PUBLISHED ARTICLE

***Citation**

Achten WMJ, Maes WH, Reubens B, Mathijs E, Singh VP, Verchot L, Muys B 2010. Biomass production and allocation in Jatropha curcas L. seedlings under different levels of drought stress. Biomass & Bioenergy 34, 667-676.

DOI: 10.1016/j.biombioe.2010.01.010

Authors: W.M.J. Achten[a†,*], W.H. Maes[a†], B. Reubens[a], E. Mathijs[b], V.P Singh[c], L. Verchot[d] and B. Muys[a,*]

[a] Katholieke Universiteit Leuven, Division Forest, Nature and Landscape, Celestijnenlaan 200 E Box 2411, BE-3001 Leuven, Belgium
[b] Katholieke Universiteit Leuven, Division Agricultural and Food Economics, Willem de Croylaan 42 Box 2424, BE- 3001 Leuven, Belgium
[c] World Agroforestry Centre (ICRAF) Regional office for South Asia, CG Block, 1st Floor, National Agricultural Science Centre, Dev Prakash Shastri Marg, Pusa, New Delhi - 110 012, India
[d] Centre for International Forestry Research, P.O. Box 0113 BOCBD, Bogor 16000, Indonesia

[†]Authors equally contributed to this work
[*]Corresponding authors:
ir. Wouter MJ Achten, Prof. dr. ir. Bart Muys,
Phone: +32 (0) 16 329721; Fax: +32 (0) 16 329760;
E-mail address: wouter.achten@ees.kuleuven.be (WMJ. Achten);

bart.muys@ees.kuleuven.be (B. Muys)

Title

Biomass production and allocation in *Jatropha curcas* L. seedlings under different levels of drought stress

Authors

W.M.J. Achten[a†,*], W.H. Maes[a†], B. Reubens[a], E. Mathijs[b], V.P Singh[c], L. Verchot[d] and B. Muys[a, *]

Affiliations

[a] Katholieke Universiteit Leuven, Division Forest, Nature and Landscape, Celestijnenlaan 200 E Box 2411, BE-3001 Leuven, Belgium

[b] Katholieke Universiteit Leuven, Division Agricultural and Food Economics, Willem de Croylaan 42 Box 2424, BE- 3001 Leuven, Belgium

[c] World Agroforestry Centre (ICRAF) Regional office for South Asia, CG Block, 1st Floor, National Agricultural Science Centre, Dev Prakash Shastri Marg, Pusa, New Delhi - 110 012, India

[d] Centre for International Forestry Research, P.O. Box 0113 BOCBD, Bogor 16000, Indonesia

---

[†] Authors contributed equally to this work

[*] Corresponding authors. Tel.: +32 (0) 16 329721; Fax: +32 (0) 16 329760;

E-mail address: wouter.achten@ees.kuleuven.be (W.M.J. Achten);

bart.muys@ees.kuleuven.be (B. Muys)

Abstract

In a greenhouse experiment we applied three levels of drought stress and monitored growth variables and biomass production of *J. curcas* seedlings propagated from three seed accessions. We determined biomass allocation, allometric relationships and plant traits. Well-watered *J. curcas* seedlings grew 0.81±0.15 cm day^{-1} in length and produced 1.49±0.31 g dry biomass day^{-1}. Under medium stress (40% plant available water) the plants maintained a similar stem shape, although they grew at lower rate (stem length: 0.28±0.11 cm day^{-1}; dry biomass production: 0.64±0.18 g day^{-1}). Seedlings under extreme drought stress (no irrigation) stopped growing, started shedding leaves and showed shrinking stem diameter from the 12th day after the start of the drought treatment. The drought treatment did not influence the wood density (0.26 g cm^{-3}). The root/shoot ratio of the wet treatment was 0.27, which is low compared to other tropical trees. Both the biomass allocation and root/shoot were significantly influenced by drought. Plants of the different accessions were uniform in biomass production and plant traits. The allometric relationship predicting total aboveground biomass (B) with the stem diameter (D) ($B=0.029\times D^{2.33}$; $R^2=0.89$) fits well in universal scaling models in which the exponent is expected to converge to ~2.67 at plant maturity. Based on a small validation data set from mature *J. curcas* individuals this hypothesis could be confirmed. A second regression model predicts the total leaf area (LA) as a function of stem diameter ($LA=2.03\times D^{2.41}$; $R^2=0.95$). The estimated transpiration crop coefficient K_{cb} ranged from 0.51 to 0.60 for the well-watered plants.

Keywords

allometric relation; leaf area; leaf mass; leaf size; Physic nut; plant traits; root/shoot; transpiration crop coefficient; water stress

1. Introduction

Jatropha curcas is receiving a lot of attention as a biodiesel feedstock. Expectations of project managers, investors and farmers are triggered by the plant's alleged potential to simultaneously reclaim wastelands, enhance socio-economic development and conserve and/or restore soil fertility in degraded areas [1,2]. These promising characteristics of the "*Jatropha* system" have resulted in numerous *Jatropha* plantation initiatives in the (semi-arid) tropics [3]. Although currently new scientific knowledge on the utility of *Jatropha* biomass [4,5] and yield projection models [6] is emerging and substantial progress is made in selection and diversity assessments of *J. curcas'* genetics [7-9], there is a persistent lack of knowledge on the basic agronomic properties. More particular, the biomass production and the growth response to environmental factors of *J. curcas* is not understood [10,11]. Such knowledge gaps imply that strong expansion of large scale plantations are not without socio-economic and ecological risk [12-14].

