Vitis **40** (3), 117–121 (2001)

Physiological responses of grapevine leaves to Bordeaux mixture under light stress conditions

J. M. Moutinho-Pereira[1)], N. Magalhães[1)], L. F. Torres de Castro[1)], M. Manuela Chaves[2)], and J. M. Torres-Pereira[1)]

[1)] ICETA/Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal
[2)] Instituto Superior de Agronomia, Lisboa, Portugal

Summary

The effect of Bordeaux mixture on the physiology of leaves of *Vitis vinifera* L., cv. Touriga Nacional, growing under field conditions in the Douro Wine Region, was evaluated. Especially in late summer, this fungicide modifies the light microclimate and leaf physiological characteristics, namely stomatal aperture and photosynthesis. Leaves treated with Bordeaux mixture showed higher reflectance, whereas transmitted photon flux density and temperature were lower compared to control leaves. Photosynthetic rates of treated leaves increased due to a lowering of both, stomatal and non-stomatal limitation. In addition, transpiration rates were higher, but neither the intrinsic efficiency of water use nor leaf water potentials were affected. Delay of leaf senescence of grapevines sprayed with Bordeaux mixture inhibited scorching of clusters and, consequently, led to higher yields per plant.

K e y w o r d s : Bordeaux mixture, photosynthesis, transpiration, stomatal conductance, light stress, semi-arid conditions.

Introduction

Leaves severely infected with powdery mildew, caused by *Plasmopara viticola*, indicate reduced rates of photosynthesis resulting in decreased plant vigour and severe crop loss. To control this disease various fungicides have been developed, the Bordeaux mixture, containing copper sulphate and slaked lime, being pioneer. This contact fungicide, discovered by chance in October 1882 by Millardet (1885) in the Médoc region (France), is still applied by many grape growers, especially after veraison.

Grape growers of Douro empirically discovered that an application of Bordeaux mixture not only controls downy mildew and other diseases, but also minimises extreme defoliation in the basal zone of the shoots in summer, thus preventing excessive light stress of clusters.

The aim of this work was to evaluate the influence of Bordeaux mixture particles (classic formulation) on photosynthesis and transpiration of vines cultivated under environmental stress conditions, *i.e.* strong light associated with high temperature and drought.

Material and Methods

P l a n t m a t e r i a l a n d t r e a t m e n t s : Grapevines of a common Portuguese cultivar, Touriga Nacional (*Vitis vinifera* L.), grafted on 1103P, were used. The experiments were performed in a commercial vineyard (Quinta do Seixo, 41°10' N latitude, 7°33' W longitude, 100 m above mean sea level), located in the Douro Demarcated Wine Region of Northern Portugal, in the Upper Corgo sub-region, in 1997. The vineyard is located on a steep hill, following the main slope and vines are trained as bilateral cordons. They were 12 years old at the start of the experiment. The soil is a typical schist. Summers are characterised by drought, high temperatures and clear sky. The vines were kept unirrigated. Two groups of 40 plants each were studied: one group received no treatment (control, C) while in the other group the grapevines were sprayed on 11 July, 1997, soon after veraison, with 2 % Bordeaux mixture (BM). To prepare this fungicide 2 kg of copper sulphate ($CuSO_4$) dissolved in 50 l of water were combined with 2 kg of hydrated (slaked) lime [$Ca(OH)_2$] mixed with water; this was then poured together through a strainer and used as soon as possible.

E n v i r o n m e n t a l c o n d i t i o n s a n d w a t e r r e l a t i o n s : Climatic data were obtained from a meteorological station (Delta-T Devices, UK) installed in the experimental vineyard. Leaf transmission and leaf reflection in the visible range of the spectrum (400 to 700 nm) were determined on a photon flux density (PPFD) basis in the field at clear sky near solar noon using a quantum sensor (Quantum Q102, Macan, Scotland). Transmitted radiation was measured normal to the plane of and immediately under the leaf, positioned with its surface perpendicular to the sun. At the same leaf, reflected radiation was measured 1 cm above the leaf by placing the sensor at an angle of 45° from the perpendicular, according to the protocol outlined by Schultz (1996). Leaf absorbance was determined by subtracting the transmitted and reflected radiation from that incident at the leaf surface. Leaf temperature was measured with an infrared thermometer (Infratrace KM800S, England) with a 15° field view. The average temperature of randomly selected leaves in each plot was obtained by holding the thermometer at about 1 m above the foliar surface. The emissivity of the canopy was assumed to be 0.97 (Heilman *et al*. 1994). Leaf water potential (Ψ) was determined with a pressure chamber

