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BRIEF COMMUNICATION

Black leaf-clips of a commercial fluorometer increased leaf temperature during dark adaptation under high solar radiation

P. GIORIO*,+, V. NUZZO**, G. GUIDA*, and R. ALBRIZIO*

*National Research Council of Italy, Institute for Agricultural and Forestry Systems in the Mediterranean (CNR–I.S.A.FO.M.), Via Patacca, 85, – 80056 – Ercolano (NA) Italy**
*University of Basilicata, Department of Crop Systems, Forestry and Environmental Science, Viale dell'Ateneo Lucano, 10, -85100- Potenza – Italy***

Abstract

The use of black leaf-clips for dark adaptation under high solar radiation conditions is reported to underestimate the maximum quantum yield of PSII photochemistry (F_v/F_m) measured by the continuous-excitation fluorometer *Pocket PEA*. The decrease in F_v/F_m was due to a rise in minimum fluorescence emission (F_o), probably resulting from increased leaf temperature (T_l). In field-grown tomato and pepper, fluorescence parameters and T_l in the region covered by the black leaf clip were measured in clipped leaves exposed to solar radiation during dark adaptation (clipped-only leaves) and in clipped leaves protected from solar radiation by aluminium foil (shrouded clipped leaves). Results confirmed significant F_v/F_m underestimates in clipped-only leaves primarily due to increased F_o. In one tomato experiment, T_l increased from 30 to 44.5°C in clipped-only leaves, with a negligible rise in shrouded clipped leaves. In two respective pepper experiments, T_l in clipped-only leaves increased from 27 to 36.2°C and 33 to 40.9°C. Based on the results of this study, a clip-effect parameter (P_{CE}) on fluorescence emission is proposed as the difference for F_v/F_m (or $–F_o/F_m$) between shrouded clipped leaves and clipped-only leaves, which resulted to be 0.706 for tomato, and 0.241 and 0.358 for the two pepper experiments.

Additional key words: leaf-clip effect; leaf temperature; parameter; pepper; photochemical efficiency; tomato.

Introduction

Chlorophyll *a* (Chl *a*) fluorescence induction (FI) permits the assessment of the maximum quantum yield of PSII photochemistry in dark-adapted leaves (F_v/F_m) (Kitajima and Butler 1975, Lazár 1999, 2006); where F_v, the variable fluorescence, is the difference between the maximum (F_m) and the minimum (F_o) fluorescence emission. In light-adapted leaves, the effective (or actual) quantum yield [$(F_m' – F')/F_m' = \Delta F/F_m'$; Genty *et al.* 1989] can be determined from the steady-state (F'), and the maximum (F_m') fluorescence emission. Continuous-excitation fluorometers detect (at very short intervals, *e.g.* 10 µs) transient light-induced O-J-I-P fluorescence elicited by a light pulse (Lazár 1999, 2006; Strasser *et al.* 2000). If the induction light pulse does not reach saturation, a fluorescence emission peak value (F_p) lower than F_m or F_m' is achieved (Lazár 1999, 2006). Upon linear heating, *e.g.* at a rate of 3-4 °C per min of a leaf segment placed in a heating device, the response of fluorescence

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+Corresponding author: tel.: +390815746606, fax: +390817718045, e-mail: pasquale.giorio@cnr.it

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Abbreviations: Chl *a* – chlorophyll *a*; F' – Chl *a* fluorescence emission of leaves at steady-state under actinic light; F_m – maximum Chl *a* fluorescence emission in dark-adapted leaves; F_m' – maximum Chl *a* fluorescence emission in light-adapted leaves; F_o – minimum Chl *a* fluorescence emission in dark-adapted leaves; F_o/F_m – minimum to maximum Chl *a* fluorescence emission ratio in dark-adapted leaves; F_v – variable fluorescence in dark-adapted leaves; F_v/F_m – maximum quantum yield of PSII photochemistry in dark-adapted leaves; FI – fluorescence induction; FTC – fluorescence temperature curves; PAR – photosynthetically active radiation; PEA – plant efficiency analyser fluorometer; PSII – photosystem II; R_g – global radiation; RH – relative humidity; SD – standard deviation; T_{air} – air temperature; T_c – temperature at start of F_o sharp increase in FTC; T_l – leaf temperature; T_m – temperature of maximum F_o in FTC; $\Delta F/F_m'$ – effective quantum yield of PSII photochemistry in light-adapted leaves; Ψ_l – leaf water potential.

