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

J. M. Moutinho-Pereira¹, N. Magalhães¹, L. F. Torres de Castro¹, M. Manuela Chaves², and J. M. Torres-Pereira¹

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.

Key words: 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

Plant material and treatments: 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 (CuSO₄) dissolved in 501 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.

Environmental conditions and water relations: 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 absorptance 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
According to Scholander et al. (1965) at predawn ($\Psi_{pp}$) and at midday ($\Psi_{md}$) (between 14.00 and 15.00 h) on sunlit leaves in the middle of shoots. Care was taken to minimize water loss during transfer of the leaf to the chamber, by enclosing it in a plastic bag immediately after excision. Leaf osmotic potential ($\Psi_p$) was determined by an osmometer (H. Roebling, Type 13/13DR, Berlin, Germany), using the leaves of the $\Psi_{pp}$ 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. $\Psi_p$ 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, $F'_m$, and maximum fluorescence, $F_m$ at open and closed reaction centres of PSII, respectively, and half rise time from $F'_m$ to $F_m$ represent $t_{1/2}$ 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 Wäss (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.

### 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>
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<tr>
<th>Date</th>
<th>Time</th>
<th>Air temperature</th>
<th>Leaf exposition</th>
<th>n</th>
<th>Leaf temperature (°C)</th>
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<td>(h)</td>
<td>(°C)</td>
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<td>55</td>
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<td>36.9 ± 0.4</td>
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<tr>
<td>14.00-14.30</td>
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<td>sun</td>
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<td>34.5 ± 0.2 ***</td>
</tr>
<tr>
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<td>11.00-11.30</td>
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<td>34.9 ± 0.3 ***</td>
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<tr>
<td>16.00-16.30</td>
<td>36.5</td>
<td></td>
<td>sun</td>
<td>35</td>
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<td>36.0 ± 0.3 ***</td>
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### Table 1

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>BM</th>
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<tr>
<td>Reflectance</td>
<td>96 ± 0.3</td>
<td>145 ± 0.4 a</td>
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<tr>
<td>Absorbance</td>
<td>84.6 ± 0.3</td>
<td>81.9 ± 0.4 a</td>
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<tr>
<td>Transmittance</td>
<td>58 ± 0.1</td>
<td>36 ± 0.1 a</td>
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</table>

### 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).
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 (FAQRHAR 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; DURING 1992).

$\Psi_{PD}$ 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; DURING et al. 1996). According to DURING 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 $\Psi_{PD}$, 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; DURING 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-
Table 3

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Chl a/b</th>
<th>Chl tot</th>
<th>Car</th>
<th>Car/Chl tot</th>
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</thead>
<tbody>
<tr>
<td>C</td>
<td>2.92±0.08</td>
<td>0.98±0.03</td>
<td>2.97±0.02</td>
<td>3.90±0.10</td>
<td>0.82±0.02</td>
<td>0.210±0.003</td>
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<tr>
<td>BM</td>
<td>3.26±0.08</td>
<td>1.12±0.03</td>
<td>2.90±0.02</td>
<td>4.39±0.11</td>
<td>0.87±0.02</td>
<td>0.199±0.002</td>
</tr>
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</table>

*** *** ** *** ** ***
Acknowledgements

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Literature


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