Studies on $^{18}O_2$-Uptake in the Light by Entire Plants of Different Tobacco Mutants

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Z. Naturforsch. **37 c,** 93 – 101 (1982); received September 23, 1981

$^{18}O_2$-Uptake, Photorespiration, Tobacco Mutants, Glycolate Metabolism, Mehler Reaction

Photorespiratory activity was measured in entire plants of five tobacco variants. These tobacco variants are: the green type *N. tabacum* var. John William's Broadleaf (*su/su Aur/aur* or *su/su Aur/Aur*) the chlorophyll-deficient tobacco mutant *Su/su* (*Su/su Aur/Aur*) and the chlorophyll-deficient mutant *Su/su* var. *Aurea* (*Su/su Aur/aur*). Furthermore, two recently characterized phenotypes originating from *N. tabacum* var. Consolation namely "consolation green" (*Aa Bb*) and "consolation yellow-green" (*aa bb*). In entire plants of these phenotypes photorespiration was measured as $^{18}O_2$-uptake in the light. This uptake was compared with the enhancement of *CO_2-fixation* in the Warburg effect *i. e.* when the oxygen partial pressure is lowered from 21% O_2 to 3% O_2. The principal conclusion from these measurements is firstly that under the assay conditions which are identical for all 5 phenotypes (330 ppm CO_2, 14000 lux white light and 25 °C) all five phenotypes yield considerable differences in photorespiratory activity. Furthermore, we were able to show that in the different phenotypes the global O_2-uptake in the light is repartitioned to different degrees among different metabolic pathways. Thus, in JWB which is under the assay conditions the only fast growing species, only half of the measured $^{18}O_2$-uptake belongs to glycolate metabolism or photorespiration proper, the other half belongs to a Mehler type reaction in which excess reducing power is eliminated apparently already at the level of photosynthetic electron transport. In the chlorophyll-deficient mutant *Su/su*, however, the observed $^{18}O_2$-uptake in the light belongs under the assay conditions exclusively to glycolate metabolism (no Mehler type reaction). The chlorophyll-deficient mutant *Su/su* var. *Aurea* behaves more like JWB, that is, part of its $^{18}O_2$-uptake is due to a Mehler type reaction and only the remainder is involved in CO_2-metabolism, which has been already found out previously by genetic analysis. In addition photorespiration depends in *Su/su* more on the temperature than in the other phenotypes tested. One of the implications of our results could be that it makes a difference to the plant whether excess reducing power is disposed of at the level of the photosynthetic electron transport chain (via a Mehler type reaction) or at the level of CO_2-metabolism.

Introduction

For the attempt of measuring what is called photorespiration only a few methods are available: One method is the measurement of the Warburg effect [1, 2] *i. e.* the increase of photosynthesis under low oxygen partial pressure which is due to the inhibition of oxidative processes. Uptake of $^{18}O_2$ in the light is certainly another readily available method which is thought to be the best one by some investigators [3, 4] but not so appreciated by others [5]. Measurement of the $^{14}CO_2$-evolution into CO_2-free air has been studied by the Zelitch group [6, 7] but its validity has been questioned by others [8, 9]. And indeed, none of these methods yields results which are comparable neither amongst different authors nor between the methods themselves [6, 9, 10]. This is apparently due to the fact that photorespiration is a complex phenomenon and any of the cited methods measures finally only a detail of the phenomenon which in turn means that the phenomenon as a whole is not all understood. In this state of mind we have attempted to start a series of investigations in which we measured $^{18}O_2$-uptake and other growth parameters in an elaborate growth chamber system described earlier by André and co-workers [11, 12]. We used *entire plants,* normal low CO_2 concentration in air (0.03%)

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0341-0382/82/0100-0093 $ 01.00/0

and on purpose the same *low* light intensity of 14000 lux for all plants tested. The plant material used were tobacco mutants and the corresponding wild types, repeatedly described in previous papers by our laboratory [13–15] and by others [6, 9, 10, 16].

