

GROWTH AND PHOTOSYNTHESIS OF CLOSELY RELATED C₃ AND C₄ GRASSES, AS INFLUENCED BY LIGHT INTENSITY AND WATER SUPPLY

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SUMMARY

Growth of Indonesian C₄ and C₃ grasses was compared in field experiments in Indonesia and in growth chamber experiments in the Netherlands. Two C₄ species, *Axonopus compressus* (Swartz) Beauv. and *Setaria plicata* (Lamk.) T. Cooke, were used and one C₃ species, *Oplismenus compositus* (L.) Beauv.

Both the field and the growth chamber experiments showed no large differences in relative growth rate between the C₃ and the C₄ grasses, grown at high light intensities, neither with a high, nor with a low water supply.

Net photosynthesis of the C₃ species was lower than that of the C₄ species at all growth and experimental conditions. Whenever this resulted in a lower unit leaf rate of the C₃ species, this disadvantage was overcome by formation of a larger leaf area.

At very low light intensities, the C₄ species *Axonopus* had a lower relative growth rate than the C₃ species *Oplismenus*. This may explain the absence of this C₄ grass in the deep shade.

The absence of the C₃ species in open areas cannot be explained by a lower relative growth rate of this species at high light intensities. Other factors as a low shoot/root ratio, a high soluble sugar content and a high initial growth rate may give C₄ grasses a high competitive value over C₃ grasses in open and dry areas. Some of these factors may depend indirectly upon the C₄ mechanism of photosynthesis.

1. INTRODUCTION

Since the discovery of the C₄ metabolism, the purpose of this metabolism was discussed. A strongly competitive value was ascribed to C₄ plants, since many weeds are C₄ plants (BLACK et al. 1969). Adaptation to high temperature, to high light intensities and to drought is given as an explanation for the occurrence of this syndrome. Most of the C₄ plants indeed are found in tropical countries under conditions of high light intensity and high temperature. BJÖRKMAN et al. (1974), however, observed, upon comparison of a C₄ species from a hot climate and another species from a cool climate that the advantage of the C₄ mechanism can only be realized if other adaptive mechanisms, essential for growth in hot environments are also present.

Earlier research on the island of Java, Indonesia, revealed that hardly any C₄ species was found in shady environments, or at high altitudes (above 2000 metres), thus at lower temperatures. In open areas at low altitudes, 30 to 50% of the studied plants consisted of C₄ species (HOFSTRA et al. 1972). Further

examination of the weeds in arable fields on Java supported these findings. In the Netherlands a C_4 plant is rarely found and then only in conditions of drought and a high light intensity (Hofstra, unpublished results).

We were interested to see if next to the C_4 metabolism other factors were of importance to explain the occurrence of C_4 plants in Indonesia in open areas. Growth experiments with tropical C_3 and C_4 grasses were performed in growth chambers at two regimes of light intensity and water supply. In addition these grasses were grown under more natural conditions in Indonesia at different light intensities. In this way we investigated whether a C_3 and C_4 grass could be grown under conditions adverse to their natural habitats.

2. MATERIAL AND METHODS

For the experiments C_3 and C_4 grasses were used, all of them belonging to the group of the Paniceae (BROWN 1958) and all of them endogenous on Java. *Oplismenus compositus* (L.) Beauv., a C_3 species, is found in moderate to heavy shade. *Setaria plicata* (Lamk.) T. Cooke and *Axonopus compressus* (Swartz) Beauv., both C_4 species, are found in open and occasionally in slightly shaded areas. All these grasses are found in slightly humid habitats.

Field experiments. The plants were propagated vegetatively. Three-leaf cuttings were set rooting in tap water at about 10% daylight and at 25°C. The rooted cuttings were placed in soil in pans (surface 500 cm², depth 17 cm), four per pan, at respectively 4, 20, 40 and 100% of the normal daylight. Reduction of the light intensity was obtained with cheesecloth. The plants were supplied with ample water and fertilizer. The experiment was performed once in the wet season (I) and once in the dry season (II). In Bogor (Java) the maximum and minimum temperature in the dry season are 32° and 20°C respectively, and in the wet season 29° and 22°C. The mean relative humidity is 80% in the dry, and 85% in the wet season. The mean light intensity is in the dry season 0.39×10^3 Watt.m⁻² and in the wet season 0.37×10^3 Watt.m⁻².

