PHOTOSYNTHESIS OF TROPICAL PASTURE PLANTS

III.* LEAF AGE

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Abstract

Grass and legume plants were grown under near-optimum conditions in controlled-environment cabinets. Changes in net photosynthetic rate, dark respiration rate, and carbon dioxide transfer resistances during leaf ontogeny, and variability between leaves on grass tillers and legume runners were studied under controlled conditions in an open gas analysis system.

As leaves aged dark respiration rate, light compensation point, and light saturation point declined progressively, whereas the initial slope of the light response curve and net photosynthetic rate measured at 10,000 f.c. increased to a maximum before declining. Immediately after unfolding, grass leaves photosynthesized at a rate more closely approximating the maximum than legume leaves which had a relatively low rate initially. For both grasses and legumes, the lower photosynthetic rate of young leaves was accompanied by high mesophyll resistances, but the decline from the maximum rate as leaves aged was associated with increases in both stomatal and mesophyll resistances. Stomatal resistance exerted a major control over the net photosynthetic rate of grasses, whereas mesophyll resistance predominated for legumes.

The influence of chlorophyll concentration on the relationship between net photosynthesis and leaf age is discussed.

I. Introduction

It is known that net photosynthetic rate and dark respiration rate change during leaf ontogeny, and differ from leaf to leaf on the same plant. Although there is considerable evidence of a decline in metabolic activity and photosynthetic enzyme and protein synthesis during senescence (Smillie 1962; Hardwick, Wood, and Woolhouse 1968), and some evidence of differences in stomatal resistance (Holmgren, Jarvis, and Jarvis 1965; Begg and Jarvis 1968; Brown and Rosenberg 1970) and mesophyll resistance (Woolhouse 1968; Ryle and Hesketh 1969) between leaves of different age, there does not seem to have been a systematic study of the simultaneous changes of carbon dioxide exchange and transfer resistances as leaves age.

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In previous papers (Ludlow and Wilson 1971*a*, 1971*b*), we have shown how environmental factors affect carbon dioxide exchange and transfer resistances. In this paper, we show how a physiological factor, leaf age, affects net photosynthetic rate (P_N), dark respiration rate (R_D), and stomatal (r_s) and mesophyll (r_M) resistance to carbon dioxide transfer and some other photosynthetic characteristics measured under standard environmental conditions. This was done in two ways; firstly, by studying these parameters during ontogeny of particular leaves, and secondly by studying the variability between leaves of different age on the same plant.

II. Materials and Methods

The procedures, apparatus, and calculation of resistances were similar to those described by Ludlow and Wilson (1971*a*). In the calculation of r_M, it was assumed that previously determined values of the CO_2 compensation concentration (Γ, 0 and 40 μl l^{-1} for grass and legume leaves, respectively) applied to all stages of ontogeny and for all positions on the plant. The consequences of this assumption will be discussed.

(*a*) *Changes during Leaf Ontogeny*

When plants of glycine [*Glycine wightii* (R. Grah. ex Wight & Arn) Verdcourt cv. Cooper], calopo (*Calopogonium mucunoides* Desv.), *S. almum* (*Sorghum almum* Parodi cv. Crooble), and elephant grass (*Pennisetum purpureum* Schum. Q5088) were 30 days old, two leaves, which were on different plants and which unfolded on the same day, were labelled for each species. Light response curves of net photosynthesis (relationship between illuminance and net photosynthetic rate) were determined on day 2 and every 4 days thereafter until the leaves of one species (elephant grass) died, 40 days from unfolding. The chamber accommodated the entire trifoliate leaves of legumes but only the apical portion of grass leaves. Area of grass leaves, therefore, has little meaning in the present context and will not be presented. Tillers and runners other than those containing the leaves being studied were prevented from developing.

(*b*) *Differences between Leaves on the Same Plant*

Light response curves of leaves were obtained from runners of four legumes [calopo; glycine; *Phaseolus atropurpureus* D.C. cv. Siratro; *Vigna luteola* (Jacq.) Benth. cv. Dalrymple] and tillers of five grasses (elephant grass; *Brachiaria ruziziensis* Germain & Evrard cv. Kennedy; *Melinis minutiflora* Beauv; *Panicum maximum* Jacq. cv. Hamil; *S. almum*) but because the results and conclusions are similar to those obtained for the ontogenetic study, only some of the data from one grass (*S. almum*) and one legume (*V. luteola*) and some mean values of the data for all species are presented. Measurements were made on 4–6-week-old plants, and leaves were numbered consecutively from the youngest to the oldest.