Correspondence to: Prof. Dr. J. Moutinho Pereira, ICETA/Universidade de Trás-os-Montes e Alto Douro, Apartado 202, 5001-911 Vila Real, Portugal. Fax: +351-259-350480. E--mail: moutinho@utad.pt

(ELE International, England), according to SCHOLANDER *et al.* (1965) at predawn (Ψ_{PD}) and at midday (Ψ_{MD}) (between 14.00 and 15.00 h) on sunlit leaves in the middle of shoots. Care was taken to minimise water loss during transfer of the leaf to the chamber, by enclosing it in a plastic bag immediately after excision. Leaf osmotic potential (Ψ_p) was determined by an osmometer (H. Roebling, Type 13/13DR, Berlin, Germany), using the leaves of the Ψ_{PD} measurements; after freezing the leaf blades with liquid N_2 cell sap was pressed on by a syringe. After centrifugation (12,000 x *g*, 3 min), 100 µl of the cell sap were used for measurements in the osmometer. Ψ_p values were not corrected for dilution of cell sap with apoplastic water (DÜRING 1984).

Gas exchange and chlorophyll fluorescence measurements: Net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and internal CO_2 concentration (C_i) were determined under field conditions on intact, sun-exposed and fully expanded leaves at the middle of shoots, using a portable IRGA (ADC-LCA-3, Analytical Development Co., Hoddesdon, England), operating in the open mode; calculation followed the equations of VON CAEMMERER and FARQUHAR (1981). The leaf chamber clip (ADC-PLC, surface: 6.25 cm^2, volume: 16 cm^3) incorporates a quantum sensor and temperature and humidity sensors. Intrinsic water use efficiency was calculated as the ratio of A/g_s. Values for mesophyll conductance to CO_2 (g_m) were calculated in accordance with CANDOLFI-VASCONCELOS and KOBLET (1991). Chlorophyll fluorescence parameters (photochemical efficiency of PSII of dark-adapted leaves, F_v/F_m, minimum, F_o, and maximum fluorescence, F_m, at open and closed reaction centres of PSII, respectively, and half rise time from F_o to F_m, $t_{1/2}$) were determined on attached intact leaves similar to those used for gas exchange measurements, using a portable chlorophyll fluorometer (Plant Stress Meter, BioMonitor S.C.I. AB, Sweden) as described by ÖQUIST and WASS (1988). Before measurements were started, leaves were adapted to dark for 30-45 min, using a clamp cuvette.

Pigment analyses: Leaf discs (3.14 cm^2) were punched out from sunlit leaves at the middle of the shoots, frozen in liquid N_2 and stored at –80 °C. Chlorophyll *a* and *b* were quantified spectrophotometrically from leaf extracts with 80 % acetone (SESTÁK *et al*. 1971). Carotenoids were extracted with chlorophyll and determined using the equations proposed by LICHTENTHALER (1987).

Statistical analyses: Values were compared by a one-way ANOVA test. All means were compared at the 0.05, 0.01 and 0.001 level of significance.

Table 1

Leaf reflectance, absorbance and transmittance of untreated (control, C) leaves and leaves treated with Bordeaux mixture (BM) measured on 24 July 1997. Values are expressed in % of incident PPFD and are the mean ± S.E. of measurements on 44 different leaves. Within one line, means marked by different letters are significantly different at p<0.001

	C	BM
Reflectance	9.6 ± 0.3 b	14.5 ± 0.4 a
Absorbance	84.6 ± 0.3 b	81.9 ± 0.4 a
Transmittance	5.8 ± 0.1 b	3.6 ± 0.1 a

Results and Discussion

Application of Bordeaux mixture to grapevines resulted in the formation of a whitish dry residue on the exposed leaves of the canopy, which significantly diminished light absorbance and transmittance, while the reflector capacity increased substantially (Tab. 1). The values of absorbed and transmitted light of control leaves (C) of Touriga Nacional are in the same order of magnitude as those reported by SCHULTZ (1996) and MABROUK *et al.* (1997) for fully expanded leaves of cvs Riesling and Merlot.