Recent research has shed a new light on the water relations and water requirements of *J curcas*. In its natural distribution area, the species grows most commonly in tropical savanna and monsoon climates (A_m, A_w) and requires a minimal annual rainfall of 944 mm year^{-1} [15]. *J. curcas* is a deciduous stem succulent species with a clear drought avoidance strategy in its leaves, a relatively high water use efficiency [16] and most probably a relatively low water footprint [17,18]. However, more experimental and field data are required on this important issue [16], as so far no studies have focused on how growth affects the biomass growth and allometric relations of the species. Descriptions of biomass allocation patterns or empirical allometric models are not available for *J. curcas*.

The aim of this paper is to describe the height growth, biomass production and leaf area evolution of *J. curcas* seedlings germinated from different accessions grown under different levels of drought stress. Based on fresh and dry biomass measurements we determined biomass allocation, allometric relationships and some important plant traits of *J. curcas* seedlings. Hypotheses based on the acquired allometric relationships are then validated with data of mature plants. Furthermore we present an estimation of the *J. curcas'* transpiration crop coefficient K_{cb}. This is a crop specific coefficient which indicates the ratio of the crop transpiration over the reference evapotranspiration when water availability is not limiting transpiration [19]. This information can feed into stand biomass and water use modeling of *J. curcas* plantations.

2. Material and Methods

We established an experiment in a tropical compartment of the K.U.Leuven university greenhouse complex at Heverlee, Belgium, which ran from 2 July, 2007 (sowing date) (summer) till early November 2007 (autumn). *J. curcas* seeds originating from (*i*) Ethiopia (Arba Minch), (*ii*) India (Lucknow) and (*iii*) Thailand (Nakhon Pathom) (further called 'accessions') were individually sown in the center of pots (height: 20.5 cm; volume: 6.5 liter) filled with a 2:1 river sand:peat mixture of which the pF-curve was established. Eighty one pots (9 replications of 3^2 combinations of treatments: three levels of Accession and three levels of Drought stress; see 2.1) were randomly arranged in a Latin square design [20]. The distance between columns and rows was 40 cm. This setup was also used to derive the plant-water relations and growth strategies of *J. curcas* (see [16]).

2.1. Growth conditions

In July and August 2007, all plants were allowed to grow for 64 days in optimal conditions (further called growth phase or GP). Diurnal variation range in air temperature was controlled and kept between 17°C and 27°C. The relative humidity was 70% and the pots were watered with a solar radiation-dependent drip irrigation system, keeping them at field capacity (determined from the pF-curve). The water contained a balanced nutrient mixture (N: 153.6 mg kg^{-1}; P: 29.6 mg kg^{-1}; K: 99.3 mg kg^{-1}; Ca: 188.1 mg kg^{-1}; Mg: 48.5 mg kg^{-1}).

After the growth phase (GP), the treatment phase (TP) started from 3 September till 25 October 2007. The relative air humidity was lowered to 30-40%, while the temperature was kept as during the GP. In this artificial environment the CO_2 concentration averaged around 500-600 ppm during the whole experiment. During the TP following drought treatments were applied: (*i*) 'dry' (pots were not watered), (*ii*) 'medium' (pots were irrigated up to 40% Plant Available Water (*PAW*), an average threshold value at which gas exchange response to water deficit appears [21]) and (*iii*) 'wet' (the control treatment where pots were watered up to field capacity). Based on the obtained pF-curve of the substrate a target soil volumetric water content (θ_v) for each treatment was calculated. The relationship between the pot weight and θ_v was established and the target weight per pot calculated. Water evaporation from the substrate was minimized by covering the soil surface and the bottom of the pots with aluminum foil [22,23]. In order to control the target pot mass the plants were manually watered standing on a balance. Watering was performed three times per week (on Monday, Wednesday and Friday) throughout the TP, with the same nutrient-enriched water used in the GP. The θ_v was measured along with the growth measurements (see 2.2) with a TRIME-FM3 Field Portable TDR Meter equipped with a P3 Probe of 15cm length (Imko, Ettlingen). These measurements allowed to check the target pot mass and to recalculate the target mass at different moments during the experiment to correct for biomass increment. Infection by pests or diseases was regularly checked, and two seedlings were removed from the experiment after infection by Spider mite (*Tetranchyus* sp.).

2.2. Measurements

On day 64, 78, 92 and 116 growth was monitored by recording the number of leaves (>1cm^2), number of branches, stem length (measured vertically from substrate surface till apical meristem) [cm], stem diameter at several fixed heights [mm], branch length [cm] and diameter [mm] at the base and top of the branches. Fresh stem volume and total wood volume (stem + branches) [cm^3] were calculated using Smalian's sectional volume formula [24]. The form factor of each individual was calculated as the ratio of its stem volume to the volume of a solid cylinder with similar basal diameter and height [24]. Water use was monitored by recording the pot mass before and after watering. During the GP an extra group of nine plants (3 of each accession) was grown to practice the irrigation method (they were all kept wet) and measurements. For this subset (further referred to as 'practice plants') the leaf dimensions (leaf length (LL) and width (LW)) were measured every two weeks.

