Planta (1981) 153:273–278

Planta

Secondary fluorescence kinetics of spinach leaves in relation to the onset of photosynthetic carbon assimilation

D.A. Walker
A.R.C. Research Group on Photosynthesis, Department of Botany, University of Sheffield, Sheffield S10 2TN, U.K.

Abstract. When spinach leaves are re-illuminated, after dark periods of 90 s or less, an initial fluorescence peak is observed which rapidly gives way to a much lower terminal value. After 2 min or more in the dark, however, there is a secondary rise, at about 50–70 s, which then gives way, more slowly, to approximately the same low terminal value as before. The secondary rise is eliminated or disguised by feeding D,L-glyceraldehyde (a specific inhibitor of photosynthetic carbon assimilation) and by mannose, 2-deoxyglucose and glucosamine, all of which are believed to sequester cytoplasmic orthophosphate. This secondary rise in fluorescence is discussed in relation to photosynthetic induction and the manner in which these compounds may modulate fluorescence by their effect on the availability of orthophosphate and their consequent impact on the adenylate status of the stroma.

Key words: Chlorophyll fluorescence – CO_2 assimilation, photosynthetic – Photosynthesis (induction) – *Spinacia.*

Introduction

When green leaves are illuminated they fluoresce. Most of the fluorescence emanates from chlorophyll a in photosystem II (for recent reviews see Papageorgiou 1975; Lavorel and Etienne 1977) and constitutes that part of the excitation energy which cannot be dissipated through other channels, such as electron transport to NADP. An early step in electron transport is the reduction of Q, so called because (in its oxidised state) it constitutes a sink for electrons and thereby quenches fluorescence (Duysens and Sweers 1963). Accordingly DCMU, which blocks electron transport between Q and plastoquinone and the subsequent electron carriers which precede photosystem I, can cause a marked increase in fluorescence (relaxation of fluorescence quenching) by preventing reoxidation of Q. Conversely, in the absence of DCMU, reoxidation of Q, which is governed (in turn) by reoxidation of the plastoquinone pool, makes an important contribution to the slow decline in the fluorescence peak which is rapidly established upon first illumination (Baker and Bradbury 1981; Bradbury and Baker 1981). In addition Krause (1973) and Krause et al. (1980) have shown that fluorescence is also quenched when the energy status of the thylakoid membrane is high (see also Lavorel and Etienne 1977) and distinguish between "Qu(Q)" quenching which involves the reduction of Q and "Qu(E)" quenching which depends upon the degree of acidification of the thylakoid compartment and, therefore indirectly, upon the adenylate status of the chloroplast (see Table 2). A third factor is the phosphorylation of chloroplast membrane proteins, by ATP, which also results in a decrease of fluorescence yield and is probably the basis of State I/State II transitions (Bennett et al. 1980; Horton and Black 1980, 1981; Horton et al. 1981). Although ATP and NADPH formation starts more or less immediately upon illumination, maximal utilisation of these compounds is linked to the onset of photosynthetic carbon assimilation. Accordingly it seemed desirable to re-examine the kinetics of slow fluorescence (Kautsky phenomena) during photosynthetic induction when both Qu(Q) and Qu(E) might be expected to undergo pronounced changes. (In this context, "induction" is defined as the lag in carbon assimilation which occurs, to a larger or smaller degree, when a leaf is re-illuminated after a dark interval – see Walker 1976, 1980a).

Particular attention was paid to a major secondary rise in fluorescence which could be induced by illumination after dark intervals of 2–10 min. With spinach,

Abbreviations: DCMU = 3(3,4-dichlorophenyl)-1,1-dimethylurea; CCCP = carbonylcyanidchlorophenylhydrazon

