The Photo-assimilation of Acetate by *Pyrobotrys* (*Chlamydobotrys*) *stellata*

By K. H. GOULDING AND M. J. MERRETT

School of Applied Biology, University of Bradford, Bradford, 7

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SUMMARY

Alcohols, amino acids, organic acids and sugars were tested as carbon sources for the growth of *Pyrobotrys* (*Chlamydobotrys*) *stellata* in the light and dark. Growth was only recorded with acetate in the light. A carbon balance sheet of ^{14}C -acetate assimilation showed a greater incorporation of ^{14}C into polysaccharide and less released as $^{14}\text{CO}_2$ in the light, compared with the dark. The primary products of ^{14}C -acetate assimilation into the soluble fraction of the organisms were identified; after 10 sec. 40 % of the total ^{14}C present in this fraction was in succinic acid, 15% in citric acid and 16 % in malic acid. The percentage of total ^{14}C in this fraction present in succinic and malic acids decreased consistently with time, while that in citric acid initially increased before decreasing. After 10 sec. the specific activity of succinic acid was more than twice that of citric acid. 4×10^{-3} M-monofluoroacetate (MFA) effectively inhibited the incorporation of ^{14}C -acetate into tricarboxylic acid cycle intermediates and related compounds, and markedly inhibited $^{14}\text{CO}_2$ evolution. 10^{-6} M- N^1 -(3-4,dichlorophenyl)- N,N -dimethyl urea (DCMU) did not significantly inhibit ^{14}C -metabolism. The key enzymes of the glyoxylate cycle, isocitrate lyase (E.C.4.1.3.1) and malate synthetase (E.C.4.1.3.2) were found to be present in *P. stellata*, and did not disappear in the absence of acetate, but even so growth was not recorded on acetate in the dark.

INTRODUCTION

Many algae will utilize acetate as a sole source of carbon for growth (Pringsheim, 1946). The most specialized type of algal acetate nutrition appears to be that shown by *Pyrobotrys* (*Chlamydobotrys*) *stellata*, which can only grow on acetate in the light and was unable to utilize any other carbon source for growth tested by Pringsheim & Wiessner (1960). The growth of *P. stellata* on acetate appears to differ in several important respects from the growth on acetate of other algae investigated, particularly *Chlorella pyrenoidosa* (Syrett, Merrett & Bocks, 1963; Syrett, Bocks & Merrett, 1964; Goulding & Merrett, 1966; Merrett & Goulding, 1967). *Chlorella pyrenoidosa* can assimilate and grow on acetate in the dark, growth being dependent on the induction of glyoxylate cycle enzymes (Syrett *et al.* 1963, 1964). Growth in the light is not dependent on the presence of glyoxylate cycle enzymes (Syrett *et al.* 1963; Goulding & Merrett, 1966). Unlike *C. pyrenoidosa* the glyoxylate cycle is implicated in the growth of *P. stellata* on acetate in the light (Wiessner & Kuhl, 1962), but *P. stellata* will not grow on acetate in the dark. Thus, the regulation of the glyoxylate cycle enzymes, isocitrate lyase (E.C.4.1.3.1) and malate synthetase (E.C.4.1.3.2) may be different in *P. stellata* from that in *C. pyrenoidosa*. The aerobic assimilation of

acetate in the light by *P. stellata* was unaffected by N^1 -(3,4-dichlorophenyl)- N,N -dimethyl urea (DCMU) (Wiessner & Gaffron, 1964) a specific inhibitor of non-cyclic photophosphorylation (Losada & Arnon, 1963). In *C. pyrenoidosa* DCMU induced a pattern of acetate assimilation in the light similar to that in the dark (Goulding & Merrett, 1966). In the presence of DCMU, in the light, less acetate carbon was incorporated into the organisms, particularly into the lipids, polysaccharide and protein, and more was released as carbon dioxide, while isocitrate lyase and malate synthetase, normally repressed in the light, were induced (Goulding & Merrett, 1966). It would appear that these two algae differ in their enzyme complements and in their dependence on the light reactions of photosynthesis for acetate photo-assimilation. This being so we decided to investigate the products of aerobic acetate photo-assimilation in *P. stellata* although these have already been described for *C. pyrenoidosa* (Merrett & Goulding, 1967).