emission to leaf temperature (fluorescence temperature curves, FTC) demonstrated that above a critical leaf temperature (T_c), F_o exhibits a sharp increase, with a consequent low F_v/F_m (Kuropatwa *et al.* 1992, Nauš *et al.* 1992, Pospíšil *et al.* 1998, Kouřil *et al.* 2004, Weng and Lai 2005). Several fluorometers use a lightweight leaf clip to cover a portion of the leaf lamina for dark adaptation studies, and can process a large number of leaf samples. The continuous-excitation fluorometer *Handy Plant Efficiency Analyser (Handy PEA*, *Hansatech Instruments*) uses white leaf-clips for dark adaptation (*Hansatech Instruments* 1999). For this fluorometer, Weng (2006) reported significant F_v/F_m underestimates after 20 min of dark adaptation under high solar radiation in field-grown rice leaves, which were associated with increased leaf temperature. The clip effect was not observed when clipped leaves where covered to prevent solar radiation. Giorio (2011) reported a marked F_v/F_m reduction in pepper and tomato leaves grown under open field conditions in southern Italy using the continuous-excitation fluorometer *Pocket PEA* that requires the use of black leaf-clips for dark adaptation (*Hansatech Instruments* 2006). The clip effect on fluorescence emission for clipped leaves under high solar radiation conditions during dark adaptation (clipped-only leaves) was not observed for clipped leaves covered with aluminium foil (shrouded clipped leaves). These results suggested heat accumulation on leaf tissue underneath the black leaf-clip, and a consequent rising in the leaf temperature, causing the clip effect on fluorescence emission (Giorio 2011). As the author did not measure leaf temperature, in this study we tested the hypothesis that the leaf-clip effect on fluorescence emission under high solar radiation conditions is due to an increase in leaf temperature when using the *Pocket PEA*/black leaf-clip fluorometer.

Materials and methods

Meteorological data: Air temperature (T_{air}) and relative humidity (RH) data were obtained by a radiation-shielded mini weather station (*Watchdog 450 datalogger*, *Spectrum technologies Inc.*, Plainfield, IL, USA) that also monitored global radiation (R_g [W m^{-2}]) measured in the waveband 0.3–1.1 µm by a silicon pyranometer (*3670I, Spectrum Technologies Inc.*, Plainfield, IL, USA). Instantaneous data were recorded at 5-min intervals. The weather station was located approximately 10 m from the plants under investigation.

Experimental site and plants: Experiments were carried out in 2011 under field conditions on nonirrigated (rainfed) tomato (*Solanum lycopersicum* L. cv. "*Principe di Borghese*") plants, transplanted on 6th May, at the commercial farm "Casa Barone" in Massa di Somma (Naples, Italy; 40°50'47"N, 14°22'51"E, 220 m a.s.l.), and on irrigated pepper (*Capsicum annuum* L., cv. "*Papaccella napoletana gialla*") plants, transplanted on 7th April, at the European Environmental Company (EURECO) experimental station in Acerra (Naples, Italy; 40°57'54"N, 14°25'43" E, 26 m a.s.l.). Soil of the tomato experimental site had 54% (w/w) sand, 41% (w/w) silt and 5% (w/w) clay while soil of the pepper experimental site had 48% (w/w) sand, 37% (w/w) silt and 15% (w/w) clay.

Three experiments were conducted: (*1*) on tomato on 20 June 2011 at 13:20 h; (*2*) on pepper on 21 June 2011 at 10:00 h; (*3*) on pepper on 21 June at 11:55 h. The 30 cm tall tomato plants had 3rd inflorescence flowering and first fruit of first cluster having reached typical size, whereas the 40 cm tall pepper plants were at start of the reproductive phase with only the 1st flower open. Plants of both species appeared in good nutritional and growth conditions.