0032-0935/81/0153/0273/$01.20

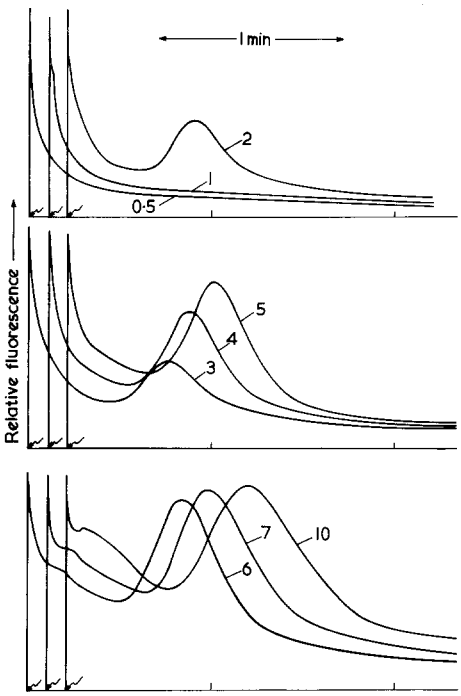

Fig. 1. Development of secondary rise in fluorescence as a function of the length of the preceding dark period. A leaf was taken from laboratory light and illuminated by the Kautsky apparatus for 3 min. It was then illuminated for further periods of 3 min with dark intervals of 30 s to 10 min as indicated. No secondary rise was observed after 30 s and 1 min but after 2 min the secondary rise was already well established. In other experiments the S → M transition (Fig. 2) usually attained its maximal development when preceded by a 3–5 min dark interval. Longer dark intervals often led to an increase in both S and M. It should be noted that if such a cycle of illumination and dark intervals was immediately repeated, the secondary rise tended to occur somewhat sooner than before. In general, the position of the peak and the peak height depended on the immediate pre-treatment of the leaf

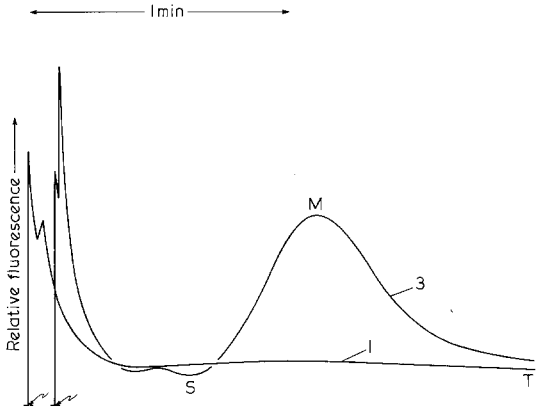

Fig. 2. Fluorescence kinetics characteristic of those displayed by untreated leaf discs in the feeding experiments illustrated in Figs. 4–7 and Table 1. Illumination in the Kautsky apparatus was comprised of 6 periods of 3 min with dark intervals of 1, 3, 10, 1 and 3 min respectively, in that order. The figure illustrates the fluorescence kinetics observed during illumination following the last cycle of 1 and 3 min dark intervals. In most instances these kinetics were very similar to those displayed after the first cycle of 1 and 3 min dark intervals. The secondary rise which developed after 3 min darkness can be described as an S → M → T transient in accord with previous practice (see text)

in air at 20° C, this normally occurred some 50–60 s after the first fluorescence peak. This secondary rise appears to have many features in common with second or third "waves", or peaks, of fluorescence which have previously been reported in a number of species (e.g. Wassink and Katz 1930; McAlister and Myers 1940; Van der Veen 1951; Baker and Bradbury 1981).

Materials and methods

Spinach. Yates hybrid 102 was grown in water culture (for details see Walker 1980b) in a glasshouse at the Research School of Biological Sciences, A.N.U., Canberra, Australia and plants were used at an early stage of development when the first leaves were 5–10 cm in length.

Fluorescence. A portable Kautsky apparatus (Schreiber et al. 1975) manufactured by Richard Branker Research Ltd. (Plant Productivity METER SF-10) was used to monitor fluorescence. This incorporates a photodiode sensor and a light emitting diode which was employed to provide "monochromatic" illumination at a 670 nm maximum and 10 W m^{-2} for periods of 3 min duration. A Corning CS 7-69 cut-off filter behind the LED excludes stray light, reflected light and fluorescence at wavelengths below 710 nm while still allowing a large fraction of photosystem II fluorescence to reach the sensor. The output was monitored on a Riken Denshi peninecorder at a chart speed of 10 cm min^{-1}. Except where stated, measurements were made at 20° C air but it should be noted that, because the Kautsky apparatus only illuminates a very small area of leaf (about 10 mm^2), respiratory metabolism in the surrounding darkened area would almost certainly raise the CO_2 concentration above atmospheric and the enhanced CO_2 might favour secondary fluorescence peaks (see e.g. McAlister and Myers 1940). During measurements leaves or leaf discs were placed (with their upper surface uppermost) on damp Miracloth resting on a temperature controlled surface and covered by polythene film. The Kautsky probe was placed directly on the polythene film.