METHODS

Organism

Pyrobotrys stellata Korschikoff (L 10-1e) (= *Chlamydotrys stellata*) was obtained from the Algensammlung Pflanzenphysiologisches Institut, Göttingen, Germany. The organism was grown in the culture medium of Wiessner (1962), stock cultures being maintained in 20 ml. medium contained in cotton-wool plugged tubes kept at room temperature (20°) with a light intensity of 200 lumens/ft.².

Growth. Organisms for experimental use were grown in 125 ml. of medium in Dreschel bottles. They were inoculated with organisms from 2-day stock cultures to give a population density of 500-organisms/mm.³. The cultures were grown at 25° ± 0.2° at a light intensity of 300 lumens/ft.² and air containing 5 % (v/v) carbon dioxide was bubbled through the cultures at a rate of 4 l./hr. After 3 days organisms were harvested by centrifugation at 500 g for 10 min., washed twice and resuspended in 0.067 M-phosphate buffer (pH 6.7) to give a concentration equivalent to 8 mg. dry-wt organism/ml., the concentration being estimated turbidimetrically by the use of an appropriate calibration curve. For kinetic experiments organisms were washed and resuspended in distilled water to give a concentration equivalent of 4 mg. dry-wt organism/ml.

Pyrobotrys stellata normally utilizes acetate as the carbon source for growth in the light; other carbon compounds were tested to see whether they supported growth. Samples of growth medium (50 ml.) lacking acetate were dispensed into 250 ml. conical flasks and supplemented with carbohydrate to a final concentration 1 % (w/v) or alcohols, organic acids or amino acids to a final concentration 0.2 % (w/v). The media were adjusted to pH 6.5. The flasks were aseptically inoculated with organisms from stock cultures, the dry weight of the inoculum being estimated directly on separate samples. Cultures were kept in the light (intensity 200 lumens/ft.²) or in the dark at 25° for 2 weeks, then the dry weight of organism estimated directly, and the viability determined by inoculating stock culture tubes.

Radiochemical experiments

Acetate assimilation by *Pyrobotrys stellata* was followed by using ¹⁴C-acetate. A suspension of *P. stellata* was equilibrated in the light or dark for 1 hr in the main compartment of large photochemical Warburg Flasks (light intensity 1200 lumens/ft.²;

temperature 25°). ^{14}C -acetate was then tipped from the side arm. After 1 hr the organisms were killed either by the addition of 10 N-sulphuric acid through the side arm, or samples of suspension were placed in ice-cold centrifuge tubes which were rapidly centrifuged and the deposited organisms killed by immersion of the tubes in boiling water for 2 min. The preparation of fractions from these organisms and the determination of ^{14}C in the fractions was as described previously (Goulding & Merrett, 1966). $^{14}\text{CO}_2$ evolved was absorbed by hydroxide of Hyamine (Packard Inc., Wembley) in detachable centre wells. These were transferred to glass counting-vials containing 10 ml. of 0.5 % (w/v) 2,5-diphenyloxazole (PPO) + 0.03 % (w/v) 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (POPOP) in toluene, mixed well and counted in a Tricarb Liquid Scintillation Spectrometer with automatic background subtraction, corrections being made for quenching. The amount of ^{14}C -acetate added in experiments was determined by persulphate combustion (Katz, Abraham & Baker, 1954).

In kinetic experiments 20 ml. of suspension containing organism equivalent 80 mg. dry-wt in distilled water were transferred to rapid sampling apparatus (Syrett *et al.* 1964) and maintained at 25° at a light intensity of 1200 lumens/ft.². After equilibration 30 μ moles sodium acetate were added, followed at zero time by 10 μ moles sodium- ^{14}C -acetate containing 50 μc . Organisms were sampled at intervals, extracted with ethanol and water, labelled compounds separated by two-dimensional chromatography, located by radio-autography, compounds identified, and ^{14}C in each compound determined, all as described previously (Merrett & Goulding, 1967).

Preparation of cell-free extracts

Organisms were washed and resuspended in ice-cold 0.1 M-phosphate buffer pH 7.5 and passed through an ice-cold French pressure cell at 15,000 lb./in.² (Milner, Lawrence & French, 1950). The extract was centrifuged at 500 g at 2° for 5 min., followed at 13,000 g for 20 min., and the supernatant fluid used for enzyme assays. The protein content was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951), with bovine albumin as a standard.

Enzyme assays

Isocitrate lyase activity was estimated by the method of Kornberg & Madsen (1958) usually on cell-free enzyme preparations, but occasionally by using frozen whole-organism suspensions (Syrett *et al.* 1963).

