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VARIATIONS INVOLVED IN FIELD MEASUREMENTS OF TRANSPIRATION RATES AND STOMATAL DIFFUSIVE RESISTANCE OF *HEVEA BRASILIENSIS* MUELL. ARG. CLONES AND THE RELATIONSHIP OF THE LATTER WITH NET PHOTOSYNTHETIC RATES

By

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ABSTRACT

Variance components and their percentage contribution to total variation during field measurements of stomatal diffusive resistance (DR) and transpiration rates (TR) were studied for Hevea clones. Variance from the plant component was much higher than other components viz., leaves of the same plant and determinations of the same leaf. DR, TR and coefficient of variation for DR and TR varied significantly with leaf age and time of the day. The three leaflets in a leaf were similar in respect to DR and TR. Within a whorl, the comparatively small leaves at the top differed significantly with respect to DR and TR from the rest. DR and TR of clones RRIC 100, RRIC 103, RRIC 45, PB 86 and IAN 710 were significantly different at 0.1% level. Initial studies show stomatal diffusive resistance to have a tendency to be correlated with net photosynthetic rates (NPR). Other possible factors that might bring about clonal differences in NPR are discussed.

INTRODUCTION

The CO_2 influx to leaves depends on these factors (a) its rate of fixation at chloroplasts, (b) the total diffusive resistance and, (c) the opposing current of respiratory CO_2 (Zelitch, 1971 a). Stomata provide a major resistance to CO_2 influx (Zelitch, 1971 b; Strimstri, 1963; Bange, 1953). The magnitudes of stomatal, mesophyll and carboxylation resistance could be estimated as described by Gastra (1952) or Gale and Poljakoff -- Mayber (1968). It can also be measured using appropriate instruments.

Samsuddin and Impens (1979 a, 1979 b) estimated the resistance to CO_2 influx in *Hevea brasiliensis* Muell. Arg. clones and seedlings. They found the internal resistance (mesophyll plus carboxylation resistances) to be larger than the boundry layer and stomatal resistance. The internal resistance was 3 — 7 times higher than stomatal resistance. However, internal resistances showed smaller variations between clones when compared to stomatal resistance. The latter showed much higher and significant correlation with Net Photosynthetic Rates (NPR) at saturation radiant energy.

Plants used in the above studies had been grown in glass houses with controlled environment. Samsuddin (1980) measured stomatal conductances to water vapour diffusion in the field (using ventilated diffusion porometer — Lamda instrument) on hardened shoots where the physiological age of leaves was equivalent to Leaf Blade Class (LBC) 9 and they received a photon flux of more than 300 μ moles m^{-2} s^{-1}. Statistical analysis have shown that the mean stomatal conductances of the seven *Hevea* species studied are significantly different. However, the differences observed between clones RRIM 600, RRIM 612, GT 1 and Tjir 1 were not significant. NPR and stomatal conductances of leaves of three *Hevea* clones subjected to water stress were measured using a portable battery operated infra-red gas analyser (IRGA) and a porometer (LI-COR, Model LI 60). Plants were grown in plastic bags in an open glasshouse. Both NPR and stomatal conductance showed declining sigmoid curves in response to increasing water stress depicting their close relationship (Ceulmans, Impens and Ng, 1983).

Environmental and plant variables that may complicate comparative measurements of stomatal opening must be controlled or taken into account to make stomatal measurements meaningful. Temperature, CO_2 concentration humidity, leaf age, nutritional status, diseases, endogenous rhythms and water stress are some important factors affecting stomatal opening and hence stomatal diffusive resistance (DR) to CO_2. Studies were undertaken to determine sample size to compare DR and transpiration rates (TR) in *Hevea* clones grown under field conditions. DR of clones studied were compared with NPR (Nugawela, unpublished) of same clones to study whether a correlation exist between them.

MATERIALS AND METHODS

Plants were grown from budded stumps in the field. No selection was done for, uniformity of planting material at this stage. TR and DR were measured simultaneously when plants were approximately 1 year old using a LI — COR (Model LI — 1600) Steady State Porometer. All measurements were done at ambient relative humidity (RH).

Leaflet to leaflet variation

Four plants each from clones, PB 86 and LCB 870 were selected and two adjacent whorls were chosen from each selected plant. A random sample of four leaves were taken from each whorl and two TR and DR observations were taken from each leaflet of a single leaf.

Leaf to leaf variation within a whorl

Two plants each from clones PB 86, RRIC 100 and IRCI 2 were taken. A whorl with healthy leaves was selected from each plant. Two DR and TR observations were taken from the centre leaflet of every leaf in the whorl.