Growth chamber experiments. Three leaf cuttings were set rooting in a mixture of peat and sand, at about 0.40×10^2 Watt.m⁻² and 25°C in a humid atmosphere in the greenhouse. The rooted cuttings were placed in pots (Ø 10 cm) in compost, one per pot and acclimatized to the growth chamber for one week at 0.10×10^3 Watt.m⁻². The plants were illuminated by white fluorescent TL tubes (Philips TL/35 and red fluorescent TL tubes (Philips, TL/15) in a ratio of 8/1. Daylength and temperature were in accordance with those in the field experiments, a daylength of 12 hours, and a day/night temperature of 28/20°C, whereas the relative humidity was about 50%.

It was difficult to start the experiment with plants of about equal size and weight, as *Axonopus* rooted much faster than *Oplismenus*. In the second experiment, therefore, *Axonopus* was set rooting one week later and in the third experiment even two weeks later than *Oplismenus*.

After one week the plants were divided in four groups and placed in the different conditions:

HL,HW: $0.20 \times 10^3 \text{ Watt.m}^{-2}$, high water supply

HL,LW: $0.20 \times 10^3 \text{ Watt.m}^{-2}$, low water supply

LL,HW: $0.20 \times 10^2 \text{ Watt.m}^{-2}$, high water supply

LL,LW: $0.20 \times 10^2 \text{ Watt.m}^{-2}$, low water supply.

The high water supply was obtained by ample irrigation. This amounted to twice the gift of water at the low water supply and in later stages of growth even four times this value. Twice a week all plants were given a nutritional supply of 25 ml Hoagland solution (normal strength).

At regular intervals, two plants of each condition were harvested, in experiment I for a period of 8 weeks, in experiment II for a period of 6 weeks. In the third experiment, instead of pots, large pans were used for the growth of the plants, in which they could tiller more freely than in pots. Four rooted cuttings were placed in one pan (surface about 1600 cm²), in each corner one. The pans were placed at each of the above mentioned conditions and the plants were harvested once after a growth period of seven weeks.

Fresh and dry weight of shoots and roots were determined and the total area of the leaves was measured. Relative growth rate (RGR), unit leaf rate (ULR), leaf area ratio (LAR) and leaf weight ratio (LWR) were calculated after EVANS (1972).

RGR: $(\ln W_2 - \ln W_1) \cdot (T_2 - T_1)^{-1}$

ULR: $(W_2 - W_1) \cdot (1/2 L_1 + 1/2 L_2)^{-1} \cdot (T_2 - T_1)^{-1}$

LAR: $L \cdot W^{-1}$

LWR: (Leaf dry weight) $\cdot W^{-1}$

W_1 and L_1 are plant dry weight and leaf area at time T_1 respectively, and W_2 , L_2 at time T_2 .

Photosynthesis. Net photosynthesis was measured, using an infrared gas-analyser (URAS) in an open system (HESKETH & MUSGRAVE 1962). The first fully developed leaves were, still attached to the plant, placed in a perspex photosynthesis chamber and illuminated with two incandescent lamps (500 Watt). The distance plant/lamp varied, giving light intensities from 20–400 Watt.m⁻². The temperature in the chamber was kept between 29° and 32°C.

Sugars. Soluble sugars were determined in an 80% ethanol extract with the anthrone reagent after FALES (1951). The insoluble sugars (starch) were determined similarly after hydrolysis of the starch with 3% HCl and subsequent treatment with a 10% Al(OH)₃ suspension.

3. RESULTS AND DISCUSSION

Photosynthesis. Net photosynthesis of *Axonopus*, the C₄ species, was higher than that of *Oplismenus*, the C₃ species. *Axonopus* showed its C₄ pathway of photosynthesis at all growth and experimental conditions (fig. 1). Photosynthesis was influenced by the light conditions during growth, 100% of daylight giving the highest value for *Oplismenus* as well as for *Axonopus*. Growth conditions of 100% and 34% daylight gave about the same values but a remarkable decrease showed when plants were grown at 10% and 4% of day-