III. Results

(*a*) *Changes during Leaf Ontogeny*

As data for duplicate leaves were qualitatively similar, only one set are presented for each species. Net photosynthetic rate of grass leaves did not become light saturated except in the later stages of senesence, whereas legume leaves were always light saturated at the highest illuminance, and the light saturation point decreased with leaf age (Fig. 1).

The initial slope of the light response curve (determined from five observations between 0 and 1000 f.c. and based on incident rather than absorbed radiation) increased to a maximum, which occurred at different times for the different species, before declining (Fig. 2). Except for elephant grass in the final stages of senescence, initial slopes for grasses were always higher than those for legumes. Light com-

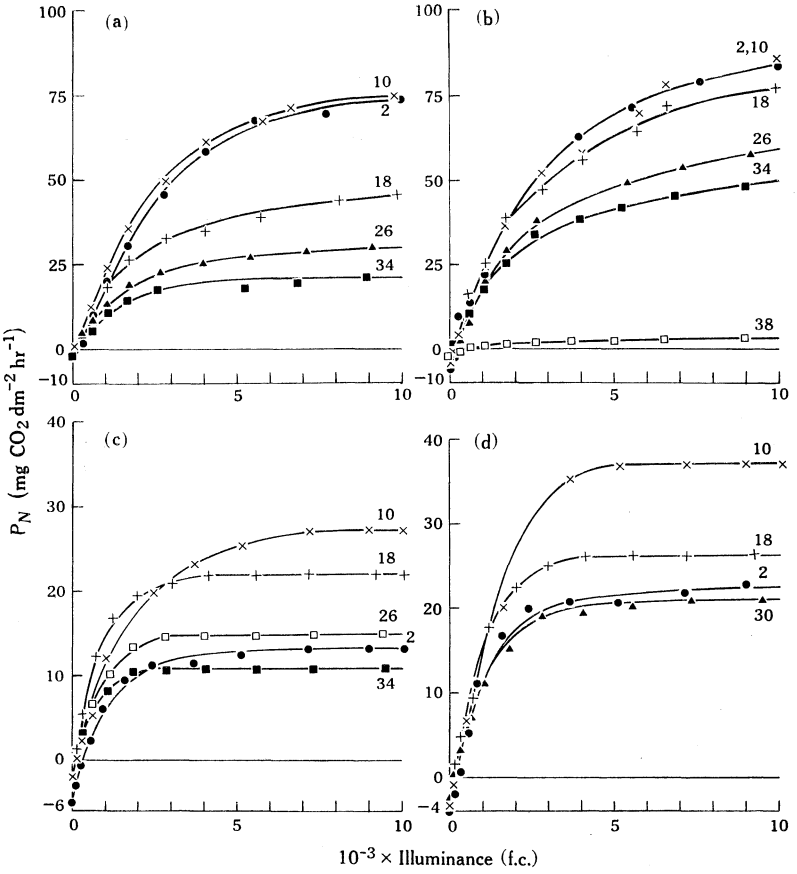

Fig. 1.—Relationship between illuminance and net photosynthetic rate (P_N) of (*a*) *S. almum*, (*b*) elephant grass, (*c*) glycine, and (*d*) calopo leaves at various times (days) after unfolding. Conditions: 300 ± 5 μl l^{-1} CO_2, $30 \pm 0{\cdot}1$°C leaf temperature, and 17 ± 3 mmHg leaf–air vapour pressure difference.

pensation points (Fig. 3) declined sharply after leaves unfolded until about day 30, but then subsequently increased slightly. Although dark respiration rate also declined during ontogeny (Fig. 4), it was not associated with an increase in stomatal resistance measured in the dark.

