Planta (1986) 168:563–570

Planta

Nitrogen supply as a factor influencing photoinhibition and photosynthetic acclimation after transfer of shade-grown *Solanum dulcamara* to bright light

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Abstract. We have compared the ability of shade-grown clones of *Solanum dulcamara* L. from shade and sun habitats to acclimate to bright light, as a function of nitrogen nutrition before and after transfer to bright light. Leaves of *S. dulcamara* grown in the shade with 0.6 mM NO_3^- have similar photosynthetic properties as leaves of plants grown with 12.0 mM NO_3^-. When transferred to bright light for 1–2 d the leaves of these plants show substantial photoinhibition which is characterized by about 50% decrease in apparent quantum yield and a reduction in the rate of photosynthesis in air at light saturation. Photoinhibition of leaf photosynthesis is associated with reduction in the variable component of low-temperature fluorescence emission, and with loss of in-vitro electron transport, especially of photosystem II-dependent processes.

We find no evidence for ecotypic differentiation in the potential for photosynthetic acclimation among shade and sun clones of *S. dulcamara*, or of differentiation with respect to nitrogen requirements for acclimation. Recovery from photoinhibition and subsequent acclimation of photosynthesis to bright light only occurs in leaves of plants provided with 12.0 mM NO_3^-. In these, apparent quantum yield is fully restored after 14 d, and photosynthetic acclimation is shown by an increase in light-saturated photosynthesis in air, of light- and CO_2-saturated photosynthesis, and of the initial slope of the CO_2-response curve. The latter changes are highly correlated with changes in ribulose-bisphosphate-carboxylase activity in vitro. Plants supplied with 0.6 mM NO_3^- show incomplete recovery of apparent quantum yield after 14 d, but CO_2-dependent leaf photosynthetic parameters return to control levels.

Key words: Nitrogen nutrition and photosynthesis – Photoinhibition of photosynthesis – Photosynthesis (acclimation) – *Solanum* (photosynthetic acclimation).

Introduction

The physiological and biochemical properties of shade-grown plants which predispose them to light-dependent damage in bright light (photoinhibition) and which determine the potential for acclimation (an increase in light-saturated rate of photosynthesis in air), have been described by many authors and reviewed by Björkman (1981). In herbaceous species, the level of nitrogen nutrition provided to plants grown in the sun seems to determine whether or not photosynthetic acclimation takes place (Takano and Tsunoda 1971; Medina 1971; Osmond 1983). Leaf nitrogen budget is likely to be an important determinant of shade-to-sun acclimation because an increase in the amount and activity of the principal leaf protein, ribulose bisphosphate carboxylase-oxygenase (RuBPCase; EC 4.1.1.39), almost invariably accompanies acclimation to bright light (Björkman 1981). It can also be argued that, if certain components of the leaf nitrogen budget predominate in the control of shade-to-sun acclimation, redistribution of nitrogen among key leaf proteins may be reflected in changes of photosynthetic enzyme and electron-transport activities in leaves after transfer from shade to sun.

Gauhl (1976) reported that some clones of *Solanum dulcamara* from shaded habitats were unable to acclimate to bright light, whereas other clones

Symbols and abbreviations: F_o = initial level of fluorescence at 77 K; F_m = maximum level of fluorescence at 77 K; F_v = variable components of fluorescence at 77 K ($F_v = F_m - F_o$); PSI, PSII = photosystem I and II, respectively; RuBP = ribulose-1,5-bisphosphate; RuBPCase = ribulose-1,5-bisphosphate carboxylase-oxygenase (EC 4.1.1.39)

from sunny habitats were capable of shade-sun acclimation. The former showed substantial decreases in chlorophyll, quantum yield, photosynthesis in air and little change in soluble protein after transfer to bright light, whereas in the latter, chlorophyll and quantum yield were unchanged, and photosynthesis in air and soluble leaf protein increased. We have used Gauhl's clones to examine whether differences in nitrogen utilization might account for the difference in shade-to-sun acclimation. We have examined in-vivo and in-vitro properties of photosynthesis after exposure of shade plants to bright light under conditions of adequate and limiting nitrogen nutrition. The data thus describe the effects of nitrogen on the extent of photoinhibition, and on recovery from photoinhibition, during shade-sun acclimation. Some of these data have been presented at a meeting (Osmond and Ferrar 1984) and a progress report presented in 1983 is in press (Ferrar and Osmond 1986).