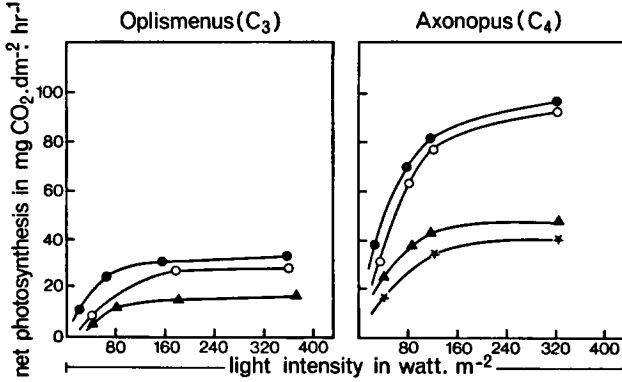


Fig. 1. Net photosynthesis, measured at different light intensities, of *Axonopus* and *Oplismenus* plants, grown in Indonesia.

Growth conditions: 100% day light: ●—●, 34%: ○—○, 20%: ▲—▲, 2%: ★—★.

light. The difference in net photosynthesis between growth conditions was clearest at high light intensities and tended to decrease at lower light intensities (fig. 1).

Growth. Data about growth are given in table 1.

a. RGR. In the growth chamber experiments the RGR of *Axonopus* and *Oplismenus* differed slightly at the high light intensity. At the low light intensity, however, the RGR of *Oplismenus* surpassed that of *Axonopus*. The RGR of *Axonopus* might even have been much lower when the plants had been grown from the start at a low light intensity without any treatment at 0.10×10^3 Watt.m⁻². This low growth rate may, therefore, account for the absence of *Axonopus* in the deep shade. In all experiments the RGR values were lower under dry conditions, for *Axonopus* as well as for *Oplismenus*.

Growth in large pans (experiment III) indeed resulted in a higher growth rate at HL, for *Oplismenus*. It was even somewhat more beneficial for *Axonopus*, this grass being more stoloniferous. At LL the RGR did not increase by using large pans.

In the wet season (field experiments) *Axonopus* had, as in the growth chamber experiments, the lowest RGR value; in the dry season the values for the three species were about equal.

b. ULR and LAR. Two components may be distinguished in the relative growth rate: a physiological one, the unit leaf rate and a morphological one, the leaf area ratio (EVANS 1972). At high light intensities the higher net photosynthesis of the C₄ plants resulted in a higher unit leaf rate (table 1, ULR). In the field experiments the difference was clearest between *Oplismenus* and *Setaria*. With decreasing light intensities the values shifted, giving at the lowest light intensity somewhat higher values for *Oplismenus*. This grass could overcome the disadvantage of its lower net photosynthesis at high light intensities by formation of a larger leaf area (table 1, LAR). All three species were capable

Table 1. Relative growth rate (RGR), unit leaf rate (ULR) and leaf area ratio (LAR) of *Oplismenus* (O), *Axonopus* (A) and *Setaria* (S), grown in different conditions of light intensity and water supply. RGR in $(\ln W_2 - \ln W_1) \cdot \text{week}^{-1}$; ULR in $\text{mg} \cdot \text{cm}^{-2} \cdot \text{week}^{-1}$; LAR in $\text{cm}^2 \cdot \text{g}^{-1}$ and calculated from the values at the end of the experiment. The values for RGR and ULR are calculated for $T_1 =$ the start and $T_2 =$ the end of the experimental period.