Whorl to whorl variation within a plant

Ten plants of clone PB 86 each with three whorls of leaves were taken; from each whorl three leaves, one from the bottom, middle and top of the whorl were selected, and two DR and TR observations were made on the centre leaflet. The experiment was repeated three times in three different days using the same set of plants to assess the consistancy of results.

Variance components and the time of sampling

A random sample of six plants from clone IAN 710 were selected, and from each plant one whorl with healthy leaves was chosen. Five leaves were sampled at random from each whorl and were labelled. Two DR and TR observations were made on the centre leaflet of the leaves sampled at different times of the day *viz*, 0800h, 1000h, 1200h, 1400h and 1600h. The experiment was repeated with clones RRIC 100 and PB 86.

Comparison of diffusive resistance and transpiration rates of *Hevea* clones

Five *Hevea* clones with 20 replicates were planted within the experimental area, for this purpose, using budded stumps. A random sample of 12 plants (1-year old) from each clone were taken and the second whorl from the top was chosen for measurements; the top whorl had mature leaves. Measurements were completed in 2 days and in 1 day 6 plants from a clone were sampled. Measurements started at 0930h and completed approximatly in 1 h each day.

RESULTS

DR and TR from a sample of 24 leaves from the clones PB 86 and LCB 870 are given in Table 1.

Table 1. TR ($\mu g\ cm^{-2}\ s^{-1}$) $\pm$ SE, DR ($s\ cm^{-1}$) $\pm$ SE in the three leaflets of a leaf

Clone	Leaflet	TR	DR
PB 86	Left	4.643 $\pm$ 0.171	1.93 $\pm$ 0.090
	Middle	4.495 $\pm$ 0.158	2.01 $\pm$ 0.090
	Right	4.410 $\pm$ 0.178	2.06 $\pm$ 0.100
LCB 870	Left	3.581 $\pm$ 0.206	3,510 $\pm$ 0.250
	Middle	3.933 $\pm$ 0.268	3.30 $\pm$ 0.240
	Right	3.756 $\pm$ 0.235	3.450 $\pm$ 0.280

There is no significant difference in DR and TR between the three leaflets of a leaf.

Within a whorl, the small leaves at the top, exhibited unusally high DR and low TR (Table 2). In Table 2, the leaf number 1 refers to the lowest leaf of the whorl.

Table 2. TR ($\mu g\ cm^{-2}\ s^{-1}$) $\pm$ SE and DR ($s\ cm^{-1}$) $\pm$ SE in different leaves of the same whorl of three Hevea *clones*

Clone	Leaf No.	TR	DR
RRIC 100	1 — 4	3.801 $\pm$ 0.067	5.38 $\pm$ 0.52
	5 — 8	2.944 $\pm$ 0.360	6.24 $\pm$ 0.77
	9 — 12	3.087 $\pm$ 0.125	6.98 $\pm$ 1.0
	13 — 16	2.084 $\pm$ 0.371	17.45 $\pm$ 9.23
IRCI 2	1 — 4	5.583 $\pm$ 0.119	2.75 $\pm$ 0.06
	5 — 8	7.436 $\pm$ 0.106	2.10 $\pm$ 0.05
	9 — 12	6.740 $\pm$ 0.702	2.22 $\pm$ 0.19
	13 — 16	3.418 $\pm$ 1.067	5.92 $\pm$ 1.95
PB 86	1 — 4	6.404 $\pm$ 0.779	2.35 $\pm$ 0.27
	5 — 8	6.193 $\pm$ 0.744	2.67 $\pm$ 0.62
	9 — 12	6.518 $\pm$ 1.600	2.49 $\pm$ 0.60
	13 — 16	5.320 $\pm$ 0.428	2.98 $\pm$ 0.01

The DR and TR of the leaves of the top and middle whorl were not different significantly, but they were significantly different from DR and TR of the bottom whorl (Table 3). Though not significant, a smaller coefficient of variation was observed in the middle whorl.

Table 3. Comparison of the first three whorls of PB 86 plants with respect to TR ($\mu g\ cm^{-2}\ s^{-1}$) (C. O. V. %) and DR ($s\ cm^{-1}$) (C. O. V. %)

Experiment	Whorl	TR		DR	
1	Top	7.218	(31.1)	2.23	(29.9)
	Middle	6.891	(29.6)	2.38	(30.7)
	Bottom	5.075	(32.3)	3.49	(41.8)
2	Top	6.298	(32.5)	3.36	(36.9)
	Middle	5.803	(23.0)	3.35	(23.3)
	Bottom	3.530	(32.3)	9.21	(87.1)
3	Top	6.654	(32.5)	3.04	(70.9)
	Middle	6.410	(33.9)	2.90	(31.6)
	Bottom	3.970	(22.3)	5.21	(37.7)

Note:— Any pair of means not connected by a vertical line within the experiment concerned, is significantly different

The DR varied significantly with time of sampling (Fig. 1).