Materials and methods

Plant growth and treatments. Plants were raised from three virus-free clones of *Solanum dulcamara* L. prepared as described in Osmond (1983). These clones were originally selected by Gauhl (1976, 1979) from an open habitat (clone Ni) and shaded habitats (clones Sh and Gr). Cuttings were propagated in 15-cm pots using standard University of California sandy humus potting mixture (Matkin and Chandler 1957). The clones were grown in a glasshouse under a shade-cloth enclosure (here termed shade) that transmitted 6% of the sunlight incident on the the glasshouse, and the soil was flushed three times weekly in the evening with Hewitt's nitrate-type nutrient solutions containing 0.6 mM NO_3^- and other nutrients (specified in Osmond 1983). They were irrigated with tap water each morning and again on evenings when no nutrient solution was supplied. Results of three sets of experiments based on nine different treatment series are summarized here. Experiment 1 was done in the summer (November–January) of 1982–83 with clones Ni, Sh and Gr. Experiment 2 was done in the autumn (March–April) of 1983 with clones Ni and Gr in two separate sets of treatments with each clone. Experiment 3 was done in the summer (December–January) of 1983–84 with clones Ni and Sh. At the 10- to 12-leaf stage (after seven to eight weeks) some plants were transferred to another unshaded glasshouse which transmitted 85% of incident sunlight (here termed bright light). The glasshouse was controlled to 25° C day (12–14 h) and 15° C night (10–12 h).

Maximum sunlight on a clear day in this glasshouse ranged from 1500 to 2000 μmol quanta $\cdot m^{-2} \cdot s^{-1}$. Average total daily incident radiant energy during each experiment was calculated from records made at the nearby CSIRO Phytotron. Averages were prepared for the initial 2 d after transfer to bright light, and for the subsequent 12 d of the recovery period. There was a range of nearly twofold between experiments which was in part a consequence of daylength and season. During the initial 2 d after transfer to bright light, average incident radiant energy ranged from 16.8 to 31.8 MJ $\cdot m^{-2} \cdot d^{-1}$ (mean 19.3). Across all experiments the extent of inhibition of photosynthesis in air 1–2 d after transfer from shade to sun was well correlated with total incident radiant energy ($r = 0.74$). During the subsequent 12 d recovery period the range of average daily incident radiant energy experienced was 16.0 to 29.4 MJ $\cdot m^{-2} \cdot d^{-1}$ (mean 21.7). Considering all experiments, light conditions during these two phases of the plant response were thus comparable. It was found that acclimation of photosynthetic rate in air, which only occurred in those plants provided with 12.0 mM NO_3^-, was not correlated with total incident radiant energy, days 2–14 ($r = 0.15$). The variations in radiation require that each data set be considered separately. For some purposes, normalization is the best method of comparison between experiments. It has to be emphasized that these shortcomings resulting from variation in radiation between experiments are offset by the fact that the experiments were conducted under conditions which approximate those in the natural environment, rather than in low-irradiance controlled-environment cabinets.

Sampling procedures and measurements of leaf photosynthetic parameters. Populations of plants from each clone were selected for uniformity and fully developed leaves were chosen for study. In expt. 1 changes in leaf gas-exchange parameters were measured on the same leaf throughout the experiment. Leaves of the same age were selected on two plants of each genotype and analyzed before transfer from shade to bright light, in the unshaded glasshouse, and then analyzed again 1, 7, and 14 d after transfer. In expts. 2 and 3, three adjacent leaves on each plant were tagged and a new plant was chosen for analysis at each time interval (before, 2, 7, and 14 d in expt. 2; before, 2 and 14 d in expt. 3). In expt. 3, plants were grown in the shade with either 0.6 mM or 12.0 mM NO_3^-. After transfer to bright light in each experiment with each clone, half the population was irrigated with 0.6 mM NO_3^-, and half with 12.0 mM NO_3^- nutrient solution.