Leaf water potential: Plant physiological status on each experiment day was measured by assessing leaf water potential (Ψ_l) on an individual set of five plants using a Scholander-type pressure bomb (*SAPS II, 3115, Soilmoisture Equipment Corp*., Santa Barbara CA, USA). Following cutting, the leaf was inserted into the pressure bomb within 30 s, and pressure was increased at a rate of 0.2 MPa min^{-1}.

Fluorescence emission and leaf temperature measurements: A continuous excitation *Pocket PEA* fluorometer equipped with black leaf-clips (*Hansatech, Instruments Ltd.*, King's Lynn, Norfolk, England) was used following Strasser *et al.* (2000, 2004) and Lazár (2006). The *Pocket PEA* excitation red light pulse for FI was emitted by a 627 nm light diode source, and applied for 1 s at the maximal available intensity of 3,500 µmol(photon) of photosynthetically active radiation (PAR) m^{-2} s^{-1}. F_o [relative units, r.u.], and F_p [r.u.] as a viable F_m [r.u.] approximation (Giorio 2011), and therefore F_v/F_m were assessed in dark-adapted leaves. In light-adapted leaves (before initiating dark adaptation), the same protocol was used to obtain a F' [r.u.] approximation (Giorio (2011) and F_m' [r.u.], and consequently $\Delta F/F_m$'. *See* Giorio (2011) for detailed methodology.

In each experiment, a single young fully expanded sun-exposed leaf was chosen from the upper region of 18 individual plants. Fluorescence measurements were conducted on the adaxial leaf lamina before and after 40–60 min of dark adaptation. Leaf temperature monitoring was the primary aim of this study, therefore the dark-adaptation time was longer than the typical canonical 20–30 min. The clipped leaves were exposed to solar radiation (nine clipped-only leaves), or covered with radiation-reflecting aluminum foil (nine shrouded clipped leaves) during dark adaptation (Giorio 2011).

A type E (chromel-contantan) thermocouple (*5SC-TT-*

E-40-36, Omega Engineering, Inc., Stamford, CT, USA) was inserted between the abaxial leaf surface and the leaf-clip for leaf-temperature measurements (T_l). The 18 thermocouples were connected to a solid-state multiplexer (*AM25T, Campbell Scientific, Inc.*, Logan, UT, USA), and monitored by a data-logger (*CR10X, Campbell Scientific, Inc.*, Logan, UT, USA). The data-logger was programmed to detect each thermocouple temperature at 2-s intervals, and to store the mean and standard deviation (SD) every 3 min.

Statistical analysis: Data were analysed with the software package *GraphPad Prism ver. 5.0* for Mac (*GraphPad Software Inc.*, San Diego, CA, U.S.A.) to assess statistically significant differences between variable means of the two clipping treatments using a Student's *t*-test. Null hypothesis was rejected at $p \leq 0.05$.

Results

Weather conditions and plant water status: In all experiments, T_{air} ranged from 26.5 to 29.6°C, RH from 48 to 60% and R_g from 656.0 to 946.2 W m^{-2} (Table 1). In both species Ψ_l was higher than –1.20 MPa (Table 1).

Table 1. Air temperature (T_{air}), relative humidity (RH) and global radiation (R_g) during dark adaptation, and leaf water potential (Ψ_l) for the three experiments (Exps.) reported in Fig. 1 and Table 2. Mean ± SD (*n* = 8–12, T_{air}; *n* = 8–12, RH; *n* = 8–12, R_s; *n* = 5, Ψ_l).

Species	T_{air} [°C]	RH [%]	R_g [W m^{-2}]	Ψ_l [MPa]
Tomato	29.1 ± 0.6	48.1 ± 1.6	946.2 ± 16.0	–1.02 ± 0.06
Pepper Exp. 1	26.5 ± 0.6	60.0 ± 2.6	656.0 ± 54.0	–1.20 ± 0.07
Pepper Exp. 2	29.6 ± 0.5	48.2 ± 2.0	880.4 ± 21.0	*see* Exp. 1

