

Development of Leaf Blade Class Concept for the Characterisation of *Hevea brasiliensis* Muel. Arg. Leaf Age

ZAHAR SAMSUDDIN*, MOHD. KHIR ABD. RAHMAN** and IVAN IMPENS*

The technique to characterise Hevea brasiliensis leaf age in days is described. The technique is based on the measurement of the angle formed between the middle leaflet and the leaf stalk expressed in terms of Leaf Blade Classes (LBC). The increase of the angle was found to be linear with days, and the number of days the leaves of the various clonal seedlings take to reach the maximum LBC is estimated.

The development of *Hevea brasiliensis* foliage follows a specific pattern, viz. the emergence of a flush which has six to ten leaves. The development of the leaves in the flush is followed by a dormant period when there is no shoot expansion or formation of new flush. This dormant period is then followed by the breakage of the terminal bud to initiate shoot expansion and the formation of a new flush. Detailed phenomena taking place during this cycle were reported by Halle and Martin¹. A similar pattern of development was reported by Baker and Hardwick² and Greathouse *et al.*³ for cocoa plants.

Halle and Martin¹ showed that the period between successive budbreak was controlled by the root:shoot ratio. If the shoot growth rate is significantly higher than the root growth rate over a long period, a temporary check on shoot growth is necessary to maintain balanced growth. Shoot growth rhythm is then internally controlled. Borchert⁴ simulated the growth rhythms of cocoa and *Hevea* using root:shoot ratio and the results compared well with the measured growth.

Due to the shoot growth habit, the Leaf Plastochron Index (LPI) as developed by

Erickson and Michelini⁵ could not be applied to *Hevea* to get an approximation of the leaf age for physiological studies requiring this parameter. Young leaves of *Hevea* hang down parallel to their stalks; this is attributed to the few supporting tissues¹. As the leaves get older, they form angles with the stalks till they reach the maximum angle of 180°, at which they remain until they senesce. This habit is taken advantage of to characterise the leaf age and the technique for determining the leaf age is described in this paper.

MATERIAL AND METHODS

Plants were germinated from seeds in a greenhouse. When the plants had two flushes of leaves, they were transferred to a phytotron with 12 h photoperiod, relative humidity of 70%–80% during the day and 80%–90% at night and the temperature was kept at 27°C ± 3°C day and night. The plants were allowed to form the third flush. Only the third and subsequent flushes were used in this experiment to ensure that the leaves had the same pre-treatment.

Four to five leaves from each flush of five plants from each of the five clonal seedlings (RRIM 600, GT 1, Tjir 1, RRIM 501 and PB 5/51) were measured. Measurements were made alternate daily on two flushes per plant

*Universitaire Instelling Antwerpen, 2610 Wilrijk, Belgium.

**State University of Ghent, Coupure Links, 533, 9000 Gent, Belgium.

for a period of three months. For the determination of the Leaf Blade Class (LBC), the maximum angle (180°) was divided into classes of 20° each, making in total nine classes. A chart was drawn with the classes numbered from LBC 1 to 9. The first LBC of 0°–20° is denoted by LBC 1 and for the class of 160°–180° it is denoted by LBC 9. For the measurement of LBC, the horizontal line was placed adjacent to the leaf stalks. The class into which the middle trifoliolate fell was the LBC of the leaf. In cases where the middle trifoliolate fell into two or three classes, the class into which the proximal portion of the leaflet fell was taken to be the LBC of the leaf.

For the establishment of the relationship between LBC and the age of leaves, a linear relationship $y = a + bx$ was proposed where the LBC (measured on discrete scale) was transformed to a continuous scale of 1–17. The values 1 and 17 that are the class modes in term of angle (10°–170°) represent the first and the final class 9. The age of the leaves, x , measured in days, is the independent fixed variable. Regression lines, $y = a + bx$, were computed globally for all the leaves of each clonal seedling. The lines were tested for zero intercept. For every clonal seedling, an estimate of the number of days (age) the leaf takes to reach the maximum class was computed using the inverse prediction of x from y , viz.

$$x = \frac{1}{b}(y - a)$$

The confidence limits for such predicted values were computed using formulae:

$$L = \frac{\bar{x} + {}^b_{yx} (17 - \bar{y})}{D} \pm H$$

where $D = b^2_{yx} - t^2_{\alpha(k-2)} s^2_b$

$$\text{and } H = t_{\alpha(k-2)} \sqrt{\frac{s^2_{yx} \left[D \left(\frac{1}{n} + \frac{1}{k} \right) + \frac{(17 - \bar{y})^2}{\Sigma(x - \bar{x})^2} \right]}{D}}$$

where b = regression coefficient of y on x ,

$$s^2_b = \frac{S^2_{yx}}{\Sigma(x - \bar{x})^2} = \text{standard error of regression coefficient}$$

$$s^2_{yx} = \text{mean square of deviation from regressions}$$

k = number of groups

n = sample size

α = significant level chosen.