In expt. 1, leaves of the same age on different plants were used for leaf photosynthesis measurements and subsequently dried for determining specific leaf weight. Dried leaves were used for determination of reduced-N by Kjeldahl digestion in sulphuric acid with selenium-K_2SO_4 as catalyst. Ammonia was determined colorimetrically at 660 nm (as the green salicylate complex) using a Technicon autoanalyzer (Technicon Inst. Inc., Tarrytown, N.Y., USA). Electron-transport and carboxylase activities, and chlorophyll, were measured on leaves of the same age on plants transferred from shade to sun at the same time. In expt. 2 the middle leaf was selected for measurement of leaf photosynthetic parameters, specific leaf weight and N analysis, as in expt. 1. The leaf above was used for fluorescence analysis, and the leaf below was extracted for analysis of in-vitro photosynthetic properties and chlorophyll. In expt. 3 the procedure was the same as in expt. 2 except that low-temperature fluorescence was not measured.

Leaf photosynthesis was measured using a custom-made CO_2/H_2O vapor-exchange system similar to that described in Osmond (1983). Photosynthesis was measured first as a function of photon flux density (PFD) in a gas stream containing 350 μbar CO_2, 210 mbar O_2, and then as a function of different external CO_2 partial pressures at approx. 1500 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ and at 25°C. The leaf-to-atmosphere water-vapor-pressure gradient was kept at 12 ± 2 mbar. Leaf intercellular partial pressure of CO_2 was computed using equations described by Farquhar et al. (1980).

Fluorescence properties of leaves were measured at 77 K. Leaves were dark-adapted and frozen in a device designed to measured photosystem I (PSI) and photosystem II (PSII) fluorescence emission at 77 K, similar to that described by Powles and Björkman (1982). The low-temperature fluorescence transients were followed with a high-speed chart recorder which resolved F_o, the initial level of fluorescence at 77 K. The recorder was then slowed to measure F_m, the maximum level of fluo-

Table 1. Specific leaf weights and reduced-nitrogen levels of leaves from two clones of *S. dulcamara* grown in the shade with 0.6 mM NO_3^- and then transferred to bright light with 0.6 mM or 12.0 mM NO_3^- (expt. 2, means of two leaves)

Clone and time	Specific leaf weight ($g \cdot m^{-2}$)		Reduced nitrogen			
			($mg \cdot (g \cdot DW)^{-1}$)		($g \cdot m^{-2}$)	
	0.6 mM	12.0 mM	0.6 mM	12.0 mM	0.6 mM	12.0 mM
Ni Day 0	11.4	11.4	66	66	0.75	0.75
2	16.3	16.7	61	66	0.99	1.10
7	19.9	23.6	58	67	1.15	1.58
14	23.8	29.5	50	67	1.19	1.98
Gr Day 0	10.0	10.0	77	77	0.77	0.77
2	15.9	14.1	75	70	1.19	0.98
7	17.4	20.7	73	74	1.27	1.53
14	22.6	22.1	40	63	0.90	1.40

rescence at 77 K, and F_v, the variable level of fluorescence, was calculated as $F_m - F_o$.

Measurement of photosynthetic reactions in vitro. Leaves were homogenized in 5 ml Sörensen's phosphate buffer (50 mM, pH 7.5) with 50 mM NaCl, 1 mM ethylenediaminetetracetate (EDTA), 0.5% bovine serum albumin (BSA) and 1% (w/v) Polyclar (insoluble polyvinylpyrolidone, Sigma P-6755). The extract was centrifuged for 1 min in a bench centrifuge to remove the Polyclar and cell debris, then centrifuged 10 min at $10000 \cdot g$ to sediment thylakoid membranes. The supernatent was used for RuBPCase assay and the pellet, washed once with 50 mM Sörensen's phosphate buffer (pH 7.5) containing 50 mM NaCl and 0.1% BSA, was used for measurements of electron transport. The pelleted, washed thylakoids were measurements of electron-transport. The pelleted, washed thylakoids were resuspended in a minimum volume of 50 mM 4-(2-hydroxyethyl)-1-piperazine-N′-2-ethanesulphonic acid (Hepes) (pH 7.6) with 0.5 mM $MgCl_2$ and 1 mM EDTA.

