Dynamics of Leaflet Elongation and its Relationship with Plant Performance in Rubber (Hevea brasiliensis) Trees under Dry Sub-humid Climatic Conditions

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Leaf growth dynamics may have a bearing on plant performance as they are the major organs of carbon acquisition. Aim of this work was to model leaflet elongation pattern and to determine relationship of function parameters/derived growth quantities with growth performance in Hevea trees. Data were collected from randomly selected six leaflets of three trees from each of nine clones and Richards function was fitted. Growth quantities, critical points and their coordinates were computed and correlated with girth of clones. Leaflet elongation followed a similar pattern irrespective of clone and its duration was short in stress-free period than stress period. Most of the correlations were negative except that of second derivative, duration of linear and plateau phases. None of the stress period correlations were significant. Variation in leaf elongation rates of individual leaflets appeared to be due to variation in rate of cell division and/or cell elongation. All the sampled leaves of a clone followed a very similar growth pattern suggesting common duration in leaf emergence to full leaf expansion of all the leaves in a given whorl irrespective of leaf size. Significant correlation observed for y-axis intercept of phase change point from lag to linear might be useful in identifying high growth clones.

Keywords: Natural rubber; Hevea brasiliensis; leaf modelling; Richards function; leaf elongation; leaf growth

Hevea brasiliensis (Willd. ex. Adr. Juss.) Muell. Arg., is one of the important industrial tree crops grown mainly in tropical climates between 12º latitude on either side of the equator. In India, the crop is largely cultivated in areas between 8º 15' N and 12º 52' N latitudes. Hevea rubber is the major source of natural rubber, a product used in the manufacture of thousands of products of which the pneumatic tyre is the most popular. Rubber trees belong to the genus Hevea under the family Euphorbiaceae. Though ten species have been recognised in the genus, Hevea brasiliensis is the only species

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cultivated for obtaining rubber, a product of commercial importance\textsuperscript{1}. The trees are erect with a straight trunk and in the wild they grow to over 40 m with a life span of more than 100 years. Like in other crops, studies on growth are of fundamental importance in understanding the interaction between plant and their environmental conditions. Leaves, representing the power generation facility and aerial environmental sensing units of plants, ultimately determine the canopy development and plant performance\textsuperscript{2–4}. In \textit{Hevea}, most of the available studies on leaf function have focussed mainly on photosynthesis, light interception and response of leaves to environmental stress\textsuperscript{5–7}. A lot of information is also available on intra-annual and inter-annual girth growth of trees and its modelling\textsuperscript{8–12}. However, leaf growth modelling and characterisation of its dynamics have not been studied so far. The objective of this paper was to fit a suitable model to leaflet elongation patterns so as to describe the different phases of elongation as a function of time and to determine whether function parameters/derived growth quantities have any relationship with the growth performance of clones.

\section*{MATERIALS AND METHODS}

\subsection*{Study Location, Plant Material and Weather and Soil Moisture Data}

Data for this study were collected from a clone trial laid out in 1982 at the Rubber Research Institute of India Regional Research Station at Dapchari (20.04°N, 72.04°E, 48 m MSL) in the Thane District of Maharashtra state. The trial consisted of 12 clones planted in a randomised block design with two replications. Each plot of a clone consisted of 25 plants in square planting at a spacing of 4.9 m $\times$ 4.9 m. For a few years all the plants survived well but in later years many of the plots dried out due to poor soil depth. The unaffected plots representing nine clones were used in this study. More details on the trial can be found in Chandrasekhar \textit{et al.}\textsuperscript{13} also climate and weather characteristics in Chandrasekhar \textit{et al.}\textsuperscript{10,11}. The clones studied were Gl 1, GT 1, PB 235, PR 107, RRII 300, RRIM 501, RRIM 600, RRIM 612 and Tjir 1. Meteorological data on rainfall, minimum and maximum temperature and sunshine duration and evaporation for the study period were collected from the observatory maintained in the station. Monthly soil moisture variations both inside the plantation as well as in the open area for three depths namely, 0-30, 30-60 and 60-90 cm were determined by gravimetric method with three replications for each depth in each recording.