Within one week after the final growth measurement (day 116, 25 October 2007), all seedlings were harvested by cutting the stems at substrate level. The roots were exposed by washing down the substrate. The fresh mass of the leaves, stem + branches and roots (excluding fine roots) were determined separately. Dry mass of all leaves, stems + branches and roots was determined after oven-drying at 105°C until constant weight. Similar data were previously collected on five younger plants (23-41 days old), growing in the same conditions, as part of a parallel running experiment [25]. The base of the stem was visibly woodier than the rest of the stem + branches. For a subset of 27 plants (three individuals of nine combinations of factor levels) of the 79 seedlings, the fresh mass and volume and dry mass of this woodier stem part were determined separately. The total leaf area (*LA* [mm^2]) was recorded for the same subset with a LI-COR leaf area meter (LI-COR, Nebraska) (accuracy: 0.1 mm^2). From the leaves of these 27 plants, 89 leaves were randomly selected for measurement of leaf length (*LL*) and width (*LW*) prior to the measurement of the leaf size (LS) with the LI-COR leaf area meter. This information permitted to calculate the *LA* of the nine practice plants at the different *LL* and *LW* measurement moments (see above) and to include these data in the modeling of the *LA* (see further).

2.3. Biomass allocation

Root/shoot ratio (= dry root biomass divided by total dry aboveground biomass [26]) and mean dry leaf mass (= total dry leaf mass divided by number of leaves) were calculated for each individual. Stem density (= dry stem + branch mass divided by total wood volume), wood density (= dry mass of woodier stem part divided by fresh volume of that part) and mean leaf size are calculated for the 27 plants in the subset.

We calculated the dry biomass allocation to leaves, stem + branches and roots as the proportion of the total dry biomass invested in these plant parts. The leaf biomass used to calculate the allocation to leaves included that of leaves shed before the end of the experiment. This was determined by multiplying the amount of shed leaves with the mean leaf mass of the corresponding drought treatment.

2.4. Allometry

Stem diameter is often used as a predictor variable in allometric relationships to estimate the aboveground dry biomass [27]. Generally these empirical relationships are analytically expressed as power functions, because it has long been noted that a growing plant maintains the proportions between different parts [27].

$$B = a \times D^b \qquad \text{(Eq. 1)}$$

where *B* is the total aboveground dry biomass, *D* the diameter (at the base or at breast height), and *a* and *b* the scaling coefficient and exponent, respectively [24,27]. Following Pilli et al. [27] empirical relationships were only determined for actively growing plants. As the seedlings of the dry treatment had stopped growing aboveground they were not included in the dataset. In order to have a representation for a wider range of diameters, the data of the five young plants of the parallel running experiment were included. From the total of 55 records in this dataset (two outliers were removed), 41 records (75%) were randomly selected to determine the empirical allometric relationship using model II regression analysis. The remaining 14 records formed a dataset for model validation.

The relationship between *LS* and *LL*, *LW* and *LL*×*LW* was investigated fitting linear, square and power functions [28] on 89 randomly selected leaves from dry, medium and wet seedlings in the subset of 27 plants. The best relationship was selected using R^2, *F*-value and residual plots as criteria.

The allometric relationship between stem diameter and *LA* was only established for the wet treatment plants. This is because insufficient records of seedlings in the other drought treatments (nine per treatment) were available to establish separate relationships. Due to different leaf loss rates (see further) it was not possible to combine different Drought treatments in one relationship. The LA-database of these nine wet treatment plants was complemented with the LAs of the nine practice plants, which were calculated for two moments (32 days old and 52 days old) based on the measured relationship between *LL*×*LW* and *LS* (see above). The *D-LA* relationship was calculated fitting a power function. Similar to the aboveground dry biomass estimation, a randomly selected subsample of 75% of the records formed the base for the empirical model, while the other 25% served as a validation dataset.

2.5. Validation of allometry on mature plants

A small set of data on mature *J. curcas* plants was collected from three *J. curcas* plants of three years old (spacing: 2×4 m^2) and three individuals of 12 years old (spacing: 4×4 m^2) in the plantations of the CCS Haryana Agricultural University (Bawal Regional Research Station, Haryana State, India). All plants were propagated from seed in the univeristy nursery and planted in the field at an age of six months . For the six trees the stem diameter at ground level, the base diameter of all first order branches and the total aboveground fresh and dry weight were determined. With this data a validation was made on the allometric finding of the experiment (see 4.4).

2.6. Transpiration crop coefficient K_{cb}

The transpiration crop coefficient K_{cb} can be calculated as [19]

$$K_{cb} = \frac{ET_{pot}}{ET_0} \quad \text{(Eq. 2)}$$

where ET_{pot} is the potential crop evapotranspiration under full water availability and ET_0 is the reference crop evapotranspiration. As during the experiment water evaporation from the substrate was minimized to negligible level, ET_{pot} was assumed equal to the plant transpiration [22,23]:

$$ET_{pot} = T = DWU_i - L_{lost} \quad \text{(Eq. 3)}$$

where DWU_i is the daily water use at measuring time i and L_{lost} is the fresh mass of leaves shed between time i-1 and i. DWU_i was calculated as the difference between the pot mass before watering on time i and the pot mass at time $i-1$ (= the time immediately after the previous watering), divided by the number of days between $i-1$ and i. Converting the DWU dimension from g day^{-1} to mm day^{-1} was based on a mean surface cover per plant of $40 \times 40 cm^2$ (according to plant spacing after canopy closure). To estimate L_{lost} the estimated number of leaves lost in that period was multiplied by the mean fresh leaf mass per drought treatment. The number of leaves was counted every two weeks, and it was assumed that leaves were shed linearly during this fortnight, which allowed estimation of leaf loss during the period between $i-1$ and i. ET_0 was estimated with the FAO Penman-Monteith equation [19]. Air temperature (T_a, [°C]), relative air humidity (RH, [%]) and Photosynthetically Active Radiation (PAR [$W \cdot m^{-2}$]) in the greenhouse were measured every thirty minutes. Because air currents are hard to measure in a greenhouse environment, arbitrarily set wind velocities (u_1=0.1 m s^{-1} and u_2=0.5 m s^{-1}), corresponding with minimal and maximal possible wind velocity in a greenhouse, were used. As such we could calculate the range between which the true K_{cb} lies. The transpiration crop coefficient K_{cb} was calculated per plant and per watering day. This was only done for the plants of the wet treatment as ET_{pot} could only be measured when water is not limiting transpiration. The mean K_{cb} per plant was determined over a two-month period after canopy closure, excluding observations of five days on which water availability was limiting due to high PAR.

