in a greenhouse and in the field in this study was consistently lower than that of  $\text{C}_3$  plants. Due to temperature-dependent changes of the solubility of gases, photorespiration is increasing with increasing tissue temperature thereby reducing the quantum yield (Ehleringer and Pearcy, 1983). The QY decreased linearly by  $0.0013 \text{ mol CO}_2 (\text{mol quanta})^{-1} \text{ }^\circ\text{C}^{-1}$  (Ehleringer and Pearcy, 1983). A QY of approximately 0.03, which we measured in our study, would be reached at an unrealistic high leaf temperature of  $48^\circ\text{C}$ . Considering that leaf temperatures during our measurements ( $25\text{-}36^\circ\text{C}$ ) were not extremely high for this tropical plant, heat-stress induced enzyme deactivation (Law and Crafts-Brandner, 1999; Salvucci and Crafts-Brandner, 2004) can, therefore, as well be excluded as a factor explaining the low QY. Excluding temperature effects on the quantum yield as a possible explanation, inherently low values of light absorbance or partial CAM type photosynthesis deserve further attention in order to explain our experimental findings.



Leaf age effects render the prediction of canopy photosynthesis and leaf activity. The increase of CO<sub>2</sub> assimilation to a maximum rate followed by a decrease towards leaf senescence is typical for evergreen and deciduous plants (Pate and Layzell, 1981; Chabot and Hicks, 1982; Kitajima et al., 2002) and has been reported by Young et al. (2010) for jatropha seedlings. Leaves of dicotyledonous plants form a carbon sink from leaf emergence to 30-60% of full leaf expansion (Turgon, 1989). The decline in photosynthetic capacity and the shift from carbon sink to source of fully expanded leaves is accompanied by re-allocation of nitrogen to more productive parts in the canopy and corresponding leaf-age related changes of SPAD values over a range of leaf age classes (Pate and Layzell, 1981; Nelson and Dengler, 1997). Our data support this leaf age pattern of CO<sub>2</sub> assimilation rates with higher rates recorded during the expansion stages of leaf development and a decline at full expansion stage. However, SPAD values steadily increased from leaf emergence to full expansion, while assimilation rates declined. The low SPAD values during early leaf expansion stage of jatropha and the reddish leaf colour indicate high foliar anthocyan levels (Fig. 5). A study on *Rosa sp.* and *Ricinus communis L.* showed that anthocyan did not affect CO<sub>2</sub> assimilation rate but served to protect shade-adapted chloroplasts from brief high-light exposure (Manetas et al., 2002). Rather than explaining the decline of assimilation rate at full expansion stage by senescence-related decline in photosynthetic capacity (Sobrado, 1994; Kakani et al., 2004), we suggest a contribution of carbohydrate feedback on carbon fixation which needs clarification by combining leaf gas exchange with metabolite measurements. The same line of argumentation may apply to the observed effect of time of measurement on assimilation rate (Table 4 and 6) as carbohydrate accumulation over the day is known to repress photosynthetic enzyme activity (Winter and Huber, 2000). Further study on of carbon sink and source quantification of jatropha is therefore essential. A model-based crop growth analysis will consequently require the definition of sucrose distribution, environmental factors as triggers for leaf appearance and seed production and the mechanistic approach of feedback inhibition of photosynthesis.



**Figure 5:** The reddish colour of young jatropha leaves indicating high foliar anthocyan levels.

Measurements of instantaneous rates of leaf gas exchange of different leaves subject to variable VPD in this study illustrated that  $A$  and  $g_s$  were correlated in a hyperbolic pattern (Fig. 4a), and that  $g_s$  declined with increasing VPD. However, the response of  $g_s$  to VPD differed between the four experiments (Fig. 4b). A hyperbolic response function between  $g_s$  and  $A$  can be expected from the increasing substrate saturation of Rubisco with increasing stomatal opening (Jones, 1992), while other experimental work indicated a strictly linear relationship between  $g_s$  and  $A$  (Grantz, 1990). Our findings are similar to that of Tezara et al. (1998) on *Jatropha gossypifolia*, whereas Young et al. (2010) reported a linear relationship between  $A$  and  $g_s$  for 3 varieties of *Jatropha curcas* L. Further clarification is obviously required as the linear or hyperbolic response pattern between  $g_s$  and  $A$  have implications for the instantaneous water use efficiency which is expected to decrease with increasing  $g_s$  if  $A$  and  $g_s$  are not linearly correlated (Jones, 1992; Hetherington and Woodward, 2003; Damour et al., 2010).

Analysis of stomatal responses to variation in VPD is usually performed by investigating leaf gas exchange activity of a single leaf (or canopies) under variable VPD (Monteith, 1995). This approach has consistently shown that  $g_s$  declined with increasing VPD in a linear (Aphalo and Jarvis, 1991) or invert logistic function (Webb, 1990) both which allow for the calculation of a maximal stomatal conductance. These measurements are considered to reflect the response of  $g_s$  to VPD, and indeed, as shown with our data which were measured on many different leaves and VPD conditions,  $g_s$  declined with increasing VPD. However, the results presented in this study indicated that responses of  $g_s$  to VPD differed between the experiments with data of Exp. 2 deviating from the general trend obvious in the other experiments. The discrepancy in response of Exp. 2 and the partially large variation in the  $g_s$  VPD response of Exp. 3 and 4 may reflect genotypic differences in hormonal signal responses to atmospheric drought (Asch et al., 2009) or may have been related to factors such as time of measurement and leaf age. This would render the application of single response functions unrealistic. However, applying the inverse logistic function of Webb (1990), and by excluding Exp. 2, the maximal stomatal conductance of *Jatropha* was in the range of  $382 \text{ mmol m}^{-2} \text{ s}^{-1}$  and  $g_s$  is predicted to be close to zero at 6 kPa. Effects of VPD, via stomatal conductance, by preventing high transpiration rates, have been demonstrated to be decisive on water use efficiency (Sinclair et al., 1983; Chaves and Oliveira, 2004, Lawlor and Tezara, 2009). With stomatal closure, water use efficiency is increasing while carbon gain and thereby growth is adversely affected. From our data it appears that, independent of the water supply, atmospheric drought can substantially reduce photosynthetic carbon gain of *Jatropha* in arid environments. Additionally, a robust approach of predicting  $A$  via  $g_s$  responses to VPD appears feasible. The outcome further indicates favourable conditions at which stomatal opening is

high and thereby allowing for biomass formation. This information should be considered in approaches which aim at quantifying leaf activity of field-grown bushes which are characterized by spatially highly diverse conditions in microclimate. Finally, our data indicate that light absorption characteristics of single leaves and leaf carbohydrate status parameters should be investigated further in order to explain the low QY and the pronounced diurnal variation observed during the studies.

