The effect of temperature and light (PAR) on the induction of Chla fluorescence *in situ*. 2. Diurnal changes in stinging nettle (*Urtica dioica*) and red currant (*Ribes* spp.)

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ABSTRACT: The phenomenon of a midday decrease in quantum and all other efficiencies of photosynthesis has been acknowledged. Quantum efficiency of PSII in nettle is known to be lower at midday than in the morning or evening. On other hand, the parameters of induction of Chla fluorescence in stinging nettle (*Urtica dioica* L.) during the daytime were shown to depend on temperature alone. An increase in temperature also slowed down processes in reaction centres (RCs) and on the acceptor side of PSII, which showed its effect on electron transport in PSII and overall photosynthesis. A similar situation was found for red currant (*Ribes* spp.). The temperature effect was considered to be transient thermal inhibition of photosynthesis caused by diurnal changes in temperature.

Key words: *Urtica dioica* L., *Ribes* spp., diurnal rhythm, thermal inhibition of photosynthesis

Abbreviations: PAR: photosynthetically active radiation; RC PSII: reaction center of photosystem two; R/FR acclimation: acclimation of plants to ratio of red and far red light; Chla: chlorophyll a; CET: Central European Time.

INTRODUCTION

Photosynthesis as the process of energy conversion depends on the intensity and quality (R/FR acclimation) of light (photosynthetically active radiation, PAR). PAR is the most important variable environmental factor affecting plants (Björkman 1981). The factor varies depending on season, time of day and habitat. According to Björkman (1981) plants acclimate to the prevailing light regimes, either to sunlight or to shade. Temperature also affects photosynthesis as a significant ecological factor (Berry & Björkman 1980). This acts as a series of enzymatic reactions under Michaelis-Menten kinetics (Edwards & Walker, 1983), such as occur in photosynthetic reactions in the stroma ("dark phase of photosynthesis"). Temperature also affects the photosynthetic processes in membranes ("light phase of photosynthesis"), which mostly depend on the status of thylakoid lipids (Nishida & Murata 1996, Havaux 1998). PAR and temperature normally change during the course of a day in a regular diurnal rhythm, and plant reactions respond to these changes (Demmig-Adams & Adams 1992, Long *et al.*

1994, Larcher 2003). Thylakoid membrane reactions are associated with photosynthetic processes in the stroma (Edwards & Walker 1983, Geiger & Servaites 1994), so that by monitoring the "light phase" the overall efficiency of photosynthesis can be assessed. The method of Chla fluorescence (Krause & Weis 1991) is commonly used for assessments of membrane reaction and total photosynthesis under different ecological conditions (Björkman & Demmig 1987, Genty et al. 1989, Burke 1990, Demmig-Adams & Adams 1992, Oberhuber & Edwards 1993). Ecophysiological studies of the effect of PAR and temperature on photosynthesis in situ frequently include PAM (Pulse Amplitude Modulated) fluorimetry (Maxwell & Johnson 2000). The method of unmodulated fluorimetry has had less application in ecophysiological research so far. In the present study, we assessed the effects of temperature and light on Chla fluorescence parameters as measured by unmodulated fluorometry during diurnal changes of ecophysiological parameters in stinging nettle (Urtica dioica) and red currant (Ribes spp.).

MATERIALS AND METHODS

Induction of Chla fluorescence was monitored using a Handy-PEA portable fluorometer (Hansatech, UK), which operates on the principle of unmodulated Chla fluorescence. The device has a software for computing, numerical presentation and recording the parameters of Chla fluorescence. The following parameters were measured: F_0, Fm, Fv/Fm and Fv/F_0 as defined by Krause & Weis (1991), Strasser et al. (1995) and Maxwell & Johnson (2000), and the Pindex (Performance index or index of relative vitality of photosynthesis), Tfm (msec; time in which the Fm level of fluorescence is reached, a measure of the reduction rate of the Q_A acceptor of PSII, i.e. the rate of electron transport in PSII) and A (bms; area above the fluorescence induction curve between F_0 and Fm, an indicator of the plastoquinone pool in PSII)