The ontogenetic pattern of P_N measured at 10,000 f.c. for all species is shown in Figure 4. Apical portions of grass laminae were fully expanded by day 2 which appeared to slightly precede the maximum P_N. The subsequent decline of P_N was

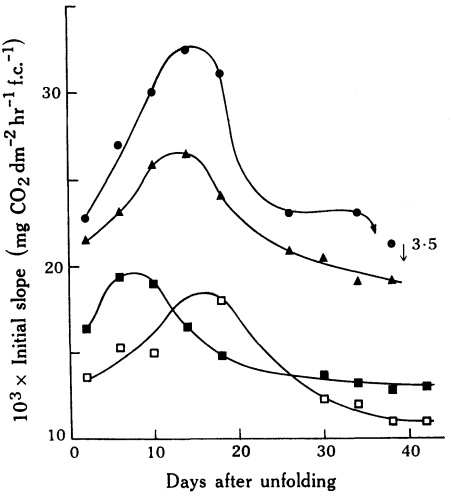

Fig. 2.—Changes in the initial slope of the light response curve of leaves of elephant grass (●), *S. almum* (▲), calopo (■), and glycine (□) during ontogeny. Conditions: 300±5 μl l^{-1} CO_2, 30±0·1°C leaf temperature, and 17±3 mmHg leaf–air vapour pressure difference.

associated with increases in both r_s and r_M. On day 40 when elephant grass leaves were dead, *S. almum* leaves were still capable of carbon dioxide uptake even though the margins were necrotic.

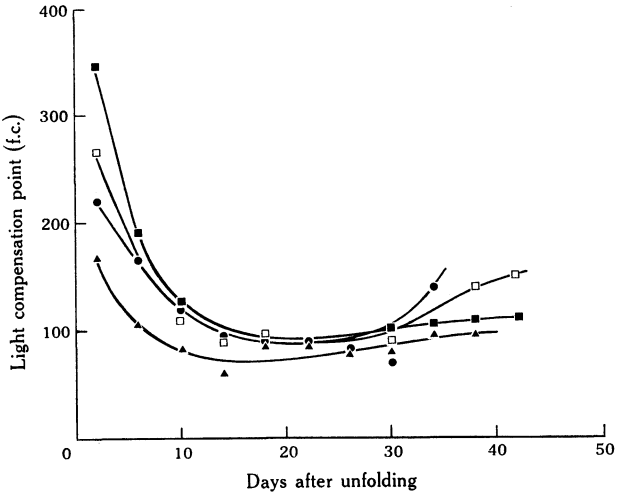

Fig. 3.—Changes in the light compensation point of leaves of elephant grass (●), *S. almum* (▲), calopo (■), and glycine (□) during ontogeny. Conditions: 300±5 μl l^{-1} CO_2, 30±0·1°C leaf temperature, and 17±3 mmHg leaf–air vapour pressure difference.

At unfolding, the P_N of legume leaves was low and r_M was high. Mesophyll resistance fell sharply as P_N approached a maximum which coincided with the cessation of leaf expansion. Increases in both r_s and r_M accompanied the subsequent decline of P_N, which was still positive at the end of the experiment when chlorophyll concentration was visibly reduced; glycine leaves, for example, were completely

chlorotic on day 35. The decrease in P_N of calapo between days 38 and 42 was associated with an increase in r_s, but stomatal resistance changed little during the ontogeny of glycine leaves.