## **Acknowledgments**

This study is a part of the project «Jatropha Anbau in Madagaskar». We gratefully acknowledge the funding of this work by EnBW-Regenwaldstiftung, Stiftung Energieforschung Baden-Württemberg, project No. 08A26908. This work was carried out in partnership with Jatrogreen S.A.R.L., Antananarivo, Madagascar.

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## Appendix III

Effect of pruning history on growth and dry mass partitioning of jatropha on a plantation site in  
Madagascar

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2011

Published in: Biomass and Bioenergy, Volume: 35, Issue: 12, December 2011, Page:  
4892-4900, <http://dx.doi.org/10.1016/j.biombioe.2011.10.017>

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

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# Effect of pruning history on growth and dry mass partitioning of jatropha on a plantation site in Madagascar

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## ARTICLE INFO

### Article history:

Received 23 February 2011

Received in revised form

14 October 2011

Accepted 16 October 2011

Available online 8 November 2011

### Keywords:

Allometry

Dry matter allocation

*Jatropha curcas* L.

Leaf area density

Leaf area projection

## ABSTRACT

While technical aspects of oil processing of seeds of jatropha are under intensive investigation, comparably little is known about the performance of jatropha in the field. We investigated the effects of water availability (rainfed versus irrigated) and pruning-induced differences in plant stature on growth, biomass partitioning, and canopy size at a plantation site in Madagascar in 2010. Plants of different pruning types differed in trunk height (43 versus 29 cm) and primary branches total length (171 versus 310 cm). The two pruning types had effects on dry mass formation and leaf area projection (LAP) during the vegetation period. Trees which had a shorter trunk and longer lateral branches produced more biomass and had a higher LAP. Total dry mass formation varied from 489 to 912 g m<sup>-2</sup> and LAP from 3.26 to 7.37. Total aboveground biomass increased from 2.3 ± 0.5 to 4.89 ± 1.4 kg tree<sup>-1</sup> and from 4.6 ± 1.8 to 8.9 ± 1.0 kg tree<sup>-1</sup> for the pruning types with shorter and longer lateral branches, respectively. Growth of twigs and leaves was positively correlated with total length of branches. Relative dry mass allocation to branches, twigs and leaves, length of twigs per cm of branches and specific leaf area (13.57 ± 0.72 m<sup>2</sup> kg<sup>-1</sup>) were not affected by pruning and water supply. Trees with shorter branches had higher LAD. Results indicate that pruning type should be considered as a management tool to optimize biomass production. Detailed studies on effects of canopy size and shape on radiation interception and growth are required to improve the productivity of jatropha.

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## 1. Introduction

In view of the limited fossil oil reserves, actors in clean development mechanisms and policy makers are attracted to *Jatropha curcas* L. as it is considered as a promising renewable energy source [1–3]. Additionally, competition with food production is supposedly low, as jatropha is often cultivated on unproductive wastelands and severely degraded sites [4,5]. However, little is known about the resource requirements of jatropha in terms of profitable plantation management such as fertilizer application, irrigation or pruning, owing to

jatropha being cultivated rather recently. Considering the planting density of jatropha which usually ranges from 1100 to 3300 plants ha<sup>-1</sup> [6], pruning is a management practice aiming at an optimal spatial arrangement of branches and the spatio-temporal dynamics of leaf area. Plant architecture analyses highlighted the importance of geometrical features of a plant and the partitioning of biomass between different organs [7] in terms of light interception and productivity [4,8]. In commercial fruit tree plantations, pruning is one of the principle means to control tree size, canopy structure, and shape, improving the efficiency of resources acquisition,

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0961-9534/\$ – see front matter © 2011 Published by Elsevier Ltd.

doi:10.1016/j.biombioe.2011.10.017

productivity and profitability for growers [9,10] and the same should apply to jatropha as well.

At present, field-related research focuses on yield potential of jatropha [6], water use [11,12], genetic variation and prospects for improving jatropha by breeding [13,14] and the ability of jatropha to grow on marginal land [4,15]; this information is required to conveying benefits of jatropha plantation for rural socio-economy and ecology. Physiological studies have been conducted on leaf gas exchange, leaf area and dry weight partitioning under different environmental conditions but mostly used seedlings [4,11,16,17]. To what extent results from seedling studies are related to the performance of adult plantation plants remains open.

Data on seasonal dynamics of biomass partitioning in jatropha are rare and modeling approaches aiming at yield potential assessment are, therefore, partially based on assumed data [6]. Jatropha is claimed to invest only 25% of its annual carbohydrate gain into stems, and with proper pruning, the majority of carbohydrates will be used for the growth of twigs, leaves, flowers and fruits [4]. It is not known to what extent biomass partitioning and canopy size are affected by water availability and pruning. The objective of our work was to assess the biomass partitioning and growth dynamics of field-grown, 4-year old jatropha plants during the vegetation period. In order to study these aspects, we assessed the biomass formation and canopy characteristics over a vegetation growth period on a plantation site where two different pruning types were found. We additionally imposed two water treatments and recorded structural parameters of the tree (e.g., branch number and length) and the constitution of newly formed biomass in leaves and twigs. The results of this study can be used in model-assisted yield potential studies including structural features of branches and twigs and in terms of improved plantation management, specifically pruning.

