Influence of leaf age

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Summary

The present study examined the electron transport rate of photosynthesis in response to light for leaves of different age following the imposition of water stress in *Vitis vinifera* during the growth period when leaf carbon gain is largely used for sugar accumulation into the berries. Vines grown in pots were gradually dehydrated to soil moisture levels of 100, 60, 40 and 25 % of field capacity and at each soil moisture level the photosynthetic light response of selected leaves was recorded using chlorophyll fluorescence analysis. Light response curves (LRC) were established by fitting a model of the form of a non-rectangular hyperbola to the data. Changes in quantum yield (<D), the convexity of LRC (8) and the maximum electron transport rate (J_{Tsat}), following adjustment to water stress and as a result of leaf aging, were analysed and their contribution towards the formation of LRCs was assessed. F varied little due to leaf aging and as a result of falling soil moisture content, whereas J_{Tsat} declined strongly following soil dehydration and the decline was exacerbated by leaf aging. Such behaviour suggests that biochemicallstomatal limitations played a more important role in determining the water stress-related decline of photosynthesis than biophysical processes of energy transfer and electron transport. 8 of LRCs, under well-watered conditions was highest for basal leaves with a leaf plastochron index (LPI) of about 16.6 but only small changes were obvious during adjustment to water stress. The most important adjustment in total photosynthetic electron flow following the onset of water deficits resulted from a depression in J_{Tsat} and was most obvious in leaves of a basal (LPI 16.6) and medium (LPI 6.9) position on the shoot. Results suggest that young, fully mature leaves are an important source of carbohydrate assimilation during berry ripening.

K e y w o r d s a n d a b b r e v i a t i o n s : photosynthetic electron transport rate (J_T), PSII quantum efficiency (llF/F_m'), reduction status of Q electron acceptor (1-qp), quantum yield (<!), convexity (8) and maximum electron transport rate (J_{Tsat}) of light response curves (LRC), leaf plastochron index (LPI).

Introduction

Plant responses to water stress in the genus *Vitis* have been extensively studied by many authors (SMART and COOMBE 1983, SCHULTZ 1996 a). Generally, a wide variability in the physiological behaviour within the genus has been reported (DÜRING 1986). For example, ZAMBONI *et al.* (1986) found that *V. riparia* closes its stomata as soon as water becomes limiting while *V. berlandieri* adjusts the leaf osmotic potential in order to maintain cell turgor. Recently, TACONO *et al.* (1998) showed that the choice of rootstock for a given scion strongly influence net photosynthesis in response to a drying soil and under high light intensities. They concluded that the observed reduction in net photosynthesis resulted from biochemical/stomatal limitations and photochemical changes in the photosynthetic apparatus.

Photosynthesis in C_3 plants is known to be very sensitive to drought stress and this is largely ascribed to rapid stomatal closure and a parallel reduction in the uptake and availability of CO_2. HAVAUX and LANNOYE (1985) proposed PSTT functioning, estimated by chlorophyll fluorescence, as a measure of water stress and drought resistance in crop species. HAVAUX (1992) suggested that water stress has little effect on the functioning of PSTT. KICHEVA *et al.* (1994) and HE *et al.* (1995) reported that PSTT quantum efficiency of mildly stressed wheat was unchanged. Similar results were obtained by MAURY *et al.* (1996) on sunflower and by JEFFRIES (1994) on field-grown potato. A loss in the PSTT quantum efficiency was noticed only under severe water stress or under mild water stress in combination with high irradiance. When lupin leaf water content fell from 85 to 60 % MEYER and DE KOUCHKOVSKY (1995) reported an inactivation of PSTT that was probably due to protein alterations. The same occurred in olive trees when leaf water potential declined to -6 MPa (AGELOPOULOS *et al.* 1996). Fluorescence analysis using the saturation pulse method indicates that during rapid wilting of detached leaves of *Hedera canariensis* to about 50 % RWC, energization of the photosystems and the thylakoid membrane remain unimpaired at low photon flux densities, *i.e.* below about 1000 !Jmol m^{-2} s^{-1} (BILGER and BJÖRKMAN 1990). However, once photon flux densities were above 1000 !Jmol m^{-2} s^{-1} water deficits in leaves rapidly led to lowered rates of open-chain electron transport and photophosphorylation (BJÖRKMAN and POWLES 1984). DÜRING (1998) reported that in grapevine water stress associated with high irradiance caused a reduction in quantum efficiency of PSTT resulting in photoinhibition, but that the damage to PSTT was reversed during the night.

A number of internal and external factors can influence vine response to water stress. Leaf age is one such factor. There are a number of studies that investigated the influence of leaf age on photosynthetic performance in grapevines (KRIEDEMANN 1968, KRIEDEMANN *et al.* 1970, TNTRIERI

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et al. 1992, Schultz 1995). However, little is known on how age impacts on photosynthesis of water stressed leaves and how age and water stress interact with regard to the response of photosynthesis to light. Quantification of these factors is important in determining their limitation to CO_2 assimilation and provides valuable information for the development of simulation models of vine carbon gain.

The mathematical description of the response of photosynthesis to light is often described as a non-rectangular hyperbola (Thornley 1976) of the form below (foot-note).

The maximum quantum yield of photosynthesis is similar across a wide range of C_3 species (Ögren and Evans 1993) and the maximum photosynthetic rate in the presence of saturating CO_2 partial pressures can be related to the electron-transport capacity of the leaf (Evans 1987). The convexity or rate of bending (8) describes a transition between those limitations to photosynthesis that are mainly related to quantum yield and those determined by the maximum Rubisco activity. Ögren and Evans (1993) found that this transition, and therefore 8, is strongly dependent on CO_2 partial pressure. The quantitative meaning of the parameters and their significance for modelling photosynthetic light response as applied to maize has been discussed by Stirling *et al.* (1994).