Tomato experiment: At the beginning of the experiment, in both clipped-only and shrouded clipped leaves T_l was approximately 30°C (Fig. 1*A*), and $\Delta F'/F_m'$ was 0.6 (Table 2). During dark adaptation, T_l of clipped-only leaves gradually increased by 14.5°C and stabilized, while in shrouded clipped leaves it increased by only 3.1°C (Fig. 1*A*). At the end of the dark-adaptation period, F_v/F_m was significantly higher in shrouded clipped leaves (0.737 ± 0.048; $p<0.0001$) relative as compared to clipped-only leaves (0.031 ± 0.027) (Table 2). The low F_v/F_m in clipped-only leaves was due to the substantial contribution of F_o (189 ± 18), which was proximal to F_m as indicated by F_o/F_m = 0.969 ± 0.027 (Table 2). F_o and F_o/F_m were significantly higher ($p<0.0001$) in clipped-only than in shrouded clipped leaves. In shrouded clipped leaves, the latter parameter remained as low as 0.263 ± 0.048 (Table 2).

Pepper experiments: At the onset of the first experiment, T_l was approximately 27°C in both two clipping treatments, with increase of only 3.2°C in shrouded clipped leaves during dark adaptation (Fig. 1*B*). Conversely, in clipped-only leaves, T_l gradually increased to 36.2°C within 30 min, and stabilized for the remaining 30 min (Fig. 1*B*). In the second pepper experiment, leaf temperature exhibited patterns similar to those observed in both the tomato and the first pepper experiment, with a maximum 40.9°C in the clipped-only leaves (Fig. 1*C*).

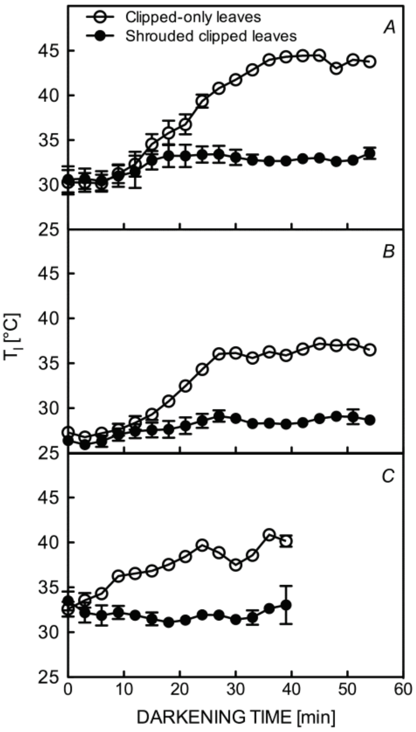

Fig. 1. Leaf temperature (T_l) during dark adaptation for tomato at 13:20 h on 20 June 2011 (*A*); pepper on 21 June 2011 at 10:00 h (Exp. 1, *B*) and 11:55 h (Exp. 2, *C*), for clipped-only, and shrouded clipped leaves. Mean ± SD (*bars*) (*n* = 9).

Consistently with the data found in tomato, in both pepper experiments, and for the two clipping treatments, $\Delta F'/F_m'$ was 0.6 (Table 2). F_v/F_m for the shrouded clipped leaves was 0.76, a value significantly higher ($p<0.0001$) than clipped-only leaves. However, F_v/F_m in the latter clipping treatment was not lower than 0.403 (Table 2).

Discussion

In this study, we tested the hypothesis that high F_o and low F_v/F_m occur in leaves clipped with black leaf clips, and exposed to high solar radiation during dark adaptation, because of an increase in leaf temperature.

Three experiments were conducted (one on tomato and two on pepper) under high light intensity and warm conditions, with both species exhibiting reasonable water-status conditions (Table 1).

Lazár and Nauš (1998) showed that fluorescence parameters do not have a normal (Gaussian) data distribution. However, we considered the use of medians, quantiles and nonparametric statistical tests not strictly necessary because of the strong black-clip effect on the fluorescence emission (Giorio 2011). The tomato experiment results showed a notably lower F_v/F_m in clipped-only leaves compared with shrouded clipped leaves, due to F_o proximal to F_m (Table 2). In agreement with our results, Giorio (2011) demonstrated a dramatic reduction in F_v/F_m when clipped leaves were exposed to high solar radiation during dark adaptation. We found the clip effect on fluorescence parameters was associated with an increase in T_l, which did not exceed 44.5°C (Fig. 1*A*). Our results are congruent with FTC curves data reported for barley and rice leaves, which indicated a sharp F_o increase as leaf temperature reached a critical threshold (T_c) in the range of 40–55°C (Ilík *et al.* 1995), resulting in a F_v/F_m decrease (Kuropatwa *et al.* 1992, Nauš *et al.* 1992, Kouřil *et al.* 2004, Weng 2006). In the absence of shrouding during dark adaptation, a significant, although moderate F_v/F_m underestimate was observed in pepper (18% and 32%, for the first and second experiment, respectively) due to increased F_o (Table 2), as leaf temperature had a moderate 8–9°C increase (Fig. 1*B*,*C*). Consistently with these results, Giorio (2011) reported a greater and earlier F_v/F_m clip effect in tomato than in pepper.