Field Experiments					Growth Chamber Experiments							
Exp. Cond.		Exp. Cond.			I		II		III			
Light	Water	O	A	S	Light	Water	O	A	O	A	O	A
<i>RGR</i>												
100%	HW I	0.66	0.51	0.78								
	II	0.66	0.67	0.66								
40%	HW I	0.58	0.45	0.71	HL	HW	0.45	0.34	0.46	0.43	0.60	0.64
	II	0.62	0.63	0.64		LW	0.38	0.21	0.35	0.33	0.42	0.44
20%	HW I	0.56	0.42	0.66								
	II	0.40	0.45	0.50								
4%	HW I	0.44	0.25	0.45	LL	HW	0.31	0.15	0.32	0.16	0.26	0.13
	II	0.29	0.28	0.31		LW	0.26	0.13	0.26	0.11	0.20	0.10
<i>ULR</i>												
100%	HW I	4.9	3.6	8.2								
	II	3.1	3.3	7.7								
40%	HW I	4.6	4.2	9.2	HL	HW	3.1	5.7	2.7	4.6	2.9	3.0
	II	2.5	3.2	3.9		LW	3.2	4.2	2.5	4.6	4.4	5.3
20%	HW I	4.3	1.6	5.3								
	II	1.3	2.8	1.9								
4%	HW I	2.6	2.1	1.8	LL	HW	1.0	0.7	1.0	0.8	0.6	0.6
	II	1.3	1.0	1.0		LW	0.9	0.8	1.0	0.4	0.4	0.4
<i>LAR</i>												
100%	HW I	67	81	45								
	II	112	111	45								
40%	HW I	67	74	39	HL	HW	69	32	102	56	94	90
	II	146	113	90		LW	64	25	88	49	45	40
20%	HW I	73	192	66								
	II	228	96	176								
4%	HW I	111	131	152	LL	HW	214	212	242	220	336	226
	II	182	187	273		LW	212	159	220	198	348	202

of increasing their LAR at low light intensities. *Axonopus*, however, could not compensate its lower ULR in this way, at least not in the growth chamber experiments.

Air humidity and water supply caused changes in the distribution of dry matter in the leaves. Comparison of the leaf area ratio and the leaf weight ratio (LAR and LWR) revealed that only small differences existed in the LWR between the two conditions, although the LAR differed sometimes considerably (tables 1 and 2). A decrease in light intensity, however, gave a decrease in RGR, but here LAR as well as LWR increased, while the decrease in RGR was due

Table 2. Leaf weight ratio (LWR) of *Oplismenus* (O), *Axonopus* (A) and *Setaria* (S), grown in different conditions of light intensity and water supply, calculated from the dry weight values at the end of the experiment. Exp. period: Ind. exp. 5 weeks, exp. III 7 weeks.

Field Experiments					Growth Chamber Experiments			
Exp. Cond.					Exp. Cond.		Exp. III	
Light	Water	O	A	S	Light	Water	O	A
100%	HW I	0.30	0.24	0.25				
	II	0.40	0.22	0.22				
40%	HW I	0.33	0.27	0.26	HL	HW	0.32	0.29
	II	0.33	0.24	0.24		LW	0.31	0.27
20%	HW I	0.41	0.37	0.27				
	II	0.43	0.41	0.44				
4%	HW I	0.43	0.49	0.39	LL	HW	0.49	0.51
	II	0.45	0.49	0.39		LW	0.51	0.47

to a sharp fall in ULR, thus to the already mentioned decrease in net photosynthesis (*fig. 1*).

In *fig. 2* the ratio Ax./Opl. is given for RGR, ULR and LAR. The value of the RGR was at all harvests and under all conditions below 1. The LAR ratio increased only at LL, HW to a value of about 1. In the first stages of growth *Axonopus* has a higher ULR, as could be expected. Remarkable is the observed decrease in ULR ratio with time, even at high light conditions which is due to a decreasing ULR of the C₄ plant.

These results give evidence that the higher photosynthetic capacity of a C₄ plant does not imply a big advantage in growth rate over a C₃ plant, even when species are compared, as closely related as *Axonopus*, *Setaria* and *Oplismenus*. C₄ plants have a high photosynthetic water use efficiency which may give these plants an advantage over C₃ plants during water stress, but even in dry conditions the RGR of *Axonopus* and *Oplismenus* was about equal. Both species may be adapted more to humid than to dry conditions, as they are usually found in slightly humid habitats.

It must be emphasized that the data from the field and from the growth chamber experiments should not be compared with regard to high or low water supply. Temperature, light intensity and relative humidity may have differed in the wet and in the dry season (section: Material), but all plants had an ample water supply. In the growth chamber experiments the plants had either a high or a low water supply.