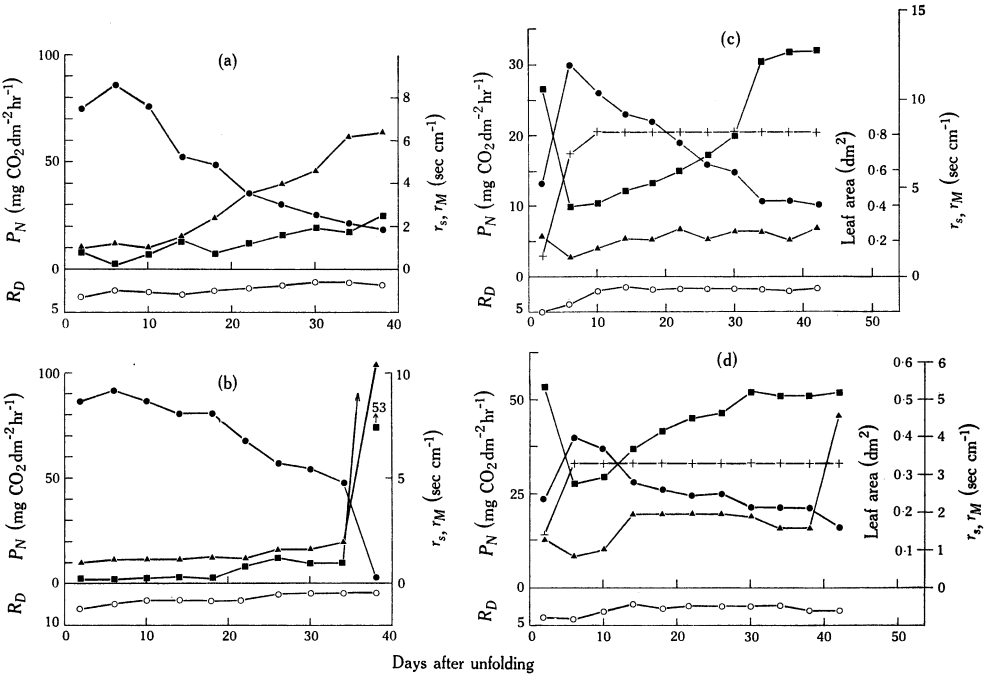

Fig. 4.—Changes in net photosynthetic rate (P_N,●), stomatal (r_s,▲) and mesophyll (r_M,■) resistance to carbon dioxide transfer, dark respiration rate (R_D,○), and leaf area (+) of (*a*) *S. almum*, (*b*) elephant grass, (*c*) glycine, and (*d*) calopo leaves during ontogeny. The apical portion of grass leaves on which gas-exchange measurements were made were fully expanded by day 2. Conditions: 10,000 f.c., 300 ± 5 μl l^{-1} CO_2; $30 \pm 0{\cdot}1$°C, 17 ± 3 mmHg leaf–air vapour pressure difference, and $r_a = 0{\cdot}9$ sec cm^{-1}.

(*b*) *Differences between Leaves on the Same Plant*

Photosynthetic characteristics differed with leaf number in a way similar to that during ontogeny (Fig. 5; Table 1), except that there was no difference between the initial slope of the light response curves for leaves of different age on runners or tillers. This probably reflects the insensitivity of the technique which was only based on observations at 0 and 400 f.c. However, the mean value for 45 grass leaves ($0{\cdot}026$ mg CO_2 dm^{-2} hr^{-1} f.c.$^{-1}$) was significantly greater than the mean for 74 legume leaves ($0{\cdot}017$ mg CO_2 dm^{-2} hr^{-1} f.c.$^{-1}$).

As only the tip of the first *S. almum* leaf had emerged, the surrounding sheaths were removed and the pale yellow lamina unrolled before being placed in the chamber. Therefore, the low P_N and light saturation of this leaf is not representative of exposed leaves, but it demonstrates the relatively large photosynthetic capacity of grass leaves even before they have unrolled and while the chlorophyll concentration is visibly low.

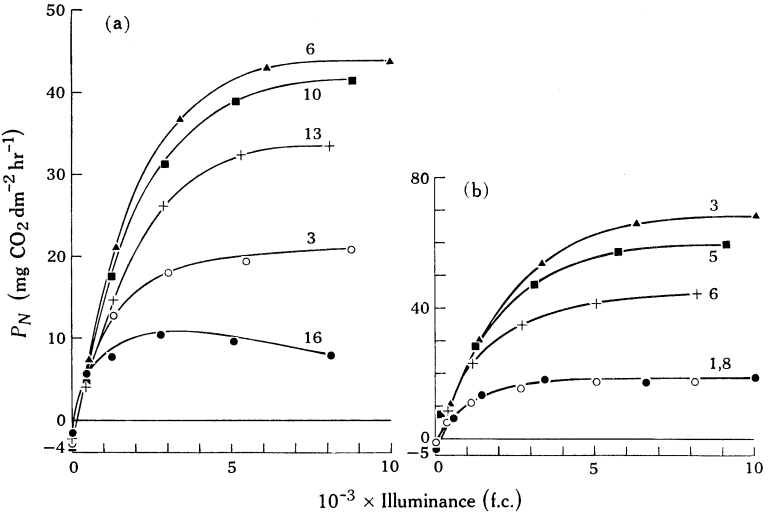

Fig. 5.—Relationship between illuminance and net photosynthetic rate (P_N) of leaves from different positions on (*a*) a *V. luteola* runner, and (*b*) a *S. almum* tiller. Leaves are numbered consecutively from the youngest to the oldest. Conditions: 300±5 μl l^{-1} CO_2, 30±0·1°C leaf temperature, and 17±3 mmHg leaf–air vapour pressure difference.