A non-rectangular hyperbola can be employed to model the response of electron transport of photosynthesis to light in a similar way as that of net photosynthesis to light. To that end P and P_m must be substituted with J_T and J_{Tsat}, where J_T is the total light-driven electron flow of photosynthesis and J_{Tsat} represents the light saturated value. Total electron transport through photosystem TT (PSTT) can be readily assessed by *in vivo* chlorophyll fluorescence measurements of intact leaves (Genty *et al.* 1989). Data reported by Tacono and Sommer (1996, 1999), Valentini *et al.* (1995) respectively for *V. vinifera* and *Quercus cerris*, showed that J_T is a reliable estimator of the photosynthetic capacity of leaves. They also demonstrated that changes in photorespiration, which constitutes an alternative sink for light-induced electron flow, did not modify the relationship between total electron transport rate (J_T) and net photosynthesis.

The present study examined the electron flow of photosynthesis in response to light for leaves of different age following the imposition of water stress. The aim was to quantify effects due to leaf age and water stress on electron transport of photosynthesis during the late vegetative growth period of *V. vinifera* when leaf carbon gain is largely used for sugar accumulation into the berries. At this relatively late stage water stress in the field can easily occur and leaf age may be an important determinant in the efficient use of water and accumulation of sugar into the berry. The parameters that define the shape of the non-rectangular hyperbola in relation to water deficits and leaf age were quantified under different light regimes and their role in relation to leaf photosynthesis is discussed.

Material and Methods

E x p e r i m e n t a l l a y o u t : One-year-old rootlings of cv. Cabernet Sauvignon were planted in 9 l high-density polyethylene pots using a standard potting mix (40 % sand, 20 % silt, 20 % peat moss, 20 % perlite v/v). After planting, pots were maintained in the glasshouse under natural light conditions and a day/night temperature regime of approximately 30/15 °C. Plants were always well-watered at 26 % soil moisture content (SMC) and were fertilised to ensure good growth. The mean mid-day irradiance in the glasshouse was around 650 W m^{-2} and the mean daily photon flux during the growth period was approximately 33 mol m^{-2} d^{-1}. Plants were pruned to 1 shoot per vine and lateral leaves were removed immediately after they appeared. When primary shoot growth ceased, as indicated by a loss in the curvilinear shape of the vegetative apex, leaves were 23-24 nodes long. Leaf plastochron index (LPT) was estimated according to the method described by Schultz (1993) to ensure that only leaves of defined developmental stages were measured. On three plants, 3 leaves per plant with an LPT of 17, 7 and 2 respectively were selected. The selected leaves were positioned on approximately the 4th, 14th, and 20th node from the base of the shoot and their chronological age was respectively, 85, 70 and 30 d from the time of unfolding. The range in age should be representative of that found in the field shortly before veraison.

After the first measurement, soils were gradually dehydrated to moisture levels of 100, 60, 40 and 25 % of field capacity, corresponding to 26, 17, 10 and 7 % respectively of volumetric SMC. A period of about 10 d was required to dehydrate the potted vines from near field capacity (26 % SMC) to near wilting (7 % SMC). At each soil moisture level, light response curves (LRC) of chlorophyll fluorescence were determined for selected leaves, using a PAM-2000 fluorometer (Walz, Effeltrich, Germany). Plants had been maintained in the shade before a series of measurements was induced. Soil moisture was monitored using a TDR instrument (TRASE, Soilmoisture Equipment Corp. Santa Barbara, CA, USA). The instrument is equipped with a 20 cm long 'buriable' waveguide that was inserted into the soil in a vertical direction. Plants similar to those whose light response was regularly measured were maintained in the glasshouse under the same environmental conditions. They were used to determine the relative water (RWC) content of leaves of a similar age (LPT) as those used for monitoring light response. Leaf discs were punched from selected leaves at dawn and their RWC was determined according to the method of Barrs and Weatherley (1962).

G e n e r a t i o n o f l i g h t - r e s p o n s e c u r v e s (LRC) : Plants were taken from the glasshouse and were maintained under dark conditions for at least 2 h before a measuring cycle was initiated. At the beginning of each measurement, maximal (F_m), minimal (F_0) and variable

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(1) $P = (cD * PFD + P_m - ((cD * PFD + P_m)^2 - 4 * 8 * cD * P_m * PFD)^{0.S})/2 * 8$

where P is the photosynthetic rate, cD is the maximum quantum yield, PFD is the incident irradiance, P_m is the light-saturated photosynthetic rate and 8 is a parameter which characterises the convexity of the curve.

fluorescence ($F_v = F_m - F_0$) were determined on dark-adapted leaves using a PAM-2000 fluorometer. Leaves were then exposed to a pre-programmed sequence of photon flux densities (PFD) ranging from 10 to 1600 !Jmol m^{-2} s^{-1} (10, 15, 20, 30, 50, 70, 100, 150, 250, 350, 500, 1200 and 1600). Leaves were exposed for a period of five minutes at each flux density, long enough to reach steady state of F_t. Prior to the end of each measurement a single saturating pulse (0.8s) was applied to determine F_m' and F_t. The saturation light pulse was followed by a far red light pulse in order to determine F_0', which was used to calculate the quenching coefficient for chlorophyll fluorescence according to BILGER and BJÖRKMAN (1990). The reduction status of the PSTT electron acceptor ($1-q_p$) was calculated as $1- (F_m'-F)/F_m'$ and non-photochemical quenching (NPQ) as $(F_m/F_m')-1$. These coefficients were chosen because they are less sensitive to variations in F_0. The value of F_0', recorded after turning off the actinic light, was used to calculate F_v' from F_m' of which the latter was obtained by applying a saturating pulse in the preceding light period. Analysis of PSTT quantum yield also provided an estimate of the total electron transport rate (J_T) according to GENTY et al. (1989) and VALENTINI et al. (1995). J_T in turn was used to quantify photosynthetic light response of vine leaves under the imposed experimental treatments.