Malate synthetase was determined by the method of Syrett *et al.* (1963).

Organic acids

Organic acids were eluted from chromatograms and estimated by fluorescence of their resorcinol derivative by the method of Frohman & Orten (1953). Fluorescence was measured with a Locarte Fluorimeter with LF/3 blue filter, standard calibration curves being prepared for each acid estimated.

RESULTS

Utilization of carbon compounds for growth

A wide range of carbon compounds were tested to see whether they supported growth of *Pyrobotrys stellata*; alcohols, organic acids, amino acids and carbohydrates

were tested. Of all the compounds tested only acetate supported significant growth in the light. Carbon dioxide and compounds closely related to acetate, such as ethanol, did not support growth. The mean generation time for *P. stellata* growing on acetate in the light at 25° was 12.3 hr, while the final yield of organism was 27,750/mm.³.

The assimilation of ¹⁴C-labelled acetate

Organisms were equilibrated in Warburg flasks in light or dark, then 10 μ moles ¹⁴C-acetate containing 2 μ c were added from the side arm. After metabolism of acetate had ceased (this was longer in the dark than in the light) organisms were sampled and distribution of ¹⁴C in the various fractions determined (Table 1). When the organisms metabolized acetate in the dark far more acetate ¹⁴C was released as CO₂ as compared with metabolism in the light (Table 1). The increased yield of ¹⁴CO₂ in the dark was correlated with the incorporation of less acetate into the acid hydrolysate (polysaccharide + nucleic acids) fraction (Table 1). Although there was some increase in ¹⁴C incorporation into the ethanol fraction in the light (Table 1), it was not as great as with *Chlorella pyrenoidosa*, where the bulk of the acetate carbon is incorporated into lipid in the light (Syrett *et al.* 1964; Goulding & Merrett, 1966).

Table 1. *The recovery of ¹⁴C added as ¹⁴C-1-acetate or ¹⁴C-2-acetate to Pyrobotrys stellata in the light and in dark*

For experimental details see materials and methods and text

	m μ c carbon-14/8 mg. dry-wt cells			
	Light		Dark	
	¹⁴ C-1-acetate	¹⁴ C-2-acetate	¹⁴ C-1-acetate	¹⁴ C-2-acetate
¹⁴ CO ₂	246.3 $\pm$ 7.9*	189.7 $\pm$ 2.1	1175.2 $\pm$ 10.2	930.4 $\pm$ 7.1
Supernatant	22.1 $\pm$ 1.2	21.0 $\pm$ 1.0	21.0 $\pm$ 0.2	25.0 $\pm$ 1.7
Water soluble	225.9 $\pm$ 8.9	352.0 $\pm$ 2.3	249.7 $\pm$ 3.7	423.5 $\pm$ 8.3
Ethanol soluble	279.6 $\pm$ 8.7	290.1 $\pm$ 6.1	117.2 $\pm$ 2.9	150.6 $\pm$ 7.1
Acid hydrolysate	841.9 $\pm$ 21.2	892.9 $\pm$ 11.7	123.4 $\pm$ 3.1	206.0 $\pm$ 0.9
Residue	31.8 $\pm$ 2.7	48.6 $\pm$ 2.7	39.6 $\pm$ 2.8	53.2 $\pm$ 3.1
Total recovered	1747.6 $\pm$ 50.6	1793.4 $\pm$ 25.9	1726.1 $\pm$ 22.9	1788.7 $\pm$ 28.2
¹⁴ C-added in acetate	1902.8	1931.2	1902.8	1931.2
(determined by combustion)				
% Recovery	91.8	92.9	90.5	92.7

*Figures are the means of duplicate flasks.

Short-term products of ¹⁴C-2-acetate assimilation

The results of an experiment investigating ¹⁴C-2-acetate assimilation in the light are given in Fig. 1. In the earliest sample (10 sec.) 40 % of the ¹⁴C-2-acetate assimilated into compounds present on the chromatogram was in succinic acid, 15 % in citric acid and 16 % in malic acid. The percentage of total ¹⁴C on the chromatogram in succinic and malic acids decreased consistently with time, while there was a slight increase in the percentage of the total ¹⁴C present in citric acid before a decrease (Fig. 1). The total counts/min. present in succinic acid was greater than that in citric acid and so was the specific activity (Table 2). Glutamic acid rapidly incorporated ¹⁴C from ¹⁴C-2-acetate, while aspartic acid, serine, glycine, alanine, glutamine and

threonine became labelled in later samples but not to the same extent as glutamic acid (Fig. 1).