## 2. Materials and methods

### 2.1. Location

The field experiment was carried out from December 2009 to May of 2010 on the plantation of JatroGreen S.A.R.L, Antananarivo, Madagascar located in the South-Western part of Madagascar (21 °43'35.58"S, 46 °23'05.63"E, altitude 750 m.a.s.l.) where natural occurrence of jatropha has been reported [13]. This region is characterized by tropical climate with an annual minimum and maximum monthly temperature of 17.4 and 25.3 °C, respectively and total rainfall of 597 mm typically occurring from November to April [18]. In 2010, mean daily and nocturnal temperatures at the experimental sites were 32.5 °C and 23.4 °C respectively, and total rainfall from November to April was 491 mm (Fig. 1).

The study was performed on one site within the plantation where jatropha trees were 4-year old. The site was flat with slopes <5%. Soil physic-chemical properties were based on 6 replicates. The soil was sandy loam (topsoil; 0–20 cm) to loam (subsoil 20–40 cm) with bulk density of  $1.51 \pm 0.00$  and  $1.47 \pm 0.01$ , respectively. The topsoil contained 64% sand, 14% silt and 22% clay. The soil is moderately acid with an average soil  $\text{pH}_{(\text{water})}$  of 5.05. Cation exchange capacity ( $\text{CH}_3\text{COONH}_4$ )

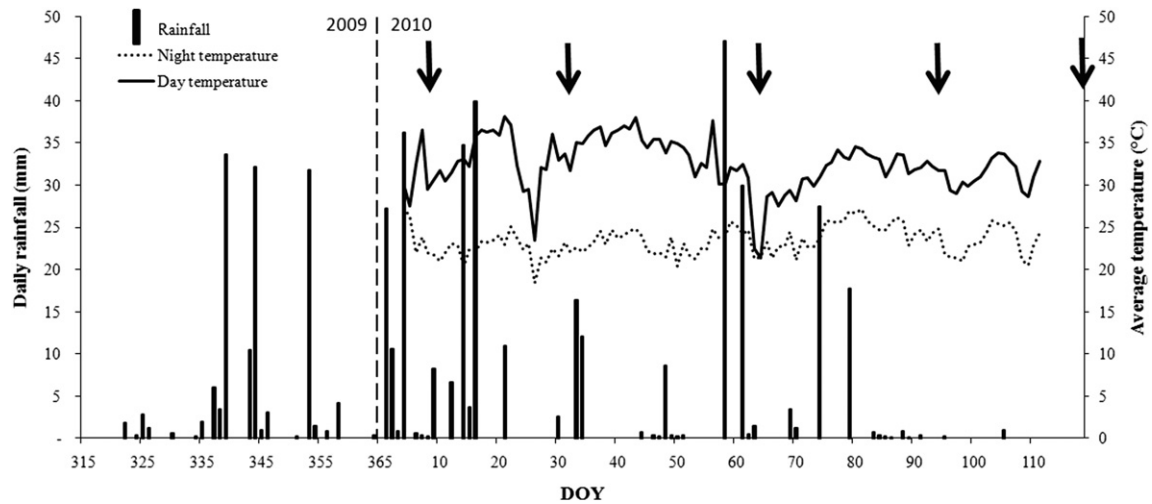
was dominated by calcium (56%) and magnesium (34%), with aluminium contributing with <2%. Total carbon and nitrogen contents and plant available P were very low (Table 1) indicating a serious limitation of plant growth by N and P availability. Plant available potassium was comparably high. Micro elements were very low except of iron content of the top soil.

### 2.2. Plant establishment and irrigation

Jatropha seedlings were transplanted from the nursery to a field site of 5 ha on the plantation in November 2005 with a planting density of 1250 plants  $\text{ha}^{-1}$  (2 m × 4 m). Seedlings were planted by removing the grass cover, loosening the soil of an area of 50 × 50 cm and breaking the subsoil hardpan by digging down to a depth of 50 cm. Fertilizer was applied five times during the following years. 180 g bat Guano and 40 g of NPK (11-22-16) per plant were applied in November 2005 and 20 g of urea in February 2006. 200 g of NPK were applied in December 2008 and 140 g of NPK and 140 g of manure in December 2009. Finally 20 g of urea, 40 g of NPK, 20 g of KCl (33) and 100 g of mono-ammonium phosphate (11-52) were applied in March 2010. The total amounts of nutrient elements applied over five years were roughly 100 kg of N, 120 kg of K and 150 kg of P per hectare. Plants were treated with fungicides every 3 months containing either mancozeb, or parathion, or hexaconazole, and insecticides (dimethoate or deltamethrine).

As seedlings from the nursery were propagated from seeds collected from a wide variety of jatropha trees in the near surroundings of the plantation, plants were assumed to be genetically different. However, the phenotype of jatropha plants at this site was visually quite similar in terms of leaf shape and color. Pruning began in the dry season of 2007, resulting in differences in tree form and size at two sub-areas of the field. In the first year when seedlings were transplanted to the field, pruning of twigs differed between the two sub-areas. At one sub-area, twigs at the lower insertion of the stem were removed and only 3–5 upper twigs were left for re-growth in the next season (pruning A). Twigs were cut at a height of approximately 0.4 m at the end of the growing season. At the other sub-area upper and lower positioned twigs were allowed to remain on the trunk and twigs were cut at approximately 0.3 m (pruning B). At both areas, plant size was controlled by pruning to approximately 1 m height during the following years. Pruning type A, therefore, resulted in long tree trunks and fewer and shorter primary branches. Pruning type B resulted in shorter trunks with more and longer primary branches.