Materials and Methods

Plant material

Two tobacco mutant sets were used. The first set is that described by Okabe *et al.* [14] which originated from a selfed seed lot of the dominant tobacco aurea mutant *Su/su* [17]. The three phenotypes within this set were due to two independent nuclear gene factors *su* and *aur*, which in the proper combination yielded green plants (*su/su Aur/aur; su/su Aur/Aur*), yellow green plants (*Su/su Aur/Aur*) and yellow plants (*Su/su Aur/aur*). The second mutant set originated from a selfed variegated plant observed in a seed lot of selfed yellow-green *N. tabacum* var. Consolation [18]. This seed lot gave rise to three phenotypes which were due to combinations of two interdependant nuclear gene factors (4): green plants (*AaBb*), yellow-green plants (*aabb*) and yellow plants (*AaBbCc*). The latter phenotype has the same gene combination as the green one plus a labile gene factor in heterozygous condition (*Cc*). The plants were grown in a green-house in clay pots to the stage where they had 3 to 4 leaves and were thereafter transferred to the growth chamber C_23A [11]. After a few days of acclimatisation to the new environment the measurements were carried out.

Gas exchange measurements

Gas exchange measurements were carried out with entire plants in the growth-chamber C_23A described by André *et al.* [11, 12]. The measuring system involved an infrared CO_2 analyzer (Hartmann and Braun, URAS-IIA) and a mass spectrometer (Riber QMM17) which permitted the simultaneous measurement of CO_2, $^{16}O_2$, $^{18}O_2$ and Ne. The device used for our small plants was the so-called mini-cell with an experimental volume of 2095 ml. After the injection of the gas mixture of $^{18}O_2$ and Ne ($^{18}O_2$:Ne 1:1) into the closed measuring system in which normal air was circulated, the plants were illuminated (14000 lux) and the measurements started. The day length was 14 h, the dark time 10 h with the ambient temperature held at 24 °C/20 °C (day/night). During day time 50% CO_2 diluted with N_2 was pulse-injected into the system whereas respiratory CO_2 was trapped in the night by an alcaline absorbant [11] in order to maintain the CO_2 concentration constant at 330 ppm. One injection pulse corresponded to 82 µl CO_2 and one second of trapping to 4.6 µl CO_2. The pulse number and the trapping time and the concentrations of $^{18}O_2$, $^{16}O_2$ and Ne were recorded hourly and stored in a computer [11, 12]. These raw data for a 24 h time lapse are recorded in Figs. 1 to 3. Gas exchanges in the system caused by soil and roots was measured in the condition when the plants were cut off. The values of Tables I and II were corrected for soil and root respiration. All experiments have been repeated several times.

Calculation of the gas exchange rates

From the type of raw data shown in Figs. 1 to 3 the following values with the denotation used by Gerbaud and André [19] were calculated: P_{CO_2} which is apparent CO_2-uptake derived from the number of CO_2-pulse injections; P_{O_2} which is apparent O_2-evolution in the day calculated by the increase of $^{16}O_2$; R_{CO_2} which is CO_2-evolution *in the night* as calculated from the trapping time; R_{O_2} which is O_2-uptake *in the night* as measurable from $^{16}O_2$-decrease. U_L and U_D which is O_2-uptake in the light (day) and O_2-uptake in the dark (night) as calculated by the $^{18}O_2$-decrease (see also sketch of Fig. 6). *Warburg effect* was measured in leaf sections (27 × 16.5 mm²) of the indicated growth stage in a closed system in 3% O_2 or normal 21% O_2 by means of an IR-Gasanalyzer (Hartmann and Braun URAS-II A). Results of Tables III and IV and Fig. 4 have been obtained with leaf sections.

Results

In entire plants of the tobacco mutant set described earlier [14] which originated from the Connecticut cigar variety John William's Broadleaf (JWB) we measured simultaneously $^{18}O_2$-uptake, $^{16}O_2$-evolution and CO_2-fixation. Fig. 1 shows the automatic hourly measurements of these parameters

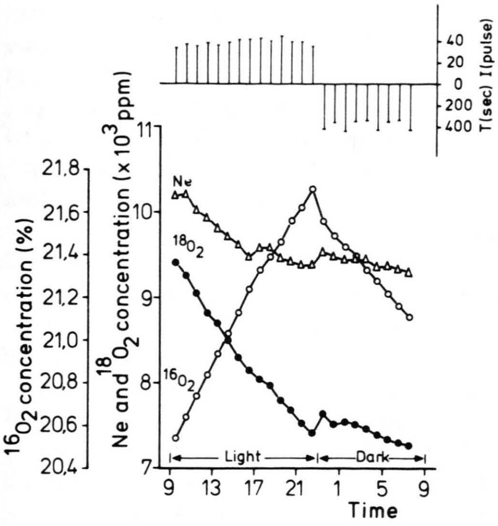

Fig. 1. Gas-exchange measurements of $^{18}O_2$, $^{16}O_2$, Ne and CO_2 in entire plants of *N. tabacum* var. John Williams Broadleaf in the growth chamber "C_23A". Assay temperature 25 °C. Constant CO_2 concentration 330 ppm. Light intensity 14000 lux. The number of CO_2 pulses and the absorption time of CO_2 in the night permit the calculation of photosynthetic rates as CO_2-fixation and dark (night) respiration (upper part of the picture). The numbers on the time axis give the actual time: light period from 9 a.m. to 11 p.m. (23^{00}); dark from 11 p.m. (23^{00}) to 9 a.m., *i.e.* a 14 h light/10 h dark cycle.