TABLE 1

NET PHOTOSYNTHETIC RATE (P_N), CARBON DIOXIDE TRANSFER RESISTANCES (r_s AND r_M), DARK RESPIRATION RATE (R_D), AND STOMATAL RESISTANCE IN THE DARK (r_s*) OF LEAVES FROM A *S. ALMUM* TILLER AND A *V. LUTEOLA* RUNNER

Leaves are numbered from the youngest to the oldest. Conditions: 9000 f.c., 300±5 μl l^{-1} CO_2, 30±0·1°C leaf temperature, 17±3 mmHg leaf-air vapour pressure difference. r_a = 0·8 sec cm^{-1}. Units for r_s, r_M, and r_s* as for r_a; units for P_N and R_D mg CO_2 dm^{-2} hr^{-1}

Leaf No.	*V. luteola*					*S. almum*				
	P_N	r_s	r_M	R_D	r_s*	P_N	r_s	r_M	R_D	r_s*
1	—	—	—	—	—	19	3·1	6·6	3·4	7
2	—	—	—	—	—	65	1·5	0·4	2·7	13
3	13	2·4	11·3	3·2	0·9	68	1·4	0·4	2·6	33
4	—	—	—	—	—	68	1·6	0·3	2·6	24
5	21	1·6	5·9	3·0	3·4	60	2·2	—	3·5	51
6	44	0·8	2·5	3·4	1·3	45	2·0	1·2	1·8	29
7	42	1·0	2·5	4·0	0·8	—	—	—	—	—
8	40	1·1	2·7	3·1	2·2	18	4·0	6·4	1·3	57
9	—	—	—	—	—					
10	42	1·3	2·2	3·3	4·0					
11	30	1·8	3·4	1·8	7·1					
12	34	1·6	2·6	2·5	4·2					
13	33	1·0	3·3	2·7	2·8					
14	24	3·2	3·4	3·7	10·5					
15	17	2·0	7·4	1·8	12·6					
16	8	3·9	17·9	1·8	11·5					

IV. Discussion

Although differences in net photosynthesis and carbon dioxide transfer resistances between successive leaves on the same plant do not necessarily reflect quantitatively the difference due to age (Saeki 1959; Hopkinson 1964; Peat 1970), changes in these parameters during leaf ontogeny and with leaf number were qualitatively similar in the present work. Therefore, both sets of data will be discussed collectively on the basis of leaf age.

The shape of the light response curve of both grasses and legumes changed with leaf age (Figs. 1 and 5). Contrary to other data (Drury and Park 1968; Woolhouse 1968) the initial slope of the light response curve changed during leaf ontogeny (Fig. 2). Peat (1970) has reported similar trends in the initial slope of a well-fertilized tomato leaf. The smaller values for young and old leaves may be due to a lower photochemical efficiency but they must, in part at least, have been due to inability to absorb incident radiation, particularly in the final stages of senescence when some leaves were chlorotic.

The rapid decline in the light compensation point after unfolding and the increase after day 30 (Fig. 3) were associated with a rapid increase and a decrease, respectively, of the initial slope of the light response curve (Fig. 2). Although R_D decreased with leaf age in the usual manner, the marked increase which occurs in the later stages of senescence of some species (Kusumoto and Shinosaki 1954; Hardwick, Wood, and Woolhouse 1968) was not observed. Metabolic activity decreases during leaf senescence (Woolhouse 1967) but it is not known whether this is a cause or an effect of the lower respiratory activity.

At 10,000 f.c., P_N of grass and legume leaves followed the usual pattern with age (Fig. 4), rising rapidly to a maximum and then declining slowly (Saeki 1959; Hopkinson 1964; Hiroi and Monsi 1966; Wada 1968). The relative P_N soon after unfolding and the rate at which the maximum P_N was attained, reflect the different patterns of leaf expansion. Grass leaves have an intercalary meristem, and unrolled parts of the leaves are fully expanded and have photosynthetic rates more closely approaching the maximum. On the other hand, in legumes considerable leaf expansion occurs after unfolding (Fig. 4; Wilson and Ludlow 1968; Ludlow, unpublished data), and the maximum P_N and the cessation of leaf expansion occur simultaneously.