We randomly selected 50 trees from pruning type A and B, which allowed for the destructive harvesting of plants subjected to 2 irrigation treatments at 5 harvest dates with 5 replicates. In order to investigate the effects of water availability on plant performance, for each pruning type, of the 50 trees, 25 were rainfed and 25 were daily irrigated with 10 mm of water from DOY 1 to 126. Trees were randomly selected for the water treatments before onset of irrigation (January 5, 2010). In order to avoid effects of ground cover on water infiltration and use, grasses were removed from an area of 2 × 2 meters around the trunk and kept bare during the vegetation growth period. Rainfed and irrigated trees received 491 and 1750 mm of water over the season, respectively.



**Fig. 1** – Average day and night temperatures and daily rainfall at the experimental site from November 17, 2009 to April 29, 2010. Arrows indicate harvest dates.

### 2.3. Measurements

Minimum, maximum and average temperature and relative humidity were measured on two bare soil plots ( $3 \times 3$  meters) established at both sub-areas of the field, recorded with Tinytag data loggers (Type TGP-4500, Gemini data loggers, Chichester, UK). Rainfall amount was quantified with a rain gauge from November 2009 to May 2010.

Height and diameter of tree crowns and trunks were recorded at December 29 2009. Branches were classified into primary, secondary, and tertiary branches (Fig. 2) and the number of branches per tree were recorded. Five harvests were made in 2010 at January 9, February 2, March 6, April 6 and May 7. Height and radius of the trees, height and basal diameter of trunks, number, lengths and basal diameter of branches and twigs were recorded at each harvest date. Trees were separated into leaves, twigs, primary, secondary and tertiary branches and trunk. Fresh masses were recorded and samples air-dried under an open sky to constant weight. Depending on the climatic conditions this took up to one week. The allometric relationship between average diameter ( $D$ ) of primary branches and total aboveground biomass ( $DW$ ) [19], and between total length of structural components and the weight of leaves and twigs were calculated. Stem wood density was calculated from stem fresh volume and that dividing the dry mass of a stem [20].

In order to determine specific leaf area ( $SLA$ ,  $m^2 kg^{-1}$ ) 50 randomly selected leaves were collected from each tree at each harvest date. Images of single leaves were taken with a 12 Megapixel camera and millimetre paper as a background below the leaf. The leaf area was analyzed with Mesurim Pro software, version 3.3 (Académie d'Amiens, Amiens, France). The areas of the 50 leaves of one tree were summed, dry mass determined and  $SLA$  calculated from total leaf area and dry mass. Leaf area of a tree was calculated from the total leaf dry mass of single trees and the  $SLA$  determined from the 50 leaves. Leaf area projection ( $LAP$ ,  $m^2 m^{-2}$ ) was calculated from leaf area and the projected ground area (derived from the average crown diameter measured along two horizontal, perpendicular axes). Leaf area density ( $LAD$ ,  $m^2 m^{-3}$ ) was calculated as the ratio of the total leaf area over the canopy density (derived from the average horizontal crown diameter and the crown depth measured vertically along the trunk axis to the upper canopy height and considering the canopy as ellipsoid).

### 2.4. Statistical analysis

The influence of treatment factors were analyzed with the statistical software package SAS version 9.00 (SAS Institute Inc., Cary, NC, USA). ANOVA of plant traits were performed with a three-factorial (pruning type, water supply and harvest

**Table 1** – Soil chemical properties of the experimental site. Total carbon ( $C_{tot}$ ) and nitrogen ( $N_{tot}$ ) contents, cation exchange capacity (CEC), Bray-I extractable P and K, diethylenetriaminepenta- acetic acid extractable Cu, Fe, Mn and Zn, and bulk density (BD).

Depth (cm)	$C_{tot}$ ( $g kg^{-1}$ )	$N_{tot}$ ( $g kg^{-1}$ )	CEC ( $mmol kg^{-1}$ )	P ( $mg kg^{-1}$ )	K ( $mg kg^{-1}$ )	Cu ( $mg kg^{-1}$ )	Fe ( $mg kg^{-1}$ )	Mn ( $mg kg^{-1}$ )	Zn ( $mg kg^{-1}$ )	BD ( $g cm^{-3}$ )
0–20										
Means	8.52	0.67	36.90	2.72	89.33	2.81	69	386	0.55	1.51
SE	0.40	0.03	1.16	0.41	9.08	0.20	2	13	0.04	0.00
20–40										
Means	4.12	0.45	43.36	0.98	34.17	1.45	33	146	0.30	1.47
SE	0.17	0.02	1.66	0.16	2.87	0.28	1	10	0.05	0.01