for the wild type JWB in a 14 h light 10 h dark cycle. The graph clearly shows that $^{18}O_2$-uptake is larger in day time than in the dark hours. Neon was used as a reference gas for the mass spectrometer. The reason for this being that the growth-chamber "C_23A" [11, 12] having a CO_2-injection device for keeping the CO_2-pressure in the cell constant. The number of CO_2 pulses (Each pulse has a defined volume) gives the CO_2-fixation rate in the chamber (upper picture of Fig. 1). But since the gas volume has to be kept constant, with every pulse a certain amount of the chamber gas mixture is swept out. This is measured by the dilution of a trace of Neon (mass 20). The $^{16}O_2$ and $^{18}O_2$ curves, shown in Figs. 1–3, are corrected for this gas loss by means of this reference gas. The same plot for the tobacco aurea mutant *Su/su* (Fig. 2) shows also an enhanced $^{18}O_2$-uptake in day time compared to that in night time. If the $^{18}O_2$-uptake curve really measures photorespiration the effect seems not so high as expected for the *Su/su* mutant. The literature repeatedly says that this mutant has an exceptionally high photorespiration when compared to JWB [6, 7, 10], or JWB is even supposed to have an especially low photorespiration [6]. However, in Figs. 1 and 2 the $^{18}O_2$-uptake rates are very much alike. Fig. 3 shows the data for *Su/su* var. *Aurea*. Here, $^{18}O_2$-uptake in the day is barely different from that in night, hence it looks as if there was no or only little photorespiration in that mutant. However, it should be noted that since we tested all three plants under the same light conditions (*i.e.* 14000 lux white light), the very chlorophyll-deficient plant *Su/su* var. *Aurea* showed very low rates of photosynthesis (see upper part of the graph with the CO_2-pulses in Fig. 3). Due to

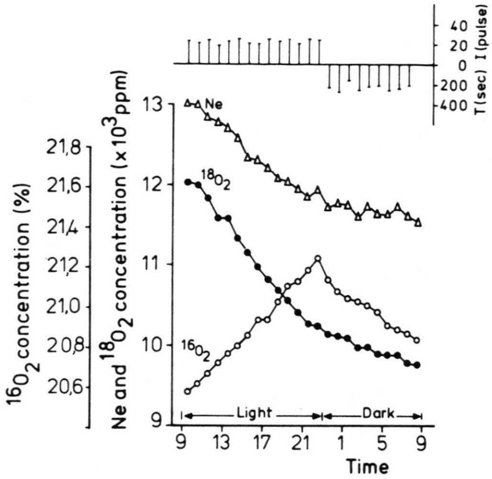

Fig. 2. Gas exchange measurements as in Fig. 1 for entire plants of the tobacco mutant *Su/su*.

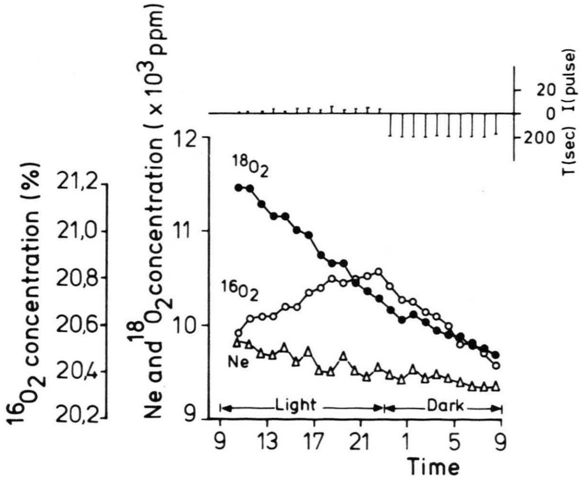

Fig. 3. Gas exchange measurements as in Fig. 1 for entire plants of the tobacco mutant *Su/su* var. *Aurea*.

Table I. Comparison of the photorespiratory activity measured as $^{18}O_2$-uptake in different tobacco plants.