A compound found in an investigation of ^{14}C -acetate assimilation by *Chlorella pyrenoidosa*, at present unidentified and recorded as compound *X* by Merrett & Goulding (1967), was also found in *Pyrobotrys stellata*, where it accounted for 8.4 % of total ^{14}C assimilated after 1800 sec. Glucose was the major product of acetate assimilation after 1800 sec. and contained 19 % of the total ^{14}C (Fig. 1).

Table 2. *The specific activity of acids of the tricarboxylic cycle during ^{14}C -2-acetate assimilation by *Pyrobotrys stellata* in the light*

For experimental conditions see legend to Fig. 1.

Time of sampling from addition of ^{14}C -acetate (sec.)	Counts/min incorporated			Concentration of acid $\text{m}\mu\text{mole}/7.2 \text{ mg. dry-wt}$			Specific activity $\text{m}\mu\text{C}/\mu\text{mole acid}$		
	Suc-cinic	Citric	Malic	Suc-cinic	Citric	Malic	Suc-cinic	Citric	Malic
10	1927	721	.	71.2	63.5	.	148.9	62.9	.
30	2398	1136	1002	72.9	67.7	62.9	181.0	91.5	87.4
60	2460	1544	1254	73.7	65.6	62.9	183.1	129.5	109.6
120	2508	1747	1270	76.3	68.8	64.5	180.8	139.5	108.5
300	2980	2593	1527	83.1	70.3	66.1	197.3	201.9	127.0
1800	2879	2625	3386	86.4	71.9	66.1	182.8	200.2	281.3

Table 3. *The effect of monofluoroacetate (MFA) on ^{14}C -acetate metabolism by *Pyrobotrys stellata* in the light*

Organisms were incubated in the light for 60 min., some in the presence of $4 \times 10^{-8} \text{ M}$ -MFA: 80 μmoles ^{14}C -1-acetate containing 2 μC were added and after 1 hr organisms were sampled and fractionated as in Methods. Values given are means of duplicate flasks.

	$\text{m}\mu\text{C } ^{14}\text{C}/8 \text{ mg. dry-wt organisms}$	
	Control	$4 \times 10^{-8} \text{ M-MFA}$
$^{14}\text{CO}_2$ evolved	42.1	2.4
Supernatant fluid	7.8	11.3
Water soluble	14.1	4.3
Ethanol soluble	8.1	2.9
Acid hydrolysate	16.8	2.4
Residue	1.6	0.6
Total	90.5	23.9

The effect of inhibitors on the photoassimilation of acetate

The effect of monofluoroacetate (MFA). The addition of MFA may result in the accumulation of fluorocitrate and the inhibition of aconitase (Treble, Lamport & Peters, 1962) and also the inhibition of malate synthetase (Dixon, Kornberg & Lund, 1960). Thus, MFA inhibits respiration proceeding by the tricarboxylic acid cycle and the glyoxylate by-pass. The effect of MFA on acetate metabolism by *Pyrobotrys stellata* was investigated by incubating organisms with MFA for 60 min., then adding ^{14}C -acetate and sampling the organisms after a further 60 min. (Table 3). Inhibition of ^{14}C -2-acetate incorporation into the ethanol, water-soluble fraction and acid-hydrolysate fraction was particularly marked, and $^{14}\text{CO}_2$ evolution was decreased to a very low value.

The effect of MFA was also investigated by determining the rate of incorporation of ^{14}C -2-acetate into the soluble fraction of the organisms. After 10 sec. the pattern of acetate assimilation was similar to that found in the absence of the inhibitor except that glutamic acid was not labelled; after 10 sec. the total counts incorporated increased only slightly throughout the rest of the experiment (Fig. 2). The incorporation of ^{14}C into succinic, citric and malic acids was strongly inhibited, while other compounds labelled in the absence of the inhibitor (Fig. 1) did not incorporate ^{14}C in detectable amounts (Fig. 2).