\subsection*{Leaflet Elongation Measurements}

The leaves of \textit{Hevea} are palmately compound with long petioles at the end of which are attached three leaflets. The leaflets have short petiolule (5-10 mm) and are folded back at emergence but subsequently assume various positions from reclinate through horizontal to nearly erect\textsuperscript{1}. Leaflet elongation measurements were made non-destructively on randomly selected six leaflets from three trees in each of the nine clones. In each tree two well exposed young expanding leaflets on different branches were selected and continuous measurements were made on the length of blade (from tip to petiolule base) until two to three consecutive measurements were identical that indicated the cessation of elongation in length. Data were collected during stress-free (September/October) and stressful (December/January) periods. September-October in the study location is the end of monsoon season and the December-January period is the annual flushing period of \textit{Hevea} trees wherein the trees shed their leaves completely by wintering. This is followed by bursting of terminal buds leading to the
production of new leaves. Leaflet length was measured on 23, 25, 27, 29, 1, 3, 5, 7, 9, 11 and 13 during September/October 1989 and on 27, 29, 1, 3, 5, 8, 11, 14 and 16 during December/January 1989-90.

**Growth Function Used**

It is now widely known that growth of plants and their parts usually follow a sigmoidal pattern. There are many mathematical models that are in use to describe the growth of plants and animals over time. The models are based on general deterministic differential equation of the form $dy/dt = f(y,t)$. Raw profiles of leaflet elongation data indicated that the pattern of elongation followed a sigmoid pattern. In this study, Richards function which is widely used in plant ecology and forestry was used. This function belongs to the exponential decline family of growth models that seek biological interpretation\cite{14-18}. The function, unlike the straight line models, provides a convenient framework for organisation and generalisation of information for sigmoidal growth pattern with parameters of the model having clear geometric meaning\cite{14,19,20}. There are many forms of this function. The form used in this study was:

$$y = A(1 + (m - 1)e^{-k(t-d)})^{\frac{1}{m}} \quad \ldots 1$$

where $y$ is the observed length (or weight, height etc.) at age $t$ (expressed in this study in days), $A$ is the maximum asymptotic limit when $t$ approaches infinity and it indicates the maximum possible value of the dependent variable determined by the genetic capacity of the clone and the influence of the environment. Parameter $k$ is a function of the ratio of maximum growth rate to mature size, normally referred to as maturing rate. It serves both as a measure of growth rate and of rate of change in growth rate. Coefficient $d$ is the time to inflection where absolute growth rate is the maximum. The parameter $m$ is the dimensionless shape parameter that permits a variable point of inflection of the curve on the $f$-axis at a proportion $P = m^{(1/(1-m))}$ of final size and thus, controls the shape of the curve. It determines whether the maximum growth occurs early or late for $m<1$ maximum slope of the curve is when $f(t) < A/2$ and when $m > 1$ maximum slope of the curve is when $f(t) > A/2$.

**Estimates of Growth Quantities, Coordinates of Critical Points and their Derivatives.** The following growth quantities were worked out for each clone as well as pooled data using respective curve parameters\cite{19}.

- Maximum elongation rate ($AER_x$) = $Akm^{1/m}$\((1-m)\)
- Mean absolute elongation rate ($AER_m$) = $Ak/(2m+2)$
- Mean relative elongation rate ($RER_m$) = $k/m$
- Duration of elongation ($DE = t_f = time to asymptotic maximum$) = $(2m+2)/k$.