One of the direct effects of the application of the Bordeaux mixture was a reduction of leaf temperature by 1-3 °C, under conditions of strong incidence of the solar radiation (Tab. 2).

Table 2

Leaf temperature on three different days of untreated (control, C) leaves and leaves treated with Bordeaux mixture (BM). Values are the mean ± S.E.; ns, not significantly (p>0.05), *, significant (p<0.05), ***, highly significant (p<0.001)

Date	Time (h)	Air temperature (°C)	Leaf exposition	n	Leaf temperature (°C) C	BM
23.07.97	14.00-14.30	32.2	sun	55	39.0 ± 0.3	38.0 ± 0.3 *
04.08.97	12.00-12.30	30.5	sun	35	36.9 ± 0.4	33.5 ± 0.3 ***
	12.00-12.30	30.5	shade	10	28.3 ± 0.2	28.2 ± 0.3 ns
	14.00-14.30	34.0	sun	35	36.5 ± 0.4	34.5 ± 0.2 ***
05.08.97	11.00-11.30	29.2	sun	35	34.9 ± 0.3	31.7 ± 0.4 ***
	11.00-11.30	29.2	shade	10	28.8 ± 0.2	28.5 ± 0.2 ns
	14.00-14.30	34.1	sun	35	37.5 ± 0.4	34.9 ± 0.3 ***
	16.00-16.30	36.5	sun	35	38.6 ± 0.3	36.0 ± 0.3 ***

Measurements of gas exchange on a clear summer day indicate significantly higher g_s, A and E values for treated leaves as compared to control leaves (Fig. 1). A/g was not significantly different between the two treatments at high PPFD, VPD and air temperature (14.00-15.00 h). In the same period, internal CO_2 concentration (C_i) was also not significantly different between treatments while the mesophyll conductance (g_m) was higher in treated than in control leaves, indicating that both stomatal and non-stomatal limitations of photosynthesis decreased in treated leaves as compared to controls (Downton *et al.* 1987). Moreover, the fact that A/g of control leaves was similar to that of treated leaves strengthens the hypothesis that if limitation of photosynthesis is essentially due to stomatal closure, the magnitude of this parameter tends to be relatively more raised (David *et al.* 1998; Flexas *et al.* 1998). In general terms, our results do not support the conclusions reported by Sawada and Hayakawa (1984), showing a depression of net photosynthetic rate at almost saturating irradiance, although their assays were performed with potted apple trees.

The higher stomatal conductance in treated leaves may have been the result of a cooling effect of Bordeaux mixture. For the conditions of Portugal Chaves *et al*. (1987) reported an optimal temperature for stomatal conductance and net photosynthesis of about 30-35 °C for cv. Tinta Amarela, a native cultivar of the Douro region. Under severe summer stress conditions, Clímaco (1997) showed for cv. Piriquita, in the Portuguese Estremadura region, that leaf temperatures of 1-2 °C above air temperature of about 36 °C led to an important reduction in g_s and A (about 18 % and 20 % of maximum values, respectively). The lower temperature of treated leaves may have decreased photorespiration (Farquhar and Sharkey 1982; Wu *et al*. 1991). This may explain the fact that the midday values of C_i in the two treatments were identical. However, these values, calculated from gas exchange data, may include some uncertainties due to the non-uniform aperture of stomata over the leaf surface (patchiness; Downton *et al*. 1988; Düring 1992).