The large r_M which accompanied the low P_N of legume leaves soon after unfolding may, in part, have resulted from an underestimate of the carbon dioxide compensation concentration (Γ), because Γ of newly unfolded leaves is sometimes greater than that of fully expanded leaves (Kriedemann 1968). In the present experiment, it was assumed that Γ was equal to the fully expanded value during leaf ontogeny. Mesophyll resistance may also be large because one or more of its components are large:

$$r_M = r_m + r_e + r_x, \tag{1}$$

where

$$r_e = C_c/\epsilon I, \tag{2}$$

and where r_m is the physical transfer resistance, r_e is excitation resistance, r_x is carboxylation resistance, C_c is the carbon dioxide concentration at the chloroplast surface, ϵ is photochemical efficiency, and I is illuminance (Monteith 1963). Carboxyla-

tion resistance may have been large following unfolding because of the low activity of photosynthetic enzymes (Bradbeer 1969) or inability to utilize an unstable product of the light reaction (Pearce 1967), but little is known about the other two resistances. However, it is evident that the smaller initial slope (Fig. 2), and presumably photochemical efficiency, would contribute to a higher excitation resistance (equation 2). The same reasons can be used to explain the high r_M of rolled grass leaves (Table 1).

The progressive decline of P_N from a maximum value of both grass and legume leaves was accompanied by increases in both r_s and r_M (Fig. 4). Increases in r_s with age have been reported (Holmgren, Jarvis, and Jarvis 1965; Begg and Jarvis 1968; Brown and Rosenberg 1970) and may result from a lower kinin activity (Livne and Vaadia 1965). There is some evidence that r_M increases with leaf age (Woolhouse

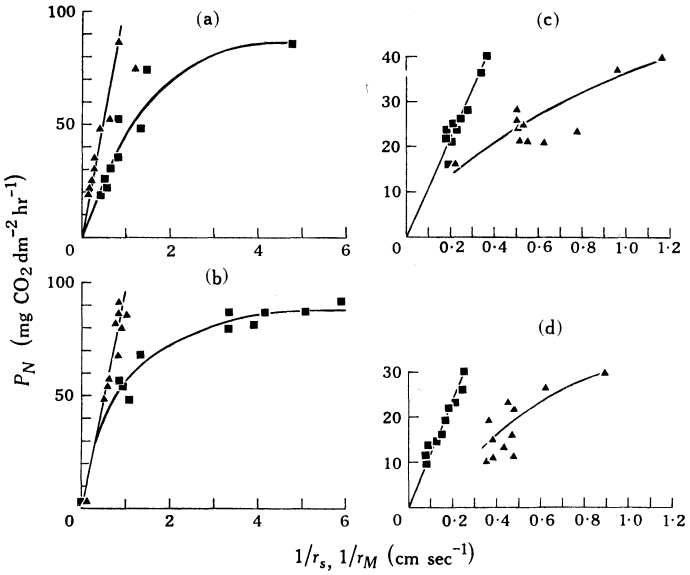

Fig. 6.—Relationship between net photosynthetic rate (P_N), and stomatal ($1/r_s$,▲) and mesophyll ($1/r_M$,■) conductance of (*a*) *S. almum*, (*b*) elephant grass, (*c*) glycine, and (*d*) calopo leaves during ontogeny. Resistance data from Figure 4 are replotted as conductances.

1968; Ryle and Hesketh 1969) which is supported by indirect evidence from isolated chloroplasts (Clendenning and Gorham 1950), *Chlorella* (Rabinowitch 1951), and flashing-light experiments (Pearce 1967). The assumption that Γ remained constant after the leaf had ceased expanding seems justified, except for elephant grass after day 35, because Γ of red clover and sunflower change little with age until death is imminent (Pearce 1967; Whiteman and Koller 1967). Therefore, it is possible to attempt to explain the increase in r_M with age in terms of its three components (equation 1). It is not known how r_m or r_e changed with age, but from equation 2 it can be seen that r_e would have increased with age as the initial slope (and probably ϵ) declined (Fig. 2) if C_c either remained constant or increased. There is no direct evidence of an increase in r_x during senescence but there is indirect evidence of

two kinds. Firstly, there is a decrease in the overall enzymatic capacity for photosynthesis (Smillie 1962) and of the enzyme carboxydismutase (Woolhouse 1967). Secondly, in aging leaves P_N is correlated with RNA and protein content (Hardwick, Wood, and Woolhouse 1968), with protein metabolism (Richardson 1957; Rhodes and Yemm 1963), and with protein nitrogen content (Miyazaki and Tatemichi 1968; Tatemichi 1968). Furthermore, kinin analogues which prevent net protein breakdown also prevent the decline in P_N with age (Natr 1967).