Estimation of photosynthetic electron transport rate (J_T) : Photosynthetic electron transport rate (J_T) is normally estimated as the product of

(2) $J_T = \Phi F/F_m' * 0.5 * PFD * a$

where $\Phi F/F_m'$ is PSTT quantum efficiency measured through the fluorescence assay, 0.5 denotes that the transport of one electron requires absorption of two quanta as two photosystems are involved, a is the leaf absorption coefficient (normally 0.87) and PFD the photon flux density. The relationship between fluorescence yield ($\Phi F/F_m'$) and quantum yield of carbon fixation (FCO$_2$) was determined for well-watered vines comprising leaves representative of the spread in age. Under photorespiratory conditions the latter was estimated as:

(3) $cDCO_2 = (P + PH + Rd) / PFD$

where P, PH and Rd are net CO_2 assimilation rate, photorespiration and day respiration rates, respectively. Photorespiration and day respiration rates were estimated as reported in TACONO and SOMMER (1996). The aim was to test if leaf age had an influence on the relationship between the two variables. Net assimilation rate using gas exchange (LT-COR 6200 system, Lincoln, NE, U.S.A.) was measured on the same leaves that were used to determine light response curves of J_T. Light response curves were generated by exposing plants for 30-60 min to each level of a sequence of PFDs, approximately 0, 25, 50, 100, 200, 400, 800, and 1600 !Jmol m^{-2} s^{-1}. Placing plants under shade cloth of increasing density generated the range in light levels. The period of 30-60 min was chosen in order to achieve gas exchange steady state but to avoid photo-inhibition that occurs when grapevine leaves are exposed to high irradiance for a prolonged period of time (TACONO and SOMMER 1996).

An estimate of the parameters of the relationship between J_T and Fe$^-$, as described by VALENTINI et al. (1995) for *Quercus cerri* L., was derived as follows:

(4) $J_T = PFD * cDe^-$;

where

(5) $cDe^- = 4(\Phi F/F_m' - b) / k$;

4 denotes the number of electrons needed per CO$_2$ fixed, k and b respectively are the slope and y-intercept of the regression line that fits the relationship of $\Phi F/F_m'$ and cDCO$_2$. A combination of equations (4) and (5) yields the following expression:

(6) $J_T = PFD * 4(\Phi F/F_m' - b) / k$

Tts combination with equation (2) provides the following solution for k, assuming an ideal relationship between $\Phi F/F_m'$ and cDCO$_2$, where b equals 0 and a equals 0.87:

(7) $k=4/(0.5*a)$; k= 9.2.

D a t a   a n a l y s i s : A non-linear procedure based on a Marquardt algorithm (WILKINSON 1990) was used to find the least-squares solution and thus obtain the values of the parameters cD, θ and J_{Tsat} of the light response curves. The curves were fitted for each set of measurements and the parameters averaged in order to obtain the mean values for different leaf age and water stress treatments. Estimates of the parameters of linear and non-linear models fitted to the LRC and ANOVA procedures were performed in SYSTAT (WILKINSON 1990).

Results

P l a n t   w a t e r   s t a t u s ,   l e a f   a g e   a n d   l i g h t   r e s p o n s e   c u r v e s : Basal leaves, positioned on node 4, had a mean LPT of 16.6 and were around 85 d from unfolding. Medium leaves, positioned on node 9, were around 70 d from unfolding with an LPT of 6.9. Apical leaves, positioned on node 20 had an LPT of 2.3 and were 30 d from unfolding. The pre-dawn relative water content (RWC) in respect to leaf age was similar within the imposed watering regimes. Therefore, age did not appear to influence the hydration status of leaves, neither for well-watered nor for water-stressed leaves (Tab. 1). There was a strong positive relationship between RWC and volumetric soil moisture (RWC = 91.747+0.173 SMC; $r^2 = 0.87$ *** - data not shown). During the imposition of water stress, soil moisture declined steadily from 26.6, 16.8, 10.5 to 6.7%, respectively corresponding to 100, 65, 40 and 25% of field capacity and to 96.3, 94.7, 93.6 and 92.8 % pre-dawn RWC.

The relationship between quantum yield of PSTT ($\Phi F/F_m'$) and quantum yield of carbon fixation (FCO$_2$) was unaffected by leaf age (LPT) when plants were well-watered (Fig. 1). A single line fitted the trend and the resulting relationship was used for the computation of photosynthetic electron transport rate (J_T) of all leaves irrespective of age:

(8) $J_T = PFD * Fe^-$;
      where Fe$^-$ = 4($\Phi F/F_m'$ - 0.0089) / 14.644, since
      DF/F$_m'$ = 0.0089 + 14.644 * FCO$_2$ ($r^2 = 0.98$***)

The non-rectangular hyperbola provided a good fit to the relationship of total electron transport rate (J^T) *vs* PFD irrespective of watering regimes and leaf age, with coefficients of determination (r^2) ranging from 0.881 to 0.925. Tn concert with a decline of soil moisture from 26 to 7 %, J_T declined irrespective of LPT, but more so in older, basal leaves