Ψ_{PD} values of untreated and treated leaves were not significantly different (P>0.05) (Fig. 2), in spite of significant differences of stomatal conductance (Fig. 1). This may mean that stomata responded to leaf temperature and VPD rather than to leaf water relations (Correia *et al*. 1990; Düring *et al.* 1996). According to Düring and Loveys (1996), a higher sensitivity of stomata may be explained by the heterobaric anatomy of grape leaves, which provokes, throughout the day, the development of water deficits in given portions of the leaf. Although untreated and treated leaves did not differ with respect to Ψ_{PD}, the osmotic potential was different, values being more negative in control leaves (Fig. 2). These data suggest that the higher light absorbance and transmittance of control leaves might be associated with osmotic adjustment (Downton 1983; Düring 1984; Rodrigues *et al*. 1993).

The relative increase of the photosynthetic rate in treated leaves may also be due to an improvement of the PSII photochemical efficiency. In fact, in treated leaves, in the hotter period of the day (Fig. 3, post midday values), the $t_{1/2}$ values were smaller and the decline of F_v/F_m was less pronounced, as a result of smaller increments of F_o and a significant de-

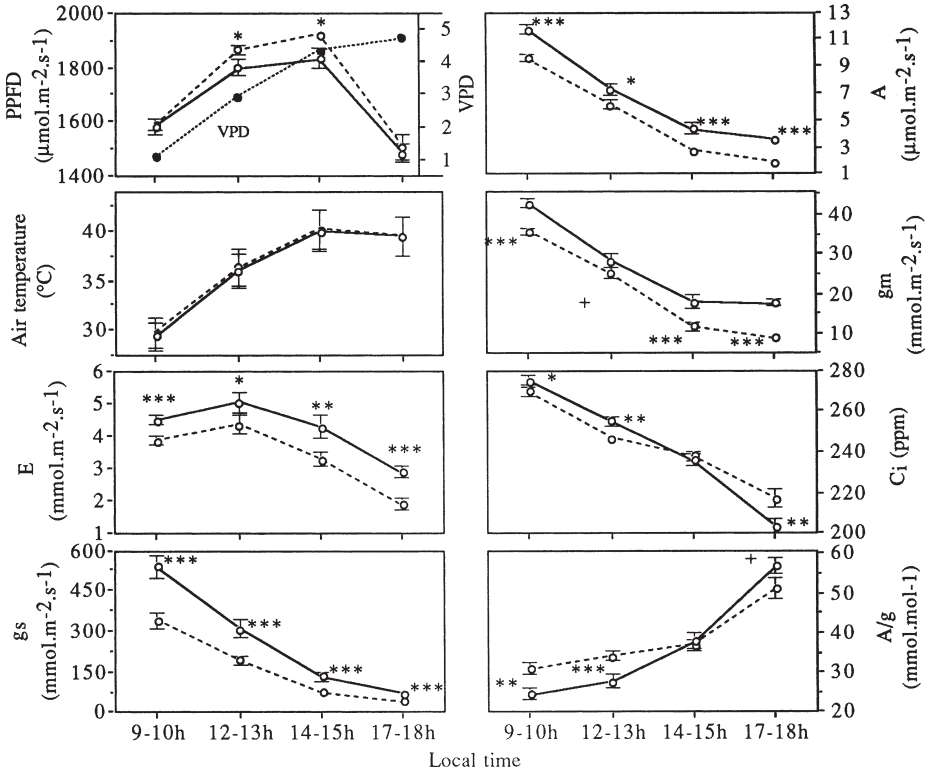

Fig. 1: Diurnal changes of net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), internal CO_2 concentration (C_i), intrinsic water use efficiency (A/g), mesophyll conductance to CO_2 (g_m), photosynthetic photon flux density (PPFD), air water vapour pressure deficit (VPD), and air temperature measured on 25 July 1997 for Bordeaux mixture-treated (solid line) and control (broken line) grapevines. Each point is an average and vertical bars represent S.E. of measurements on 24 different leaves. +, *, ** and *** denote statistically significant differences between treatments at p<0.10, p<0.05, p<0.01 and p<0.001, respectively.