Tobacco	Apparent rate of photosynthesis µmol O_2-evolved · mg Chl^{-1} h^{-1}	True rate of photosynthesis µmol O_2-evolved · mg Chl^{-1} h^{-1}	% Photorespiration
John William's Broadleaf (JWB)	81	157	94
Su/su	176	272	54.5
Su/su var. *Aurea*	314	726	131
Consolation green	73	126	72
Consolation yellow-green	155	243	56

True rate of photosynthesis is assumed to be the sum $^{18}O_2$-uptake and $^{16}O_2$-evolution. Light intensity 14000 lux white light (non saturating). CO_2-concentration 0.03%. Temp. 24 °C day.

this, soil respiration might have played a stronger role than in the other cases.

The comparison of $^{18}O_2$-uptake measurements and $^{16}O_2$-evolution, *i.e.* apparent photosynthesis, shows that at the same low light intensity of 14000 lux considerable differences exist between the tobacco mutants and the corresponding green controls. Table I shows the per cent increase of the apparent rate of photosynthesis ($^{16}O_2$-evolution) by the $^{18}O_2$-uptake phenomenon under the assumption that the true rate of photosynthesis is the sum of $^{16}O_2$-evolution rate and the $^{18}O_2$-uptake rate. The obvious observation is that there are consistent differences in all 5 tobaccos tested, with the amazing fact being that photorespiration is lowest in *Su/su*. If a difference in photorespiration by a factor of two is appreciable than the notion by Andrews and Lorimer [20] that photorespiration is an inherent property of all C_3 plants and therefore should always be the same seems not to apply in our case. On the other hand the objection indeed could be that in *Su/su* not enough light intensity was offered (here 14000 lux) and higher light intensity would have increased % photorespiration to the JWB-values. This, however, seems not necessarily be the case since *Su/su* var. *Aurea* is certainly not light saturated [14] in 14000 lux but nevertheless shows a 131% increase of the apparent rate of photosynthesis provided that, as already emphasized, the $^{18}O_2$-uptake is considered as being really a photorespiratory phenomenon.

Table II shows the simultaneously measured parameters in entire plants. All plants were of comparable size. Oxygen gas exchange was measured by mass spectrometry ($^{18}O_2$ and $^{16}O_2$). On the basis of unit leaf area clearly JWB shows the largest photoconsumption of $^{18}O_2$. The ratio of photoconsumption of $^{18}O_2$ to photosynthetic rate (U_L/P_{O_2}) provides an estimate of photorespiratory activity in relation to photosynthetic performance. In this case as in Table I *Su/su* shows the lowest activity with a ratio of 0.6 in comparison to JWB with 0.95 or *Su/su* var. *Aurea* with 1.28. Hence, if $^{18}O_2$-photoconsumption reflects photorespiratory activity, *Su/su* exhibits the lowest activity in comparison to the

Table II. CO_2 and O_2-gas exchange rates in entire plants of two sets of tobacco mutants.

	Photosynthesis		Ratio	$^{18}O_2$-uptake in Light	$^{18}O_2$-uptake in Dark	Ratio	Dark Respiration *		Plant Size Leaf Area
	P_{CO_2}	P_{O_2}	P_{O_2}/P_{CO_2}	U_L	U_D	U_L/P_{O_2}	R_{CO_2}	R_{O_2}	
	[ml CO_2 or O_2/dm²/h]			[ml O_2/dm²/h]			[ml CO_2 or O_2/dm²/h]		[cm²]
John Williams Broadleaf (JWB)	4.56	4.61	1.01	4.42	1.00	0.95	1.09	1.12	91
Su/su	3.12	3.30	1.07	1.97	0.81	0.60	0.82	0.67	79
Su/su var. *Aurea*	0.87	1.31	1.54	1.67	0.47	1.28	0.35	0.42	95
Consolation green	3.65	3.50	0.96	2.54	0.97	0.73	0.53	0.72	155
Consolation yellow-green	3.76	3.75	1.00	2.14	0.59	0.58	0.49	0.82	115

* Dark respiration was measured during night time. The values are the averages of two measurements. Average chlorophyll content (mg/dm²): JWB, 2.57; *Su/su*, 0.87; *Su/su* var. *Aurea*, 0.19; Consolation green, 2.14; Consolation yellow-green, 1.10.

Table III. Warburg effect in three tobacco phenotypes under different light intensity measured as CO_2-fixation.