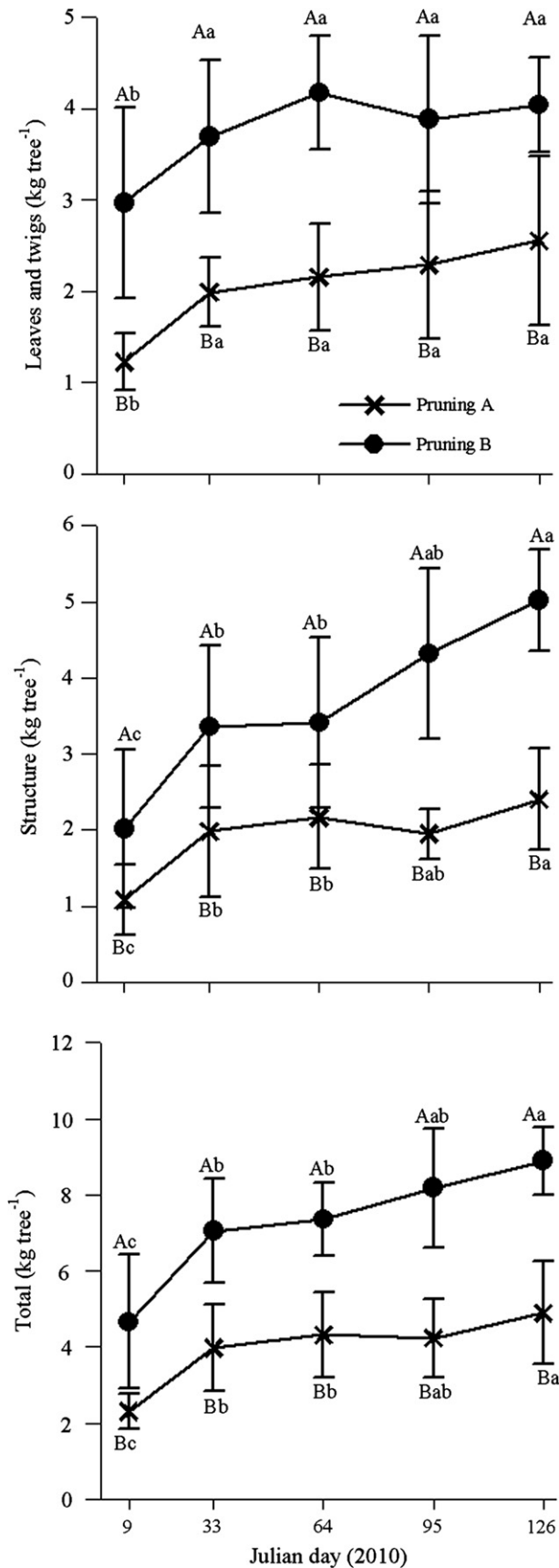


Fig. 3 – Dry weight of leaves and twigs, structural organs, and total dry weight of *Jatropha curcas* L. trees under pruning type A (—x—) and B (—●—) at 5 harvest dates. Data

branches was greater under pruning B compared to A. Average length of primary branches under pruning A ( $40 \pm 12$  cm) was shorter than under pruning B ( $48 \pm 9$  cm). Differences in number and length of tertiary branches were not significant.

Dry weight of leaves, twigs and structure (trunk and branches) and total dry weight were significantly different between pruning types and harvest dates, but not affected by water supply (Table 3, Fig. 3). Dry weight of leaves and twigs only increased from harvest 1 to 2, while no significant further increase in dry weight was recorded later on. Over all harvest dates, dry weight of leaves and twigs was higher for pruning B compared to pruning A. Structural dry weight was affected by interaction of harvest dates and pruning type. Dry weight of structure and total dry weight increased over the experimental period, was consistently higher under pruning B than A with less pronounced increases in dry weight over time under pruning A. Total aboveground biomass increased from  $2.3 \pm 0.5$  to  $4.89 \pm 1.4$  kg tree<sup>-1</sup> ( $n = 10$ ) and from  $4.6 \pm 1.8$  to  $8.9 \pm 1.0$  kg tree<sup>-1</sup> ( $n = 10$ ) for pruning type A and B, respectively.

Stem wood density (SWD) is a parameter which is required for calculating the construction costs of tissue in modeling of trees. In a greenhouse study with seedling plants SWD was not affected by water supply and genotype and varied between 0.25 and 0.26 g cm<sup>-3</sup> [16]. Our data with SWD of primary branches of  $0.25 \pm 0.01$  are comparable to these values and were not affected by pruning, water supply and harvest dates.

Pruning type and irrigation had no effects on the relative dry mass allocation of leaves, twigs and the structural components (ANOVA results not shown). Relative allocation to structural dry mass (trunks and branches) increased from harvest date 1 to 5, while that of twigs did not change over time (Fig. 4). Relative allocation of dry mass of leaves was higher at harvest dates 1 and 2 and decreased toward harvest date 5.

### 3.2. Allometric parameters

The analysis of allometric relationships indicated that regression at harvest 3 and 4 showed a better goodness of fit ( $y = 0.05 L^{1.19}$ ,  $r^2 = 0.36$  and  $y = 0.02 L^{1.39}$ ,  $r^2 = 0.44$ , respectively) compared to other harvest dates (Fig. 5). The relation between the total length of structural components (L) and the dry weight of leaves and twigs (DW) resulted in a positive linear regression ( $DW = 0.78 + 0.47 L$ ,  $r^2 = 0.73$ ) (Fig. 6). We further analyzed the relationship between the lengths of newly formed twigs per unit length of already existing structure ( $L_T/L_S$ ) of 1st to 3rd order branches. Statistical analysis of this ratio indicated an interaction of harvest dates and pruning (Table 3). This interaction was due to a decrease and increase at harvest 2 of the ratio for pruning A and B, respectively (Fig. 7). At the other four harvest dates the ratio was similar under both pruning techniques with an average value of  $4.26 \pm 1.25$  cm of newly formed twigs per cm of already existing branches.

are pooled over water treatments. Capital and small letters indicate differences between pruning type and harvest dates, respectively (Pr > F: 0.001).