Table 2. $\Delta F/F_m'$ measured prior to the onset of dark adaptation, and F_v/F_m, F_o and F_o/F_m measured at the end of dark adaptation for the three experiments reported in Fig. 1. Mean ± SD ($n = 9$). F_o is scaled by a multiplier of 0.01. For each parameter, significant differences among means between clipped-only and shrouded clipped leaves (Student *t*-test) are indicated by **** ($p \leq 0.0001$), or ns (not significant). r.u. – relative units.

Species	$\Delta F/F_m'$		F_v/F_m		F_o [r.u.]		F_o/F_m	
	clipped-only	shrouded	clipped-only	shrouded	clipped-only	shrouded	clipped-only	shrouded
Tomato	0.611 ± 0.056	0.656 ± 0.035^{ns}	0.031 ± 0.027	$0.737 \pm 0.048^{****}$	189 ± 18	$71 \pm 8^{****}$	0.969 ± 0.027	$0.263 \pm 0.048^{****}$
Pepper Exp. 1	0.637 ± 0.036	0.633 ± 0.079^{ns}	0.523 ± 0.126	$0.764 \pm 0.036^{****}$	85 ± 13	$61 \pm 6^{****}$	0.477 ± 0.126	$0.236 \pm 0.036^{****}$
Pepper Exp. 2	0.601 ± 0.034	0.601 ± 0.036^{ns}	0.403 ± 0.195	$0.761 \pm 0.023^{****}$	104 ± 25	$60 \pm 4^{****}$	0.598 ± 0.195	$0.239 \pm 0.023^{****}$

FTC reported in the literature (*e.g.* Kouřil *et al.* 2004, Kuropatwa *et al.* 1992) indicate, for the 40 to 50°C temperature range, a sharp F_o rise, and a moderate F_m drop leading to $F_o = F_m$ at the temperature of the first peak of FTC, which results in $F_v/F_m = 0$, and $F_o/F_m = 1$. These values are also reached when the clip effect is at the maximum. On this basis, it is here proposed by P.G. a clip-effect parameter (P_{CE}) defined as the difference for F_v/F_m (or $-F_o/F_m$) between shrouded clipped leaves and clipped-only leaves. Our results indicated P_{CE} was 0.706 for the tomato experiment, and 0.241 and 0.358 for the first and second pepper experiment, respectively (data not shown).

We measured abaxial leaf surface temperature, and Chl fluorescence as monitored from the adaxial leaf surface. Fluorescence systems detect fluorescence emission in the most external mesophyll cells (Lichtenthaler *et al.* 2005), proximal to the leaf lamina adaxial side in our study. A temperature gradient could exist between leaf lamina abaxial and adaxial sides. Therefore, our data may be affected by a temperature effect bias on fluorescence emission. It should also be noted that we monitored leaf temperature for a longer period of time typically considered sufficient to achieve dark adaptation. However, time needed for dark adaptation depends on species, stress history, and components of nonphotochemical quenching mechanism under investigation, which relaxation can take from minutes to many hours (Maxwell and Johnson 2000). Moreover, under open field conditions in southern Italy (or elsewhere), crops can experience weather conditions quite hotter than in our study. Consequently more rapid effects of solar exposition on fluorescence emission (*i.e.* within 20–30 min) can occur as reported by Giorio (2011). It may also be challenging to manage many clipped leaves without a delay in canonical darkening time.

We conclude the clip effect on fluorescence emission was caused by heat build-up, leading to an increase in leaf tissue temperature underneath the black leaf clip exposed to high solar radiation during darkening.

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