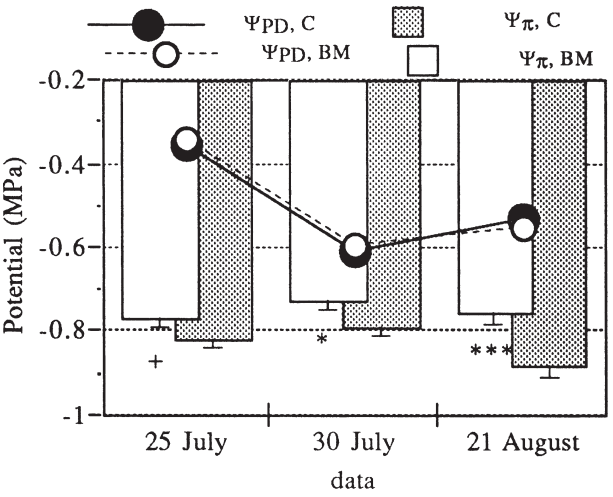

Fig. 2: Predawn leaf water potential (Ψ_{PD}) and the corresponding leaf osmotic potential (Ψ_{π}) of Bordeaux mixture-treated (BM) and control (C) vines. Points or columns are means and vertical bars represent S.E. of measurements on 32 different leaves. For symbols see Fig. 1.

cline of F_m and F_v. A decline of F_v/F_m, if associated with an increase in F_o, means that a photoinhibitory damage in the PSII might have occurred (KRAUSE and WEIS 1991; BALL *et al.* 1994). Simultaneously, the smaller $t_{1/2}$ in leaves whose F_v/F_m was lower suggests an impoverishment of the pool size of electron acceptors at the reducing side of PSII, including plastoquinone (KRAUSE and WEIS 1991; BALL *et al.* 1994).

During summer the degradation of chlorophyll *a* and *b* was less evident in treated leaves compared to control leaves (Tab. 3). On the other hand, in control leaves chlorophyll *b* was more damaged than chlorophyll *a*, leading to a higher chl *a/b* ratio than in treated leaves. Application of Bordeaux mixture also led to an increase of total carotenoids. In various species carotenoids have been shown to play a decisive role in the dissipation of excess excitation energy; this has been reported also for grapevines (DEMMIG-ADAMS and ADAMS III 1992; CHAUMONT *et al.* 1994, 1995; DÜRING 1999).

Conclusion

The results confirm the hypothesis that the application of Bordeaux mixture has a beneficial effect on grapevine physiology, particularly if high irradiance and temperature limit photosynthesis. It is interesting to note that the assumed antitranspirational effect of Bordeaux mixture could not be detected. On the contrary, application of Bordeaux mixture led to a lowering of leaf temperature, to higher stomatal conductance and to a higher photochemical efficiency of PSII.