The percentage contributions to the total variance from the plants, leaves and determinations at different times of sampling were calculated as for a nested classification (Snedecor and Cochran, 1980). See Tables 5 and 6.

Table 4. Variance components and their percentage contribution to the total variance in TR determination of three Hevea *clones.*

Clone	Time (h)	Plants of the same clone	Leaves of the same plant	Determinations of the same leaf	C.O.V. %
IAN 710	0800	22.631 (72.7%)	6.657 (21.4%)	1.844 (5.9%)	38.5
	1000	17.299 (76.8%)	4.392 (19.5%)	0.848 (3.8%)	74.3
	1200	36.407 (81.8%)	7.198 (16.2%)	0.921 (2.1%)	87.6
	1400	17.475 (77.5%)	4.549 (20.2%)	0.516 (2.3%)	91.4
	1600	17.597 (81.6%)	3.252 (15.1%)	0.707 (3.3%)	108.0
RRIC 100	0800	45.549 (85.4%)	7.121 (13.3%)	0.678 (1.3%)	186.1
	1000	2.6031 (78.3%)	0.561 (16.9%)	0.162 (4.9%)	118.0
	1200	32.626 (85.2%)	5.294 (13.8%)	0.388 (1.0%)	158.7
	1400	4.949 (69.7%)	1.939 (27.3%)	0.215 (3.0%)	148.3
	1600	16.791 (82.2%)	3.387 (16.6%)	0.260 (1.3%)	155.0
PB 86	0800	17.185 (69.4%)	6.042 (24.4%)	1.528 (6.2%)	72.9
	1000	39.076 (81.9%)	8.160 (17.1%)	0.495 (1.0%)	94.4
	1200	34.230 (57.4%)	22.992 (38.6%)	2.381 (4.0%)	75.9
	1400	21.663 (52.0%)	18.459 (44.3%)	1.534 (3.7%)	88.5
	1600	15.708 (55.3%)	11.480 (40.4%)	1.211 (4.3%)	70.9

Note: Percentage contributions are given within brackets

Table 5. Variance components and their percentage contribution to the total variance in DR determination of three Hevea *clones.*

Clone	Time (h)	Plants of the same clone	Leaves of the same plant	Determination of the same leaf	C. O. V.%
	0800	0.074 (73.0%)	0.019 (19.0%)	0.008 (7.9%)	62.3
	1000	9.766 (59.2%)	6.215 (37.7%)	0.517 (3.1%)	183.5
IAN 710	1200	31.271 (58.0%)	21.017 (39.0%)	1.662 (3.1%)	265.4
	1400	38.231 (60.9%)	23.056 (36.7%)	1.476 (2.4%)	209.4
	1600	193.470 (58.5%)	135.390 (41.0%)	1.690 (0.5%)	300.1
	0800	101.860 (64.8%)	53.908 (34.3%)	1.435 (0.9%)	259.5
	1000	311.250 (60.5%)	198.260 (38.5%)	5.060 (1.0%)	221.4
RRIC 100	1200	1610.800 (49.1%)	1657.200 (50.6%)	9.768 (0.3%)	407.1
	1400	3821.700 (55.9%)	2940.90 (43.0%)	72.038 (1.1%)	338.2
	1600	1264.100 (52.7%)	1087.00 (45.4%)	45.368 (1.9%)	340.8
	0800	29.941 (56.4%)	21.971 (41.4%)	1.220 (2.3%)	310.3
	1000	19.020 (54.6%)	15.737 (45.8%)	0.073 (0.2%)	205.0
PB 86	1200	82.622 (47.0%)	89.350 (50.8%)	3.794 (2.2%)	367.8
	1400	108.050 (52.2%)	96.642 (46.7%)	2.460 (1.2%)	337.1
	1600	131.100 (46.6%)	128.400 (45.6%)	21.885 (7.8%)	423.0