The relative importance of r_s and r_M as determinants of P_N during leaf ontogeny can be seen from the relationship between P_N and stomatal ($1/r_s$) and mesophyll ($1/r_M$) conductance (Figs. 6 and 7). The relationship between P_N and $1/r_M$ is steeper and therefore more important in legumes, whereas $1/r_s$ predominates in grasses: these being a consequence of the relative magnitudes of the conductances in legumes and grasses.

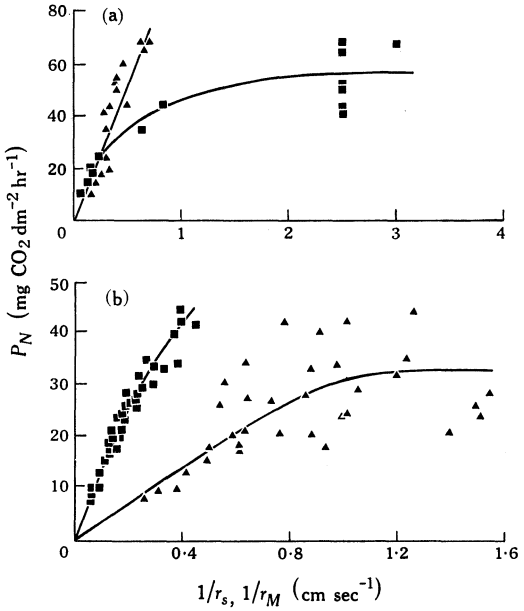

Fig. 7.—Relationship between net photosynthetic rate (P_N) and stomatal ($1/r_s$,▲) and mesophyll ($1/r_M$,●) conductance of leaves from various positions on (*a*) grass tillers and (*b*) legume runners. Resistance data from Table 1 and data for the other three legumes and four grasses are plotted as conductances.

The decline of net photosynthesis during senescence is accompanied by a decline in chlorophyll concentration and a causal relationship between these parameters has been proposed (Sestak and Catsky 1962). However, because a linear relationship between P_N measured at high illuminances and chlorophyll concentration is seldom found (Sestak 1966; Hardwick, Wood, and Woolhouse 1968; Treharne, Cooper, and Taylor 1968), changes in chlorophyll concentration may merely parallel changes in P_N and the relationship may not be causal. The extent to which changes in chlorophyll concentration influenced the decline in P_N of grass and legume leaves was investigated using the equation describing P_N:

$$P_N = (C_a - \Gamma)/(r_a + r_s + r_m + r_e + r_x), \quad (3)$$

where C_a is the ambient carbon dioxide concentration. A change in chlorophyll concentration could only have a direct effect on ϵ in r_e (equation 2). If it is assumed

that the change in chlorophyll concentration was the sole cause of the change in the initial slope of the light response curve, and that the change in the initial slope reflects a corresponding change in ϵ (both of which are unlikely), the maximum effect which chlorophyll concentration could be expected to have on P_N can be estimated. Excitation and carboxylation resistances were calculated as described by Ludlow (1970) assuming r_m was 0·1 and 1·0 sec cm^{-1} and Γ was 0 and 40 μl l^{-1} CO_2, respectively, for grasses and legumes. Even though the assumptions made tend to maximize the influence of chlorophyll concentration, the 40% decrease in initial slope (and by assumption, in chlorophyll concentration) between days 14 and 34 could only account for 18% of the decrease in P_N of elephant grass, and the corresponding figures for calopo between days 6 and 38 were 60% and 18%. Therefore, although chlorophyll concentration is positively related to P_N, it probably plays a subsidiary role to such factors as photosynthetic enzyme activity in determining P_N as leaves age. The reasons given by Sestak (1966) for the lack of linearity between chlorophyll concentration and P_N, and the absence of a direct relationship between initial slope of the light response curve and P_N (Figs. 3 and 4) are consistent with this suggestion. Furthermore, the relatively high P_N of the first, pale yellow *S. almum* leaf (Table 1) and of chlorotic glycine leaves [Fig. 4(*c*)] are inconsistent with a direct causal relationship between P_N and chlorophyll concentration.

V. Acknowledgments

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