

ARTICLE

Photosynthetic electron transport activity of light and shade-acclimated field grown grapevine leaves

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ABSTRACT The light response curves of relative electron transport rate (ETR) in field grown grapevine (*Vitis vinifera* L.) leaves were studied on different canopy exposure. The daily course of incident photosynthetic photon flux density (PPFD) was measured by means of ceptometer configured in segmented mode and inserted through the canopy. We compared the photosynthetic activity of shaded leaves and leaves exposed to sunlight on the eastern and western sides of canopy by North-South row direction. Moreover, we studied the seasonal influence of change in daily mean temperature and precipitation frequency on ETR light response curves. The different sides of canopy showed significant differences with regard the incident PPFD value, the western side received with 30% higher maximum PPFD values compared to eastern leaves. However, despite these differences we did not find any significant differences in the ETR light response curves between east and west-positioned leaves. The favourable water condition with lower daily mean temperature (<20°C) resulted in significant lower ETR values in all leaf types. Based on seasonal fluctuation of ETR light curves we can hypothesized that low temperature have higher impact on photosynthetic electron transport activities of grapevine leaves than moderate water deficit.

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KEY WORDS

grapevine,
sun and shade leaves,
electron transport rate,
light response curves

Canopy microclimate is one of the most important with regard to fruit composition and wine quality, in which the effects of soil, climate and cultural practices are summarized (Smart 1985). In general, commercial vineyards are characterized by high plant density, large shoot numbers with closed canopy (Smart 1985; Bertamini and Nedunchezian 2002). In the different cordon training systems the shoots of individual vines are positioned vertically and include leaves forming a continuous canopy wall in the rows. During the canopy development the leaves are exposed to different light conditions (Smart 1988), the young leaves expand at direct sunlight and later they are shaded through shoot growing and the development of neighbouring immature leaves (Bertamini and Nedunchezian 2003). Shade conditions can result rapid senescence of interior leaves or photosynthetic performance is acclimate to shade and the senescence process follows the natural way (Schultz 1991; Wells 1991). Generally used operations in canopy management, e.g. defoliation, significantly alter light distribution (Mabrouk et al. 1997), photosynthetic performance (Candolh-Vasconcelos et al. 1994; Poni et al. 2006) and growth compensation (Hunter 2000) of the canopy system. These studies concluded that removed leaves from different canopy sides are not all the same. We must select those leaves for the defoliation that represent insignificant share from the total photosynthetic performance of canopy.

It seems to be clear that chlorophyll fluorescence measurements are very useful tools for descriptions and estimation of grapevine leaf photosynthesis in field studies (Bálo et al. 1992; Flexas et al. 2000; Iacono and Sommer 2000; Ortoidz and Düring 2001; Cifre et al. 2005; Christen et al. 2007). Recent studies are emphasizing important differences between leaf photosynthesis on the southern and northern canopy sides at common East-West row direction (Mabrouk et al. 1997; Escalona et al. 2003), but light distribution of canopy wall and photosynthetic activity of different leaf exposures for the North-South row direction (on steep slopes) are less well understood. In this paper we characterized the light interception of external and internal sides of canopy by the way of daily course measurements of PPFD at North-South row direction and described photosynthetic activity of light (on eastern and western exposures) and shade-acclimated leaves using ETR light response curves during the growing season.

Materials and Methods

Plant material and experimental site

The study was carried out in the Szentmiklós vineyard of Research Institute for Viticulture and Oenology Pécs, in Hungary. The experimental site is located on steep slopes of Mecsek Hills (latitude: 46°07' N, longitude: 18°17' E, 230-260 m above sea level) in non-irrigated field conditions. The eight-year-old vines of cultivar Sauvignon blanc (*Vitis*