2.7. Statistical analysis

The influence of the fixed factors, Accession (three levels) and Drought treatment (three levels) on growth and plant traits was investigated. The plant structure characteristics were analyzed with a type III multifactor multivariate repeated measures ANOVA with factors Accession and Drought treatment (within-subject factor time, four levels). The influence of the fixed factors on the plant traits was analyzed with a type III univariate ANOVA. The data on the biomass allocation, stem and wood density were analyzed using a type III multifactor multivariate repeated measures ANOVA.

The performance of the allocation models was checked by plotting the relative residuals (the differences between the estimated and measured values of the validation dataset) against the diameter at stem base. Linear regression analyses were used to check if the residuals showed a trend.

All statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL). Mean values are given ± standard deviation, except indicated otherwise.

3. Results

3.1. Growth

The θ_v measurements and the repeated measures ANOVA showed that the Drought treatment served its purpose (data not shown). In less than two weeks the *PAW* significantly ($P < 0.001$) differed between the three Drought treatments. On the days the plants were watered, the *PAW* in the wet treatment was always higher than 40%, the medium treatment had reached 40% after two weeks and afterwards was watered up to this threshold, while the dry treatment passed this threshold after one week of treatment.

The growth trends of *J. curcas* seedlings under different drought treatments are shown in Fig. 1. Results from the ANOVA analysis are given in Table 1.The Drought treatment significantly influenced the growth variables, while Accession had no significant effect. Time (i.e. plant age) and the Time × Drought interaction significantly ($P < 0.001$; Table 1) influenced stem length, number of leaves, diameter at the stem base, stem volume, form factor and total volume (volume stem + branches).

The seedlings of the wet treatment kept growing steadily (length, diameter at stem base and volume) throughout the entire period. From the age of 78 days onwards, when the target *PAW* values were reached, wet- and medium drought-treatment plants grew 0.81±0.15 and 0.28±0.11 cm day^{-1} in length and increased their total wood volume with 3.72±0.86 and 1.33±0.38 cm^3 day^{-1}, respectively. During the treatment period (52 days) wet treatment plants produced an average of 1.49±0.31 g dry biomass per day, medium treatment plants 0.64±0.18 g day^{-1}. Over the whole growing period of 116 days the wet and medium treatment seedlings produced 109±16 and 66±9 g of dry biomass. Medium and wet treated plants clearly differed in biomass production rate. During the treatment period the stem length increase of seedlings in the wet treatment (86%) was twice as much as in the medium treatment (43%), and total woody volume increment was 2.7 times higher (wet: 223% and medium: 90% increase). This corresponds to a dry biomass production rate which is 2.3 times higher in the wet treatment plants than the plants of the medium treatment. The form factor for wet and medium treatments decreased during the treatment period.

The dry treatment plants showed a different growth pattern. After two weeks of treatment (78 days old) the height growth of the plants halted (mean length growth = 0.03±0.03 cm day^{-1}) (PAW had decreased to 12%) and the number of leaves was at its maximum. From then on the diameter of stem base and stem volume decreased (mean volume increase = -0.20±0.49 cm^3 day^{-1}), leveling the form factor from 78 days onwards (Fig. 1). Between days 78 and 116 the dry plants on average lost 1 leaf every two days (-0.48±0.15 leafs day^{-1}).

(Insert Table 1)

(Insert Fig. 1)

3.2. Biomass characteristics

Stem density (0.20±0.01 g cm^{-3}) and wood density (0.26±0.03 g cm^{-3}) did not differ significantly between the Drought treatments or Accessions. The root/shoot ratio was significantly different for the three different Drought treatment levels and was the highest for the dry treatment and the lowest for the wet treatment (Table 2), while Accession had no effect. The root/shoot ratio calculated with total aboveground dry woody biomass (= stem + branches) did not show any significant difference between the different factor levels (Table 2).

(Insert Table 2)

The Drought treatment significantly influenced the biomass allocation ($P < 0.001$), while Accession did not. In Fig. 2 the estimated marginal means (means adjusted for the covariates, if any) and the 95% confidence intervals (Bonferroni) of the portion of the different plant parts relative to the total dry biomass are shown. The medium and wet treatment plants showed similar biomass allocation (roots: ~21%; wood: ~45% and leaves: ~34% of total biomass) (Fig. 2). About 42-44% of the total aboveground dry biomass was allocated to the leaves. Compared to the wet and medium plants, the seedlings of the dry treatment stored significantly more biomass in their woody parts (stem+branches) (medium: $P = 0.03$; wet: $P < 0.001$) and significantly less in their leaves (medium: $P = 0.01$; wet: $P < 0.001$). Note that the mass of shed leaves was included in these calculations. The root portion of the plants in the dry treatment was the highest, and was only significantly different from the wet treatment plants ($P = 0.03$).

(Insert Fig. 2)

The black bars in Fig. 2 show the relative loss of leaf mass during the treatment period. At the end of the experiment the plants in dry treatment had lost 58% of the biomass allocated to the leaves. The medium treatment plants lost 28% of the total produced leaf biomass, while the wet treatment lost 12% of the total produced leaf biomass.