The activity of RuBPCase was measured by ^{14}C incorporation in assays containing 100 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), pH 8.1, 20 mM $MgCl_2$, 18.4 mM $NaH^{14}CO_3$ and 0.9 mM ribulose-1,5-bisphosphate (RuBP) at 28° C. The extract (20 µl) was preincubated in the assay medium (0.5 ml) for 10 min before addition of RuBP, and the assay was stopped after 60 s by addition of HCl. Acid-stable radioactivity was measured by scintillation counting. All electron-transport activities were measured in saturating light from a quartz-iodide source. Photosystem II electron-transport was measured as 2,5-dimethyl-*p*-quinone (DMQ)-dependent O_2 evolution. The assay was done in an O_2-electrode (Rank Bros. Bottisham, UK) in a buffer containing 50 mM Hepes, pH 7.6, 0.5 mM $MgCl_2$, 1 mM EDTA and 2 mM DMQ. Aliquots of the thylakoid suspension (approx. 50 µg chlorophyll) were added, and the light-dependent O_2 evolution measured with 2.5 mM NH_4Cl as uncoupler. Whole-chain electron-transport was measured by methyl-viologen-dependent (0.07 mM) O_2 evolution in the thylakoid resuspension buffer, using about 50 µg chlorophyll and uncoupled in the same way. Photosystem I electron-transport was measured by dichlorophenol-indophenol (DCIP) ascorbate-to-methyl viologen-dependent O_2 evolution in the same buffer with 0.03 mM DCIP, 0.3 mM ascorbate and 0.07 mM methyl viologen, and uncoupled in the same way. Chlorophyll content of fresh leaves, crude extracts and thylakoid preparations were determined spectrophotometrically in 80% acetone at 645, 663 and 710 nm.

Results and discussion

Leaf properties. In all experiments there was a substantial increase in specific leaf weight when shade-grown *S. dulcamara* clones were transferred to bright light (Table 1). There were no reproducible differences between clones in this response, and it occurred independently of the level of nitrogen supply in the nutrient solution. The increase in specific leaf weight was accompanied by a decrease in leaf nitrogen levels, except in Ni plants provided with 12 mM NO_3^- in the nutrient solution (Table 1). These trends might reflect the observations of Medina (1971) that large amounts of starch accumulate in leaves of plants grown in bright light with low NO_3^-. Although not measured in our experiments, starch accumulation could account for the increase in specific leaf weight and decline in total N concentration in plants supplied with 0.6 mM NO_3^- (Table 1). Increased specific leaf weight in plants supplied with 12.0 mM NO_3^-, at more or less constant total N level, presumably means that protein levels in these leaves increased. In all treatments, transfer of plants from shade to sun resulted in an increase in the level of reduced-nitrogen in leaves, independent of nitrogen supply (Table 1). Presumably nitrogen was mobilized from other leaves, as indicated by enhanced sensescence of lower leaves compared with controls kept in shade. However, it is clear from Table 1 that high-nitrogen treatments after transfer to sun led to a 60% increase in the concentration of reduced-nitrogen on a leaf-area basis, compared to low nitrogen treatments.

Leaf photosynthesis in shade and sun clones in relation to nitrogen nutrition. Clones of *S. dulcamara* from sun and shade habitats did not differ in leaf

photosynthetic parameters when grown in potting mixture in the shade with high (12 mM) or low (0.6 mM) levels of NO_3^- in the solutions used to irrigate the plants. Light-saturated photosynthesis in air of shade-grown plants before transfer to bright light averaged 7.3 ± 1.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over all experiments. The apparent quantum yield, measured in air at 21% O_2 and not corrected for reflectance and transmission, was the same at both levels of nitrogen. However, after shade-grown plants were transferred to bright light, the level of nitrogen supplied had marked effects on changes in photosynthetic parameters. We were unable to distinguish differences in the response of the sun clone Ni from those of shade clones Sh and Gr.