Table 1

Reduction status of Q acceptor (1-q_p) and non-photochemical quenching (NPQ) as influenced by soil moisture content (SMC), predawn relative water content (RWC), leaf age (LPI) and photon flux density (PFD). Values for Q and NPQ are means of 3 plants. Values for ± standard deviation of the mean are given in brackets, where n = 33, 3 and 3 for PFD < 500, 500-1500 and > 1550, respectively

| | | | | PFD (μmol Q m^{-2} s^{-1}) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | <500 | 500-1500 | >1500 | <500 | 500-1500 | >1500 |
| SMC | RWC | LPI | | 1-q_p | | | NPQ | |
| | 96.2 (0.1) | 2.3 (0.2) | 0.1 (0.1) | 0.4 (0.1) | 0.6 (0.02) | 0.5 (0.7) | 1.6 (0.9) | 2.1 (1.0) |
| | 96.1 (0.1) | 6.9 (0.2) | 0.1 (0.1) | 0.5 (0.1) | 0.7 (0.02) | 0.2 (0.2) | 1.3 (0.3) | 1.8 (0.3) |
| | 96.6 (0.2) | 16.6 (1.3) | 0.1 (0.1) | 0.5 (0.2) | 0.7 (0.04) | 0.4 (0.3) | 1.7 (0.4) | 2.1 (0.5) |
| Mean 26.6 (0.8) | 96.3 (0.2) | 8.6 (6.0) | 0.1 (0.1) | 0.5 (0.1) | 0.7 (0.04) | 0.4 (0.3) | 1.6 (0.6) | 2.0 (0.6) |
| | 92.9 (0.5) | 2.3 (0.2) | 0.1 (0.1) | 0.5 (0.1) | 0.7 (0.02) | 0.3 (0.2) | 1.4 (0.5) | 2.0 (0.6) |
| | 93.2 (0.9) | 6.9 (0.6) | 0.1 (0.1) | 0.5 (0.1) | 0.7 (0.04) | 0.4 (0.3) | 1.7 (0.3) | 2.0 (0.6) |
| | 92.4 (0.7) | 16.6 (1.3) | 0.1 (0.1) | 0.6 (0.2) | 0.7 (0.05) | 0.5 (0.5) | 2.1 (0.2) | 2.4 (0.3) |
| Mean 6.7 (0.3) | 92.8 (0.7) | 8.6 (6.0) | 0.1 (0.1) | 0.5 (0.1) | 0.7 (0.05) | 0.4 (0.4) | 1.7 (0.4) | 2.1 (0.4) |
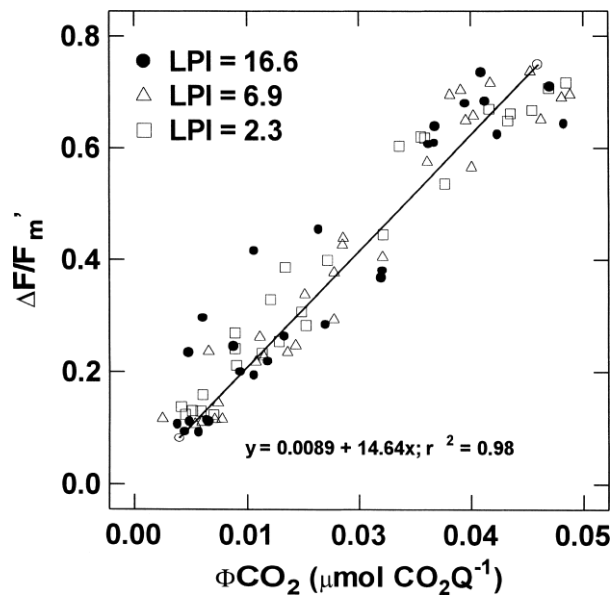
Fig. 1: Relationship between Quantum Yield of Photosystem II ($\Delta F/F_m'$) and quantum yield of carbon fixation (ϕCO_2) measured under photorespiratory conditions for well-watered plants.

Fig. 2: Light response curves (LRC) of photosynthetic electron transport rate (J_T) determined for leaves of different age (LPI= leaf plastochron index) and soil moisture levels (SMC = soil moisture content). Vertical bars represent standard errors of the population mean (n= 468).