The data found are in accordance with those of BULL (1971) and SLATYER (1970). Bull compared the growth of two C₃ plants, the sunflower and the bean, with that of two C₄ plants, maize and sugarcane. He found the highest growth rate for the sunflower, but it must be said that the plants differed strongly in morphological characteristics. Slatyer compared two *Atriplex* spe-

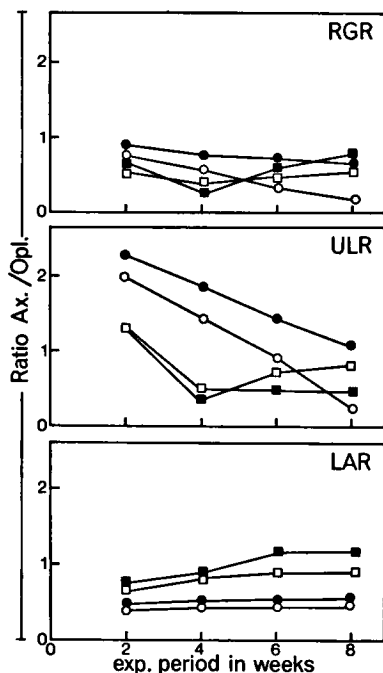


Fig. 2. Ratio *Axonopus/Oplismenus* of the relative growth rate (RGR), the unit leaf rate (ULR) and the leaf area ratio (LAR), calculated from the data of experiment I at the different harvests. Experimental conditions: HL, HW: ●—●; HL, LW: ○—○; LL, HW: ■—■; LL, LW: □—□.

cies, a C₃ and a C₄ one. The C₄/C₃ ratio of the growth rate declined during growth to values below 1, whereas both ULR and LAR ratios had values below 1 at the end of the experiment, as in our experiments. He grew the plants, however, at one light intensity and with an ample water supply.

Competitive factors. The absence of C₃ grasses in open areas in the tropics, as found earlier (HOFSTRA et al. 1972), cannot be explained by the data found. The plants, however, did grow in pots and were not subject to competition from other plants. Some characteristics may be of special value in competition in extreme conditions. From the data of the experiments some of these properties may be found. They will be discussed below.

In dry conditions and especially at high light intensities, the shoot/root ratio of *Axonopus* was lower than that of *Oplismenus*, giving *Axonopus* by a more extended root system an advantage under conditions of water shortage (table 3).

The soluble sugar content, as determined in shoot and root of the plants from exp. I, gave a higher value for plants in low than in high water conditions (table 4). This is in accordance with the findings of BROUWER (1968) and WESSELIUS & BROUWER (1972). It is due to the fact that leaf growth is more ham-

Table 3. Shoot/root ratio of *Oplismenus* (O) and *Axonopus* (A), calculated from the dry weight values at the end of the growth period in the growth chamber.

Exp. Cond.		I		II		III	
Light	Water	O	A	O	A	O	A
HL	LW	12.1	2.7	10.1	6.6	7.5	3.5
LL	LW	15.1	5.4	16.0	13.4	11.1	7.3

Table 4. 80% ethanol soluble carbohydrate and starch content of shoot and root of *Axonopus* and *Oplismenus*, measured at the different harvests of exp. I and calculated as g glucose.100 g dry weight⁻¹.

Exp. Cond.		Exp. Period	80% eth. sol. carbohydrate			Starch		
Light	Water		A	O	Ratio A/O	A	O	Ratio A/O
			<i>Shoot</i>					
HL	HW	2 weeks	15.0	5.0	3.0	18.2	21.8	0.83
		4 weeks	12.5	6.2	2.0	11.8	21.0	0.56
		6 weeks	11.2	11.2	1.0	8.7	28.5	0.30
HL	LW	2 weeks	17.8	5.5	3.2	19.2	24.0	0.80
		4 weeks	18.7	8.0	2.3	12.5	23.5	0.53
		6 weeks	19.0	13.5	1.4	8.2	28.5	0.32
			<i>Root</i>					
HL	HW	2 weeks	3.0	0.6	5.0	15.0	16.2	0.92
		4 weeks	5.0	2.0	2.5	14.0	16.0	0.87
		6 weeks	5.8	5.0	1.2	13.1	15.3	0.86
HL	LW	2 weeks	11.2	1.2	9.3	15.0	15.0	1.0
		4 weeks	11.2	3.7	3.0	14.1	14.5	0.95
		6 weeks	11.5	7.0	1.5	12.9	12.5	1.05

pered than photosynthesis in dry conditions. The soluble sugar content in shoot and root of *Axonopus* was higher than that of *Oplismenus*, although the starch content was lower. This might give the cells the capacity to retain more water in dry conditions. The Ax./Opl. ratio for the soluble sugars is highest at the early stages of growth, but the data should be considered as preliminary as only the plants of one experiment were examined.