Phenotype	Light intensity [klux]	Apparent photosynthesis in 21% $O_2(P_{21})$ [ml CO_2/dm²/h]	Apparent photosynthesis in 3% $O_2(P_3)$ [ml CO_2/dm²/h]	Acceleration P_3-P_{21}	Acceleration P_3/P_{21}
Wild type *N. tabacum*	7	1.81	2.28	0.47	1.26
var. John Williams	14	3.66	5.48	1.82	1.50
Broadleaf	42	6.74	8.26	1.52	1.23
(JWB)	80	7.12	8.58	1.46	1.20
N. tabacum	7	1.25	1.92	0.67	1.54
Su/su	14	2.99	4.11	1.12	1.37
	42	5.70	7.15	1.45	1.25
	80	6.46	7.92	1.46	1.23
N. tabacum	7	0	0	0	0
Su/su var. *Aurea*	14	0.77	1.60	0.83	2.07
	42	3.65	5.03	1.38	1.38
	80	6.05	8.64	2.59	1.43

The values are the averages of two measurements. Temperature during the measurement was 25 °C. Chlorophyll content: JWB, 4.12 mg/dm²; *Su/su*, 1.43 mg/dm²; *Su/su* var. *Aurea*, 0.55 mg/dm².

wild type form. This together with the result of Table I is seemingly in contrast to reports by Zelitch and Day [6] or Salin and Homann [10] who reportet higher rates of photorespiration for *Su/su* on the basis of measurements of $^{14}CO_2$-release into CO_2-free air [6] or by measuring the Warburg effect [10]. We ourselves [21] have measured $^{14}CO_2$-evolution into CO_2-free air and were able to confirm Zelitch's results but we do not appreciate this method for several not further discussed reasons. If the tobaccos "Consolation green" or "Consolation yellow-green" are assayed for the $^{18}O_2$-photoconsumption it is seen that $^{18}O_2$-uptake in relation to photosynthetic performance measured as $^{16}O_2$-evolution is higher in Consolation green (ratio 0.73) than in Consolation yellow-green (ratio 0.58) although the difference appears almost insignificant.

Further scrutiny of Table II yields the observation that in all tobaccos tested the directly measured P_{CO_2}/P_{O_2} ratio is very close to one despite the fact that $^{18}O_2$-analysis shows a simultaneous uptake of $^{18}O_2$ in the light (U_L). This is in agreement with observations of others [19]. Moreover, in the case of JWB P_{O_2} and U_L are practically identical which means that at 14000 lux with JWB we are measuring practically at the crossing point of the mirror shaped curves of Fig. 5. It is obvious that $^{18}O_2$-uptake measurements will not only include O_2-

Table IV. Effect of temperature, light intensity and plant condition (age) on the size of the Warburg effect.

Phenotype	Temperature [°C]	Photosynthesis at 14000 lux in 21% O_2	Photosynthesis at 14000 lux in 3% O_2	Diff.	Photosynthesis at 42000 lux in 21% O_2	Photosynthesis at 42000 lux in 3% O_2	Diff.
			[ml $CO_2 \cdot dm^{-2} \cdot h^{-1}$]				
John Williams	25	4.01	5.36	1.35	8.08	9.64	1.56
Broadleaf (JWB)	33	3.98	5.48	1.5	8.33	11.21	2.88
Su/su young	25	3.74	5.06	1.31	8.24	10.65	2.41
pale-green leaves	33	2.71	3.71	1.0	7.95	10.09	2.14
Su/su older	25	4.8	6.19	1.39	9.0	11.02	2.02
greener leaves	33	3.15	5.18	2.03	8.80	12.43	3.63

The definition of "young-pale-green" leaves and "older greener" leaves refers more to different leaf thickness than to age even though "older greener" leaf is somewhat older than the plant conditions we usually use for experiments. *Su/su* has the property to respond to different growth conditions by greening (see Planta **77,** 77 (1967). The reasons for this greening are not yet understood. Thus, "older greener" refers to this observation.