(LPT 16.6) relative to medium (LPT 6.6) and apical (LPT 2.3) leaves (Fig. 2 A-C). The highest values of J_T were recorded for apical leaves regardless of watering regime. J_T of medium leaves was very similar to that of apical leaves at 26 and 17 % SMC, but was lower for leaves at 10 and 7 % SMC, respectively. Regardless of water supply the lowest values of J_T were always recorded for basal leaves relative to the younger, medium and apical leaves. Obvious differences in J_T due to watering did not lead to similar differences in chlorophyll fluorescence quenching as results in Tab. 1 illustrate. Differences in the reduction status of the PSTT electron acceptor (1-q_p) and in non-photochemical quenching (NPQ) due to water supply and leaf age were never statistically significant. However, both quenching variables always increased strongly in concert with PFD.
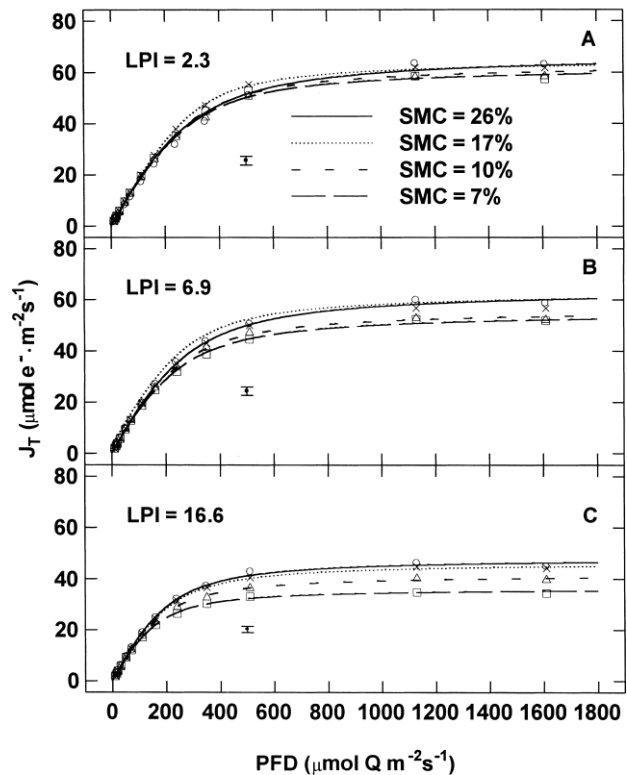
Tnfluence of leaf age and soil moisture content on cD, δ and J_{Tsat}: There was a positive linear relationship between the maximum quantum yield of LRC (cD) and SMC, even though the changes in cD were limited to a relatively small range, extending from values of 0.196 to 0.181 (Fig. 3 A). The slope and intercept of the linear regression for cD were similar for leaves of different
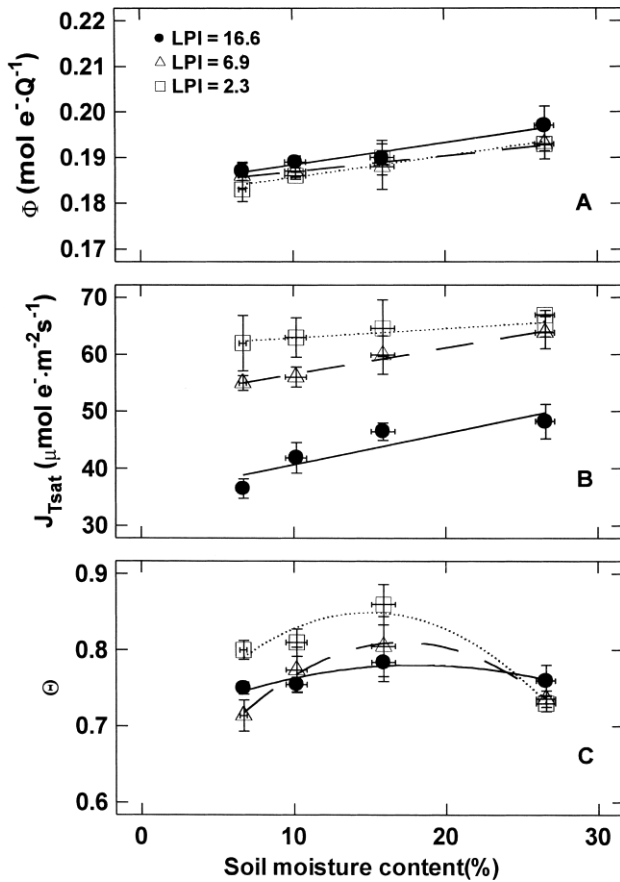
Fig. 3: Relationship between the parameters of the photosynthetic light response curves (Φ = quantum yield, J_{Tsat} = maximum electron transport rate, Θ = rate of bending) and soil moisture content, estimated for leaves of different age (LPI = leaf plastochron index). Vertical and horizontal bars represent standard error of the mean (n = 12).

age (Tab. 2) suggesting a similar behaviour regardless of leaf age. Maximum electron transport rate (J_{Tsat}) was linearly and positively related to soil moisture content but the slopes of the regression lines differed with regard to leaf age (Tab. 2;

Fig. 3 B). Maximum electron transport rate was always highest for apical leaves (LPT 2.3), regardless of soil moisture content, followed by leaves of a medium LPT (6.9) and by basal leaves (LPT 16.6) whose rate was substantially lower. Tn addition, J_{Tsat} of basal and of medium leaves declined more steeply with a decline in soil moisture relative to apical leaves. The convexity parameter (Θ), irrespective of leaf age (LPT), followed an exponential relationship reaching a maximum at around 16 % soil moisture (Fig. 3 C). Convexity of basal leaves changed very little across the observed range of soil moisture contents. Convexity of well-hydrated leaves was higher for basal than for either medium or apical leaves. Under less well-hydrated conditions, Θ of basal leaves was higher relative to medium but lower relative to apical leaves. For medium and apical leaves, Θ was optimal at soil moisture levels around 16 % but declined towards either side and, regardless of soil moisture content, Θ of apical leaves was always higher than that of medium leaves.

Discussion and Conclusion

Plants have developed protective mechanisms that enable dissipation of excess radiation from the photosynthetic system. Excess energy can be dissipated either within the photosystem by photochemical quenching, *e.g.* through photorespiration, or by non-photochemical quenching (HAVAUX 1992). This process is often associated with a reduction in the efficiency of maximum quantum yield of PSTT and therefore is termed photoinhibition. Grapevine leaves in the field may be affected by photoinhibition and young, immature leaves, less than 10-15 d from emergence, are often found to be more susceptible than mature leaves that are older than 15 d (TACONO and SOMMER 1996). The process is often exacerbated by environmental stresses such as high temperature and drought (POWLES 1984, DÜRING 1998).