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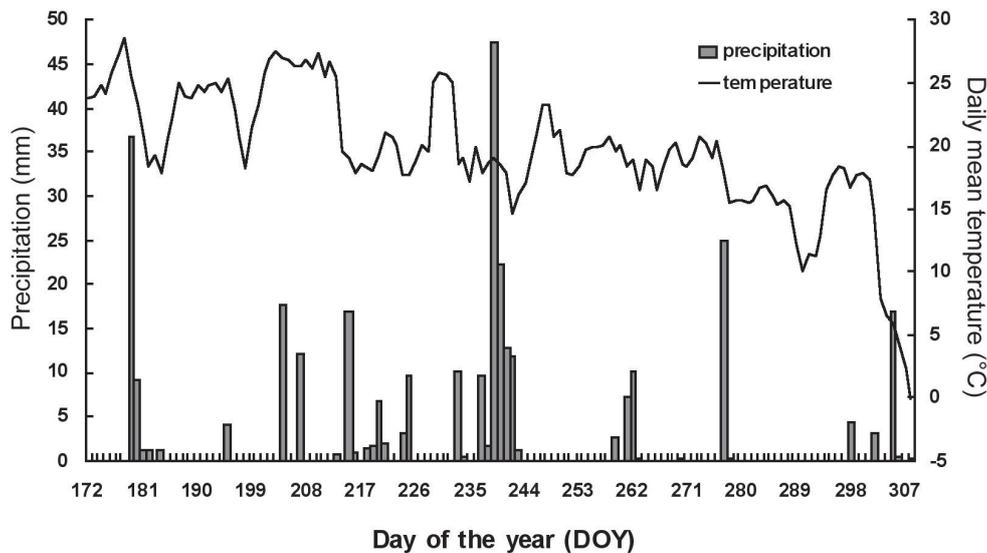


Figure 1. Precipitation frequency and daily mean temperature of air in 2006 (MARD Research Institute for Viticulture and Oenology Pécs).

vinifera L. convar. occidentalis, subconvar. gallica, provar. microcarpa) was grafted on Berlandieri x Riparia Teleki 5C rootstocks and planted in North-South row direction of steep slope. The soil is Ramann type brown forest soil on red sandstone. The training system was mid-high cordon with 2.2 x 0.8 m row and vine spacing and average pruning level was 8 buds per square metre. We measured only healthy mature leaves were taken from randomly chosen vines and 4-5* nodes of shoots near clusters. The leaf samples were collected from the eastern external side, internal side and western external side of canopy in three repetitions of different exposures between June and November in six measurement days (DOY 178,194, 223,251,286, 306).

Meteorological parameters

Weather conditions, *i.e.* precipitation frequency and air temperature were registered by means of an automatic weather station (Lufft HP-100, Fellbach, Germany) situated in the experimental vineyard. The meteorological data were recorded continuously every 12 minutes per day.

PPFD measurements in the canopy

Daily course of PPFD values on different canopy exposures were measured using a linear PAR/LAI ceptometer (Model P-80 AccuPAR, Decagon Devices, WA, USA). The ceptometer was situated on two plastic columns in the row and inserted horizontal through the canopy near the cluster zone. In the course of measurements, we used unattended mode of device and chose the segmented option (Operator's manual AccuPAR 2001). Within this option the measurements were programmed as follows: start time 7:50, stop time 20:00,

sampling interval 5 min, with night shutdown (sleep 20:30 and wake 4:30). The full length of ceptometer (0.8 m) was segmented to 3 segments like 0.20 m on the external sides and 0.40 m in the interior of canopy. Reported values were evaluated as average of five diurnal course measurements during the mid growing season.

Chlorophyll fluorescence measurements

Pulse modulated chlorophyll a fluorescence measurements were made in detached leaves using a PAM-2100 fluorometer (Heinz Walz GmbH, Effeltrich, Germany) connected to a notebook computer with data acquisition software (PamWin v 1.17, Heinz Walz GmbH, Effeltrich, Germany). The leaf samples were dark-adapted for 30 min in field conditions and detached before the end of the dark adaptation. The measurements were performed immediately after the detachment at normal temperature and pressure (20°C, 101 kPa) under laboratory conditions. For inclusions of ETR light response curves, we used the "rapid light curves" option of PamWin software with the pre-programmed operating parameters. The initial PPFD of measurements was lower than 0.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf surface. Each light response curve consist of 10 illumination steps (20 s per step) with increasing PPFD values between 0 and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The values of ETR were estimated from the last point of rapid light curves (F_{max} between 1400 and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PPFD). Minimum (F_0) and maximum fluorescence (F_m) was measured by switching on the modulated radiation of 0.6 kHz and 20 kHz, respectively and induced with a 0.8 s saturating flash of 6000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The actual PPFD values were measured on-line with a micro quantum sensor (4 mm^2 of the total 80 mm^2 measur-