Fig. 1. Short-term products of ^{14}C -2-acetate assimilation in the light by *Pyrobotrys stellata*. Twenty ml. of organism suspension (equivalent 4 mg. dry-wt./ml.) were transferred to sampling apparatus, aerated and allowed to equilibrate in the light for 1 hr: 30 μmoles sodium acetate were added 10 min. before zero time, followed at zero time by 10 μmoles ^{14}C -2-acetate containing 50 μC . Organisms sampled, extracted and chromatographed and ^{14}C determined as in methods. Percentage ^{14}C in any one compound expressed as percentage of total ^{14}C present on the chromatogram. ●, Succinate; □, malate; ▲, citrate; ■, glutamate; ×, compound X; +, glucose.

Effect of N^1 -(3,4-dichlorophenyl)- N,N -dimethyl urea (DCMU). DCMU is a powerful inhibitor of photosynthesis, inhibiting the generation of NADPH_2 and ATP by non-cyclic photo-phosphorylation (Losada & Arnon, 1963). Wiessner & Gaffron (1964) found that DCMU was without effect on aerobic acetate assimilation in the light by *Pyrobotrys stellata*, while Goulding & Merrett (1966) found that DCMU induced a dark pattern of acetate metabolism in the light in *Chlorella pyrenoidosa*, resulting in decreased lipid and polysaccharide formation and increased release of $^{14}\text{CO}_2$. The effect of DCMU on ^{14}C -2-acetate incorporation into various cell fractions of *P. stellata* (Table 4) confirmed the observation of Wiessner & Gaffron (1964) that acetate assimilation was unaffected by DCMU, and also showed that ^{14}C -2-acetate incorporation into other cell fractions was also unaffected by DCMU (Table 4).

Glyoxylate cycle enzymes in Pyrobotrys stellata

The nature of control of glyoxylate cycle enzymes in *Pyrobotrys stellata* has not hitherto been investigated. *P. stellata* organisms grown on acetate in the light were centrifuged down aseptically, resuspended in fresh medium and aerated in the presence of acetate in the dark, and in the light without acetate. The presence or absence of

Fig. 2. Short-term products of ^{14}C -2-acetate assimilation by *Pyrobotrys stellata* in the light in the presence of $4 \times 10^{-3}\text{M}$ -MFA. Twenty ml. of organism suspension (equiv. 4 mg. dry-wt/ml.) were transferred to sampling apparatus, aerated and allowed to equilibrate in the light for 1 hr in the presence of MFA to give final concentration $4 \times 10^{-3}\text{M}$: 30 μmoles sodium acetate were added 10 min. before zero time, followed at zero time by 10 μmoles ^{14}C -2-acetate containing 50 μc . Organisms sampled, extracted and chromatographed and ^{14}C determined as in methods. (a) Total counts min. per compound: $\times - \times$, total counts/min. incorporated. (b) Percentage ^{14}C in any one compound expressed as percentage of total ^{14}C present on the chromatogram. $\bullet$, Succinate; $\blacktriangle$, citrate; $\square$, malate.

CO_2 had no effect on the amounts of enzyme in the organisms. The results of these experiments (Fig. 3) suggest that malate synthetase and isocitrate lyase are constitutive in *P. stellata* but we were unable to test this experimentally because the organism will

not grow on any carbon source except acetate. Despite this, growth on acetate in the dark did not take place (as it does in *Chlorella pyrenoidosa* where these enzymes are induced when organisms are incubated with acetate in the dark; Syrett *et al.* 1963).

Fig. 3. Malate synthetase and isocitrate lyase in *Probotrys stellata*. Organisms were grown on acetate in the light, harvested aseptically and resuspended. (a) In acetate-free medium and incubated in the light; (b) in acetate-containing medium and incubated in the dark. Samples of organisms were removed at zero time and after 6, 24 and 48 hr incubation and assayed for enzyme activity.

Table 4. The effect of 10^{-6} M-DCMU on ^{14}C -1-acetate assimilation by *Pyrobotrys stellata* in the light

Organisms were allowed to equilibrate in the light in presence or absence of DCMU at time 0, 80 $\mu\text{moles } ^{14}\text{C}$ -1-acetate containing 2 μC added. Organisms sampled after 1 hr and fractionated, as in Methods.

	m $\mu\text{C } ^{14}\text{C}$ /8 mg. dry wt. organisms	
	Control	10^{-6} M-DCMU
$^{14}\text{CO}_2$ evolved	54.7	69.6
Supernatant	5.8	4.1
Water extract	14.8	13.5
Ethanol extract	8.6	7.3
Acid hydrolysate	11.0	10.8
Cell residue	0.6	1.0
Total	95.5	106.3