Feeding. Whole leaves were fed through the petiole. Leaf discs of 1 cm diameter were cut from leaves with a newly sharpened cork-borer and floated on solutions in laboratory light. In each case a small fan was used to generate an air-stream to facilitate transpiration. Sugars and related compounds were supplied at 20 mM, NH_4Cl at 50 mM and DCMU at 10 μM for periods of 30 min to 2 h but no attempt was made to determine the extent of uptake and it is probable that some concentration would occur within the leaf.

Results

Induction has been attributed to light activation of enzymes and to the build-up of intermediates depleted in the preceding dark period (Walker 1980a). With spinach, unless the leaves have been kept in the dark for many hours, induction lags at 20° C are usually in the range of 1–3 min. After very brief dark intervals, of 1 min or less, induction is negligible but is substantially re-established by dark periods of 3–10 min (see e.g. Walker 1980a). Accordingly, the Kautsky apparatus (see Methods) was used to illuminate the leaves at its maximum intensity of 10 W m^{-2}

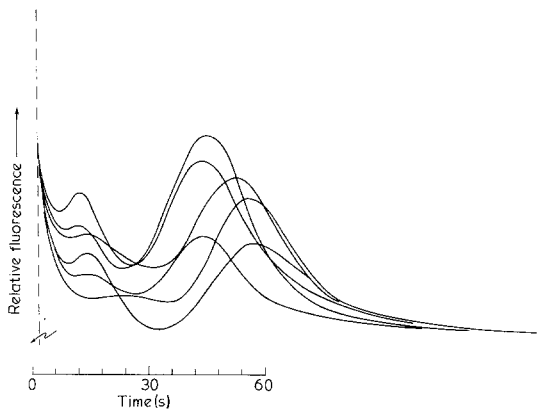

Fig. 3. Some examples of the range of secondary kinetics observed, over a period of 3 months using leaves at various stages of development. Each trace was obtained by following the procedure described in the legend to Fig. 2 and illustrates the kinetics observed following re-illumination after the last dark interval of 3 min. The kinetic pattern seemed to be governed both by the thickness of the leaf and physiological conditions but, in the absence, of unequivocal correlation, uniformity of behaviour was achieved in subsequent experiments (see methods) by using discs from leaves of similar size and age

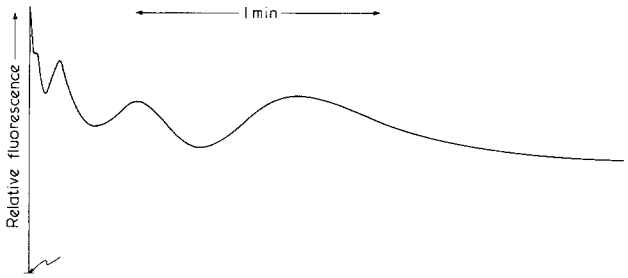

Fig. 4. An example of more complicated secondary kinetics observed at 5° C. Conditions otherwise the same as for Fig. 2

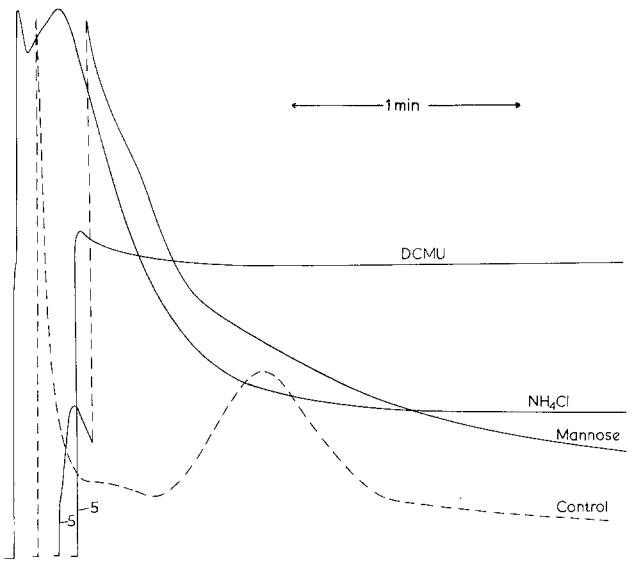

Fig. 5. Modification of fluorescence kinetics on feeding DCMU, NH_4Cl or mannose (see Methods). The fluorescence in the presence of DCMU was recorded with 5-fold scale contraction. The fluorescence in the presence of mannose was initially recorded with 5-fold scale contraction and then switched to full scale as indicated.