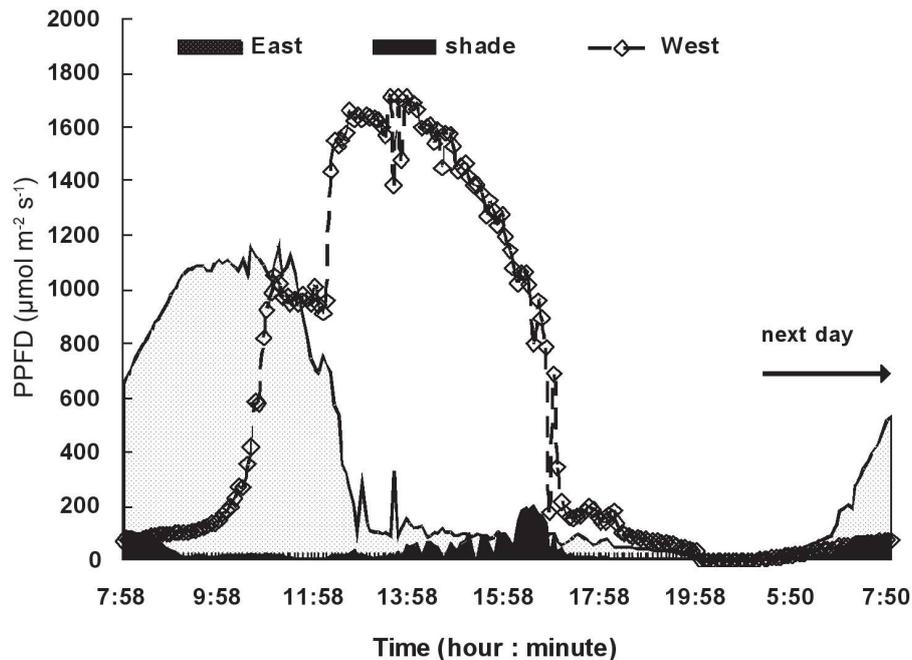


Figure 2. Diurnal course of incident PPFD values in the different leaf exposures of canopy, where East is the eastern external segment, shade is the Internal segment and West is the western external segment of ceptometer inserted through the canopy.

ing area) integrated into the Leaf-Clip Holder 2030-B. The ETR light curves were fitted on the basis of the well-known photosynthesis model (Eilers and Peeters 1988; Eilers and Peeters 1993). Electron transport rate was estimated as $ETR = F/F'_m \times PAR \times 0.5 \times ETR \text{ factor}$, where F/F'_m is the effective quantum yield of irradiated sample (variable fluorescence/effective maximum fluorescence), PAR is photosynthetically active radiation, and ETR factor corresponds to the fraction of incident radiation absorbed by green leaves (value of 0.84) (Schreiber et al. 1994).

Statistical analysis

Statistical analysis was carried out using Excel (Microsoft Corp., Redmond, USA). Paired samples t-test was performed on all data sets with 95% confidence interval between the ETR values of different leaf types.

Results and Discussion

Analysis of meteorological background

In the mid growing season the studied site received less precipitation compared to the 50 years average (Research Institute for Viticulture and Oenology Pécs 2007) after well-watered periods of April and June. Rapidly developing drought stress conditions were observed in July (DOY 182-212; Fig. 1). In this period the daily mean temperature often fluctuated above 25°C (with 28°C maximum value) and the amount of

monthly precipitation was 46% of the total long-term average. After this drought stress period, the August (DOY 213-243) showed untypical conditions, average air temperature was lower by 1.9°C and precipitation was higher with 112% than the 50 years average. We evaluated the air temperature and precipitation changes 14 and 7 days before the fluorescence measurements. These values were as follows: precipitation in the previous 14 days was 0 mm, average of daily mean temperatures in the previous 7 days was 25°C (DOY 178); precipitation in the previous 7 days was 0 mm, average of daily mean temperatures in the previous 7 days was 24°C (DOY 194); precipitation in the previous 7 days was 30 mm, average of daily mean temperatures in the previous 7 days was 19°C (DOY 223); precipitation in the previous 7 days was 0 mm, average of daily mean temperatures in the previous 7 days was 21°C (DOY 251); precipitation in the previous 7 days was 0 mm, average of daily mean temperatures in the previous 7 days was 16°C (DOY 286); precipitation in the previous 7 days was 20 mm, average of daily mean temperatures in the previous 7 days was 10°C and actual air temperature before sunrise was -0.5 °C (DOY 306).

