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Influence of enhanced concentration of carbon dioxide and moderate drought on fluorescence induction in white clover (*Trifolium repens* L.)

M. POL*, D. GOŁĘBIOWSKA*, and J. MIKLEWSKA**

Department of Physics and Department of Mathematics' Applications***,
University of Agriculture, ul. Papieza Pawla VI nr. 3, 71-459 Szczecin, Poland

Abstract

In a 5-months' experiment with white clover grown in two CO_2 atmospheres (AC = 350 and EC = 700 cm^3 m^{-3}) and two humidities (-0.2 and -0.8 MPa), changes in fluorescence induction parameters were investigated. Changes induced by EC did not coincide with those induced by moderate drought. Long-term EC significantly increased stationary fluorescence F_s and decreased the vitality coefficient Rfd. Moderate drought significantly shortened the fluorescence half-time and decreased the area above the induction curve S_c and the F_v/F_m ratio.

Additional key words: 2-factor interactions; vitality coefficient.

Introduction

Enhanced concentration of CO_2 (EC) affects various sides of plant metabolism. It stimulutes the rate of photosynthesis in C_3 plants, *e.g.*, in white clover kept under EC for a short time (Bunce 1993, Kimball *et al.* 1993, Grieu *et al.* 1995, Liang *et al.* 1995, Teodorovic *et al.* 1999). Other authors do not give clear answer concerning plant reaction to EC, especially to its long term impact (Reising and Schreiber 1992, Polle *et al.* 1993). EC also modifies tolerance of plant photosynthetic apparatus to the environmental stresses, *e.g.*, to the water shortage in bed. EC may diminish risk of that stress (Andre and Du Cloux 1993, Tyree and Alexander 1993, Pospíšilová and Čatský 1999). Stress factors damage the photosynthetic apparatus, especially the photosystem (PS) 2 (Lichtenthaler 1996). During the stress, chlorophyll fluorescence emission increases and, as a result, its yield changes (Govindjee *et al.* 1986).

We studied the influence of long-term EC (700 cm^3 m^{-3}, concentration expected in the middle of 21st century - Lindzen 1994) and moderate drought on Chl fluorescence induction in white clover.

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Fax: (+4891) 48 71 062; e-mail: fizyka@dedal.man.szcecin.pl
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Materials and methods

White clover (*Trifolium repens* L. cv. HUIA) was vegetatively reproduced from stolons. They were cultivated in a greenhouse at 20/15 °C and irradiated for 9 h every day. Five weeks after the stolons were inoculated, plants were placed in phytotrons for 5 months and subjected to CO_2 concentrations in an atmosphere of 350 (AC) or 700 (EC) $cm^3\ m^{-3}$, irradiance of 400-500 μmol $m^{-2}\ s^{-1}$ PAR, plant water supply good ($\psi = -0.2$ MPa) or moderate drought ($\psi = -0.8$ MPa), photoperiod 14/10 h, relative air humidity 70-80 %, and air temperature of 20/17 °C. Drought was applied after 46 d of growth in AC or EC.

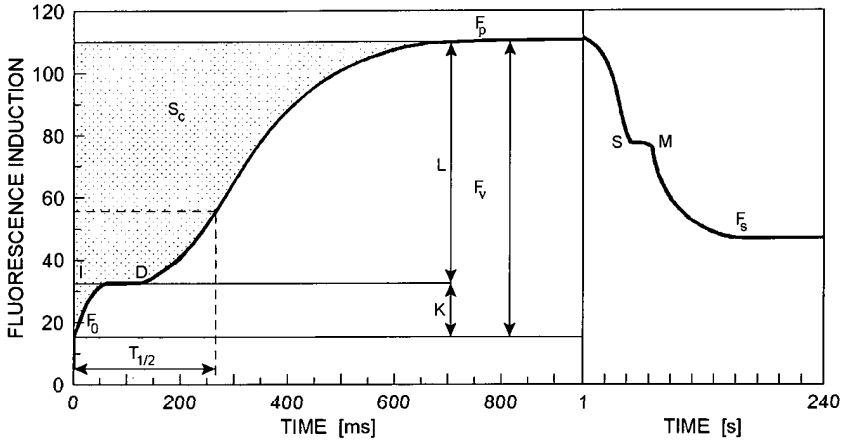

Fig. 1. Fluorescence induction curve with sensitive points chosen for fluorescence induction measurements. F_0 - initial fluorescence, characterizes the amount of chlorophyll the excitation energy of which can not be used in primary processes of photosynthesis. F_p - maximal fluorescence intensity when the primary stable acceptor Q of photosystem 2 is maximally reduced under non-saturation irradiance. S_c - area above the fluorescence curve that characterizes plastoquinone pool. $T_{1/2}$ - time for reaching half of the F_p value. F_s - stationary fluorescence.