for fixed periods of 3 min. Figure 1 shows the development of the secondary rise in fluorescence when these periods of illumination were interspersed with dark intervals which were successively increased in duration from 0.5–10 min (c.f. Fig. 1 of Van der Veen 1951). It is of interest that in this particular experiment the initial peak was independent of the length of the preceding dark interval suggesting that both Qu(Q) and Qu(E) had completely relaxed in all cases. In other experiments, of a similar nature, the first sign of a secondary rise at 20° C was usually detected after about 90 s and the height of the secondary peak above the preceding trough was usually near maximal after a dark interval of 3–5 min. The precise nature of the kinetics varied, however, with the immediate pre-history of the leaf and in order to standardise this as far as possible leaves were taken from laboratory light and given two successive cycles of illumination interrupted by 1 and 3 min dark intervals separated by a longer dark period of 10 min. Behaviour during the first and second cycles was very similar. Figure 2 is typical of the response and (in accord with Fig. 1) shows no sign of a secondary rise in fluorescence after a 1 min dark interval but a large secondary peak when re-illumination followed 3 min darkness. The initial kinetics were also more complex than those obtained when the length of the dark interval was increased in a more gradual fashion (Fig. 1). Figure 3 shows that the secondary rise varied from leaf to leaf and with the degree of development of the leaf. Under these conditions the secondary peak usually reached 30–50% of the height of the primary peak and occurred some 50–60 s later.

Slow fluorescence transients (see e.g. Papageorgiou 1975) have often been designated $S \rightarrow M \rightarrow T$ where S is a quasi steady-state, M is a secondary peak and T is terminal fluorescence. The complexity of the fluorescence kinetics which are sometimes observed in spinach (Fig. 4) is such that it reinforces the doubts of Lavorel and Etienne (1977) that this or similar terminology (see e.g. Bannister and Rice 1968), can be uniformly applied. However, the largest of the secondary peaks always immediately preceded the terminal fluorescence in untreated leaves and could therefore be designated M, or an $S \rightarrow M \rightarrow T$ transition (as in Fig. 2), without implying that corresponding peaks displayed by other species necessarily have more in common than chlorophyll a fluorescence (c.f. Papageorgiou 1975; Schreiber et al. 1975; Van der Veen 1951; Wassink and Katz 1930).

As in previous work (see e.g. Lavorel and Etienne 1977; Krause 1973; Krause et al. 1980), DCMU caused a large initial rise in fluorescence and an equal-

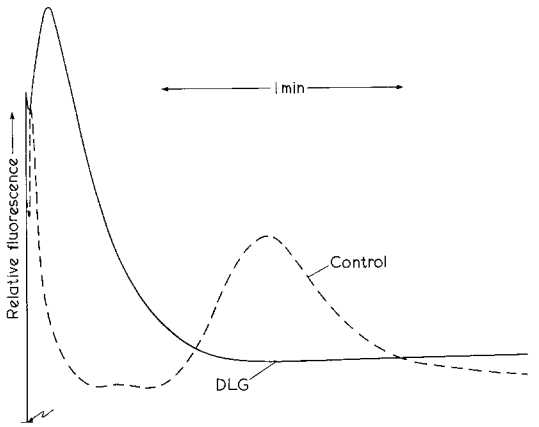

Fig. 6. Modification of secondary kinetics following D,L-glyceraldehyde feeding (see Methods). Conditions otherwise as for Fig. 2