Light interception of different leaf exposures

Diurnal course of incident PPFD values showed significant differences in the different leaf exposure of canopy. The western leaves of external canopy received the highest PPFD between 12:18 and 15:38 at local time (Fig. 2). In the morn-

ing, the PPFD values of eastern canopy side increased earlier than western side or canopy interior. This leaves intercepted efficient light conditions from 7:58 till 12:18 and duration of stage at maximum PPFD (above $600 \text{ pmol m}^{-2} \text{ s}^{-1}$) was very similar to the western side. However, on the western side a 30% higher photon flux density was measured compared to the eastern side. The initial and ending slopes of the respective curves were very similar in the external canopy sides during the diurnal PPFD course and during the initial slopes. Then they increased exponentially, but we found a stationary phase between 11:03 and 12:13 in the curve of western side (Fig. 2). After noon, the incident PPFD values showed a rapid decreasing on the eastern side because of change in actual zenith angle. The low values from 13:00 to 20:00 resulted suboptimal light conditions for the eastern leaves of external canopy. This light distribution suggested that eastern sun leaves have lower potential of the photosynthetic performance in North-South row direction. Eastern sun leaves have shorter illumination period with 1 hour from PPFD values above $200 \text{ pmol m}^{-2} \text{ s}^{-1}$ compared to the other side. We characterized the different canopy sides by means of gas exchange parameters (data not shown) and measured $12 \text{ pmol m}^{-2} \text{ s}^{-1}$ of average CO_2 assimilation rate on eastern leaves in the mid growing season. In our estimation, the shorter illumination period of 1 hour causes lower carbon assimilation yield (approximately $1 \text{ kg per } 100 \text{ vines}$) on eastern leaves when the half of average leaf area index was $2 \text{ m}^2 \text{ m}^{-2}$ and when the both leaf types were grown in the same conditions (Teszák, unpublished data). However, the estimation of canopy carbon balance is more difficult, the photosynthetic performance of external leaves on western canopy side becomes adverse via increasing air temperature and leaf surface temperature after noon. In spite of longer illumination period of western side the leaves have a disadvantaged status because the higher leaf surface temperature inhibited photosynthetic processes and the daily course of photosynthesis is postponed compared to the leaves on eastern or southern exposures. In the canopy interior, the incident PPFD values were drastically reduced due to the light absorption of leaves on external canopy surface. During the diurnal course of photosynthetically active radiation the shade-acclimated leaves received less than $100 \text{ pmol m}^{-2} \text{ s}^{-1}$ of PPFD in the internal canopy side. In the case of canopy interior, a short period to be found almost $200 \text{ pmol m}^{-2} \text{ s}^{-1}$ of PPFD between 16:13 and 16:48 (Fig. 2), but the time and length of illuminated period and the light intensity may alter because of different positions and frequency of canopy gaps. It is well known that heavily shaded leaves do not contribute to canopy photosynthesis, they turn yellow and abscise (Smart 1973; 1988; Schultz 1991). We observed similar process in shade leaves such as the first visible reaction of vine canopy to moderate drought stress in July. However, we can not leave out of consideration the function of interior leaves, because due to several steps of canopy management the available

canopy structure is altering (Candolfi-Vasconcelos et al. 1994; Mabrouk et al. 1997; Poni et al. 2006) and this change reduces the potential CO_2 assimilation surface on the external canopy sides (Smart 1985, 1988; Hunter 2000). Since the shade-acclimated leaves are functioning at lower dark respiration level over a large part of the growing season (Schultz 1991), they may play a significant role in carbohydrate balance of the total canopy. The light microclimate of shade leaves is not widely known and their important role needs further verifications, where we can recommend this application.