DISCUSSION

The incorporation of ^{14}C -acetate into the soluble fraction of the cells of *Pyrobotrys stellata* resembles ^{14}C -acetate assimilation in *Chlorella pyrenoidosa* in that succinate becomes rapidly labelled with ^{14}C (Merrett & Goulding, 1967). After 10 sec. over 40 %

of the ^{14}C present in the *P. stellata* organisms was in succinic acid while only 15 % is in citric acid (Fig. 1). This result cannot be explained on the basis of a larger pool size of succinic acid trapping more ^{14}C passing through the tricarboxylic acid cycle, since initially the specific activity of succinic acid was greater than that of citric acid (Table 2). Moreover, in a kinetic-type experiment (as in Fig. 1) initially the greatest percentage of the total ^{14}C assimilated was in the primary products, and this decreased with time as ^{14}C enters other compounds. In several experiments we always obtained the result recorded in Fig. 1, where the slope of the graph for succinic and malic acids is decreasing while that of citric acid is increasing in the early samples. Cell-free extracts of *P. stellata* showed isocitrate lyase and malate synthetase activity and, assuming that these enzymes were functioning in the intact organism in the light, succinic acid could be formed from the breakdown of isocitric acid by isocitrate lyase. However, if succinic acid is formed in this manner it is difficult to see how the specific activity of succinate can be greater than that of citrate from which it was derived via isocitrate. The rapid increase in the specific activity of malic acid (Table 2) supports the view that malate synthetase was active in the intact *P. stellata* organisms, and malic acid was being formed from acetyl-CoA and glyoxylate. There was no evidence of malic formation from carboxylation reactions to this extent; $^{14}\text{CO}_2$ incorporation into the organisms was only 10 % of carbon-14 incorporation from ^{14}C -acetate. It also seems unlikely that succinate could be formed via malic acid, because initially the specific activity of succinate was greater than that of malic acid (Table 2). Monofluoroacetate effectively inhibited the incorporation of ^{14}C -acetate into tricarboxylic acid intermediates (Fig. 2) and blocked $^{14}\text{CO}_2$ evolution (Table 3). Furthermore, the total counts of radioactivity incorporated did not increase greatly during the experiment. Citrate did not accumulate and the formation of succinate was inhibited (Fig. 2). This was not so in *C. pyrenoidosa* when metabolizing ^{14}C -2-acetate in the light in the presence of MFA, where citrate did accumulate (Merrett & Goulding, 1967), and succinate formation was unaffected. It would appear that monofluoroacetate inhibits the uptake of acetate by *P. stellata*, which may indicate that this reaction is dependent on ATP formation resulting from respiration of some of the added acetate.

The effect of DCMU upon acetate assimilation in *Pyrobotrys stellata* was insignificant (Table 4) as compared with its effect on acetate assimilation in *Chlorella pyrenoidosa* where it stimulates $^{14}\text{CO}_2$ evolution sixfold (Goulding & Merrett, 1966). Thus, it appears that the role of light in acetate assimilation by *P. stellata* and by *C. pyrenoidosa* is somewhat different. Recent work on the two light reactions of photosynthesis has led to the majority view that they function in series in which the shorter wave system (system II) forms a strong oxidant leading to O_2 evolution, while the far red system (system I) forms a strong reductant that reduces NADP (Duysens, Ames & Kamp, 1961; Hill & Bendall, 1960; Witt, Muller & Rumberg, 1961; Kok 1961). The photoassimilation of acetate by *C. pyrenoidosa* is dependent on system II and if this is blocked by DCMU, reversion to a dark metabolism of acetate occurs (Goulding & Merrett, 1966). Thus, it seems that an assimilation in light dependent on system I is not possible. *P. stellata* is a weak evolver of oxygen during photosynthesis and it seems probable that system II is deficient in it, while inhibitor experiments with DCMU confirm that acetate assimilation is dependent on system I. In contrast, under anaerobic conditions Wiessner & Gaffron (1964) found DCMU inhibits the photo-

assimilation of acetate in *P. stellata*, implying that under these conditions system II is functioning and may not be completely deficient. Wiessner (1965) showed the quantum requirement for acetate assimilation in far red light by *P. stellata* to be 8. System I is concerned with CO₂ reduction in higher plant photosynthesis, and it seems probable that *P. stellata* is unable to use this system for CO₂ reduction because of the absence of ribulose-1-5-diphosphate carboxylase from the organism (Merrett, 1967).

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