Critical points of the growth curve that indicate the phase change points from exponential to linear ($CP_1$), acceleration to deceleration ($CP_2$), linear to senescent ($CP_3$) and their coordinate points were worked out\cite{21}. $CP_1$ is the inflection point from where the growth changes from acceleration to deceleration phase. The coordinates of this point $(t_i, y_i)$ are calculated as:

$$t_i = d, \quad y_i = Am^{1-m} \quad \ldots 2$$

The first ($y'_i$) and second ($y''_i$) derivatives of this point are:

$$y'_i(mid growth rate, AER_x) = \frac{m}{Akm^{1-m}}, \quad y''_i = 0 \quad \ldots 3$$
CP\text{1} is the point of phase change from exponential to linear phase. Time point of this phase represents the duration of lag phase (DG). Its coordinate points are:

\[ DG = t_1 = t_i + \frac{1}{k} \ln \left( \frac{(m-1)L_1^{(m-1)}}{1 - L_1^{(m-1)}} \right), \quad y_1 = AL_1 \quad \ldots \quad 4 \]

where, \( t_i \) is the time point of CP\text{1} and \( L_1 \)

\[ L_1 = \left[ \frac{m(m+1) - (m-1)\sqrt{m(m+4)}}{2m(2m-1)} \right]^{1/(m-1)} \quad \ldots \quad 5 \]

Other quantities in the equation are Richards function parameters. The first \((y'_1)\) and second \((y''_1)\) derivatives of this point are:

\[ y'_1 = \frac{AKL_1}{m-1}(1 - L_1^{m-1}), \]

\[ y''_1 = \frac{AK^2L_1}{(m-1)^2}(1 - L_1^{m-1})(1 - mL_1^{m-1}) \quad \ldots \quad 6 \]

CP\text{2} is the transition point of change from linear to plateau phase. The coordinates are:

\[ t_2=t_1 + \frac{1}{k} \ln \left( \frac{(m-1)L_2^{(m-1)}}{1 - L_2^{(m-1)}} \right), \quad y_2 = AL_2 \quad \ldots \quad 7 \]

where, \( L_2 = \left[ \frac{m(m+1) + (m-1)\sqrt{m(m+4)}}{2m(2m-1)} \right]^{1/(m-1)} \).

Other quantities in the equation are as explained above. The first \((y'_2)\) and second \((y''_2)\) derivatives of this point are:

\[ y'_2 = \frac{AKL_2}{m-1}(1 - L_2^{m-1}), \]

\[ y''_2 = \frac{AK^2L_2}{(m-1)^2}(1 - L_2^{m-1})(1 - mL_2^{m-1}) \quad \ldots \quad 8 \]

Grand period of growth, \textit{i.e.} the time duration of linear phase \( DL = \Delta t (t_2 - t_1) \) was calculated as:

\[ DL = \Delta t = t_2 - t_1 = \frac{1}{k} \ln \left[ \frac{L_2^{m-1}(1 - L_1^{m-1})}{L_1^{m-1}(1 - L_2^{m-1})} \right] \quad \ldots \quad 9 \]

The duration of plateau phase (DP) was calculated as:

\[ DP = t_p - t_2, \quad \ldots \quad 10 \]

where \( t_p \) is the time to end of plateau phase when the leaflet length \( y \) equalled 95% of its final value and is considered as the end of leaflet expansion\textsuperscript{22,23}. Time to plateau phase was worked out using the following inverse equation of the fitted function:

\[ f^{-1}(y) = d + \ln \left[ \frac{(y/A)^{1-m} - 1}{(m-1)} \right]/-k \quad \ldots \quad 11 \]

\textbf{Fitting of Function and Statistical Analyses.}
All modelling work was undertaken at the clonal level using six leaflet measurements of each clone. Leaflet expansion was analysed as a function of time (measured in days) against cumulative elongation measurements of leaflets. Data analyses were carried out in the open source software R, a free software environment for statistical computing and graphics\textsuperscript{24}. Fitting of function was carried out using the nlstools package\textsuperscript{25}. Starting values for all the parameters were specified following Fekedulegn \textit{et al.}\textsuperscript{26} and Ogle\textsuperscript{27}. Graphs were made using ggplot2\textsuperscript{28}. Wherever necessary, growth quantities estimated from fitted parameters of the individual leaflets were subjected to ANOVA assuming completely randomised design as the leaflets were randomly selected for conducting measurements. Homogeneous groups were identified following orthogonal contrasts\textsuperscript{29}. Correlations of girth of clones, asymptotic length and leaflet length at end of lag phase
with various quantities extracted from fit parameters were worked out.