ETR light response curves of sun and shade leaves

There were high significant differences between sun leaves and shade-acclimated leaves for each measurement date. In spite of different light microclimate of eastern and western canopy sides ETR light response curves showed very similar values in leaves of both exposures (Fig. 3).

The ETR increased with light intensity in light and shade-acclimated leaves, maximum values of sun leaves were distinctly higher than shade leaves. On the basis of the last points of light curves (between 1400 and $1500 \text{ pmol m}^{-2} \text{ s}^{-1}$ of PPFD) Sauvignon blanc leaves showed normal ETR values that are characterized some white varieties (Ortoizze and Düring 2001). The average ETR values of shade-acclimated leaves were lower with 68% compared to the sun leaves at the first measurement date (DOY 178), after starting drought period lasting for 14 days precipitation deficit and at average 25°C of daily mean temperature (Fig. 1; Fig. 3). Photosynthetic activity of both leaf types changed with enhanced drought conditions in July (DOY 194), moderate drought resulted in lower ETR of sun leaves with 20% and increasing ETR in shade-acclimated leaves with 32% compared to the previous record. This change of potential electron transport efficiency indicated that light-acclimated leaves can not keep their photosynthetic activity at the starting level, but shade-acclimated leaves have a capacity to increase ETR when precipitation deficit is on the increase. On the other hand, the approach of the different light response curves means that the vine canopy has alternative possibility to balance of its total photosynthetic performance by means of shade-acclimated leaves. There is non-negligible importance of shade leaves and their PSII electron transport capacity considering that they are functioning hardly shaded conditions (below $200 \text{ pmol m}^{-2} \text{ s}^{-1}$ of PPFD) with short illumination period per day (Fig. 2). Our results of shade-acclimated leaf behaviour are particularly in agreement with previous research, which described chloroplast acclimation in the canopy interior with decreasing chlorophyll a/b ratio, increasing maximal fluorescence values and better light perception based on fluorescence induction curves (Bálo et al. 1992). At the third measurement date (DOY 223), light and shade-acclimated leaves showed lower ETR values with 20% and 40% compared to former