3.3. Allometry

In Fig. 3 the empirically obtained allometric relationship estimating the aboveground dry biomass from the diameter is shown. When this model was applied to the validation data set, the estimated values, on average, differed -5.5 % (±17%) from the observed values. In the scatter plot of the residuals no trend could be distinguished (R^2 = 0.001), indicating that this model gives estimates without systematic error.

(Insert Fig 3)

3.4. Leaf area

Leaf size was best modeled by a power relation with $LL \times LW$ as independent variable, shown in Fig. 4

(Insert Fig. 4)

The obtained empirical relation estimating the total *LA* of seedlings by stem diameter is shown in Fig. 5. On average, the estimated values calculated by this model differ -2% (±27%) from the observed values. In the residual plot no trend could be distinguished (R^2 = 0.03), indicating that this model gives estimates without systematic error.

(Insert Fig. 5)

After 116 days of growth the wet treatment plants had a mean *LA* of 7081±1166 cm^2. The mean *LA* of the medium and dry seedlings was 3319±930 cm^2 and 1007±426 cm^2, respectively.

3.5. Transpiration crop coefficient K_{cb}

Mean daily water use was 239 g day^{-1} or 1.5 mm day^{-1} for the wet treatment seedlings, with peak s up to 2.4 mm day^{-1}. The mean ET_0, estimated over the whole treatment period, ranged from 2.7 mm day^{-1} (at u_1=0.1 m s^{-1}) to 3.1 mm day^{-1} (at u_2=0.5 m s^{-1}). For the wet-treatment seedlings the K_{cb} coefficient ranged from 0.51±0.09 till 0.60±0.11. The K_{cb} did not differ between different Accessions.

4. Discussion

4.1. Biomass production and allocation

Accession had no effect on the studied characteristics (Table 1). This is consistent with results of leaf traits of the same experiment [16], with results concerning stem length and diameter of 11 accessions grown in the field [29] and with results from Kaushik et al. [30,31] indicating predominant effect of seed dimensions and environment on growth above genetic differences between accessions. Differences in seed dimensions would trigger high variability in the results of each accession [31]. Although the seed dimensions were not measured, the low variability within each accession for a given drought treatment (data not shown) suggests that the installed growing conditions had a bigger effect on the growth performance of the *Jatropha* seedlings in this experiment than the genetic differences between the accessions.

J. curcas can still grow at the threshold of water stress (40% PAW) without changing the form of its stem and its biomass allocation pattern. The total dry biomass at the end of the experiment (after 116 days) of both the wet treatment and the medium stressed seedlings was among the highest of earlier reported figures of 104 days old seedlings of ten tropical deciduous woody tree and shrub species [32]. This might indicate that *J. curcas*' biomass production rate, in both optimal watering conditions as with 40% *PAW*, is high in comparison to other tropical tree and shrub species, but can also be due to the high CO_2 concentration (see further). For further discussion on the physiological site of leaf physiology and growth rate of *Jatropha* seedlings we refer to Maes *et al*. [16].

In case of extreme drought (no irrigation) the plants started shedding their leaves within 14 days [16]. At the end of the experiment all plants in all treatments lost some leaves at the bottom of the crown (Fig. 1). This is probably a result of the lower light intensity in combination with the higher competition for light among the different individuals than in the initial stage of the experiment, causing the lowest leaves to drop [33].

The proportion of total dry biomass allocated to leaf biomass in wet and medium treatment seedlings (after 116 days) (0.33-0.35) corresponds with the average (0.35±0.13) of 104 days old seedlings of ten tropical deciduous woody tree and shrub species as given by Huante & Rincón [32]. This indicates that *J. curcas* makes an average or intermediate investment in leaf biomass, in comparison to other tropical trees and shrubs. The proportion of the total aboveground dry biomass represented by the leaves in *J. curcas* (0.42-0.44) is twice as high as that proportion in Mediterranean trees and shrubs [34].

4.2. Plant traits

The root/shoot ratio differed significantly between the Drought treatment levels. However, these differences can be caused by the loss of leaves, which was stronger for the plants under dry conditions than under medium and wet conditions. The root/shoot ratios calculated with total aboveground dry woody biomass (=stem + branches) showed no significant differences between the factor levels. This indicates that the leaves have triggered the significant differences in the root/shoot calculations in which the leaves were included. Although fine roots were not extracted and the root/shoot calculations were problematic because of the leaf loss, both root/shoot calculation and biomass allocation indicate that water-stressed seedlings had significantly higher root/shoot ratios than the wet treatment plants. The root/shoot ratios of the different Drought treatments (calculated with leaves: dry: 0.41; medium: 0.33; wet:0.27) are among the lower values reported for 104 days old seedlings of ten tropical deciduous woody tree and shrub species (0.44 ± 0.16) [32].

The wood density (0.26 g cm^{-3}) is consistent with reported wood densities of *J. curcas* plants of four and 12 years old (0.253 g cm^{-3}) [35]. These values are very low compared to other dryland forest trees, confirming the stem-succulent characteristics in *J. curcas* [16,36].

4.3. Allometry

The scaling coefficient and exponent (parameters *a* and *b* of Eq. 1) are reported to vary with species, site and age [27]. However, West et al. [37] suggested that *b* should scale against *D* with a universal exponent $b \approx 2.67$, because it reflects on an optimal tree architecture. Pilli et al. [27] confirmed this theoretical value for adult plants by applying empirical data from 17 datasets ($b = 2.64 \pm 0.30$). They showed that values of parameter *b* are lower for younger plants ($b = 2.08$-2.51) (36 datasets). The values obtained for *J. curcas* seedlings in this study ($b = 2.33$) agree with the results obtained from these other datasets. As such the above presented empirical allometric relationship of aboveground dry biomass fits well in a universal model [27,37]. Although the relation found in this research is primarily useful for seedlings, it is a first indication that *J. curcas* follows the universal allometric model described by West *et al*. [37]. The next section illustrates that it can be used to estimate the biomass of mature trees as well.