RESULTS

Weather and Soil Moisture Conditions

Rainfall in the region of the experimental location is predominantly of monsoonal type and is received from June to September with maximum rainfall in July and August (Table 1). October occasionally receives rainfall from post monsoon showers. In the remaining months rainfall normally does not occur. January to April receive more than 10 h of sun shine daily with the lowest of less than 4 h and maximum of 12 h, while May, October, November and December receive a daily average of less than ten hours. May through September, April and October, March and November, January, February and December had comparable mean T\textsubscript{min}. Lowest T\textsubscript{min} were in December and January and the highest during April to October. July, August and September recorded T\textsubscript{max} around 30\degree C while December and January below 25\degree C. During dry season the profile of photoperiod and temperature change is the reverse of that encountered during wet and mid seasons. In the summer months of March, April and May, evaporative demand was high and in some days maximum daily evaporation was as high as 14 mm per day reflecting the pattern of seasonal variation in temperature. July, August and September had low and the remaining months had moderate evaporative demand. Soil moisture was very low in the upper layers during January to May. Soil moisture progressively decreased from December and reached permanent wilting point levels by February. In the upper layers it was very low during January to May. In the lower layers though it was slightly better, the stress conditions were evident.

Parameter Estimates and Fit Statistics. Parameter estimates and fit statistics obtained for data of stress-free and stress conditions are given in Table 2. Asymptotic maximum values for the stress-free period ranged from 14.6 cm in RRIM 600 to 23.7 cm in GT 1. The rate parameter k ranged from 0.36 to 0.67. Maximum value of the shape parameter was 5.6 for PR 107 and the minimum was 2.5 for RRIM 501. The inflection point was maximum in Tjir 1 (10.3 days) while it was minimum in RRIM 600 (4.3 days). Estimates of the parameters for the stress period were in the range of 15.1 to 18.9 cm for A, 0.43 to 0.56 for k, 3.8 to 4.7 for m and 6.2 to 11.0 days for inflection point. All the parameters were significant as their asymptotic confidence intervals did not contain zero. Root mean square errors (RMSE) were very small and coefficient of determination (R\textsuperscript{2}) values were close to one. The trajectory of the fitted curves overlaid on the scatter plots of the leaflet (Figures 1 and 2) showed excellent fit of the Richards model to leaf elongation data from both the measurement periods. Leaflet elongation followed a similar pattern irrespective of clone.

Growth Quantities and Duration of Elongation. Growth quantities worked out for the stress-free period (Table 3) indicated maximum elongation rate in GT 1 (2.15 cm day\textsuperscript{-1}) and minimum in PR 107 (1.19 cm day\textsuperscript{-1}). Range of AER\textsubscript{m} was 0.73 to 1.36 cm day\textsuperscript{-1}. RER\textsubscript{m} was high in RRIM 600 (0.20 cm cm\textsuperscript{-1} day\textsuperscript{-1}) and low in PR 107 (0.10 cm cm\textsuperscript{-1} day\textsuperscript{-1}). In the stress period AER\textsubscript{x} ranged from 1.0 cm day\textsuperscript{-1} to 1.4 cm day\textsuperscript{-1}, while the AER\textsubscript{m} varied from 0.63 to 0.88 cm day\textsuperscript{-1}. There was not much variation in RER\textsubscript{m} as it ranged from 0.1 to 0.13 cm cm\textsuperscript{-1} day\textsuperscript{-1}. Estimated DE varied from 14.0 days in RRIM 600 to 23.8 days in PR 107 in the stress-free period while in stress period it was maximum in RRII 300 (24.62 days) and minimum in RRIM 612 (19.54 days). Increase in duration of growth from stress-free to stress period was maximum in RRIM 600 (8.1 days) and GT 1 (6.8 days). Clones Gl 1 and PR 107 did not show any
change in the duration of growth between the two periods. Pooled analysis produced a small increase in leaf growth duration from stress-free to stress period.