As already mentioned (section: Material), the initial growth rate of *Axonopus* cuttings is higher than that of *Oplismenus* cuttings. This is shown clearly in fig. 3 where the ratio Ax./Opl. of the plant dry weights in exp. II is even increasing during the first weeks of growth in contrast with exp. I in which the ratio is higher at the start of the experiment but is decreasing immediately. In exp. I the cuttings were bigger than in exp. II at the start of the experiment. This fast growth rate of young C₄ plants may be a consequence of the higher photosynthetic rate and may give the plants thus a higher competitive value in the initial stages of growth.

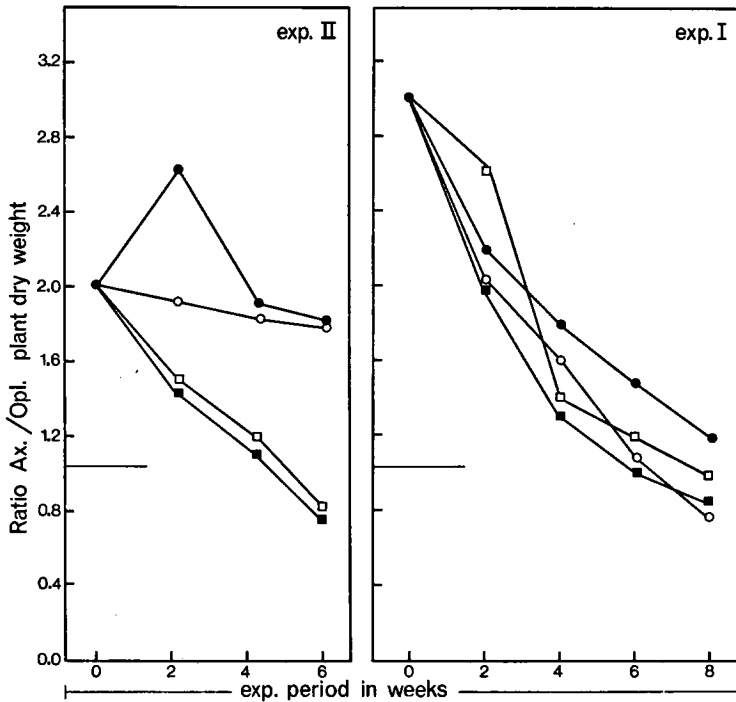


Fig. 3. Ratio *Axonopus/Oplismenus* of the plant dry weight at different harvests. Experimental conditions: HL, HW: ●—●; HL, LW: ○—○; LL, HW: ■—■; LL, LW: □—□.

It might also explain the dissimilarity between these data and those of WILSON & FORD (1971). They compared the growth of C₃ and C₄ grasses at different temperature regimes at 40,000 Lux and found at a temperature regime of 26.7/21.1°C a much higher dry weight of the C₄ grasses. They used seedlings and harvested after about three weeks of growth. The C₄ plants, therefore, might still have been in the initial stage of high growth rate which presumably may be found in seedlings as well as in cuttings. Moreover, in our opinion, their comparison was not correct since they used C₄ grasses, growing in tropical regions and C₃ grasses from temperate regions.

Apart from these differences between the C₄ and C₃ grasses in shoot/root ratio, in soluble sugar content and in initial growth rate, it must be mentioned that an allelopathic factor was isolated from the root exudate of *Imperata cylindrica* (Alang-Alang) a C₄ grass and a very noxious weed in Indonesia. This exudate inhibited germination and growth of other plant species (EUSSEN & WIRJAHARDJA 1973). This factor, if found in other C₄ grasses and then only in open areas, may influence the occurrence of C₃ grasses.

It may be concluded that the C₄ mechanism is not the sole factor for rapid growth of plants. Other factors, as e.g. the above mentioned, may play a role

to give plants a competitive value over others, especially in extreme conditions. Some of these factors may be, indirectly, the result of the C_4 pathway of photosynthesis.

The results may be influenced by the experimental conditions, but, on the whole, the field experiments are in good agreement with the growth chamber ones. It should be examined whether indeed these mentioned factors may function in other C_4 species and whether they function in the field.

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