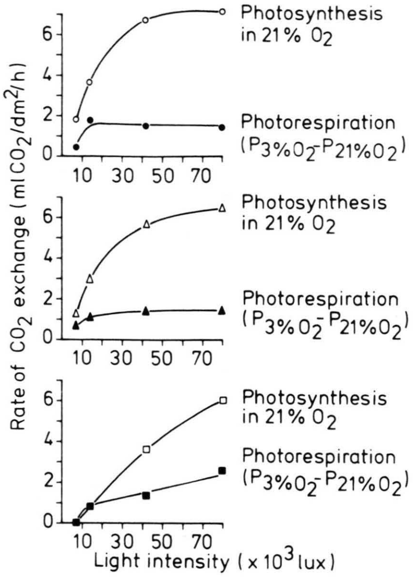

Fig. 4. Light intensity dependance of photosynthesis and photorespiration measured by IR gas analysis at 25 °C. Upper curves: *N. tabacum* var. John William's Broadleaf; middle curves: Tobacco *aurea* mutant *Su/su*; lower curves: Tobacco mutant *Su/su* var. *Aurea*.

uptake due to glycolate oxidase or RuBP-oxygenase activity but also oxygen consuming reactions which dispose of excess reducing power already at the level of photosynthetic electron transport [22]. To distinguish between oxygen uptake due to a Mehler type reaction and due to the glycolate pathway, we measured under the same conditions (*i. e.* at 14000 lux) the size of the Warburg effect (Table III).

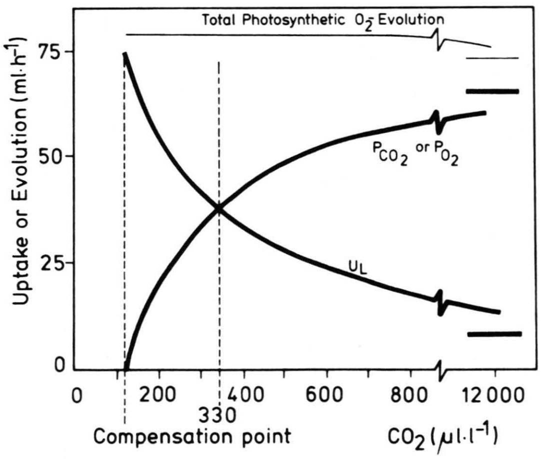

Fig. 5. Schematic illustration of the interrelationship of oxygen uptake in the light (U_L) and photosynthesis (CO_2-fixation P_{CO_2}) or O_2-evolution (P_{O_2}) in dependance on the CO_2 concentration in the ambient atmosphere. The scheme is based on the results of Radmer and Kok [23] and Gerbaud and André [19].

Under the assumption that $U_L - U_D$ (Table II) measures total O_2-uptake in the light induced by photorespiration proper (glycolate metabolism) *plus* Mehler type reactions and under the assumption that the Warburg effect or $P_{3\%} - P_{21\%}$ in (Table III) measures exclusively the influence of glycolate metabolism on the rate of photosynthesis, the comparison yields the following interesting suggestions: In the case of JWB $U_L - U_D$ is 3.42 and $P_{3\%} - P_{21\%}$ is 1.82 ml O_2 or CO_2/dm^2/h. The corresponding values for *Su/su* are 1.16 and 1.12 and for *Su/su* var. *Aurea* 1.2 and 0.83 ml O_2 or CO_2/dm^2/h. This could mean that roughly half of the $^{18}O_2$-uptake in the wild type is due to Mehler type reactions or at least not involved in glycolate metabolism. It appears that in *Su/su* practically the entire $^{18}O_2$-uptake has to do with CO_2 metabolism. On the other hand in *Su/su* var. *Aurea* roughly 40% of total $^{18}O_2$-uptake has nothing to do with CO_2-metabolism and is probably due to a Mehler type oxygen uptake. In conclusion JWB and *Su/su* var. *Aurea* behave similarly as already shown earlier [14]. The exception is *Su/su* and its CO_2-fixation. In search for an explanation for why *Su/su* plants under our actual experimental conditions gave lower photorespiratory rates than JWB we measured the light intensity dependence of the Warburg effect at 25 °C (Fig. 4). These experiments show that the Warburg effect at 14000 lux is certainly light saturated for JWB, very near light saturation in *Su/su* and not light saturated for *Su/su* var. *Aurea.* Hence, the low light intensity offers no explanation why we [21] or Zelitch [6] or Salin and Homann [10] found higher rates for *Su/su.* It rather seems that assay temperature and growth stage or rather the product of certain growing conditions yields *Su/su* plants with exceptionally high photorespiration (Table IV). "*Su/su* older greener leaves" are apparently the plant condition that Salin and Homann [10] or Zelitch [6] have described. Here, clearly *Su/su* assayed at 33 °C is highest in photorespiration.