as defined by Strasser et al. (1995). Photosynthetically active radiation (PAR; mmol m^{-2} s^{-1}) was measured using a Li-1000 datalogger (Li-Cor, USA), while temperature was measured with a BIG DIGIT Dual Thermo thermometer (measuring range from -50^{0}C to +70^{0}C). As light and temperature are indicators of the energy (light or thermal) to which plants are exposed, an Arrhenius data transformation was performed prior to statistical processing, consistent with Marković et al. (1996). The parameters measured as absolute values (F_0, Fm, Tfm and A) were transformed to natural logarithm, while PAR and absolute temperature (T; ^{0}K) are given as their reciprocals (1/PAR, 1/T). The ratio parameters (Fv/Fm, Fv/F_0 and Pindex) were not transformed. Statistical data processing (determination of means, correlation and regression analyses of the parameters of Chl fluorescence (transformed or untransformed) vs. 1/PAR or 1/T) was done using Excel software (Microsoft, USA). Measurements of Chla fluorescence were carried out on leaves of stinging nettle (U. dioica L.) as a herbaceous plant, and red currant (Ribes spp.) as a shrub species at Rudno ($\approx$1200 m altitude; 200 km south of Belgrade) in July 2006. Test plants were part of the same ecosystem in a private village yard at Rudno. The readings were taken at 06:30 h (PAR@270 mmol m^{-2} s^{-1}; T@21.5 ^{0}C), 12:00 h (PAR@960 mmol m^{-2} s^{-1}; T@30.0 ^{0}C) and 18:30 h (PAR@50 mmol m^{-2} s^{-1}; T@20.7 ^{0}C) CET, after leaf acclimation to darkness (for about 1 h) in situ with 5 replications.

RESULTS AND DISCUSSION

The parameters of diurnal induction of Chla fluorescence in stinging nettle (U. dioica L.) were found to be dependent on temperature alone (Table 1). This finding was unexpected with regard to the phenomenon of midday decrease in quantum (and all other) efficiency of photosynthesis (Demmig-Adams & Adams 1992). By measuring the parameters of induction of Chla fluorescence in nettle

Table 1. Correlation between parameters of induction of Chla fluorescence and reciprocal values of absolute temperature (1/T; ^{0}K) and photosynthetically active radiation (1/PAR; mmol m^{-2} s^{-1}) measured on nettle (Urtica dioica) leaves at Rudno diurnally during July 2006. *p<0.05 and **p<0.01.

Character	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) Time									
(2) ln F_0	0.123								
(3) ln Fm	-0.226	-0.244							
(4) Fv/Fm	-0.201	-0.765**	0.808**						
(5) Fv/F_0	-0.248	-0.727**	0.841**	0.990**					
(6) Pindex	-0.218	-0.864**	0.523*	0.848**	0.867**				
(7) lnTfm; mS	-0.060	-0.834**	0.475	0.812**	0.799**	0.809**			
(8) ln A; bmS	-0.301	-0.898**	0.568*	0.911**	0.905**	0.921**	0.918**		
(9) 1/PAR; mmol m^{-2} s^{-1}	0.497	-0.039	0.355	0.286	0.245	-0.039	0.189	0.045	
(10) 1/T; ^{0}K	0.074	-0.490	0.734**	0.796**	0.775**	0.554*	0.694**	0.665**	0.485

Table 2. Correlation between parameters of induction of Chla fluorescence and reciprocal values of absolute temperature (1/T; ^{0}K) and photosynthetically active radiation (1/PAR; mmol m^{-2} s^{-1}) measured on red currant (*Ribes spp.*) leaves at Rudno diurnally during July 2006. *p<0.05 and **p<0.01.