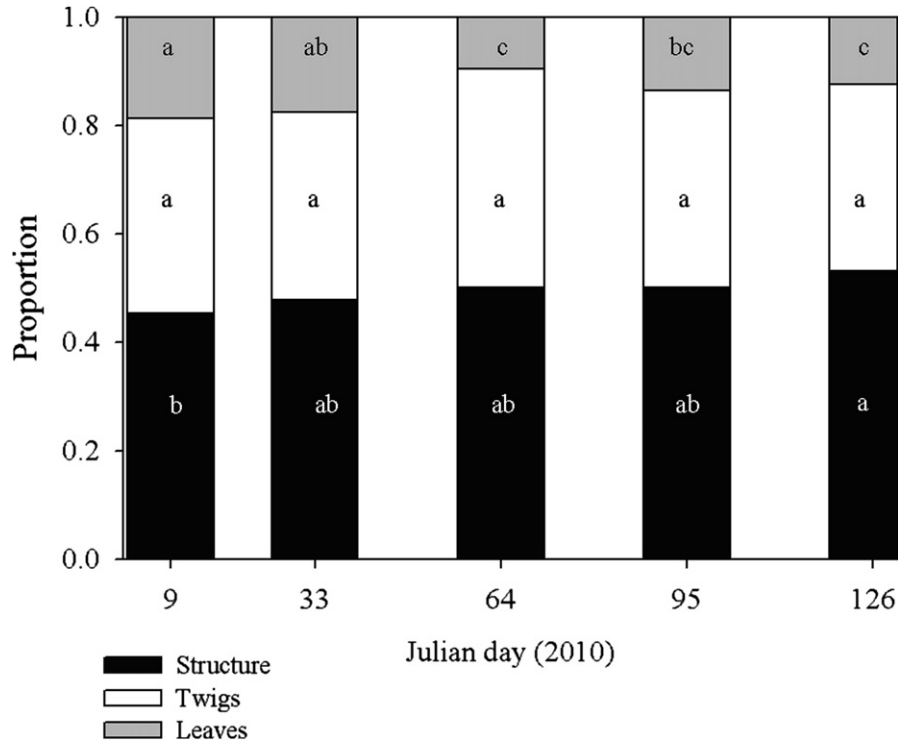


Fig. 4 – Relative allocation of dry weight to leaves, twigs and structure of *Jatropha curcas* L. trees over 5 harvest dates. Data are pooled for pruning type and water treatments. Different letters indicate significant differences between harvest dates.

### 3.3. Canopy characteristics

SLA was not significantly different between pruning techniques, harvest dates and water supply and was, pooled over treatments, on average  $13.57 \pm 0.72 \text{ m}^2 \text{ kg}^{-1}$ . LAP was larger under pruning type B than A (Table 3, 4) and increased from harvest 1 to 4 and decreased at harvest 5. Analyzing the leaf

canopy in context of the spatial dimensions by considering the tree canopy as an ellipsoid, LAD was not affected by water treatments but was affected by interaction of pruning and harvest dates (Table 3, 5). LAD increased from harvest date 1 to 4 with a more remarkable increase under pruning B compared to A and, under both pruning techniques, significantly decreased at harvest date 5.

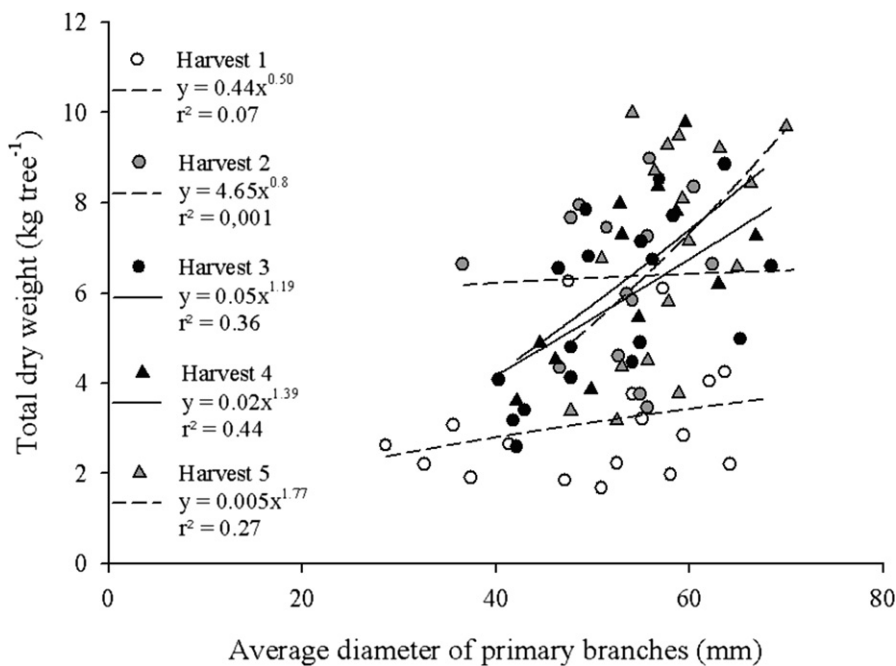
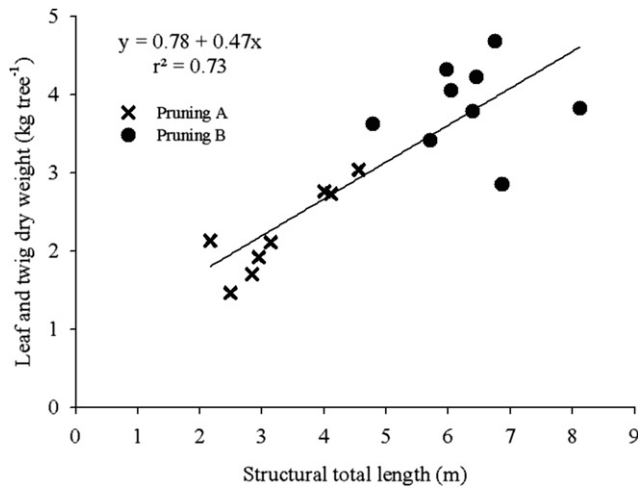


Fig. 5 – Regression between average diameter of primary branches and total dry weight of *Jatropha curcas* L. at harvest dates.



**Fig. 6 – Regression between structural total length (sum of branch lengths) and leaf and twig dry weight of *Jatropha curcas* L. at the last harvest date under the two different pruning types.**

## 4. Discussion

### 4.1. Structural parameters and dry weight increase over time

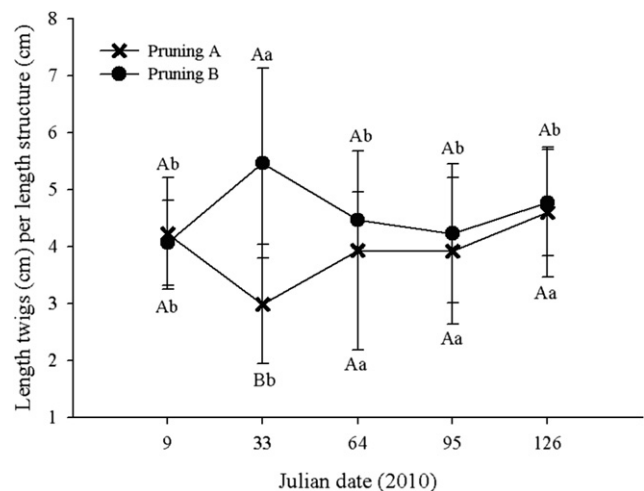
This study illustrated pronounced effects of pruning on biomass formation on a jatropha plantation in Madagascar. As indicated by soil nutrient analyses (Table 1), the plantation site was most likely limited by nitrogen and phosphate availability. In so far, the structural traits reported in this study are typical for a low-input system of jatropha plantation. As opposed to the frequently stated good performance of jatropha under conditions of low resource availability, we assume that jatropha is highly responsive to improved nutrient availability in this region of Madagascar which is not primarily limited by water availability. Although biomass growth during the season was quite high we observed a complete failure in seed production for unknown reason.