4.4. Validation of allometry on mature plants

Both the empirical seedling allometric relation ($a = 0.029$ and $b = 2.33$) and the universal mature allometric relation ($a = 0.029$ and $b \approx 2.67$) were tested for the stem diameters of the six mature plants. None of these relations predicted the measured aboveground dry weight with acceptable accuracy. This is probably due to the common practice to pinch off/cut back the terminal shoot at 30-45 cm height in the first year after planting [11] which influences the overall plant architecture. However, summing the biomass estimations based on the base diameter of each first order branch using $a = 0.03$ and $b = 2.68$ gave a good prediction (R^2=0.92) of the measured aboveground biomass excluding the stump (i.e. stem) (Fig. 6). This validation, based on a small data set, suggests that the universal allometric model applies to the first order branches of mature *Jatropha* plants. The stump mass (*SM*) [g] was estimated based on its diameter (*D*) [mm] as $SM = -5176 + 38.15 \times D$ ($R^2 = 0.92$), and summing the estimations of the branch and stump biomass gave a very good prediction (R^2=0.99) of the measured total aboveground biomass (Fig. 6).It should be noted that this relation between the stump mass and the diameter is influenced by the pruning, and in other experiments, *SM* could also be estimated based on its volume (diameter and length) and wood density (the wood density reported in this paper, 0.26±0.03 was equal to that of the mature plants, 0.26±0.01).

(Insert Fig. 6)

4.5. Transpiration crop coefficient K_{cb}

The K_{cb} estimation (0.51-0.60, for wet treatment seedlings) corresponds well with the K_{cb} of two 12 year old *J. curcas* trees in South-Africa estimated by Gush and Moodley [35] based on a limited number of sap flow measurements (K_{cb}=0.51, with peak at 0.76).

4.6. Elevated CO_2 concentration

In the greenhouse, the CO_2 level was unintentionally higher than ambient level. It is generally known that growth of young trees is enhanced under increased atmospheric CO_2 through increased carbon uptake [38] which can lead to increased plant height, stem diameter, leaf area index and fine root density [39]. The extent of increasing biomass production is species-specific and, due to lack of knowledge, can not be estimated for *J. curcas*. The absolute results on the seedling growth must be interpreted with care. With respect to biomass allocation and allometry an increase in root/shoot and proportion of leaves is often reported. However, several studies showed that increased growth does not significantly change the root/shoot ratio and is not necessarily associated with a shift in biomass allocation[38,40]. More recent meta-analyses concluded that changes in biomass allocation between leaves, stems and roots are minimal [41-43]. Furthermore, allocation differences tend to disappear after allometric analysis which indicates that eventual differences in allocation were due to size differences and not to the increased CO_2 concentration as such [43]. As previously discussed the root/shoot ratio is rather low compared to these of tropical trees and shrubs. This additionally suggests that the elevated CO_2 concentration probably did not increase these properties and that our estimates reflect true root/shoot relations at ambient levels. A discussion on the effect of elevated CO_2 on *J. curcas'* physiology can be found in [16].

5. Conclusions

With the reported greenhouse experiment on *Jatropha* seedlings we could successfully assess some important plant traits. Wood density was low (26 g cm^{-3}) and independent of Drought treatment. Similarly the generally low root/shoot ratio only increased significantly under extreme drought. The Accessions had no effect on the studied plant traits, growth or biomass allocation. The monitoring of the growth and biomass allocation showed that *J. curcas*, in optimal conditions, grows fast, produces a lot of biomass and achieves a high leaf area in comparison with other tropical deciduous woody species. At the threshold of drought stress (40% PAW) *J. curcas* can still maintain considerable growth and biomass production. Although the growth rate was lower than in wet treatment conditions, the plants maintained similar stem shape and biomass allocation pattern. Under extreme drought *J. curcas* started shedding its leaves and stopped growing. In such situation the biomass allocation showed higher investment in the roots. Well and medium watered *Jatropha* plants showed medium biomass investment in leaves and low biomass investment in roots in comparison to other tropical deciduous woody tree and shrub species.

Furthermore the experiment results in allometic relations which successfully predict aboveground dry biomass and leaf area of the seedlings based on stem diameter. These resulting relations suggest that *J. curcas* fits well into the universal allometric model, a hypothesis which was validated by a small data set of mature *J. curcas* individuals. Additionally the crop coefficient K_{cb} estimations of *J. curcas'* transpiration crop coefficient K_{cb} confirm earlier estimations from two plants in the field. *Jatropha*hese results are useful step towards modeling plantation stand biomass, water use and leaf area.

Acknowledgements

This research is funded by the Flemish Interuniversity Council – University Development Co-operation (VLIR-UDC), and is a collaboration between K.U.Leuven and the World Agroforestry Centre (ICRAF). Seeds were acquired trough Mr. Witoon Jaiphong (Kasetsart University, Thailand), Dr. Dina Nath Tewari (Utthan NGO, India) and Mr. Ywe Jan Franken (FACT, The Netherlands). Dr. Yadav, Dr. N. Kaushik, Dr. K. Kumar, Dr. Deswal and the field laborers of the Department of Forestry of the CCS Haryana Agricultural University Regional Research Centre in Bawal are greatly acknowledged for giving us the opportunity to measure allometric validation data on mature individuals. Special thanks go to ir. Poi Verwilt and the FORECOMAN staff for their support in the field work. The constructive comments provided by three anonymous reviewers are greatly acknowledged.