Note: Percentage contributions are given within brackets

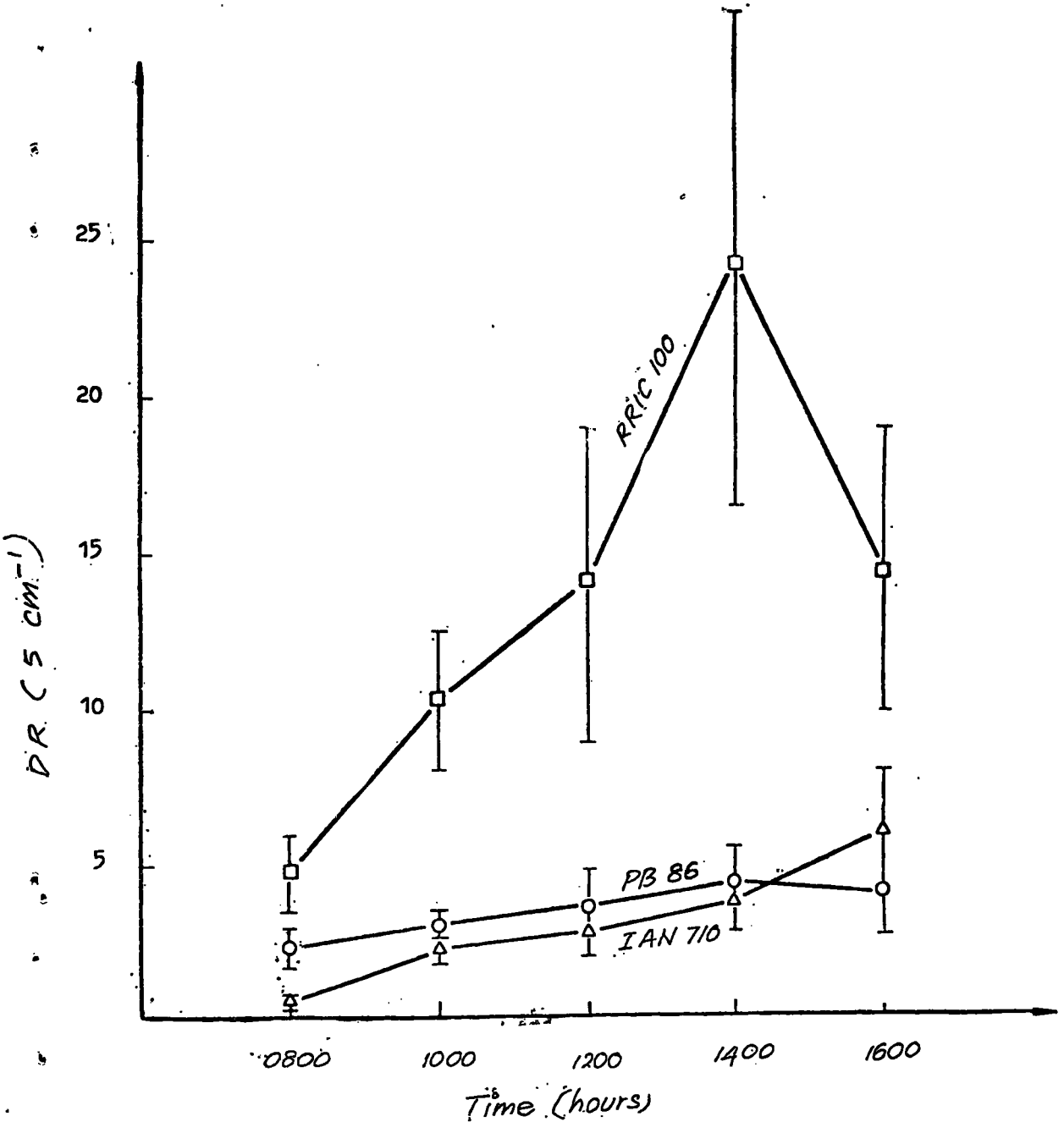
25
20
15
10
5
DR (5 cm⁻¹)
RRIC 100
PB 86
IAN 710
0800
1000
1200
1400
1600
Time (hours)

Fig. 1.

The DR and TR of clones RRIC 100, RRIC 103, RRIC 45. PB 86, and IAN 710 were significantly different (Table 7).