Tt might be argued that the interpretation of the light response curves in the present study was compromised by

Table 2

Estimated parameters (a, b, c) and coefficients of determination (r^2) of equations describing the relationship between the parameters of the light-response curve (Φ, J_{Tsat} and Θ, as Y in the equation) and percent soil moisture (as X in the equation). Parameters were estimated for leaves of different age as indicated by the leaf plastochron index (LPI; n = 12; **, *** indicates significance at P < 0.01 and 0.001, respectively)

Parameter Y	Equation	Leaf age (LPI)	a	b	c	r^2	P>F
Φ	Y = a + bX	2.3	0.180	0.00049		0.652	***
		6.9	0.180	0.00034		0.420	**
		16.6	0.183	0.00050		0.656	***
J_{Tsat}	Y = a + bX	2.3	60.96	0.215		0.519	***
		6.9	52.13	0.444		0.592	***
		16.6	35.24	0.541		0.620	***
Θ	Y = a + bX.e^{cX}	2.3	0.488	0.076	-0.078	0.814	***
		6.9	0.409	0.070	-0.065	0.968	***
		16.6	0.670	0.016	-0.058	0.704	***

photoinhibition. Our results suggest that this was not the case. The experimental protocol consisted of a pre-programmed sequence of light exposures to determine photosynthetic light response (LRC) of single leaves. The PSTT electron acceptor never attained a reduction status $(1-q_p)$ that is believed to predispose PSTT to photoinhibitory damage due to water deficits or leaf age (Tab. 1). Similarly, the behaviour of non-photochemical quenching (NPQ) also suggested an absence of photoprotective processes due to water deficits or leaf age (Tab. 1). Therefore, the observed differences in the behaviour of the parameters of the LRC relationships, *i.e.* a shift in quantum yield (cD), saturated levels of photosynthetic electron transport (J_{Tsat}) or rate of bending of photosynthetic light response curves (8), were directly attributable to the imposed water regimes or leaf age.

Several authors have shown the existence of a linear relationship between $\Diamond F/F_m'$ and the quantum yield of carbon fixation (cDCQ$_2$) established under non-photorespiratory conditions (Cornic *et al.* 1989, Genty *et al.* 1989, Cornic and Briantais 1991). Tn the present experiment parallel measurements of gas exchange and fluorescence emission were made under photorespiratory conditions and pre-defined light regimes of increasing PFD, using well-watered plants. Results showed a linear fit with a slope of around 14.6 which was different to the 'ideal relationship', with a slope of around 9.2 (see Materials and Methods). Working under non-photorespiratory conditions Genty *et al.* (1989) for *Zea mays* and Öquist and Chow (1992) for *Hordeum vulgare* determined values of around 9. The discrepancy in the slopes is probably attributable to additional sinks for light driven electrons or to uncertainties regarding the estimation of day respiration. However, the relationship between $\Diamond F/F_m'$ and cDCO$_2$ was found to be independent of leaf age suggesting that leaf absorptance did not change as a result of aging. This is in contrast to results obtained by Schultz (1996) who showed that in *Vitis vinifera* absorptance is low (60-70 %) for young apical and old basal leaves on the shoot and high for fully matured but not yet senescent leaves (85-95 %). Presumably, in the present experiment, basal leaves did not receive any reciprocal shade and the younger, apical leaves had already attained full maturity at the time the experimental treatments were imposed.

Leaf age affected the parameters that define the photosynthetic light-response curve when plants were undergoing adjustment to water deficits (Fig. 3). Results from previous studies using other plant species suggest that light saturated photosynthesis follows a tri-phasic response with respect to leaf age (Dwyer and Stewart 1986, Stirling *et al.* 1994). Tts course is characterised by a rapid increase to a maximum rate, then reaches a plateau that is followed by a non-linear decrease (Marshall and Bisoce 1980, Miranda *et al.* 1981). Unlike the typical tri-phasic response, our data would suggest maximum electron transport (J$_{Tsat}$) in relatively young, apical leaves was similar to that of leaves in a medium position on the shoot (Fig. 3 B). Tt is likely that the apical leaves in the present experiment (30 d from unfolding), in spite of their low LPT, were physiologically mature. According to Schultz (1993) apical leaves with an LPT of 2 should have displayed very low rates of J$_{Tsa}$ but instead behaved like fully expanded adult leaves. The results confirm that LPT alone is not always a suitable indicator of the physiological leaf age but should always be considered in relation to the chronological age particularly after leaf appearance has ceased.

Unlike J$_{Tsat}$ quantum yield (cD) varied little during leaf development and this behaviour is consistent with observations made by Sterling *et al.* (1994). Also, cD remained relatively constant with a fall in the level of soil hydration, whereas J$_{Tsat}$ declined strongly and even more so in basal leaves. This indicates that biochemical/stomatal limitations played a more important role than biophysical processes of energy transfer and electron transport in determining the water-stress related decline of photosynthesis.

The changes in the LRC convexity parameter (8) were less uniform (Fig 3 C). Under well-watered conditions, 8 was highest for basal leaves and only small changes were obvious during adjustment to water stress. Large gradients of light within the leaf, associated with a high chlorophyll content, could result in a gradual transition between the initial slope and upper asymptote (Stirling *et al.* 1994) but this only explains the higher values of 8 in older leaves. Tn contrast, the sudden rise of 8 in young leaves, following a slight decline in soil moisture, is more likely due to a superior elasticity of their palisade and mesophyll tissues and an associated rapid change in mesophyll conductance relative to older leaves (Loreto *et al.* 1994). Furthermore, young leaves are thinner than adult leaves and Lauteri *et al.* (1997) have shown that leaf thickening decreases CO$_2$ diffusion in the mesophyll. Tn our case, differences in leaf thickness between basal and medium compared to apical leaves may have led to a lowering of the CO$_2$ concentration and an associated decline of Rubisco activity (Renou *et al.* 1990) under conditions of water stress. This would explain the differential response of 8 in relation to leaf age when plants experience water stress. Tt also may explain the initial sharp rise of 8 followed by a steady decay due to a further decline in soil moisture. At high soil moisture, 8 of medium leaves was similar to that of apical leaves but as soil moisture declined their capacity to adjust 8 was much lower than in young apical leaves. This was probably because of a less elastic photosynthetic leaf tissue in medium and basal as compared to apical leaves. This also confirms that in our experience chronologically young leaves of about 20 d from appearance were physiologically mature. They behaved unlike newly emerged leaves, whose photosynthetic capacity is often limited by intrinsic regulatory and metabolic factors that are reflected in low values of 8 (Baker and Ort 1992).