Discussion

We have tried to characterize the photorespiratory behaviour in two tobacco mutant sets which have been analyzed for their genetic factor combi-

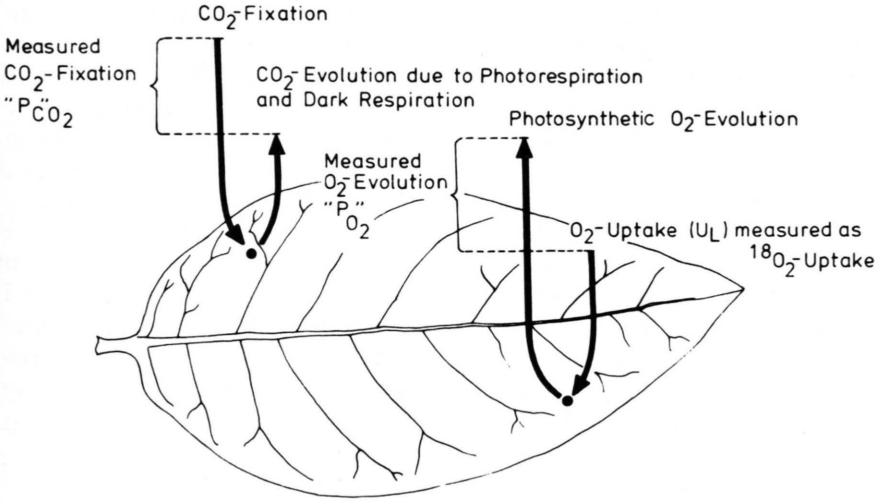

Fig. 6. Scheme to denote the meaning of the measuring parameters.