The kinetic of Chl fluorescence induction was investigated with leaf discs of 11 mm diameter, 10 replicates for each factor combination. Samples were taken from fully developed leaves (stage 1.0 according to Carlson) not showing any discolouration nor defects, from the middle part of stolon. Before measurement of fluorescence induction, samples of leaves were placed in a thermo-luminostat under irradiance of 200 μmol $m^{-2}\ s^{-1}$ PAR and temperature of 20 °C for 20 min on water surface in petri dishes. The samples were darkened for 15 min before fluorescence measurements. That was done monthly for a period of 5 months. Fluorescence was induced with irradiance of 120 μmol $m^{-2}\ s^{-1}$ PAR (LED $\lambda_{max} = 660$ nm) and measured with the prototype of fluorimeter (Murkowski 1988). Fluorescence intensity was registered with 6 ms period during the first second, and then the measurement was continued with 1 s periods until 240 s. Characteristic parameters of the fluorescence induction curve were F_0, F_p, F_s, F_v, L, K, L/K, S_c, $T_{1/2}$, F_v/F_0, F_v/F_p, S_c/F_p, SM, ID, and Rfd = $(F_p - F_s)/F_s$ (Fig. 1). All the directly measured parameters

and their differences were referred to unit leaf area. We also measured after 1 and 5 months the F_0 and F_m using the Plant Efficiency Analyser *PEA* (*Hansatech*, Norfolk, UK). In this case fluorescence was induced by an irradiance of 1200 μmol m^{-2} s^{-1}.

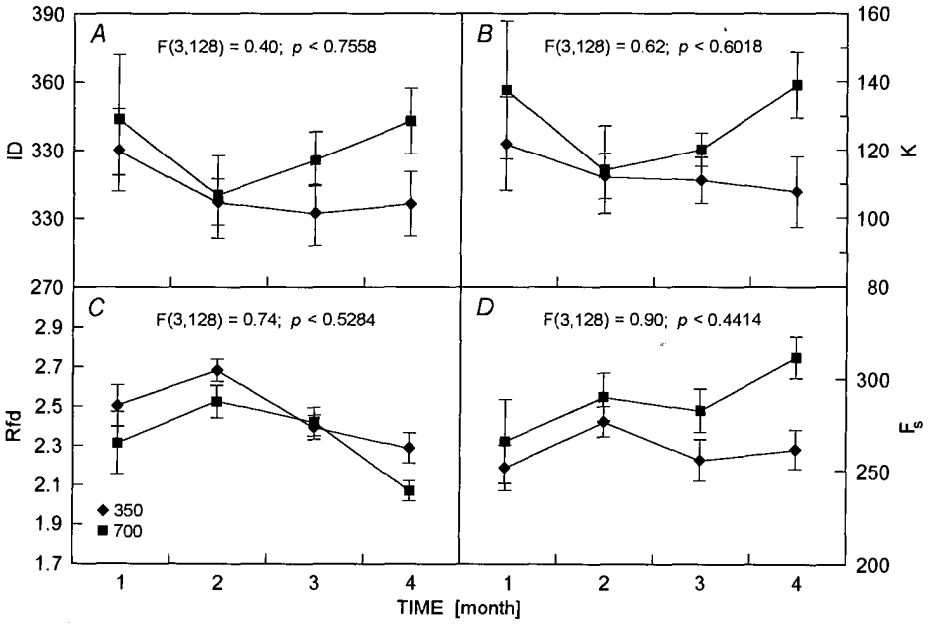

Fig. 2. Time course of group means of fluorescence induction parameters obtained in the 5-month experiment with white clover grown under ambient (350 cm^3 m^{-3}) or enhanced (700 cm^3 m^{-3}) CO_2 concentration; averages for the two humidities used (-0.2 and -0.8 MPa). Fluorescence was induced with an irradiance of 120 μmol m^{-2} s^{-1} PAR (LED λ_{max} = 600 nm). First measurements were done 2 months since the start of experiment.