Photosynthetic acclimation is usually discussed in terms of changes in the light-saturated rate of photosynthesis in air. Figure 1, in which the rate of photosynthesis in air for shade-grown plants of all clones in each experiment is set at 100%, shows that nitrogen nutrition after transfer to bright light has striking effects on acclimation. Leaves of plants supplied with low levels of nitrogen showed an initial depression of the photosynthetic rate in air after transfer from shade; the rate recovered after 7 d and remained unchanged after 14 d. If the plants were supplied with high levels of nitrogen the initial depression of the photosynthetic rate in air was smaller, recovery was faster, and acclimation was evident after 7 and 14 d (Fig. 1). These results confirm earlier observations with clones of these plants which were compared after growth in sun and shade with high and low levels of nitrogen (Osmond 1983). They also confirm previous observations with sun clones, namely that leaves developed in the shade are capable of full acclimation to bright light (Gauhl 1976).

As pointed out elsewhere (Ferrar and Osmond 1986) intercellular CO_2 partial pressure, in air at light saturation, remained more or less unchanged (270 μbar) even though photosynthesis ranged from 3.6 to 21.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during these experiments. Thus changes in leaf photosynthesis during photoinhibition and subsequent recovery are not the result of changes in stomatal conductance. Rather, it seems that stomatal conductance follows changes in leaf photosynthetic activity, as observed in other studies (Gauhl 1976; Wong et al. 1985).

The level of nitrogen nutrition clearly determines whether acclimation takes place in *S. dulcamara*, irrespective of shade or sun origin of the clones studied. The shade clones used here are the same as, or were collected from the same sites, as those used by Gauhl (clone Gr, 1976; clone Sh, Gr, 1979). In Gauhl's experiments nitrogen nutrition seems to have been abundant, but clones from nitrogen-rich habitats were excluded from comparison in these studies (C. Büchen-Osmond, University of Frankfurt, FRG; presently Virus Ecology Research Unit, Research School of Biological Sciences, Australian National University: personal communication). The shade conditions used here are comparable, but our bright-light conditions are about twice as severe as those used by Gauhl, and should have exaggerated the differences between sun and shade clones. With Clough et al. (1980, 1983) we conclude that ecotypic differentiation in the potential for photosynthetic acclimation is unlikely to be a major factor in the occupation of shade or sun habitats by *S. dulcamara*.

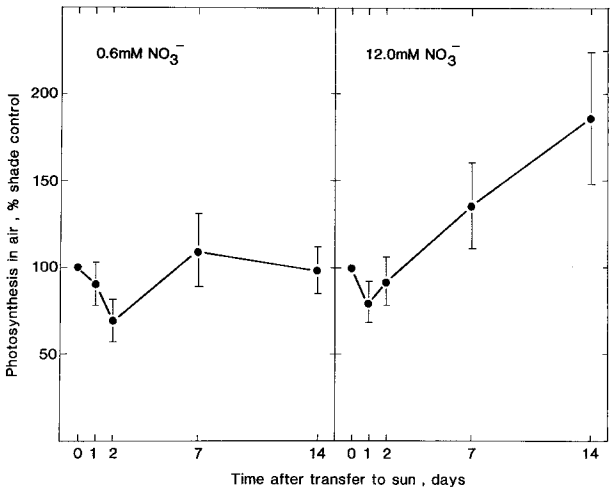

Fig. 1. Changes in leaf photosynthesis in air when shade-grown *S. dulcamara* is transferred to bright light, as influenced by nitrogen nutrition. Data are means ± SE for all three experiments and clones Ni, Sh, Gr ($n = 9$)

Changes in leaf photosynthesis during onset and recovery from photoinhibition. Analysis of other leaf photosynthetic parameters during the onset and recovery from photoinhibition after transfer from shade to bright light provides insight into the photoinhibitory process and subsequent photosynthetic acclimation. Figure 2 shows CO_2-response curves for clone Sh in expt. 3, and Fig. 3 shows the light-response curves for the same leaves, 2 d and 14 d after transfer from shade in plants provided with high and low nitrogen nutrition. In Table 2, normalized data for all experiments have been averaged to show the overall trends. Once again there were no reproducible differences between the clones.