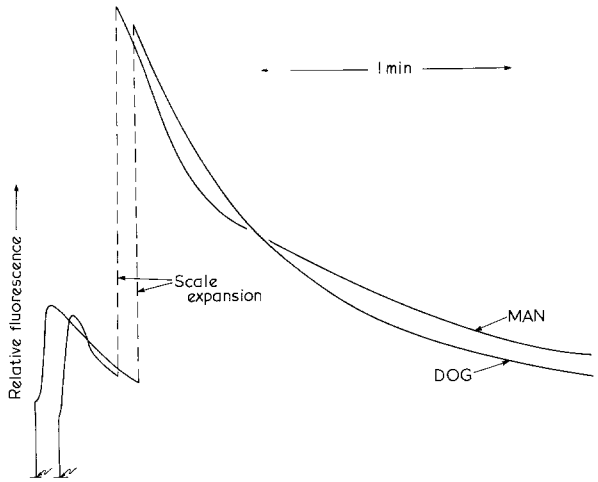

Fig. 7. Modifications of secondary kinetics following feeding of 2-deoxyglucose and mannose (see Methods). Conditions otherwise as for Fig. 2

ly high terminal value (Fig. 5). Ammonium chloride also gave a response consistent with its known action as an uncoupler and similar to that observed by Baker and Bradbury (1981) after infiltration with CCCP. It was of interest, however, that D,L-glyceraldehyde, a relatively specific inhibitor of the reductive pentose phosphate pathway (Stokes and Walker 1972; Slabas and Walker 1976) also inhibited the secondary rise (Fig. 6). Similarly, mannose, 2-deoxyglucose and glucosamine (which share the ability to act as alternative substrates for hexokinase and are thereby capable of sequestering cytoplasmic Pi – see e.g. Herold and Walker (1979) all eliminated or disguised the secondary rise in fluorescence (Fig. 7 and Table 1) whereas glucose caused only a partial diminution (Table 1). All of the above effects were observed repeatedly over a period of three months, at first on intact leaves and subsequently on leaf discs. The results reported were obtained with discs. These were preferred to intact leaves because the level of fluorescence was more reproducible. Nevertheless it was not possible to quantify the fluorescence yield on a reliable basis and the present report is therefore concerned with the nature of the kinetics rather than absolute values of fluorescence yield.

Table 1. Summary of a single series of feeding experiments carried out on discs cut from leaves of the same, age, size and appearance. The height of the secondary (S → M) transition is given in relative units. A negative value indicates a fall in fluorescence and is the difference between fluorescence values in relative units at times corresponding to S and M in controls. The control measurements were made on discs from the same leaf used in the experimental treatments. Number of observations in parenthesis. Experimental details as in "Methods"

Treatment	S → M Fluorescence	Mean
Controls (7)	9 to 60	+40
glucose (2)	12 to 16	+14
2-deoxyglucose (3)	−23 to −61	−37
glucosamine	−34	−34
mannose (3)	−20 to −107	−56
D,L-glyceraldehyde (2)	− 7 to −11	− 9
ammonium chloride	−66	−66
DCMU (2)	− 8 to −10	− 9

Discussion

When leaves are re-illuminated after relatively short dark periods they frequently exhibit changes in the rate of O_2 evolution and CO_2 fixation (see e.g. Van der Veen 1949; Walker 1980) which are reminiscent of a dampening oscillation and might easily reflect the operation of control mechanisms which have been pushed off-balance. The secondary increase in fluorescence which has been defined in the present experiments and which appears to be very similar to many earlier and contemporary observations (see e.g. Van der Veen 1951; Bradbury and Baker 1981) may be another feature of the same changes. Certainly it is difficult to explain a secondary rise and fall in fluorescence on the basis of contemporary views (Lavorel and Etienne 1977; Krause et al. 1980) without invoking corresponding and complementary changes in [NADP]/[NADPH] and [ATP]/[ADP]. Table 2 summarises present views. It shows that whereas Qu(Q) might be expected to increase as the onset of carbon assimilation provides an increasing sink for NADPH, the converse is true for Qu(E). The latter is favoured by the high energy state of the thylakoid membrane which, in turn, would tend to be diminished as ATP consumption increased ADP availability. An initial rise in fluorescence followed by a relatively uncomplicated and rapid fall to a low terminal level can readily be accommodated within the conceptual framework