**Critical Points of Growth Curve and Duration of Phases.** Period required for change from lag to linear phase (CP_1=DG) varied from 1.29 days in RRIM 600 to 7.34 days in Tjir 1 (Tables 4 and 5), while the y-axis intercepts ranged from 3.96 cm in RRIM 600 to 9.4 cm in GT 1. Maximum growth rate at this point (AER_x) was in GT 1 (1.61 cm day^{-1}) and the minimum was in PR 107 (0.93 cm day^{-1}). The second derivative (y''_1) at this point varied from 0.09 cm cm^{-1} day^{-1} in PR 107 to 0.23 cm cm^{-1} cm^{-1} in RRIM 600 and GT 1. In the subsequent inflection point, lowest t_I was noted in RRIM 600 (4.27 days) and the highest was in Tjir 1 (10.34 days). Minimum length of leaflet at inflection was 8.02 cm (RRIM 600) and the maximum was in GT 1 (15.24 cm). Growth rates at this point varied from 1.17 cm day^{-1} (PR 107) to 2.1 cm day^{-1} in GT 1. The estimates obtained for the third critical point (CP_2) indicated minimum t_2 for RRIM 600 (7.25 days) and maximum for PB 235 (13.5 days). Intercepts of y-axis varied from 12.01 cm to 20.79 cm. AER was lowest in PR 107 (0.71 cm day^{-1}) and highest in GT 1 (1.31 cm day^{-1}). Reduction in RER was low in PR 107 (-0.21 cm cm^{-1} day^{-1}) and high in GT 1 (-0.42 cm cm^{-1} day^{-1}). In the stress period, lowest t_I values were obtained for Gl 1 (2.25 days) while the highest was in RRIM 612 (7.74 days). Lowest intercept values of y-axis were noted in PB 235 (6.13 days) and highest in RRII 300 (7.48 days). AERs of this point were 0.76 to 1.06 cm day^{-1} while the RERs were 0.1 to 0.14 cm cm^{-1} day^{-1}. Gl 1 had the lowest t_I (6.18 days) and RRIM 612 the highest (11.05 days). The values for y_I ranged from 9.82 cm in PB 235 to 12.4 cm in RRII 300. Time to start of plateau phase was lowest in Gl 1 (10.1 days) and the highest for RRIM 612 (14.35 days). Intercepts of y-axis ranged from 13.32 cm to 17.11 cm while the AERs were 0.61 to 0.86 cm day^{-1}. RERs were in the range of -0.26 cm cm^{-1} day^{-1} to -0.15 cm cm^{-1} day^{-1}.

Lowest duration of lag phase (DG) was observed for RRIM 600 (1.68 days) and the highest in Tjir 1 (7.6 days) in the stress-free period (Table 5). Length of linear phase varied from 5.96 days in RRIM 600 to 8.05 days in RRIM 501 in the stress-free period. Pooled analysis produced a value of 7.86 days. In the stress period, DG varied from 3.74 days to 7.8 days. Lowest duration of plateau phase (DP) was observed in Tjir 1 (1.27 days) and the highest in RRIM 501 (4.09 days). RRIM 600 had the minimum t_p (10.2 days) to reach the end of plateau phase while the maximum was observed for PB 235 (15.2 days). Duration of DG was maximum in RRIM 612 (8.0 days) and minimum in Gl 1 (2.28 days) in the stress period. In the subsequent linear phase, minimum duration was observed for RRIM 600 (6.41 days) while the maximum was in RRII 300 (8.43 days). Plateau phase ranged from 1.67 days (RRIM 612) to 2.58 days (RRII 300). RRIM 501 took lowest number of days (12.3 days) to reach t_p while RRIM 612 took about 16.1 days.