Photoinhibition measured 1 or 2 d after

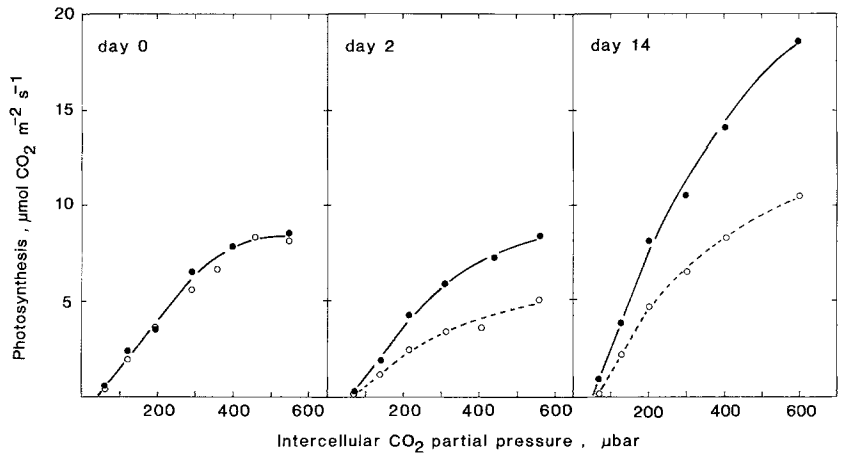

Fig. 2. Changes in the CO_2 response curve of leaf photosynthesis in shade-grown *S. dulcamara* (clone Sh; expt. 3) 2 d and 14 d after transfer to bright light with 0.6 mM NO_3^- (o—o) or 12.0 mM NO_3^- (●—●)

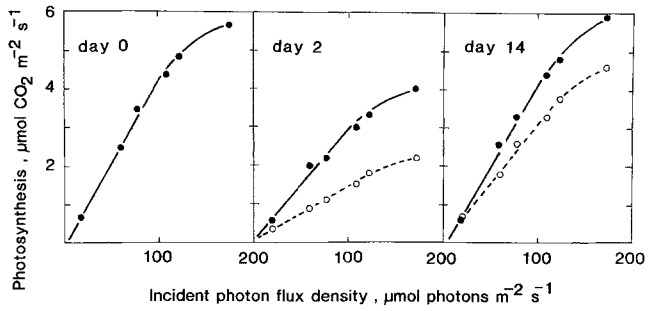

Fig. 3. Changes in the initial slope of the light-response curve of leaf photosynthesis in air for shade-grown *S. dulcamara* (clone Sh; expt. 3) 2 d and 14 d after transfer to bright light with 0.6 mM NO_3^- (o—o) and 12.0 mM NO_3^- (●—●)

Table 2. Changes in photosynthetic parameters of leaves of *S. dulcamara* grown in shade after transfer to bright light. Data are drawn from experiments with all three clones and normalized by setting the control value before transfer as 100% for each clone in each experiment. Values are means ± SE (*n*). A representative set of data is shown in Figs. 2 and 3

Parameter and time	0.6 mM NO_3^-	12 mM NO_3^-
Initial slope of CO_2-response curve		
Day 1, 2	92 ± 27 (9)	99 ± 16 (9)
Day 14	108 ± 18 (9)	227 ± 18 (9)
Apparent quantum yield		
Day 1, 2	49 ± 14 (5)	59 ± 17 (5)
Day 14	62 ± 10 (5)	107 ± 29 (5)

transfer from shade to bright light did not usually affect the initial slope of the CO_2-response curve (Table 2) although in some experiments (Fig. 2) a depression was observed in leaves of plants grown on low nitrogen. Recovery from photoinhibition and photosynthetic acclimation 14 d after transfer to bright light were both markedly dependent on nitrogen supply. The initial slope of the CO_2-response curve showed a small increase in low-nitrogen plants (Table 2) which was more pronounced in some experiments (Fig. 2) than others. However, at a high level of nitrogen supply, the initial slope showed a marked increase in slope (Fig. 2), more than doubling on average (Table 2). The changes in this parameter correspond with changes in the rate of photosynthesis in air (Fig. 1).