Table 2. Factors which affect fluorescence yield and fluorescence quenching by altering Qu(Q) and Qu(E). Qu(Q) quenching depends upon the oxidation status of Q. When Q is oxidised it can accept electrons from photosystem II and quench fluorescence (Duysens and Sweers 1963). During carbon assimilation Q is re-oxidised by passing electrons through plastoquinone and photosystem I to NADP and ultimately to CO_2. In these circumstances quenching will therefore be favoured by re-oxidation of plastoquinone and eventually by re-oxidation of NADPH. Fluorescence is also quenched by Qu(E) which depends upon the energy status of the thylakoid membrane (Krause et al. 1980). A high energy state promotes quenching and therefore fluorescence yield will tend to increase when a ready supply of ADP ensures that the high energy state is diminished by active photophosphorylation. It should be noted that, during induction, the reduction of NADP and the formation of ATP from ADP will precede the maximum rate of utilisation of these compounds in carbon assimilation. During carbon assimilation re-oxidation of NADPH should favour re-oxidation of Q and lower fluorescence. Conversely, utilisation of ATP should make ADP available for photophorylation thereby lowering the high energy state of the thylakoid and *increasing* fluorescence

Qu(E)					
Energy state	ΔH^+	ADP	ATP	Qu(E)	Fluorescence
high	high	low	high	high	low
low	low	high	low	low	high
Qu(Q)					
Q oxid	Q red	NADP	NADPH	Qu(Q)	Fluorescence
high	low	high	low	high	low
low	high	low	high	low	high

outlined in Table 2 but this response is only seen after a brief dark interval when the constraints of induction would be negligible. The fact that the secondary rise or S→M→T transition is progressively re-established after a few minutes in the dark at the same time as induction is progressively re-established (see e.g. Walker 1980; Leegood and Walker 1981) implies a causal connexion between secondary fluorescence kinetics and carbon assimilation. This notion is strengthened by the fact that the secondary rise is abolished by D,L glyceraldehyde, a potent and relatively specific inhibitor of photosynthetic carbon assimilation (Stokes and Walker 1972; Slabas and Walker 1976) which is without apparent direct effect on photophosphorylation and electron transport. Similarly the fact that the secondary rise is either abolished, disguised or completely modified by mannose, 2-deoxyglucose and glucosamine, three agents which are able to sequester orthophosphate (Herold and Walker 1979) suggests that an explanation of the secondary fluorescence kinetics may reside in the relationship between the advent of maximal carbon assimilation and its impact on the adenylate status of the stroma. For example, Lilley et al. (1977) have demonstrated an initial rise and subsequent fall in the stromal [ATP]/[ADP] ratio during induction and there is considerable evidence (see e.g. Walker and Robinson 1978; Robinson and Walker 1979) that the [ATP]/[ADP] ratio can play a crucial role in the regulation of photosynthetic carbon assimilation. The extent to which the reconstituted chloroplast system (Lilley and Walker 1979) can realistically simulate in vivo photosynthesis is a matter for argument but there is no doubt that, in the reconstituted system, a transient increase in ribulose 5-phosphate concentration can bring about a transient cessation of phosphoglycerate reduction or that, during the onset of this cessation, [ADP] and [NADPH] tend to increase simultaneously. (This is because ribulose 5-phosphate, in the presence of its kinase, acts as an extremely effective sink for ATP. The ADP so generated then stops the conversion of 3-phosphoglycerate to 1,3-diphosphoglycerate and the consequent re-oxidation of NADP – see e.g. Robinson and Walker 1979; Walker 1980a). Whether the build-up of metabolites which occurs during induction can lead to such a self-correcting imbalance between ribulose 5-phosphate and 3-phosphoglycerate remains to be established but, if it does, it could account for the frequently observed oscillations in O_2 evolution and CO_2 fixation mentioned above and contribute to secondary changes in fluorescence. Observations such as those of Bradbury and Baker (1981) that the oxidised plastoquinone pool is antiparallel with fluorescence during these transitions (i.e. more reduced at M than S) is consistent with this view. Equally our present understanding of the factors involved in quenching, as summarised in Table 2, would indicate that a simultaneous rise (or fall) in [NADPH] and [ADP] would be matched by a corresponding rise (or fall) in fluorescence. Clearly almost any inhibitor of photosynthesis would tend to dampen such oscillations but the fact that orthophosphate sequestering agents, can apparently abolish a secondary rise in fluorescence (which is itself associated with the induction phase of photosynthetic carbon assimilation) make it difficult to avoid the conclusion that the mutual interrelationship between orthophosphate, adenylate status and phosphoglycerate reduction might make an important contribution to the transients in O_2, CO_2 and fluorescence which are observed under these conditions. It would be less than circumspect, however, to suggest that an explanation of all of the complexities of slow fluorescence kinetics should be sought in these relationships on this basis. For example, increased quenching by phosphorylation of the light harvesting complex protein would also depend upon the adenylate status and on the redox state of the electron transport chain (Horton et al. 1981; Horton and Black 1980; Allen et al. 1981; Bennett et al. 1980)