Pruning of jatropha trees is used to optimize the tree architecture by maximizing the number of lateral branches for a given planting density [17]. Behera et al. [17] found that pruning the main branch of 2-years old jatropha at 30–45 cm height was ideal for maximizing growth as indicated by annual increment in plant height. However, evidence from work of da Schio [21] indicates that seed yield of non-pruned trees (with intercropping) was higher than that of pruned ones. The study we performed allowed for the comparison of jatropha trees with pronounced differences in architecture induced by pruning. Trees with smaller trunk height, bigger trunk diameter and longer primary and secondary branches (pruning B), had a higher total biomass toward the end of the growing period and a higher biomass increase over the experimental period.

Classical allometric relationships allow for the prediction of total aboveground biomass from stem diameter [22]. Achten et al. [19] proposed a regression model predicting the total aboveground biomass (excluding the trunk) of jatropha as

a function of the average base diameter ( $d$ ) of first order branches ( $DW_{tot} = 0.03 \times d^{2.68}$  for adult trees). In this study, the parameters of the regression model varied depending on the harvest dates, differed from that of Achten et al. [19], and showed a poor goodness of fit, indicating that this regression approach is not suitable for predicting total biomass of jatropha at this site. However, total length of branches appeared as a decisive parameter for biomass increase in our study (Fig. 6). This positive relationship indicates that total length of branches can be used as an indicator of biomass productivity. This can be expected from a physiological point of view, as total length of branches, more than stem diameter, is functionally related to carbon assimilation as leaves are attached to these branches. However, a prediction of productivity of jatropha plantation sites with differences in plant age might be better predicted by differences in stem diameter, as stem diameter is known to increase with plant age, whereas total length of branches of these sites might be similar due to pruning practices.

The relationship between length of already existing branches and length newly formed twigs has not been assessed in jatropha so far, but may be of relevance as length and number of branches can be manipulated by pruning. Our results suggest a more or less constant ratio between length of newly formed twigs and branches such that productivity in terms of twig formation is positively related to the length of already existing branches. The deviation from the more or less constant ratio at harvest date 2 (Fig. 7) cannot be explained and needs to be analyzed in further studies focusing on more detailed data on twig growth, leaf formation and volume growth of branches which exhibited a high seasonal dynamic. Similarly to the findings of Achten et al. [19] water supply had no effect on relative biomass allocation and did not differ between the two pruning types, indicating that allocation pattern are conserved under contrasting pruning history and water availability. However, relative allocation is not constant



**Fig. 7 – Lengths of twigs per lengths of structural organs of *Jatropha curcas* L. trees under pruning types A and B. Data are pooled over water treatments. Interaction between pruning techniques and harvest dates was significant at  $P > F: 0.05$ . Capital and small letters indicate significant differences between pruning techniques and harvest dates, respectively.**



**Table 4 – Leaf area projection (LAP, m<sup>2</sup> of leaves per m<sup>2</sup> of projected ground area) of *Jatropha curcas* L. trees under two different pruning types on a plantation in Madagascar. Data are pooled over water treatments. Capital and small letters indicate significant differences between pruning types and harvest dates, respectively.**

	Pruning A	Pruning B	Average
Harvest 1	3.26 ± 1.34	3.47 ± 1.04	3.35 ± 1.19 c
Harvest 2	3.80 ± 1.33	5.98 ± 1.81	4.83 ± 1.89 b
Harvest 4	6.00 ± 1.01	7.37 ± 1.71	6.81 ± 1.59 a
Harvest 5	3.78 ± 1.10	4.33 ± 0.75	4.05 ± 0.96 bc
Average	4.07 ± 1.53 B	5.40 ± 2.05 A	

throughout the growing season as indicated by an increased allocation of dry mass into structural components with increasing age. This temporal shift in relative allocation should be considered in modeling approaches.

Newly formed twigs are the structure on which leaves will be harbored and decide on the spatial exploitation pattern of the tree (crown shape). Assessment of canopy area in single bushes and trees is experimentally difficult as the not-closed and strongly heterogeneous canopy restricts the use of indirect methods of leaf area estimates [23]. Alternatively, canopy area can be calculated from leaf dry mass and SLA. Our data of SLA are slightly lower (20% less) than the 17 m<sup>2</sup> kg<sup>-1</sup> reported by Maes et al. [16]. SLA is known to be affected by the prevailing light regime with an increase of SLA with under low-light conditions. As pruning had no effect on SLA were not consistently observed, we conclude that, due to the wide spacing (4 m × 2 m) and high light environment, effects of light intensity on SLA were not relevant in this study.

LAI and LAP are key parameters in production ecology and are used for predicting primary production [24]. LAP increased over the first 4 harvest dates and decreased toward the end of the growing season. Our LAP data are similar to values of seasonal LAI dynamics reported by Behera et al. [23] for one-year old non-pruned *jatropha* (from 0.23 to 4.82) and 4-years old *jatropha* stands (from 3.35 to 6.81). Worth mentioning that calculation of LAI per extrapolation to the 1250 trees ha<sup>-1</sup> resulted in an increase from 0.51 to 1.69 and from 1.44 to 3.14 for pruning A and B, respectively, from harvest 1 to 4. Due to the central function of LAI, and therefore of LAP considering a low density planting, in terms of light interception, the higher LAP of *jatropha* at pruning B formed the basis of higher biomass production.