On the other hand, photoinhibition was always associated with a decrease in the apparent quantum yield which was marginally, but not reproducibly (Table 2), greater in plants grown with low nitrogen (Fig. 3). Recovery from photoinhibition during the acclimation period was accompanied by full recovery of apparent quantum yield, but only in those plants supplied with a high level of nitrogen (Fig. 3; Table 2). During expt. 2 we had access to equipment for in-vivo measurement of 77 K fluorescence at 692 nm, a technique which is believed to indicate damage to primary photochemistry following photoinhibition (Powles and Björkman 1982). Figure 4 shows that there was a good correlation between changes in apparent quantum yield, and F_v of low-temperature fluorescence.

Correlations between leaf photosynthesis and photosynthetic properties in vitro. Interpretation of the biochemical significance of these changes in leaf photosynthetic parameters has been enhanced by models which integrate the bioenergetics and regulation of carbon metabolism in photosynthesis (Farquhar et al. 1980). These models have been validated by comparison of in-vitro and in-vivo photosynthetic parameters (von Caemmerer and Farquhar 1981, 1984). We have made similar comparisons, based on measurements of fully activated RuBPCase activity in leaf extracts, and electron-transport activity in chloroplast thylakoids prepared from *S. dulcamara* throughout the above ex-

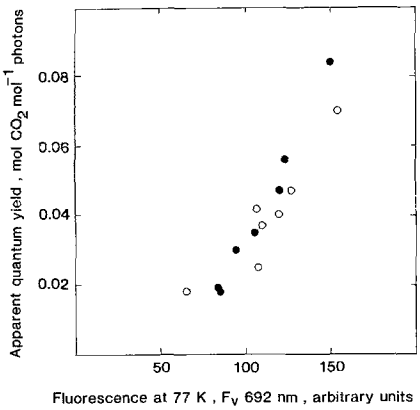

Fig. 4. Correlation between apparent quantum yield (measured from the initial slope of light-response curve in air; compare Fig. 3) and variable component of low-temperature fluorescence in adjacent leaves of *S. dulcamara* (clone Ni, o—o; clone Gr, •—•, in expt. 2). Data refer to control plants grown in the shade (highest values), photoinhibited plants transferred to bright light for 2 d with 0.6 mM NO_3^- (lowest values), and leaves of other plants with 0.6 mM and 12.0 mM NO_3^- during recovery and acclimation (intermediate values)

Table 3. In-vitro photosynthetic parameters in preparations from leaves of shade-grown *S. dulcamara* before transfer to bright light. Values refer to mean ± SE ($n=9$) for all clones

Parameter	Rate	
	(μmol·m^{-2}·s^{-1})	(μmol·(mg Chl)$^{-1}$·h^{-1})
RuBPC	18.9± 3.7	207
PSII electron transport	26.6± 5.4	292
Whole-chain electron transport	18.2± 5.2	200
PSI electron transport	66.0±26.0	724
Photosynthesis in air	7.3± 1.4	80

periments. Table 3 shows the average values for these in-vitro parameters in extracts of shade-grown plants. The RuBPCase activity, PSII electron-transport activity and photosynthesis in air in *Solanum* are closely comparable with the rates measured in shade-grown *Phaseolus* (von Caemmerer and Farquhar 1984). The carboxylase activity is also comparable to that found in *Gossypium* grown in full sunlight on the same low-nitrogen solution (Wong 1979) and PSII electron-transport activities are comparable to those of control plants of *Beta* used by Taylor and Terry (1984).

Table 4 shows that the above changes in light-saturated photosynthesis in air and in the initial slope of the CO_2-response curve are both highly correlated with the extractable RuBPCase activity. Our data set extends over only part of the range of the correlation explored by von Caemmerer and Farquhar (1981, 1984) but is essentially indistinguishable from that of *Phaseolus*, and bears the same relationship to model predictions. The same conclusion applies in comparisons with data for *Triticum* grown at different nitrogen levels (Evans 1983) or with data obtained with nitrogen-regulated senescence in *Oryza* (Makino et al. 1984). This does not necessarily establish RuBPCase activity as the sole limitation of CO_2-limited photosynthesis in vivo (Taylor and Terry 1983). However, our experiments confirm the importance of changes in RuBPCase activity during shade-sun acclimation in these clones of *S. dulcamara* (Gauhl 1976) and in general (Björkman 1981).