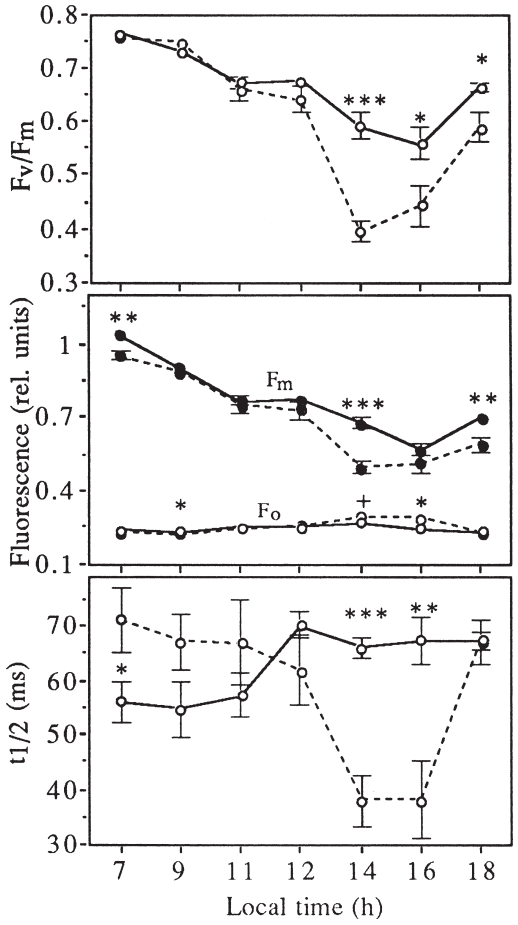

Fig. 3: Diurnal changes of the photochemical efficiency of PSII of dark-adapted leaves (F_v/F_m), minimal fluorescence (F_o) and maximal fluorescence (F_m) at open and closed reaction centers of PSII, respectively, and half rise time from F_o to F_m ($t_{1/2}$) measured on 25 July 1997 for Bordeaux mixture-treated (solid line) and control (broken line) grapevines. Each point is an average and vertical bars (not shown if smaller than symbols) represent the S.E. of measurements on 10 different leaves. For symbols see Fig. 1.

Table 3

Leaf content ($mg \cdot dm^{-2}$) of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl_{tot}) and carotenoid (Car) and the ratio chlorophyll *a*/*b* (Chl *a/b*) and carotenoid/total chlorophyll (Car/Chl_{tot}) in Bordeaux mixture-treated (BM) and control (C) grapevines approximately 40 days after Bordeaux mixture application. Values are the mean ± S.E. (n=20). For symbols see Tab. 2

Treatments	Chl *a*	Chl *b*	Chl *a/b*	Chl_{tot}	Car	Car/Chl_{tot}
C	2.92 ± 0.08	0.98 ± 0.03	2.97 ± 0.02	3.90 ± 0.10	0.82 ± 0.02	0,210 ± 0.003
BM	3.26 ± 0.08	1.12 ± 0.03	2.90 ± 0.02	4.39 ± 0.11	0.87 ± 0.02	0.199 ± 0.002
	***	***	**	***	**	***

Acknowledgements

We thank Sogrape S.A. and Joaquim Fernandes for their contribution to this study and their agronomic management of the experimental field. Financial support from UTAD and PRODEP is gratefully acknowledged. We also thank Andrey Gerry for reviewing the English version of the manuscript.

Literature

Ball, M. C.; Butterworth, J. A.; Roden, J. S.; Christian, R.; Egerton, J. J.; 1994: Applications of chlorophyll fluorescence to forest ecology. Aust. J. Plant Physiol. **22**, 311-319.

Caemmerer von, S.; Farquhar, G. D.; 1981: Some relationships between the biochemistry of photosynthesis and gas exchange of leaves. Planta **153**, 376-387.

Candolfi-Vasconcelos, M. C.; Koblet, W.; 1991: Influence of partial defoliation on gas exchange parameters and chlorophyll content of field-grown grapevines – Mechanisms and limitations of the compensation capacity. Vitis **30**, 129-141.

Chaumont, M.; Morot-Gaudry, J. F.; Foyer, C. H.; 1994: Seasonal and diurnal changes in photosynthesis and carbon partitioning in *Vitis vinifera* leaves in vines with and without fruit. J. Exp. Bot. **45** (278), 1235-1243.

- -; - -; - -; 1995: Effects of photoinhibitory treatment on CO_2 assimilation, the quantum yield of CO_2 assimilation, D_1 protein, ascorbate, glutathione and xanthophyll contents and the electron transport rate in vine leaves. Plant, Cell Environ. **18**, 1358-1366.

Chaves, M. M.; Harley, P. C.; Tenhunen, J. D.; Lange, O. L.; 1987: Gas exchange studies in two Portuguese grapevine cultivars. Physiol. Plant. **70**, 639-647.

Clímaco, P.; 1997: Influência da cultivar e do ambiente na maturação da uva e na produtividade da videira (*Vitis vinifera* L.). Tese submetida à Universidade Técnica de Lisboa para obtenção do grau de Doutor.

Correia, M. J.; Chaves, M. M.; Pereira, J. S.; 1990: Afternoon depression in photosynthesis in grapevine leaves - Evidence for a high light stress effect. J. Exp. Bot. **41**, 417-426.

David, M. M.; Coelho, D.; Barrote, I.; Correia, M. J.; 1998: Leaf age effects on photosynthetic activity and sugar accumulation in droughted and rewatered *Lupinus albus* plants. Aust. J. Plant Physiol. **25**, 299-306.