Character	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) Time									
(2) ln F_0	-0.240								
(3) ln Fm	-0.426	0.279							
(4) Fv/Fm	-0.182	-0.391	0.443						
(5) Fv/F_0	-0.265	-0.406	0.762**	0.677**					
(6) Pindex	-0.404	-0.463	0.613*	0.574*	0.911**				
(7) lnTfm; mS	-0.150	-0.367	0.389	0.037	0.616*	0.606*			
(8) ln A; bmS	-0.369	-0.279	0.652**	0.487	0.822**	0.886**	0.689**		
(9) 1/PAR; mmol m^{-2} s^{-1}	0.500	-0.231	0.095	0.099	0.220	-0.003	0.296	-0.024	
(10) 1/T; ^{0}K	0.062	-0.318	0.435	0.435	0.800**	0.605*	0.594*	0.602*	0.506
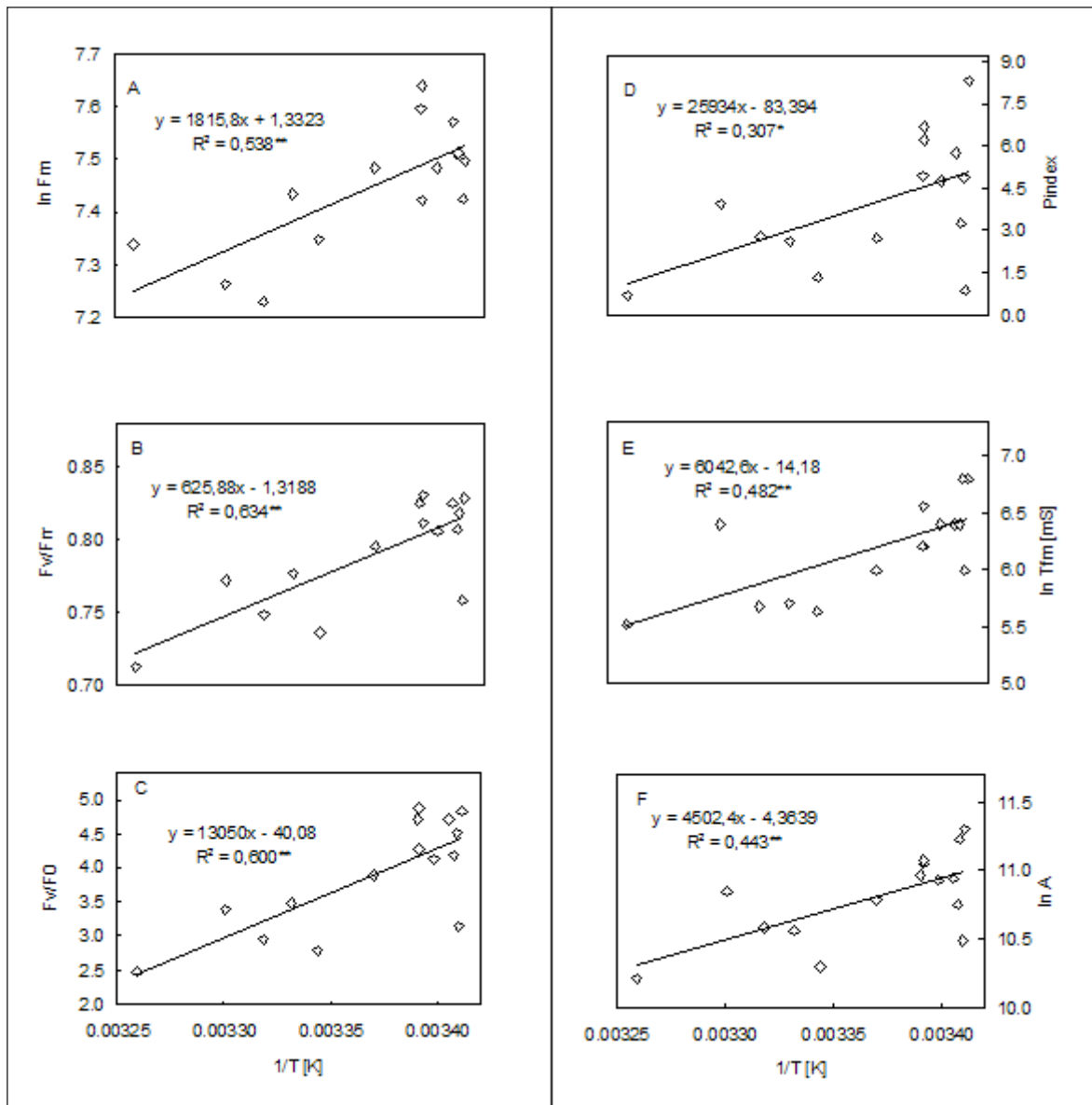
Fig. 1 a-f: Regression dependence of changes of parameters of induction Of fluorescence Chla in stinging nettle (*U. dioica*) leaves on diurnal changes of temperature at Rudno during July 2006. *p<0.05 and **p<0.01.
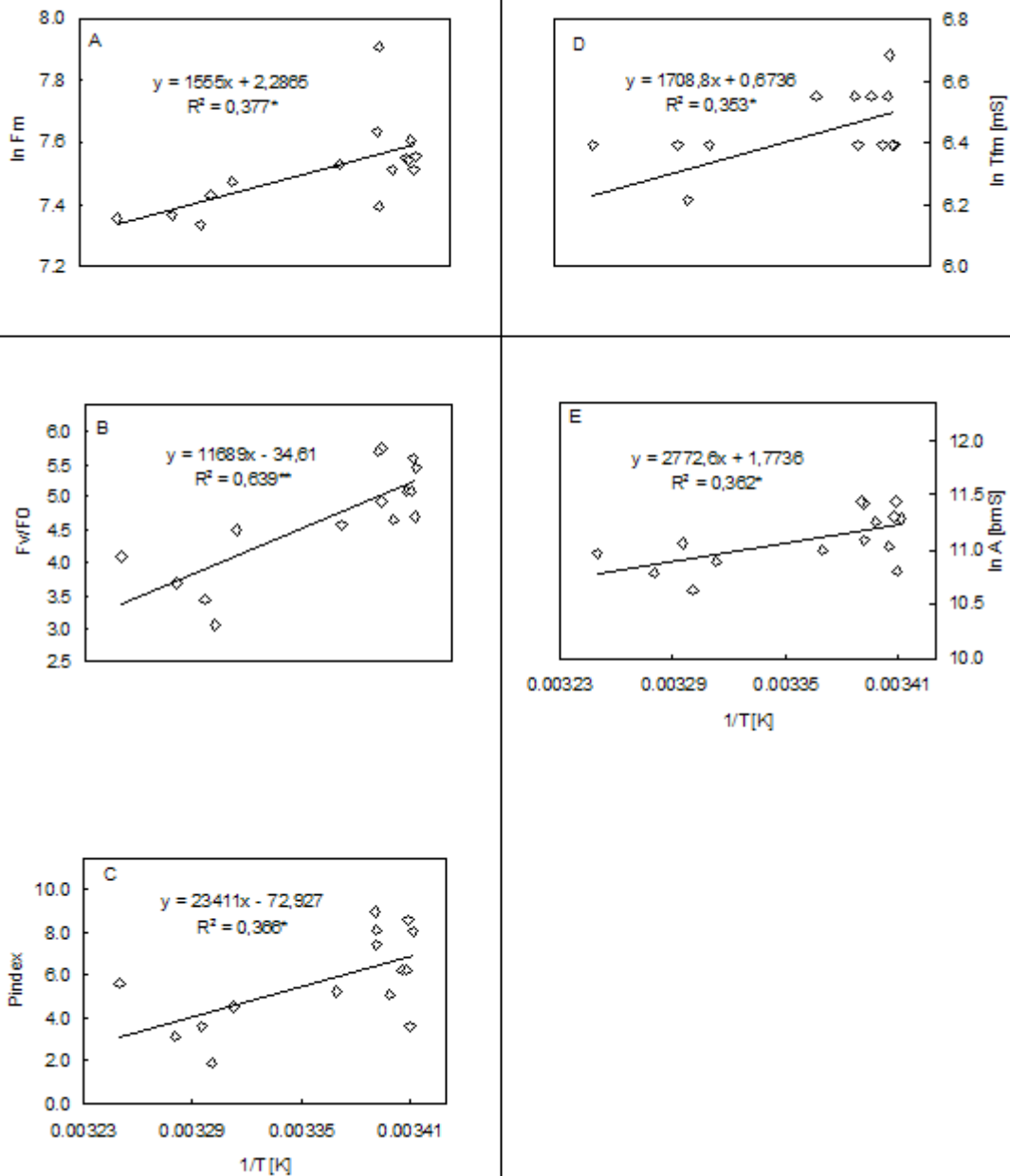
Fig. 2 a-e: Regression dependence of changes of parameters of induction of fluorescence Chla in red currant (*Ribes spp.*) leaves on diurnal changes of temperature at Rudno during July 2006. *p<0.05 and **p<0.01.