**Correlations with Girth, Asymptote and Leaflet Length at CP1.** Most of the correlations of fitted parameters and the calculated quantities with girth of the clones were negative except that of second derivatives, DL and DP (Table 6). Negative and significant (P<0.05) correlations were observed for m, x-axis intercepts of the critical points of the curve (t_1, t_I and t_2), while the same coefficients with y-axis intercepts (y_1, y_I and y_2) were also negative but were highly significant (P<0.01). None of the correlations with girth were significant in the stress period. Asymptotic length produced significant correlations with absolute rate quantities and highly significant (P<0.01) correlations with leaflet length at
\( y_1 \) and \( y_2 \). Correlations of \( y_1 \) in stress-free period followed a similar trend observed for girth but in the stress period highly significant correlations were noted for length attributes only.

**DISCUSSION**

Parameter estimates, fit statistics and the trajectory of the fitted curves overlaid on the scatter plots of the observations indicated excellent fit of the Richards model to leaf elongation measurements from both stress-free as well as stressful conditions. Time-course of leaflet elongation in all the replicate leaves followed a qualitatively similar pattern irrespective of clone. It followed a typical sigmoidal pattern encompassing the initial lag phase leading to linear phase and finally a plateau phase in which the elongation rate declined until the length reached an asymptotic maximum. It is reported that in dicotyledonous crops typically most of the cell multiplication necessary for the leaf expansion happen in the lag phase while most of the cell elongation gets completed in the linear phase\(^2,3,30–33\). Thus, it appears that cell multiplication happens very fast in clones RRIM 600 and RRIM 501 in the stress-free period. In general, cell elongation gets completed in about six to eight days in both periods. Comparison of the results obtained in both periods indicated no drastic event in the leaf elongation process. This may be due to the fact that smoothly changing environmental constraints can lead to acclimatisation process within plants that adjust their performance to the environmental conditions resulting in non-stressful changes in even when resources are limited\(^34–36\).

In *Hevea*, growth of stem is characterised by rapid elongation of the bud along with the production of 9–15 leaves in tiers/storeys/whorls arranged in a spiral phyllotaxy. In a whorl, the lowermost leaf will have longest petiole with the longest leaflets. The size of the leaflets gets decreased towards the top of the whorl. In spite of good variation in the final leaflet length, variation observed in DL and \( t_p \) were small. Thus, the observed variation in leaf elongation rates of individual leaflets may reflect variation in the rate of cell division and/or cell elongation\(^3,32,37\). From the graphs, it appears that the sampled leaves of a clone follow a very similar pattern of leaf growth. Therefore, it is possible that irrespective of the leaf size, the duration of leaf emergence to full leaf expansion may be common to all the leaves in a given whorl. Comparative analysis of the present results with other studies is not possible as there are none available.

**CONCLUSION**

The results presented here show that under dry sub humid climatic conditions, total duration of leaflet growth in clones of *Hevea* would take about 14 to 24 days with the linear phase of the elongation period varying from six to eight days. Clone RRIM 600 appeared to have comparatively better leaflet elongation parameters. Comparison of duration of elongation (DE) indicated the hastening of leaf elongation duration in the stress-free period and prolonging of duration in the moisture stress period. Highly significant correlation (close to one) observed for \( y\)-axis intercept of \( y_1 \) indicated that it should be possible to identify high growth clones using this parameter with the negative sign showing that high growth clones will have comparatively lower values of \( y_1 \). As leaves are the only organ that is capable of incorporating carbon to structural components of a plant body determining the overall performance of plant yield\(^6,36\); more studies are needed on physiological and biochemical processes driving leaf expansion.
for generating useful information aimed at improving plant yield.

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REFERENCES