There are examples in the literature that illustrate limitations to the physiological significance of possible changes in 8, cD and J$_{Tsat}$. For example, it has been pointed out that these parameters are not completely independent and 8 may interact with cD during the fitting procedure. Tn some cases this may lead to unrealistic results (Leverenz 1988) such that, when J$_{Tsat}$ declines and cD would remain constant, 8 will likely increase. One should be aware of such limitations and take them into account when trying to interpret physiological behaviour.

Additionally, since cD is related to incident radiation, rather than absorbed radiation, it may be quite different from the actual quantum yield if substantial absorption changes

occur. We did not attempt to verify potential changes in leaf absorption due to water stress. However, we did not observe any leaf wilting or yellowing due to water stress treatments. Tn addition, measurements of leaf relative water content indicated that stress was not severe and therefore was unlikely to strongly influence leaf absorption.

Our results demonstrate that vines are able to adapt their photosynthetic processes to a reduction in water availability. Tn young, mature leaves this was expressed in a higher photosynthetic capacity and a higher tolerance to water stress. Young, fully expanded leaves accounted for about 10 % of the total shoot leaf area at veraison and represented an important source of carbohydrate assimilation during berry ripening. Under conditions of water stress and high levels of PFD they probably accounted for around 15 % of the shoot photosynthetic electron transport rate. From a practical point of view it is therefore desirable to make management decisions that ensure the continued functioning of young leaves late in the season. For example, care should be taken during summer hedging in order to avoid or minimise damage to young leaves. Alternatively, training and/or management systems should be adopted that avoid excessive vegetative growth and therefore require minimal summer hedging.

References

AGELOPOULOS, K.; DICHIO, B.; XILOYANNIS, C.; 1996: Inhibition of photosynthesis in olive trees (*Olea europaea* L.) during water stress and rewatering. J. Exp. Bot. **47**, 1093-1100.

BAKER, N. R.; ORT, D. R.; 1992: Light and crop photosynthetic performance. In: N. R. BAKER, H. THOMAS (Eds.): Crop Photosynthesis: Molecular Mechanisms to the Field, 349-363. Bios Press 0xford.

BARRS, H. D.; WEATHERLEY, P. E.; 1962: A re-examination of the relative turgidity technique for estimating water deficits of leaves. Aust. J. Biol. Sci. **15**, 415-428.

BILGER, W.; BJÖRKMAN , 0.; 1990: Role of xanthophyll cycle in photoprotection elucidated by measurements of light induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth. Res. **25**, 173-185.

BJÖRKMAN, 0.; POWLES, S. B.; 1984: Inhibition of photosynthetic reactions under water stress: interaction with light level. Planta **161**, 490-504.

CORNIC, G.; BRIANTAIS, J. M.; 1991: Partitioning of photosynthetic electron flow between CO_2 and O_2 reduction in a C_3 leaf (*Phaesolus vulgaris* L.) at different CO_2 concentrations and during drought stress. Planta **183**, 178-184.

- -; LE GOUALLEC, J. L.; BRIANTAIS, J. M.; HODGES, M.; 1989: Effect of dehydration and high light on photosynthesis of two C_3 plants (*Phaseolus vulgaris* L. and *Elatostema repens* (Lour.) Hall f.). Planta **177**, 84-90.

DÜRING, H.; 1986: Testing for drought tolerance in grapevine scions. Angew. Botan. **60**, 103-111.

- -; 1998: Photochemical and non-photochemical responses of glasshouse-grown grape to combined light and water stress. Vitis **37**, 1-4.

DWYER, L. M.; STEWART, D. W.; 1986: Effect of leaf age and position on net photosynthesis rates of maize. Agric. Meteorol. **36**, 29-46.

EVANS, J. R.; 1987: The dependence of quantum yield on wavelength and growth irradiance. Aust. J. Plant Physiol. **14**, 69-79.

GENTY, B.; BRIANTAIS, J. M.; BAKER, N. R.; 1989: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta **990**, 87-92.

HAVAUX, M.; 1992: Stress tolerance of photosystem II *in vivo*. Antagonistic effects of water, heat, and photoinhibition stresses. Plant Physiol. **100**, 424-432.

- -; LANNOYE, R.; 1985: Drought resistance of hard wheat cultivars as measured by a rapid chlorophyll fluorescence test. J. Agricult. Sci. **104**, 501-504.

HE, J. X.; WANG, J.; LIANG, H.; 1995: Effects of water stress on photochemical function and protein metabolism of photosystem II in wheat leaves. Physiol. Plant. **93**, 771-777.

IACONO, F.; BUCCELLA, A.; PETERLUNGER, E.; 1998: Water stress and rootstock influence on leaf gas exchange of grafted and ungrafted grapevines. Sci. Hortic. **75**, 27-39.

- -; SOMMER, K. J.; 1996: Photoinhibition of photosynthesis and photorespiration in *Vitis vinifera* under field conditions: Effects of light climate and leaf position. Aust. J. Grape Wine Res. **2**, 10-20.

--; --; 1999: The measurement of chlorophyll fluorescence as a tool to evaluate the photosynthetic performance of grapevine leaves. In: E. H. RÜHL, J. SCHMID (Eds.): Proceedings of the First ISHS Workshop on Water Relations of Grapevines. Acta Horticulturae **493**, 31-44. ISHS Leuven, Belgium.