(*U. dioica* L.) we found the midday values of quantum efficiency parameters (Fv/Fm, Fv/F$_0$) to be lower than the corresponding morning and evening values (data not shown). However, a statistically significant correlation clearly existed only between temperature and those parameters (Table 1). Regarding the PSII processes that were more susceptible to temperature changes, there was a notable and highly significant relation between the parameters of quantum efficiency of PSII (Fv/Fm, Table 1:

r=0.796**, shown graphically in Fig. 1b: R^2= 0.634**; Fv/F$_0$, Table 1: r=0.775**, Fig. 1c: R^2=0.600**), as well as ln Fm (Table 1: r=0.734**, Fig. 1a: R^2=0.538**) and the reciprocal of absolute temperature.

These results may be interpreted as follows. The parameter F$_0$ reflects fluorescence activity in the PSII antenna system (KRAUSE & WEIS, 1991), while Fm shows the fluorescence activity of the antenna system, as well as the recombination of charges within the RC PSII, and the

parameters Fv/Fm and Fv/F_0 are associated with quantum efficiency of PSII (Krause & Weis 1991, Marković *et al.* 1996). Therefore, an increase in temperature would affect the quantum efficiency of PSII by slowing down processes in the RC PSII (charge separation, etc.). In a similar way, temperature also affected the parameters: Pindex (Table 1: r=0.554*, Fig. 1d: R^2= 0.307*), ln Tfm (msec; Table 1: r=0.694**, Fig. 1e: 0.482**) and ln A (area, bms; Table 1: r=0.665**, Fig. 1f: R^2=0.443**), while statistical significance of the relation of those parameters with 1/T was lower than the significance of the parameters Fv/Fm and Fv/F_0 vs. 1/T. These parameters reflect the processes on the acceptor side of PSII (Q_A redox status and capacity of the plastoquinone pool in PSII), meaning that temperature had an effect on electron transport in PSII, as well as on overall photosynthesis (parameter Pindex). These conclusions were substantiated by the highly significant correlation between parameters of quantum efficiency of PSII (Fv/Fm, Fv/F_0) and process indicators on the acceptor side of PSII (ln Tfm, ln A) as well as the Pindex parameter as a measure of overall photosynthetic activity (Table 1).

Similar relationships were also observed in red currant (*Ribes* spp.; Table 2, Fig. 2a-e), we assume that temperature affected the primary processes in the RC and on the acceptor side of PSII, consequently affecting electron transport as well (Krause & Weis 1991). The data indicate that the processes in the RC PSII have a crucial effect on electron transport and overall photosynthesis, at least in these two species under their given ecophysiological conditions. Possibly, the higher midday temperatures affected the status of thylakoid lipids (Marković *et al.* 1996, Havaux 1998), at least under the given conditions, both in stinging nettle and red currant. This is important as these two plant species and their life-forms (herbaceous and shrub plant species) occupy the same ecological niche, and share the temperature (and PAR) range that is optimal for photosynthesis of most plant species (Larcher 2003). These diurnal temperature changes also affected the Pindex parameter (Table 1 and 2; Fig. 1d and 2c), considered to be one of the indicators of photosynthesis (Strasser *et al.* 1995). The xanthophyll cycle is known to provide photoprotective regulation of photosynthesis under stress or strong light changes, including diurnal changes in light and temperature (Demmig-Adams & Adams 1992; Long *et al.* 1994). Such regulatory function of xanthophyll is performed during photoprotective nonphotochemical quenching of Chla fluorescence, quenching of free radical species and regulation of fluidity of thylakoid membranes (Havaux 1998). As temperature is the key factor that affects fluidity, and consequently the functioning of thylakoid membranes (Marković *et al.* 1996, Havaux 1998), the observed effects on photosynthesis may be considered as temporary thermal inhibition of photosynthesis (Laisk & Oja 1998), caused by diurnal changes in temperature.

CONCLUSIONS

Based on the findings, we inferred that the transient thermal inhibition of photosynthesis may be characteristics of the plants from a mountain ecological niche and of the conditions existing during measurements. This is consistent with conclusions made in a study by Nikolić *et al.* (2008), in which species from many different ecological habitats were analyzed.

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Botanica SERBICA

REZIME

Uticaj temperature i svetlosti (PAR) na indukciju fluorescencije chla *in situ*: 2. diurnalne promene kod koprive (*Urtica dioica*) i ribizle (*Ribes* spp.)

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Razmatran je fenomen podnevne inhibicije kvantne efikasnosti i drugih aspekata fotosinteze. Parametri kvantne efikasnosti PSII kod koprive su niži u podne nego ujutro ili uveče. Nađeno je, u oglednim uslovima, da parametri indukcije fluorescencije Chla kod koprive (*Urtica dioica* L.) tokom dana zavise samo od promena temperature. Porast temperature usporava procese u reakcionom centru (RC) i na akceptorskoj strani PII_I, što utiče na transport elektrona u PSII, kao i na ukupnu fotosintezu. Slična situacija je zapažena i kod ribizle (*Ribes* spp.). Zapaženi efekat temperature može se razmatrati kao privremena termalna inhibicija fotosinteze usled diurnalnih promena temperature. Mišljenja smo da je taj fenomen karakteristika ispitivanih biljaka iz jedne planinske ekološke niše, kao i specifičnih uslova merenja.

Ključne reči: *Urtica dioica* L., *Ribes* spp., diurnalni ritam, termalna inhibicija fotosinteze