nations and their photosynthetic capacity [4, 14]. In order to illustrate the experimental approach, the scheme of Fig. 6 is supposed to denote the different measuring parameters: $^{18}O_2$ added to the surrounding atmosphere, permits the measurement of oxygen uptake in the light (U_L); this oxygen uptake counteracts true photosynthetic oxygen evolution leading to the measured parameters "P_{O_2}" which is apparent photosynthetic oxygen evolution. Photorespiratory CO_2-evolution is not directly measurable, but in turn counteracts photosynthetic CO_2-fixation leading to P_{CO_2} which is the readily measured apparent CO_2-fixation, measurable by either infrared-CO_2-gas analysis or $^{14}CO_2$-fixation. This photorespiratory CO_2-evolution is thought to be indirectly measurable by the enhancement of CO_2-fixation when O_2-partial pressure is lowered from 21% to let us say 2–3% which is thought to inhibit oxidative processes (glycolate oxidase, RuBP-oxygenase etc.); this phenomenon is the long known Warburg effect (1). The prerequisite when measuring photorespiration is obviously the clear definition of measuring conditions of the plants used, such as temperature [7] light intensity [14], age of the plants [9, 10], CO_2- and O_2-partial pressure in the ambient atmosphere [1, 7, 14, 19] and the exact knowledge what the measured parameter (see Fig. 6) *e.g.* U_L means. As long as these conditions are not fullfilled it is obvious that comparisons of results between laboratories [6, 9] are difficult. In this sense the papers by Radmer and Kok [23] or Gerbaud and André [19] show that at low CO_2-concentration O_2 is competing with CO_2 for the reducing power. Thus, in wheat at 330 ppm CO_2-fixation equals oxygen uptake (U_L) whereas at high CO_2-concentration CO_2 outrans O_2 for reducing equivalents (scheme of Fig. 5). This is known from another aspect as the inhibition of photorespiration at high CO_2-concentrations [7, 24]. It is obvious that when comparing plants for their photorespiratory activity one must be aware of where in the mirror shaped curves of Fig. 5 the measurement is finally taking place, since photosynthetic activity is different in different plants or mutants when CO_2 [6, 19, 23], temperature [7] or light intensity [14] is changed. When taking all these aspects and considerations into account the first conclusion of our result is that there exist considerable differences in photorespiratory activity between the mutant plants tested regardless whether U_L is measured as $^{18}O_2$-uptake in the light or whether CO_2-evolution in the light is measured via the Warburg effect (Tables II and III). This is in agreement with the standpoint of other workers [6, 10] and in contrast with the standpoint that photorespiration is an inherent property of all C_3 plants and therefore within limits everywhere the same [9, 20, 24]. Apart from this principal conclusion the detailed observation of the above mentioned aspects yields with our acutal plant material and under our actual conditions results which differ from our earlier observations [21] as well as from those of Zelitch [6] and Salin and Homann [10]. Under the experimental conditions used (14000 lux white light, constant 330 ppm CO_2, entire plants) the wild type tobacco JWB performs more photorespiration than the tobacco aurea mutant *Su/su* (Tables II and III). This difference is maintained when ratios of photosynthetic activities towards photorespiratory activities are compared (Table I) or when the different reaction types such as U_L (Table II) or $P_{3\%} - P_{21\%}$ (Table III) are compared. If the different phenotypes JWB, *Su/su* and *Su/su* var. *Aurea* are compared (Tables II and III) it appears that JWB and *Su/su* var. *Aurea* behave similarly, only *Su/su* is different. This is consistent with our previous genetic analysis but leaves us again with the problem why earlier reports show higher photorespiratory activity for *Su/su* [6, 10]. The simple attempt to compare three phenotypes of tobacco for their photorespiratory behavior under identical conditions reveals that each individual phenotype exhibits a reaction combination totally different from that of the other which means that they are ultimately not comparable. Thus, under 14000 lux the green phenotype (JWB) shows a very high oxygen uptake (U_L) in the light (Table II) only half of which is, when compared to the Warburg effect (Table III), involved in CO_2-metabolism (*e.g.* glycolate metabolism). The other half of U_L is apparently an oxygen uptake phenomenon which can only be due to a Mehler type reaction. Since under these conditions (14000 lux in the C_23A) JWB is the fast growing species this disposal of excess reducing power already at the level of photosynthetic electron transport seems not to be nocious to the plant. It seems almost thinkable that the Mehler type reaction is coupled to some kind of cyclic photophosphorylation reaction and produces ATP under the experimental conditions. In comparison to that in *Su/su* the *entire* oxygen uptake in the light is linked with CO_2-metabolism as the comparison of Table II and Table III clearly shows. Obviously, no Mehler type oxygen uptake is taking place in *Su/su*. Under these conditions on the other hand in *Su/su* var. *Aurea* approximately 30% of U_L is Mehler type O_2-uptake which has nothing to do with CO_2-metabolism whereas two thirds of the oxygen uptake might be involved in glycolate metabolism. Thus, from the reactions types involved *Su/su* var. *Aurea* and JWB are very similar which has already been noted by Okabe *et al.* [14]. This result is not fully conclusive since *Su/su* var. *Aurea* is very chlorophyll-deficient in comparison to the wild type JWB and has a photosynthetic performance at 14000 lux which is considerably below that of JWB [14]. However, the result might show or indicate that CO_2 metabolism in *Su/su* is different from that in JWB and *Su/su* var. *Aurea*, causing that under the experimental conditions, CO_2 is exclusively the acceptor for the excess of reducing equivalents and not competing with O_2. From this point of view Zelitch and Day's measurements [6] of the CO_2-burst in *Su/su* and JWB have yielded relative results which might be not so aberrant as the evaluation of the used method has suggested [8, 9]. In the scheme of Fig. 5 *Su/su* is behaving at 330 ppm as if CO_2 concentration was higher than it actually is. N. B. our experimental conditions *i.e.* 14000 lux, 330 ppm hit for JWB exactly the crossing point of the U_L- and P_{O_2}-curves in Fig. 5. *Su/su* lies somewhere on the right hand side of this point. The capacity of *Su/su* to perform high photorespiration as first reported by Zelitch and Day [6] is apparently dependent on the age of the plant as Salin and Homann have shown [10]. Under our experimental conditions, exposed in the present paper, young *Su/su* plants in general do not exhibit higher rates than JWB on a leaf area basis, irrespective of the different reaction mechanisms described. This is in part certainly due to the fact that we work and anlyze at 25 °C and not at 35 °C as Zelitch repeatedly stressed [6, 7] together with the low light intensity. Thus, it was observed that photorespiration could be excessively high (Table IV) if *Su/su* was analyzed in a different growth stage which yielded amongst others differences in leaf thickness and/or chlorophyll content (compare ref. [13]). This thicker or greener *Su/su* is apparently the plant type Salin and Homann [10] or Zelitch [6] have used.

In conclusion our results might have the following implications: It makes apparently a big difference whether excess reducing power which is the reason proper of photorespiration, is disposed of at the level photosynthetic electron transport (Mehler type reaction) or at the level of CO_2-metabolism. In the latter case the disadvantage to the plant is more obvious than in the former case where additional ATP formation via cyclic or pseudocyclic electron transport might be visualized above all at high light intensities to counteract the energy loss.

Acknowledgements

The authors thank Mr. J. Vivoli, Mrs. T. Massimino and Miss. B. Alvermann for technical assistance, as well as Mr. M. André for discussions.

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