RESULTS AND DISCUSSION

The increase of the LBC is linear (*Table 1*). By inverse interpretation, the leaf age can be estimated with the knowledge of the LBC (*Table 2*).

Table 1 shows the regression equation and the correlation coefficients of the different clonal seedlings. Significant values of the intercept, a , suggests that the leaves at initiation are not completely pressed to the stalks, but form an angle (*Figure 1*). Based on these angle measurements, RRIM 501, Tjir 1, RRIM 600, PB 5/51 and GT 1 were respectively grouped under LBC 2, 2, 1, 1 and 1 at leaf initiation. The number of days to reach LBC 9 is given in *Table 2* for each of the clonal seedlings. This class is chosen because it is the final class that can be characterised by this method, after which the leaf age is characterised by the number of days the leaf has reached LBC 9. For example, a leaf that has stayed in LBC 9 for twenty days is characterised by LBC 9 + 20 days.

The knowledge of leaf age is important in comparative physiological studies, for example in trying to compare several varieties with respect to their photosynthetic rates (Pn). Several workers⁶⁻¹⁰ have shown that Pn are dependent on leaf age. Izhar⁷ remarked that

TABLE 1. LBC-AGE RELATIONSHIP FOR THE VARIOUS *HEVEA BRASILIENSIS* CLONAL SEEDLINGS AS EXPRESSED BY CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS

Seedlings	Regression equation ^a	Correlation coefficient
RRIM 501	$y = 4.88 + 0.33 x$	0.76**
Tjir 1	$y = 3.24 + 0.37 x$	0.88**
RRIM 600	$y = 1.26 + 0.46 x$	0.88**
PB 5/51	$y = 2.62 + 0.40 x$	0.82**
GT 1	$y = 2.40 + 0.46 x$	0.83**

**P = 0.01

^a All regression lines, tested for zero intercept, show significant values of k's, at P = 0.01.

TABLE 2. ESTIMATED NUMBER OF DAYS TAKEN TO REACH THE MAXIMUM CLASS (X_{17})

Seedlings	x_{17} ^a	Confident limit (10%)		Confident limit (5%)	
		Lower limit	Upper limit	Lower limit	Upper limit
RRIM 501	36.5	28.5	48.5	27.0	52.0
Tjir 1	37.0	32.5	43.0	31.5	43.5
RRIM 600	33.0	29.5	37.0	28.5	38.0
PB 5/51	36.0	30.0	43.5	29.0	45.5
GT 1	32.5	28.0	38.0	27.0	39.5

^a To the nearest 0.5 day

physiological age should be used for such a study because he observed distortion in the maximum Pn when days were used as a measure of age. Thus Dickman⁸ employed LPI to characterise physiological leaf age. Though the LPI concept has successfully been used to describe physiological leaf age and developmental pattern in *Xanthium italicum* and *Populus* spp., it fails to describe *Hevea* leaf age. This is because LPI requires regular initiation of leaves as a prerequisite. As mentioned, *Hevea* does not fulfil this condition. The concept of LBC, however, has the same ability as LPI. Data from Samsuddin¹¹ show that the changes in some physiological variables namely, photosynthetic, light and dark respiration

rates, carbon dioxide compensation concentration, stomatal and internal resistances to carbon dioxide diffusion and light utilisation efficiency have similar evolution to those species described by the LPI concept.

Though this concept is developed based on observations on seedlings at young stage, it should however, be able to describe leaf age at any stadium of growth. This is because LBC describes the physiological age of a leaf. As such it is not dependent on the number of branches on a plant for instance. Nevertheless it is dependent on the growth condition like relative humidity, temperature and nutrient supply¹¹. Hence, if this method is to be used to estimate leaf age in terms of days, the envi-