**Table 5 – Leaf area density (LAD, m<sup>2</sup> of leaves per m<sup>3</sup> of canopy volume) of *Jatropha curcas* L. trees under two different pruning types on a plantation in Madagascar. Data are pooled for the water treatment. Capital letters and small letters represent significant differences between pruning types and harvest dates, respectively.**

	Pruning A	Pruning B
Harvest 1	3.64 ± 1.81 A ab	2.03 ± 1.33 B b
Harvest 2	3.02 ± 1.32 A ab	3.80 ± 1.29 A a
Harvest 4	4.54 ± 1.07 A a	3.94 ± 1.36 A a
Harvest 5	2.82 ± 0.69 A b	2.17 ± 0.45 A b

Additionally to LAP the three-dimensional distribution of leaf area is of relevance in canopy analyses of bushes and trees which are not forming a closed canopy. Canopy structure (spatial distribution and individual canopy components) and consequently the LAD can be an indicator of plant growth and productivity [25] and could be used to define the quantity and pattern of radiation interception. Abraha and Savage [26] reported values of LAD from 0.52 m<sup>2</sup> m<sup>-3</sup> to 3.39 m<sup>2</sup> m<sup>-3</sup> at different developmental and growth stages and canopy characteristics of *jatropha*. Our LAD data were similar to these values and were similar under the two pruning types. The similarity of LAD values under both pruning types as opposed to the differences in LAP indicate that the larger leaf area under pruning B was proportionally invested in exploiting a larger crown value at the same time. In so far LAD appears as a parameter which, in this case, is not explaining pruning differences in biomass increase.

## 5. Conclusion

Investigating effects of pruning on growth, canopy size and leaf area density of *J. curcas* trees is relevant for management of a plantation. Pruning of the *jatropha* trees at a lower height of trunk and allowing for longer primary branches did not change the wood density and resulted in a higher productivity in terms of total aboveground biomass independently of the water supply. As opposed to an allometric relationship between the average diameter of primary branches and total above ground biomass suggested by Achten et al. [19], our data suggest that these traits were not constantly correlated. In order to verify this allometric relationship further data from other plantation sites would be required. Our data indicate that the length of newly formed twigs, where the leaves are attached to, can be related to the length of already established branches. LAD and relative dry mass allocation to leaves were not affected by the two pruning techniques, indicating that pruning differences in leaf area size were proportionally converted to corresponding pruning differences in the canopy volume exploited by plants. Further work should direct attention to test if differences in LAP always result in constant LAD when different eco-types of *jatropha* are compared.

The reported field experiment on 4-year old *jatropha* indicates that the biomass production and canopy size depended mainly on primary branch length but not on water supply. We therefore suggest to study in-depth the optimization of branch length and number, canopy volume and shape for an optimization of light interception, growth and profitability.

## Acknowledgment

This study is a part of the project «*Jatropha* Anbau in Madagaskar». We gratefully acknowledge the funding of this work by EnBW-Regenwaldstiftung, Stiftung Energieforschung Baden-Württemberg, project No. 08A26908. This work was carried out in partnership with Jatrogreen S.A.R.L., Antananarivo, Madagascar.

## Appendix

**Dry weight ( $T\ ha^{-1}$ ) of leaves and twigs, structure and total dry weight of *Jatropha curcas* tree under pruning type A and B at 5 harvest dates. Data are pool over water treatments. Capital and small letters indicate differences between pruning types and harvest dates, respectively ( $Pr > F: 0.001$ ).**

	Leaves and twigs DW		Structure DW		Total DW	
	Pruning A	Pruning B	Pruning A	Pruning B	Pruning A	Pruning B
Harvest 1	1.5 ± 0.4 B b	3.7 ± 1.3 A b	1.3 ± 0.6 B c	2.5 ± 1.3 A c	2.9 ± 0.59 B c	5.8 ± 2.2 A c
Harvest 2	2.5 ± 0.5 B a	4.6 ± 1.0 A a	2.5 ± 1.1 B b	4.2 ± 1.3 A b	5.0 ± 1.4 B b	8.8 ± 1.7 A b
Harvest 3	2.7 ± 0.7 B a	5.2 ± 0.8 A a	2.7 ± 0.8 B b	4.3 ± 1.4 A b	5.4 ± 1.1 B b	9.2 ± 1.2 A b
Harvest 4	2.9 ± 1.0 B a	4.8 ± 1.1 A a	2.4 ± 0.4 B ab	5.4 ± 1.4 A ab	5.3 ± 1.3 B ab	10.2 ± 2.0 A ab
Harvest 5	3.2 ± 1.2 B a	5.0 ± 0.6 A a	3.0 ± 0.8 B a	6.3 ± 0.8 A a	6.1 ± 1.7 B a	11.1 ± 1.1 A a

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## Declaration of Originality

Hereby I declare that this doctoral thesis is independently written by myself. In addition, I confirm that no other sources than those specified in the thesis have been used. I assure that this thesis, in the current or similar format, has not been submitted to any other institution in order to obtain a Ph.D. or any other academic degree.

Ich erkläre hiermit, dass ich diese Dissertation selbständig angefertigt habe. Es wurden nur die im Literaturverzeichnis aufgeführten Hilfsmittel benutzt und fremdes Gedankengut als solches kenntlich gemacht. Ich versichere, dass ich diese Arbeit in gleicher oder ähnlicher Form noch keiner anderen Institution zur Prüfung vorgelegt habe.

Hohenheim, November 2 2011

A handwritten signature in black ink, appearing to read 'Arisoa' with a stylized flourish underneath.

Arisoa Mampionona Rajaona

# Curriculum Vitae

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