and in pea leaves it has been shown that at least part of the slow fluorescence decline is due to State I/State II transitions (Barber et al. 1981). Much of the further resolution of these signals will demand experimentation at the sub-cellular level. Nevertheless the present results demonstrate that slow fluorescence kinetics can be easily and drastically modified by compounds which interfere with carbon assimilation, as well as by those which react more directly with the photochemical apparatus. It, therefore, offers the hope that feeding experiments of this nature could constitute yet another new avenue through which to approach this difficult problem.

This work was carried out at, and supported by the Research School of Biological Sciences, Australian National University and C.S.I.R.O., Canberra. I am most grateful to Professor C.B. Osmond and Dr. M.D. Hatch for their help and encouragement and for the skilled technical assistance of Prudence Kell. I would also like to thank Dr. P. Horton for his advice and criticism during the preparation of this manuscript.

References

Allen, J.F., Bennett, J., Steinback, K.E., Arntzen, C.J. (1981) Chloroplast protein phosphorylation couples redox state of plastoquinone to distribution of excitation energy between photosystems. Nature (London) **291**, 25–29

Baker, N.R., Bradbury, M. (1981) Possible applications of chlorophyll fluorescence techniques for studying photosynthesis in vivo. In: Plants and the daylight spectrum, Smith, H. (ed). Academic Press, New York (in press)

Bannister, T.T., Rice, G. (1968) Parallel time courses of O_2 evolution and chlorophyll fluorescence. Biochim. Biophys. Acta **162**, 555–580

Barber, J., Horler, D.N.H., Chapman, D.C. (1981) Photosynthetic pigments and efficiency in relation to the spectral quality of absorbed light. In: Plants and the daylight spectrum, Smith, H. ed. Academic Press, New York (in press)

Bennett, J., Steinback, K.E., Arntzen, C.J. (1980) Chloroplast phosphoproteins: Regulation of excitation energy transfer by phosphorylation of thylakoid membrane polypeptides. Proc. Natl. Acad. Sci. USA **77**, 5253–5257

Bradbury, M., Baker, N.R. (1981) Analysis of the slow phases of the in vivo chlorophyll fluorescence induction curve. Changes in the redox state of photosystem II electron acceptors and fluorescence emission from photosystem I and II. Biochim. Biophys. Acta **635**, 542–551

Duysens, L.N.M., Sweers, H.E. (1963) The mechanism of two photochemical reactions in algae as studied by means of fluorescence. In: "Studies on microalgae and photosynthetic bacteria" pp. 353–372. Jap. Soc. Plant Physiol. eds. University of Tokyo Press, Tokyo

Herold, A., Walker, D.A. (1979) Transport across chloroplast envelopes – the role of phosphate. In: Membrane transport in biology, pp. 411–439, Vol. II. Giebisch, G., Tosteson, D.C., Ussing, H.H., eds. Springer-Verlag, Berlin Heidelberg New York

Horton, P., Black, M.T. (1980) Activation of adenosine 5′-triphosphate-induced quenching of chlorophyll fluorescence by reduced plastoquinone. FEBS Lett. **119**, 141–144

Horton, P., Black, M.T. (1981) Light-dependent quenching of chlorophyll fluorescence in pea chloroplasts induced by adenosine 5′-triphosphate. Biochim. Biophys. Acta **635**, 53–62

Horton, P., Allen, J.F., Black, M.T., Bennett, J. (1981) Regulation of phosphorylation of chloroplast membrane polypeptides by the redox state of plastoquinone. FEBS Lett. **125**, 193–196