References

[1] Francis G, Edinger R, Becker K. A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India: need, potential and perspectives of *Jatropha* plantations. Nat Resour Forum 2005;29:12-24.

[2] Zahawi RA. Establishment and growth of living fence species: an overlooked tool for the restoration of degraded areas in the tropics. Restor Ecol 2005;13:92-102.

[3] GEXSI. Global market study on *Jatropha* - final report. Berlin, Germany: GEXSI LLP; 2008. http://tinyurl.com/cnyn44

[4] Gunaseelan VN. Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from *Jatropha* curcus on dry lands. Biomass Bioenerg 2009;33:589-96.

[5] Sharma DK, Pandey AK, Lata. Use of *Jatropha curcas* hull biomass for bioactive compost production. Biomass Bioenerg 2009;33:159-62.

[6] Lapola DM, Priess JA, Bondeau A. Modeling the land requirements and potential productivity of sugarcane and *Jatropha* in Brazil and India using the LPJmL dynamic global vegetation model. Biomass Bioenerg 2009;33:1087-95.

[7] Mishra DK. Selection of candidate plus phenotypes of *Jatropha curcas* L. using method of paired comparisons. Biomass Bioenerg 2009;33:542-5.

[8] Ranade SA, Srivastava AP, Rana TS, Srivastava J, Tuli R. Easy assessment of

diversity in *Jatropha curcas* L. plants using two single-primer amplification reaction (SPAR) methods. Biomass Bioenerg 2008;32:533-40.

[9] Achten WMJ, Nielsen LR, Aerts R, Lengkeek AG, Kjaer ED, Trabucco A et al. Towards domestication of *Jatropha curcas*: a review. Biofuels 2010;1:in press.

[10] Openshaw K. A review of *Jatropha curcas*: an oil plant of unfulfilled promise. Biomass Bioenerg 2000;19:1-15.

[11] Achten WMJ, Verchot L, Franken YJ, Mathijs E, Singh VP, Aerts R et al. *Jatropha* bio-diesel production and use. Biomass Bioenerg 2008;32:1063-84.

[12] Fairless D. Biofuel: the little shrub that could - maybe. Nature 2007;449:652-5.

[13] Achten WMJ, Mathijs E, Verchot L, Singh VP, Aerts R, Muys B. *Jatropha* biodiesel fueling sustainability? Biofuel Bioprod Bior 2007;1:283-91.

[14] Achten WMJ, Maes WH, Aerts R, Verchot L, Trabucco A, Mathijs E et al. *Jatropha*: From global hype to local opportunity. J Arid Environ 2010;74:164-5.

[15] Maes WH, Trabucco A, Achten WMJ, Muys B. Climatic growing conditions of *Jatropha curcas* L. Biomass Bioenerg 2009;33:1481-5.

[16] Maes MH, Achten WMJ, Reubens B, Samson R, Muys B. Plant-water relationships and growth strategies of *Jatropha curcas* L. saplings under different levels of drought stress. J Arid Environ 2009;73:877-84.

[17] Maes WH, Achten WMJ, Muys B. Use of inadequate data and methodological errors lead to an overestimation of the water footprint of *Jatropha curcas*. P Natl

Acad Sci USA 2009;106:E91.

[18] Jongschaap REE, Blesgraaf RAR, Bogaard TA, van Loo EN, Savenije HHG. The water footprint of bioenergy from *Jatropha curcas* L. P Natl Acad Sci USA 2009;106:E92.

[19] Allen RG, Pereira LS, Raes D, Smith M. Crop Evapotransiration - guidelines for computing crop water requirements. Rome, Italy: FAO; 1998.

[20] Dytham C. Choosing and using statistics - A biologist's guide. 2nd ed. United Kingdom: Blackwell Science; 2003.

[21] Sadras VO, Milroy SP. Soil-Water thresholds for the responses of leaf expansion and gas exchange: a review. Field Crop Res 1996;47:253-66.

[22] Sakuratani T. Improvement of the probe for measuring water flow rate in intact plants with the stem heat balance method. Journal of Agricultural Meteorology 1984;40:273-7.

[23] Gutiérrez MV, Harrington RA, Meinzer FC, Fownes JH. The effect of environmentally induced stem temperature gradients on transpriation estimates from the heat balance method in two tropical woody species. Tree Physiol 1994;14:179-90.

[24] West PW. Tree and forest measurement. 1st ed. Germany: Springer; 2004.

[25] Achten WMJ, Reubens B, Maes W, Mathijs E, Verchot L, Singh VP, Poesen J, Muys B. Root architecture of the promising bio-diesel plant *Jatropha*. In:

Anonymous., editors. Communication in Agricultural and Applied Biological Sciences 72(1), Leuven, Belgium, Oct. 17, 2007. Ghent: Ghent University; p. 81-85. http://www.biw.kuleuven.be/lbh/lbnl/forecoman/eng/publications.asp.

[26] Atkinson D. Root characteristics: why and what to measure? In: Smit AL, Bengough AG, Engels C, Van Noordwijk M, Pellerin S, Van de Geijn SC, editors. Root Methods - A handbook, Berlin, Germany: Springer-Verlag; 2000, p. 1-32.

[27] Pilli R, Anfodillo T, Carrer M. Towards a functional and simplified allometry for estimating forest biomass. For Ecol Manag 2006;237:583-93.