Results

An influence of EC, moderate drought, and time on changes of the studied parameters was checked using a 3-factor analysis of variance. We did not find any significant pair interactions except from time with drought for the S_c/F_p and Rfd parameters. A lack of interactions allowed a separate analysis of main effects (EC and drought) on the values of fluorescence parameters (Table 1). The EC treatment resulted in a statistically significant increase of F_s and ID. EC significantly affected F_s, ID, K, and Rfd (Fig. 2). Drought decreased $T_{1/2}$, F_v/F_0, and F_v/F_p, while a slight but statistically significant increase of S_c values occurred (Fig. 3).

Values received from *PEA* were evaluated using a 2-way analysis of variance for F_v/F_0 and F_v/F_m. A lack of significant influence of EC on these parameters was confirmed. F_v/F_m under moderate drought showed lower values than under ambient humidity. The difference was significant at $p < 0.2$.

Discussion

White clover grown under EC and moderate drought (ψ = -0.8 MPa) showed significant increase of fluorescence intensity on the ID level and of K values. F_s also increased, but F_0 was not affected. The K value relates to the rate of so-called rapid stage, OID (Fig. 1). The ID phase incline is related to the heterogeneity of PS2;

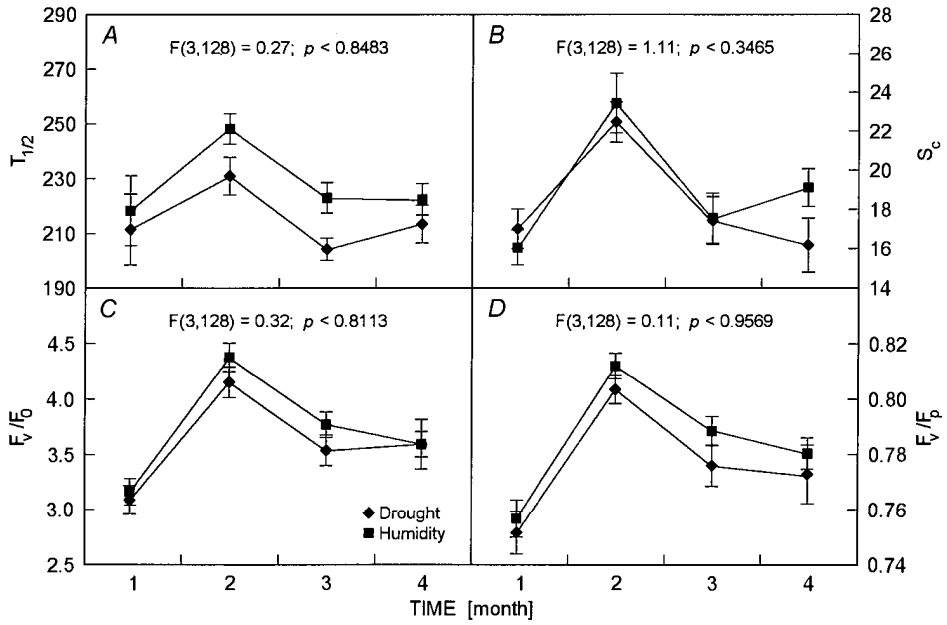

Fig. 3. Time course of group means of fluorescence induction parameters obtained in the 5-month experiment with white clover grown at two humidities (-0.2 and -0.8 MPa); averages for the two used CO_2 concentrations, ambient (350 cm^3 m^{-3}) and enhanced (700 cm^3 m^{-3}). Fluorescence was induced with an irradiance of 120 µmol m^{-2} s^{-1} PAR (LED λ_{max} = 600 nm). First measurements were done 2 months since the start of experiment.

namely, existence in PS2 of at least two kinds of reaction centres. The rapid stage OID is related to the functioning of α centres ($PS2_\alpha$) located in grana thylakoids while the slower (exponential) stage DP to the β centres ($PS2_\beta$) located in stroma thylakoids (Anderson and Melis 1983, Govindjee *et al.* 1986, Veselovskiĭ and Veselova 1990). Thus the influence of EC may be related to a settlement of new diffusion equilibrium between immediate surrounding of the PS2 centres and atmosphere.