Krause, G.H. (1973) The high-energy state of the thylakoid system as indicated by chlorophyll fluorescence and chloroplast shrinkage. Biochim. Biophys. Acta **292**, 715–728

Krause, G.H., Briantais, J.-M., Vernotte, C. (1980). Two mechanisms of reversible fluorescence quenching in chloroplasts. In: Proceedings of 5th International Congress on Photosynthesis, Kassandra-Halkidiki, September 1980. International Science Services, Jerusalem (in press)

Lavorel, J., Etienne, A.-L. (1977) In vivo chlorophyll fluorescence. In: Primary processes of photosynthesis, pp. 203–268. J. Barber, ed. Elsevier/North Holland Biomedical Press, Amsterdam

Leegood, R.C., Walker, D.A. (1981) Photosynthetic induction in wheat protoplasts and chloroplasts. Autocatalysis and light activation of enzymes. Plant Cell Environ. **4**. 59–66

Lilley, R. McC., Walker, D.A. (1979) Studies with the reconstituted chloroplast system. In: Encyclopedia of Plant Physiology (New Series) – Photosynthesis Vol. II, pp. 41–52, Gibbs, M., Latzko, E. eds. Springer-Verlag, Berlin Heidelberg New York

Lilley, R.McC., Chon, S.J., Mosbach, A., Heldt, H.W. (1977) The distribution of metabolites between spinach chloroplasts and medium during photosynthesis in vitro. Biochim. Biophys. Acta **460**, 259–270

McAlister, E.C., Myers, J. (1940) The time course of photosynthesis and fluorescence observed simultaneously. Smithson Inst. Misc. Collection **99**, No. 6, 1–37

Papageorgiou, G. (1975) Chlorophyll fluorescence: An intrinsic probe of photosynthesis. In: Bioenergetics of photosynthesis, pp. 320–366. Govindjee ed., Academic Press, New York

Robinson, S.P., Walker, D.A. (1979) Photosynthetic carbon reduction cycle. In: The biochemistry of plants: A comprehensive treatise, pp. 193–236, Vol. 8, Hatch, M.D., Boardman, N.K. ed. Academic Press, New York

Schreiber, U., Groberman, L., Vidaver, W. (1975) Portable, solid-state fluorometer for the measurement of chlorophyll fluorescence induction in plants. Rev. Sci. Instrum. **46**, 538–542

Slabas, A.R., Walker, D.A. (1976) Inhibition of spinach phosphoribulokinase by D,L-glyceraldehyde. Biochem. J. **153**, 613–619

Stokes, D.M., Walker, D.A. (1972) Photosynthesis by isolated chloroplasts. Inhibition by D,L-glyceraldehyde of carbon dioxide assimilation. Biochem. J. **128**, 1147–1157

Van der Veen, R. (1949) Induction phenomena in photosynthesis. Physiol. Plant. **2**, 287–296

Van der Veen, R. (1951) Fluorescence and induction phenomena in photosynthesis. Physiol. Plant. **4**, 486–494

Walker, D.A. (1976) CO_2 fixation by intact chloroplasts: photosynthetic induction and its relation to transport phenomena and control mechanisms. In: The intact chloroplast, Chap. 7, pp. 235–278. Barber, J. ed., Elsevier, Amsterdam

Walker, D.A., Robinson, S.P. (1978) Regulation of photosynthetic carbon assimilation. In: Photosynthetic carbon assimilation. Basic life sciences, Vol. 11, pp. 43–59. "Proceedings of a Symposium held at Brookhaven National Laboratory, Upton, New York, May/June 1978", Siegelman, H.W., Hind, G. eds.

Walker, D.A. (1980a) Photosynthetic induction. In: Proceedings of 5th International Congress on Photosynthesis, Kassandra-Halkidiki, September 1980. International Science Services, Jerusalem (in press)

Walker, D.A. (1980b) Preparation of higher plant chloroplasts. In: Photosynthesis and nitrogen fixation. Methods in enzymology Vol. 69, pp. 94–104. Academic Press, New York

Wassink, E.C., Katz, E. (1930) The initial changes of chlorophyll fluorescence in *Chlorella*. Enzymologia **6**, 145–172

Received 10 June; accepted 1 July 1981