[28] Severino LS, Vale LS, Esberard de Macedo Beltrão N. A simple method for measurement of *Jatropha curcas* leaf area. In: Proceedings of the FACT seminar on *Jatropha curcas* L. agronomy and genetics, Wageningen, The Netherlands, Wageningen: FACT Foundation;

[29] Heller J. Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. 1. PhD, Institute of Plant Genetic and Crop Plant Research, Gatersleben, Germany & International Plant Genetic Resource Institute; 1996. http://www.ipgri.cgiar.org/Publications/pdf/161.pdf

[30] Kaushik N, Kumar K, Kumar S, Kaushik N, Roy S. Genetic variability and divergence studies in seed traits and oil content of *Jatropha* (*Jatropha curcas* L.) accessions. Biomass Bioenerg 2007;31:497-502.

[31] Kaushik N, Kaushik JC, Kumar S. Response of *Jatropha* seedlings to seed size and growing medium. Journal of Non-Timber Forest Products 2003;10:40-2.

[32] Huante P, Rincon E. Responses to light changes in tropical deciduous woody seedlings with contrasting growth rates. Oecologia 1998;113:53-66.

[33] Eamus D. Ecophysiological traits of deciduous and evergreen woody species in the seasonally dry tropics. Trends Ecol Evol 1999;14:11-6.

[34] Sternberg M, Shoshany M. Aboveground biomass allocation and water content relationships in Mediterranean trees and shrubs in two climatological regions in Israel. Plant Ecol 2001;157:171-9.

[35] Holl M, Gush MB, Hallowes J, Versfeld DB. *Jatropha curcas* in South Africa: an assessment of its water use and bio-physical potential. Pretoria, South Africa: Water Research Commission; 2007.

[36] Borchert R. Soil and stem water storage determine phenology and distribution of tropical dry forest trees. Ecology 1994;75:1437-49.

[37] West GB, Brown JH, Enquist BJ. A general model for the structure and allometry of plant vascular systems. Nature 1999;400:664-7.

[38] Ceulemans R, Mousseau M. Effects of elevated atmospheric CO_2 on woody plants. New Phytologist 1994;127:425-46.

[39] Saxe H, Ellsworth D, Heath J. Tree and forest functioning in an enriched CO_2 atmosphere. New Phytologist 1998;139:395-436.

[40] Norby RJ, Wullschleger D, Gunderson CA, Johnson DW, Ceulemans R. Tree responses to rising CO2 in field experiments: implication for the future forest. Plant

Cell Environ 1999;22:683-714.

[41] Poorter H, Navas M-L. Plant growth and competition at elevated CO_2: on winners, losers and functional groups. New Phytologist 2003;157:157-98.

[42] Cornelissen JHC, Carnelli AL, Callaghan TV. Generatlities in the growth, allocation and leaf quality responses to elevated CO_2 in eight woody species. New Phytologist 1999;141:401-9.

[43] Poorter H, Nagel O. The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. Australian Journal of Plant Physiology 2000;27:595-607.

Fig. captions

Fig. 1. Growth evolution of *J. curcas* saplings under dry, medium and wet treatment presented by graphs giving the estimated marginal means (means adjusted for the covariates, if any) of growth variables at different sapling ages. Error bars represent the 95% confidence interval.

Fig. 2. Biomass allocation – mean share of total dry biomass per plant part for plants grown under dry, medium and wet growth conditions. Error bars represent the 95% confidence interval.

* Different letters indicate significant differences ($P < 0.05$).

Fig. 3. Empirical regression model for total dry above biomass ($B_{\text{tot. above}}$) in function of the diameter at the stem base ($D_{\text{at base}}$) ($F = 308.6$; $P < 0.001$; $a = 0.029 \pm 0.013$ ($P = 0.025$); $b = 2.328 \pm 0.132$ ($P < 0.001$)).

Fig. 4. Empirical regression function of the individual leaf size (LS) in function of the product of leaf length and leaf width ($LL \times LW$) ($F = 8992.1$; $P < 0.001$; $a = 0.803 \pm 0.040$ ($P < 0.001$); $b = 0.985 \pm 0.010$ ($P < 0.001$)).

Fig. 5. Empirical regression model for total leaf area (LA) in function of the diameter at the stem base ($D_{\text{at base}}$) ($F = 348.5$; $P < 0.001$; $a = 2.03 \pm 0.78$ ($P = 0.018$); $b = 2.413 \pm 0.129$ ($P < 0.001$)).

Fig. 6. Measured dry mass plotted against the dry mass estimation (● Total aboveground biomass (R^2=0.99); ○ Aboveground excluding stump (R^2=0.92)) along the bisector (y = x).

Tables

Table 1. Multivariate test results (Pillai's Trace) giving significance of factor effects on the growth variables

Effect		***df***	***F***	***P***
Between Subjects	Accession	12	1.731	0.067
	Drought treatment	12	12.415	< 0.001
	Accession × Drought treatment	24	0.716	0.834
Within Subjects	Time	18	224.985	< 0.001
	Time × Drought treatment	36	12.525	< 0.001

Table 2. Mean values ± standard deviations of *J. curcas* roo/shoot per factor level and division of homogenous subgroups per factor based on post hoc test results (Tukey HSD)

Factor	**Level**	**Root/shoot**		**Root/shoot (no leaves)**	
Drought treatment	**dry**	0.41 ± 0.12	**a†**	0.50 ±0.12	**a**
	med	0.33 ±0.09	**b**	0.51 ±0.10	**a**
	wet	0.27 ±0.05	**c**	0.48 ±0.07	**a**

† Different letters indicate significant differences (P < 0.05) between factor levels