Demmig-Adams, B.; Adams III, W. W.; 1992: Photoprotection and other responses of plants to high light stress. Ann. Rev. Plant Physiol. Plant Mol. Biol. **43**, 599-626.

Downton, W. J.; 1983: Osmotic adjustment during water stress protects the photosynthetic apparatus against photoinhibition. Plant Sci. Letters **30**, 137-143.

- -; Grant W. J.; Loveys, B. R.; 1987: Diurnal changes in the photosynthesis of field-grown grape vines. New Phytol. **105**, 71-80.

- -; Loveys, B. R.; Grant W. J.; 1988: Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis. New Phytol. **110**, 503-509.

Düring, H.; 1984: Evidence for osmotic adjustment to drought in grapevines (*Vitis vinifera* L.). Vitis **23**, 1-10.

- -; 1992: Low air humidity causes non-uniform stomatal closure in heterobaric leaves of *Vitis* species. Vitis **31**, 1-7.

- -; 1999: Photoprotection in leaves of grapevines: Responses of the xanthophyll cycle to alterations of light intensity. Vitis **38**, 21-24.

- -; Loveys, B. R.; 1996: Stomatal patchiness of field-grown Sultana leaves: Diurnal changes and light effects. Vitis **35**, 7-10.

- -; - -; Dry, P.R.; 1996: Root signals affect water use efficiency and shoot growth. Acta Horticulturae **427**, 1-13.

Farquhar, G. D.; Sharkey, T. D.; 1982: Stomatal conductance and photosynthesis. Ann. Rev. Plant Physiol. **33**, 317-345.

Flexas, J.; Escalona, J. M.; Medrano, H.; 1998: Down-regulation of photosynthesis by drought under field conditions in grapevine leaves. Aust. J. Plant Physiol. **25**, 893-900.

Heilman, J. L.; McInnes, K. J.; Savage, M. J.; Gesch, R. W.; Lascano, R. J.; 1994: Soil and canopy energy balances in a west Texas vineyard. Agric. For. Meteorol. **71**, 99-114.

Krause, G. H.; Weis, E.; 1991: Chlorophyll fluorescence and photosynthesis: The basis. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42**, 313-349.

Lichtenthaler, H.K.; 1987: Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. **148**, 350-382.

Mabrouk, H.; Sinoquet, H.; Carbonneau, A.; 1997: Canopy structure and radiation regime in grapevine. II. Modeling radiation interception and distribution inside the canopy. Vitis **36**, 125-132.

Millardet, P. M.; 1885: Traitement du mildiou et du rot. J. Agr. Prat. **2**, 513-516.

Öquist, G.; Wass, R.; 1988: A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. Physiol. Plant. **73**, 211-217.

Rodrigues, M. L.; Chaves, M. M.; Wendler, R.; David, M. M.; Quick, W. P.; Leegood, R. C.; Stitt, M.; Pereira, J. S.; 1993: Osmotic adjustment in water stressed grapevine leaves in relation to carbon assimilation. Aust. J. Plant Physiol. **20**, 309-321.

Sawada, S.; Hayakawa, T.; 1984: Effect of Bordeaux mixture on net photosynthetic rate and stomatal and intracellular resistances in apple leaves. Photosynthetica **18**, 69-73.

Scholander, P. F.; Hammel, H. T.; Bradstreet, E. D.; Hemmingsen, E. A.; 1965: Sap pressure in vascular plants: Negative hydrostatic pressure can be measured in plants. Science **148**, 339-346.

Schultz, H. R.; 1996: Leaf absorptance of visible radiation in *Vitis vinifera* L.: Estimates of age and shade effects with a simple field method. Sci. Hortic. **66**, 93-102.

Sesták, Z.; Castky, J.; Jarvis, P. G. (Eds.); 1971: Plant Photosynthetic Production. Manual of Methods. Dr. W. Junk Publ, Haia.

Wu, J.; Weimanis, S.; Heber, U.; 1991: Photorespiration is more effective than the Mehler reaction in protecting the photosynthetic apparatus against photoinhibition. Bot. Acta **104**, 283.

Received, February 5, 2001