We found that EC impacts the intensity of the rapid stage of fluorescence increase. It may also be connected with $PS2_\alpha$ centres located in grana, as follows from studies of Anderson and Melis (1983). Diffusion to grana area is surely more difficult and is settled on the lower level. Increase in CO_2 concentration may change this equilibrium stage and cause that more of $PS2_\alpha$ centres take part in the photosynthesis process while the $PS2_\beta$ system is saturated already at AC.

Table 1. Results of LSD tests. Influence of enhanced CO_2 and moderate drought on the chosen parameters of fluorescence induction of white clover cv. HUIA. Results of 5-month experiment at two levels of CO_2 (350 and 700 cm^3 m^{-3}) and two levels of humidity (-0.2 and -0.8 MPa). Fluorescence was induced with an irradiance of 120 μmol m^{-2} s^{-1} PAR (LED λ_{max} = 660 nm). p – coefficient of significance, n = 200.

Parameter	CO_2 effect			Drought effect		
	350 cm^3 m^{-3}	700 cm^3 m^{-3}	p	humidity	drought	p
F_0	197.8	204.7	0.234	198.4	204.8	0.278
ID	309.5	331.9	0.033*	320.2	321.3	0.917
K	111.7	127.2	0.032*	116.5	121.8	0.469
$T_{1/2}$	228.1	218.9	0.101	230.9	214.2	0.003**
S_c	19.2	19.0	0.889	19.8	18.2	0.042*
F_p	917.3	963.4	0.097	946.1	933.1	0.641
L	607.8	631.5	0.272	625.9	611.8	0.515
F_s	262.1	288.9	0.001***	280.2	269.6	0.194
SM	585.2	598.1	0.423	587.5	596.8	0.565
L/K	6.220	5.830	0.522	5.846	6.248	0.508
F_v	719.5	758.7	0.098	747.7	728.4	0.416
F_v/F_0	3.671	3.743	0.429	3.787	3.610	0.048*
F_v/F_p	0.782	0.783	0.729	0.788	0.777	0.016*
S_c/F_p	0.021	0.020	0.221	0.021	0.020	0.149
Rfd	2.498	2.374	0.031*	2.411	2.468	0.323

Increase of F_s intensity under stress while values of other fluorescence parameters are unchanged usually betokens weakened photosynthesis conditions. Increase of CO_2 concentration in atmosphere up to 700 cm^3 m^{-3} is not necessarily a stress factor. F_s increase is related to the statistically significant decrease of Rfd, often called the vitality coefficient. Rfd declined with time in both atmospheres, however, the decline was more pronounced under EC.

Influence of EC on the Rfd coefficient is related to changes of photosynthetic rate (Bazzaz and Fajer 1992). Increase of photosynthetic rate causes storage of excessive amount of assimilates and in this way reduces the functioning of chloroplasts. The ability of plant to produce simple sugars initially surpasses its transport ability.

F_v/F_0 and F_v/F_p ratios are commonly used parameters in the studies on stress factors affecting the photosynthetic apparatus of plants. Decrease of F_v/F_0 with unchanged F_0 that occurred in our experiment, is interpreted as an index of decrease of activity of the donor side of PS2 (Veselovskiĭ and Veselova 1990). Drought may cause that a part of the structure of primary acceptor Q can not be fully reduced and F_p stays low.

The F_v/F_p is a measure of potential efficiency of primary photochemical reactions of PS2 and its value only slightly differs for various plant species. Changes in values of F_v/F_p are usually linearly correlated with changes of maximal quantum efficiency of photosynthesis and activity of electron transport. Changes of this parameter (Fig. 3*D*) are parallel to changes of Rfd. Our results demonstrate that even drought as moderate as ψ = -0.8 MPa irreversibly changes the photosynthetic apparatus, because the adaptation of investigated leaf samples in the thermo-luminostat to the water

surface in petri plates prior to fluorescence measurements does not reverse changes caused by drought.

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