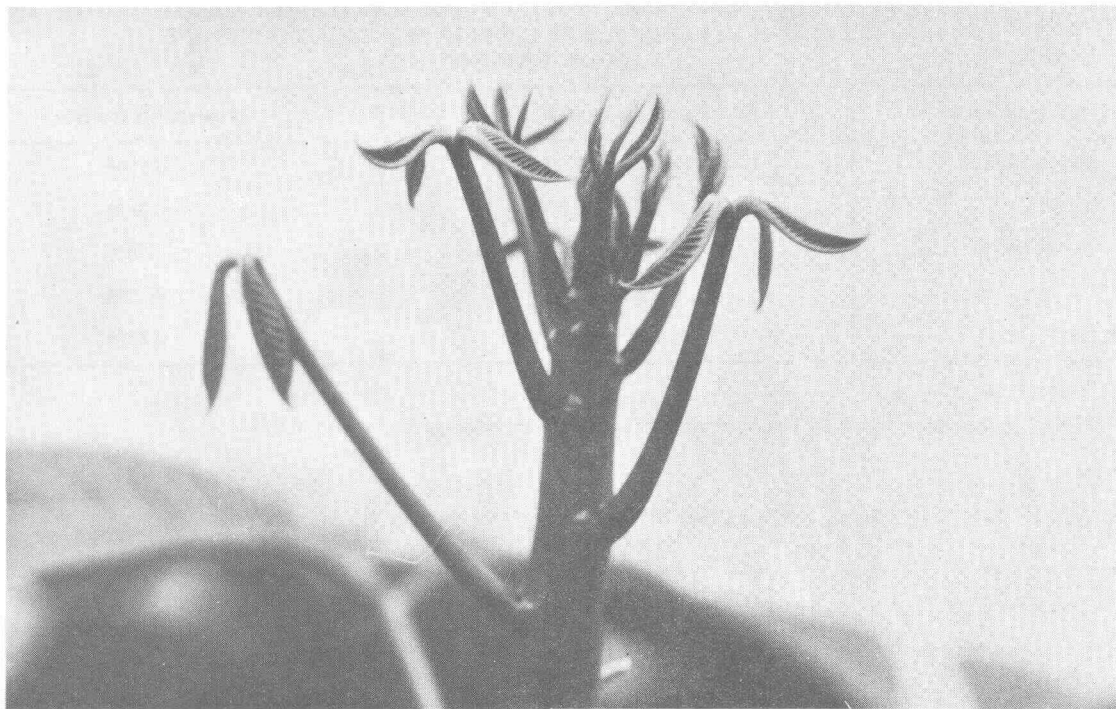


Figure 1. The pose of very young leaflets of *Hevea brasiliensis* (Tjir 1) showing the angle they made to the stalks (see text for discussion).

ronmental conditions have to be known, and a preliminary experiment has to be undertaken.

CONCLUSION

The technique for the characterisation of leaf age by this method is simple and gives an idea of leaf ontogeny which might be germane to physiological studies. Probably in trying to get more accurate estimate of the leaf age, the class limits should be decreased, hence increasing the number of LBC.

This method, however, fails to give an idea of leaf age after it has reached LBC 9. Nevertheless, leaf age after LBC 9 can be characterised by the number of days the leaves have reached LBC 9.

ACKNOWLEDGEMENT

We are indebted to the Director of the Rubber Research Institute of Malaysia for sending

us the seeds used in this experiment. The technical help of Miss Dijan Verschooren during the course of this experiment is highly appreciated.

Rubber Research Institute of Malaysia
Kuala Lumpur
September 1977

REFERENCES

1. HALLE, F. AND MARTIN, R. (1968) Etude de la croissance rythmique chez l'Hévéa (*Hevea brasiliensis* Muell. Arg. Euphorbiacées-Crotonoidées). *Adansonia, Sér. 2*, 8, 475.
2. BAKER, N.R. AND HARDWICK, K. (1976) Development of the photosynthetic apparatus in cacao leaves. *Photosynthetica*, 10, 361.
3. GREATHOUSE, D.C., LAETSCH, W.M. AND PHINNEY, B.O. (1971) The shoot-growth rhythm of a tropical tree, *Theobroma cacao*. *Amer. J. Bot.*, 58, 281.

4. BORCHERT, R. (1973) Simulation of rhythmic tree growth under constant conditions. *Physiol. Pl.* **29**, 173.
5. ERICKSON, R.O. AND MICHELINI, F.J. (1957) Plastochron index. *Amer. J. Bot.*, **44**, 297.
6. FREELAND, R.O. (1952) Effect of age of leaves upon the rate of photosynthesis in some conifers. *Pl. Physiol.*, **27**, 685.
7. IZHAR, S. (1966) Physiological and genetical studies of the net carbon dioxide exchange by individual intact leaves of several dry bean varieties (*Phaseolus vulgaris* L). Ph.D Thesis, Cornell University.
8. DICKMAN, R.O. (1971) Photosynthesis and respiration by developing leaves of cottonwood (*Populus deltoides* Bartr.). *Bot. Gaz.*, **132**, 253.
9. MALKINA, I.S. (1976) Changes in the light curves of photosynthesis with aging in leaf of Norway maple. *Sov. Pl. Physiol.*, **23**, 208.
10. CATSKY, J., TICHA, I. AND SOLAROVA, J. (1976) Ontogenetic changes in the internal limitations to bean photosynthesis. I. Carbon dioxide exchange and conductances for carbon dioxide transfer. *Photosynthetica*, **10**, 394.
11. SAMSUDDIN, Z. (1978) Private communication. Rubber Research Institute of Malaysia.