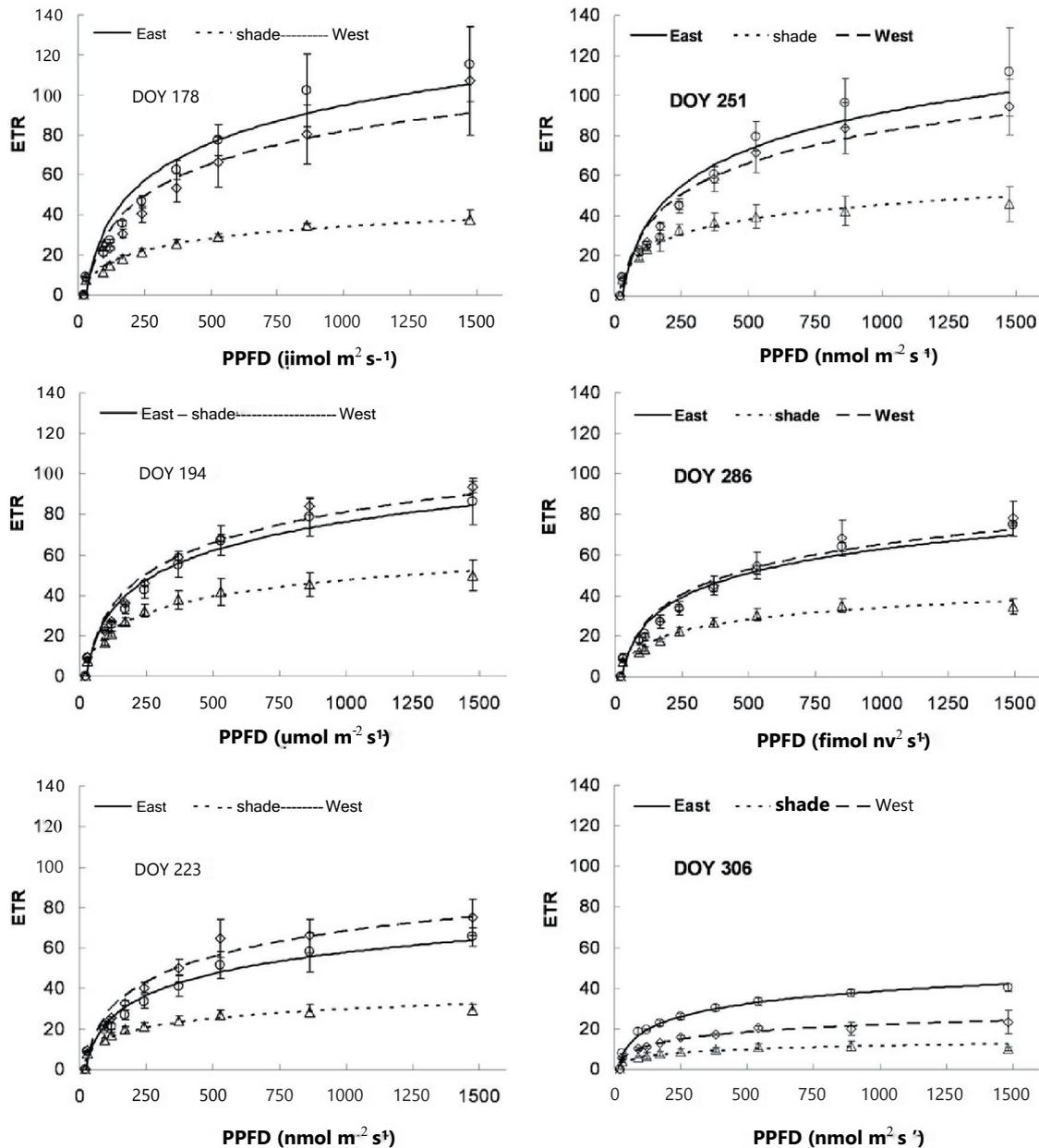


Figure 3. Light response curves of ETR in eastern external (East), interior (shade) and western external (West) leaves of canopy. DOY number indicates the days of year during the growing season and vertical bars represent standard deviation of mean (n = 3).

record. There are significant responds of photosynthetic apparatus to decreasing air temperature (7 days average of daily mean temperature was 19°C with minimum of 12 °C) under favourable water conditions (7 days average of precipitation was 30 mm). After the unusual cold and rainy period daily mean temperature increased above 20 °C with negligible precipitation values developed favourable conditions for ripening of grapes. The apparent electron transport rate showed significant increase with rising daily mean temperature (32°C

of maximum) in light (48%) and shade-acclimated (55%) leaves (Fig. 1; Fig. 3). On the basis of increasing ETR light response values (DOY 251) we can find a high reversibility of moderately depressed PSII electron flow at the phenological stage of ripening. This change suggested that shade-acclimated leaves have significant potential to enhance photochemical activity of the total canopy system. At late growing season (post-harvest period, DOY 286) there were medium low ETR_{max} in light and shade-acclimated leaves, but light-

acclimated leaves showed stronger decrease as a response to low daily mean temperature with average of 16°C and with minimum of 10°C during the previous 7 days. In this period shade-acclimated leaves showed lower ETR with 57%_{max} compared to the sun leaves. In spite of this decrease, there was no significant difference between ETR of eastern and western light-acclimated leaves indicating a similar response to low temperature. The light-acclimated leaves on the different canopy sides showed significant differences only at last measurement date (DOY 306), when we recorded a cold shock effect (Fig. 1), which resulted in severe decreasing of ETR light response curves (Fig. 3). The highest ETR_{max} value was found in the light-acclimated leaves for the eastern canopy side that indicated a slighter chilling-induced photoinactivation of PSII. The western leaves and shade-acclimated leaves had significantly lower ETR_{max} values (with 42% and with 75%) compared to the eastern leaves. These results correspond with recent studies (Bálo et al. 1986; Hendrickson et al. 2004; Bertamini et al. 2005), that reported significantly reduced ribulose-1,5 biphosphate carboxylase activity and PSII activity due to loss of D1 protein at low temperature (5°C). Based on results of ETR light response curves we can suppose that light-acclimated leaves have similar photochemical potential on the eastern and western sides of canopy in North-South row direction, but eastern sun leaves indicated lower photoinhibition for chilling temperature.

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