INTRIERI, C.; PONI, S.; SILVESTRONI, 0.; FILIPETI, I.; 1992: Leaf age, leaf position and photosynthesis in potted grapevines. Adv. Hort. Sci. **6**, 23-27.

JEFFRIES, R. A.; 1994: Drought and chlorophyll fluorescence in field-grown potato (*Solanum tuberosum*). Physiol. Plant. **90**, 93-97.

KICHEVA, M. I.; TSONEV, T. D.; POPOVA, L. P.; 1994: Stomatal and nonstomatal limitations to photosynthesis in two wheat cultivars subjected to water stress. Photosynthetica **30**, 107-116.

KRIEDEMANN, P. E.; 1968: Photosynthesis in vine leaves as a function of light intensity temperature and leaf age. Vitis **7**, 213-220.

- -; KLIEWER, W. M.; HARRIS, J. M.; 1970: Leaf age and photosynthesis in *Vitis vinifera*. Vitis **9**, 97-104.

LAUTERI, M.; SCARTAZZA, A.; GUIDO, M. C.; BRUGNOLI, E.; 1997: Genetic variation in photosynthetic capacity, carbon isotope discrimination and mesophyll conductance in provenances of *Castanea sativa* adapted to different environments. Functional Ecol. **11**, 675-683.

LEVERENZ, J. W.; 1988: The effect of illumination sequence, CO_2 concentration, temperature and acclimation on the convexity of the photosynthetic light response curve. Physiol. Plant. **74**, 332-341.

LORETO, F.; DI MARCO, G; TRICOLI, D.; SHARKEY, T. D.; 1994: Measurements of mesophyll conductance, photosynthetic electron transport and alternative electron sinks of field grown wheat leaves. Photosynth. Res. **41**, 397-403.

MARSHALL, B.; BISOCE, P. V.; 1980: A model for C_3 leaves describing the dependence of net photosynthesis on irradiance. II. Application to the analysis of flag leaf photosynthesis. J. Exp. Botan. **31**, 41-48.

MAURY, P.; MOJAYAD, F.; BERGER, J. A.; PLANCHON, C.; 1996: Photochemical response to drought acclimation in two sunflower genotypes. Physiol. Plant. **98**, 57-66.

MEYER, S.; DE KOUCHKOVSKY, Y.; 1995: Electron transport, photosystem-2 reaction centers and chlorophyll protein complexes of thylakoids of drought resistant and sensitive lupin plants. Photosynth. Res. **37**, 49-60.

MIRANDA, V.; BAKER, N. R.; LONG, S. P.; 1981: Limitations of photosynthesis in different regions of the *Zea mays* leaf. New Phytol. **89**, 179-190.

ÖGREN, E.; EVANS, J. R.; 1993: Photosynthetic light response curves. I. The influence of CO_2 partial pressure and leaf inversion. Planta **189**, 182-190.

ÖQUIST, G.; CHOW, W. S.; 1992: 0n the relationship between quantum yield of photosystem II electron, as determined by chlorophyll fluorescence and the quantum yield of CO_2-dependent O_2 evolution. Photosynth. Res. **33**, 51-62.

POWLES, S. B.; 1984: Photoinhibition of photosynthesis induced by visible light. Annu. Rev. Plant Physiol. **35**, 15-44.

RENOU, J. L.; GERBAUD, A.; JUST, D.; ANDRE, M.; 1990: Differing substomatal and chloroplastic CO_2 concentrations in water-stressed wheat. Planta **182**, 415-419. 0

SCHULTZ, H. R.; 1993: Photosynthesis of sun and shade leaves of field-grown grapevine (*Vitis vinifera* L.) in relation to leaf age. Suitability of the plastochron concept for the expression of physiological age. Vitis **32**, 197-205.

- -; 1995: Grape canopy structure, light microclimate and photosynthesis. I. A two-dimensional model of the spatial distribution of

surface area densities and leaf ages in two canopy systems. Vitis **34**, 211-215.

- - ; 1996 a: Water relations and photosynthetic responses of two grapevine cultivars of different geographical origin during water stress. In: S. Poni, E. Peterlunger, F. Iacono, C. Intrieri (Eds.): Proc. Workshop: Strategies to 0ptimize Wine Grape Quality. ISHS, Acta Horticulturae **427**, 251-266.

- -; 1996 b: Leaf absorptance of visible radiation in *Vitis vinifera* L.: Estimates of age and shade effects with a simple field method. Sci. Hortic. **56**, 93-102.

Smart, R. E.; Coombe, B. G; 1983: Water relations of grapevines. In: T. T. Kozlowski (Ed.): Water Deficits and Plant Growth, 137-196. Academic Press New York.

Stirling, C. M.; Aguilera, C.; Baker, N. R.; Long, S. P.; 1994: Changes in the photosynthetic light response curve during leaf development of field grown maize with implications for modelling canopy photosynthesis. Photosynth. Res. **42**, 217-225.

Thornley, J. H. M.; 1976: Mathematical Models in Plant Physiology. Academic Press, London.

Valentini, R.; Epron, D.; De Angelis, P.; Matteucci, G; Creyer, E.; 1995: *In situ* estimation of net CO_2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. cerris* L.) leaves: Diurnal cycles under different levels of water supply. Plant Cell Environ. **18**, 631-640.

Wilkinson, L.; 1990: SYSTAT: The System for Statistics SYSTAT Inc.: Evanston, Illinois.

Zamboni, M.; Fregoni, M.; Iacono, F.; 1986: Comportamento di specie ed ibridi di vite in condizioni di siccita. Atti del IV Simposio Internazionale di Genetica della Vite.Vignevini (Supplemento 12), 119-122.