Table 6. TR ($\mu g\ cm^{-2}\ s^{-1}$) DR ($s\ cm^{-1}$) and NPR ($mg\ CO_2\ dm^{-2}\ h^{-1}$) of five Hevea *clones*

Clone	DR s cm^{-1}	TR mg H_2O cm^{-2} s^{-1}	NPR mg Co_2 dm^{-2} h^{-1}
RRIC 100	3.3928	6.2790	8.227
RRIC 103	2.5924	7.9008	11.671
RRIC 45	2.6744	8.0492	10.338
PB 86	1.9082	10.3655	11.583
IAN 710	2.0509	9.8613	9.941
L.S.D.	0.7258	2.4938	0.736

DISCUSSION

All three leaflets of a *Hevea* leaf are of identical age. The observed DR and TR did not differ significantly between leaflets of a leaf (Table 2). It could be that leaflets of a leaf are in uniform microclimatic conditions and of similar physiological status. Hence it was decided to sample one leaflet per leaf in subsequent measurements.

Of the leaves in a single whorl the comparatively smaller leaves at the top showed significantly different DR and TR from the rest. This could be due to differences in the amount of light received. Light is a stimulus for stomatal opening (Kanemasu *et al.*, 1969; Kvet and Marshall, 1971). Leaves were sampled systematically from the top, the middle and the bottom of a whorl in our studies to overcome this. Samsuddin (1980) selected leaves receiving more than 300 μ moles m^{-2} s^{-1} of light in his studies with *Hevea* clones.

Of the 3 whorls in an immature *Hevea* plant, the lowest, the one with oldest leaves, showed reduced stomatal opening. The first two although different in age were similar in this respect. This is in agreement with Fisher's (1967) observation that it is the physiological age that is important in stomatal opening. The existance of gradients in environmental parameters along the stem could also contribute to whorl to whorl variation and hence leaves of the same physiological age and at same height of the plant were selected in our studies.

In addition to responding to environmental stimuli, stomatal opening shows endogenous rhythms (Hsiao, 1975). For clones IAN 700, RRIC 100 and PB 86, DR was monitored from 0800h to 1600h. The DR varied significantly with time of sampling, the lowest been around 0800h. Hence when clonal comparisons are made in the field, measurements should be done within a defined period of the day.

The percentage contribution to the total variance from the plants component was much higher than that of the determinations component (Tables 4 and 5). This is true for all 3 clones and for different sampling times. The maximum contribution from the determinations component to the total variance of an observation was less than 8%, and from the plants component, it was greater than 50%. The contribution from the leaves component was between these two. Hence a higher number of plants per clone needs to be sampled, while the number of determinations per leaf can be reduced. The coefficient of variation for DR and TR values were comparatively smaller when the sampling was done in the morning. The results suggest that the time interval between 0800 h — 1000 h is more suitable for sampling leaves in order to study the clonal differences with respect to DR and TR.

A pilot study to determine TR and DR of clone PB 86 was carried out using four 1-year old plants under field conditions. Estimated variances of the mean TR and DR from the pilot study and for various sampling plans are given in Table 7.

Table 7. Estimated variance of the mean transpiration rates and diffusive resistance for various sampling plans (clone PB 86). a: No. of plants, b: No. of whorls, c: No. of Leaves, d: No. of observations, e: Estimates from pilot study.

Sampling plan				Estimated variance of the mean	
a	b	c	d	TR	DR
4	2	4	6	0.115[e]	0.026[e]
6	1	3	2	0.107	0.027
8	1	3	2	0.080	0.020
10	1	3	2	0.064	0.016
12	1	3	2	0.053	0.013
12	1	3	1	0.060	0.015

Table 7 shows that approximately 50% reduction in the estimated variance of mean TR and DR could be obtained when the number of plants is increased to about 10—12 while the number of determinations per leaf were two.

The DR and TR were compared taking into account all important variables that influence stomatal opening and closing. The DR and TR values were significantly different at 0.1% level in clones RRIC 100, RRIC 103, RRIC 45, PB 86 and IAN 710.

Stomatal diffusive resistance has a greater correlation with TR than NPR (Table 6). Diffusion of H_2O and CO_2 during transpiration and photosynthesis respectively has two resistances in common namely the boundary layer and stomatal resistance. Diffusion of

CO_2 during photosynthesis has additional resistances (Zelitch, 1971a), and therefore a lower correlation with stomatal diffusive resistance. Hence clonal differences in NPR might be partly due to the clonal differences in stomatal DR to CO_2 diffusion. Specific differences in diffusibility of the intercellular spaces in the leaf, metabolic pathways concerned with CO_2 fixation and CO_2 evolution, translocation of assimillates may also contribute to clonal differences NPR (Larcher, 1969). Further studies with high and low yielding clones are necessary to establish the extent to which stomatal diffusive resistance can control the photosynthetic capacity of a leaf and hence the productivity of the plant. Such information can help breeders in breeding new *Hevea* clones for improved yields and vigorous growth.

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