Change in apparent quantum yield following photoinhibition and during acclimation, and hence change in fluorescence, is well correlated with in-

Table 4. Relationships between in-vivo photosynthetic parameters determined by leaf gas exchange and in-vitro photosynthetic parameters determined in leaf extracts (RuBPCase activity) and chloroplast thylakoids (electron-transport activities) in three clones of *S. dulcamara*

Parameter relationships	Linear polynomial regression coefficient, r^2								
	Expt. 1			Expt. 2				Expt. 3	
	Ni	Sh	Gr	Ni		Gr		Ni	Sh
				(1)	(2)	(1)	(2)		
Light-saturated photosynthesis in air versus RuBPCase activity	0.70	0.88	0.97	0.74	0.92	0.86	0.80	0.57	0.91
Initial slope of CO_2-response curve versus RuBPCase activity	0.79	0.85	0.93	0.54	0.72	0.82	0.66	0.45	0.81
Apparent quantum yield (initial slope of light-response curve in air)									
versus PSII electron-transport acitivity	–	–	–	0.46	0.75	0.61	0.94	0.70	0.83
versus whole-chain electron-transport activity	–	–	–	0.44	0.50	0.70	0.98	0.50	0.73
versus PSI electron-transport activity	–	–	–	0.49	0.47	0.56	0.82	0.44	0.60

vitro electron-transport activities. Four of six experiments in Table 5 show r^2 of 0.7 or higher, and in these PSII electron-transport activity was more highly correlated with apparent quantum yield than whole-chain or PSI electron transport. Our data confirm other studies of photoinhibition in shade-grown plants, and photoinhibition of sun-grown plants exposed to bright light in the absence of CO_2 (Powles 1984), in which electron-transport activities decline but there is little change in RuBPCase activity. For example, we found that the ratio of carboxylase to PSII electron transport increased from 0.80 in controls to 1.85 in low-nitrogen plants and to 1.30 in high-nitrogen plants, following 2 d photoinhibition in expt. 3. The change in ratio in low-nitrogen plants was solely the consequence of a relative decrease in electron-transport activity. In the high-nitrogen plants the ratio was offset by smaller decrease in electron-transport and some increase in carboxylase activity.

Following acclimation to bright light the ratio of RuBPCase to electron-transport activity increases, in all experiments, principally because of a proportionally greater increase in carboxylase activity. There are few data with which to compare these observations, and no other experiments in which these relative changes have been observed in the same leaves during shade-to-sun acclimation. However, the response is similar to that found by Björkman et al. (1972) in leaves of *Atriplex* plants grown at different light levels. In an experiment with beans, von Caemmerer and Farquhar (1984) observed a decrease in this ratio, because of more rapid decrease in carboxylase than in electron-transport, when plants were transferred to shade and during normal senescence in the light.

Conclusions

Based on the changes in the ratio of in-vitro activities in our experiments, and because RuBPCase comprises such a large proportion of soluble leaf protein, it seems that changes in this fraction will dominate leaf nitrogen budgets during photoinhibition and subsequent shade-to-sun acclimation. Under nitrogen-limited conditions the activity ratios indicate that the carboxylase protein may be conserved during photoinhibition. It is hazardous to speculate on these matters because the specific activity of RuBPCase may change during leaf aging (Friedrich and Huffaker 1980).

It is clear that the changes which occur during photoinhibition of shade plants and their subsequent acclimation to bright light are not controlled by single factors or simple interactions. In our experiments, only continued nitrogen limitation after transfer from shade to bright light leads to a response similar to that found in shade ecotypes of other species (Björkman 1981) and to that reported by Gauhl (1976, 1979) for shade clones of *S. dulcamara*. The sun and shade clones used here, and shade clones in the field, were heavily infested with viruses and had to be made virus-free for our experiments (Gibbs 1983; Osmond 1983). It is reasonable to speculate that in Gauhl's experiments, heavy virus infection (without visible morphological symptoms) could have disturbed the leaf nitrogen budget during shade-to-sun acclimation, leading to results similar to those obtained with plants kept on limiting nitrogen.

We are grateful to Dr. S.C. Wong for help with maintenance of the gas-exchange system and for construction of, and access to, the 77 K fluorescence equipment.

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Received 27 January; accepted 2 April 1986