Stomatal behaviour, photosynthesis and leaf anatomy in grapevines grown at an elevated CO$_2$ concentration in the field

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Abstract

Physiological and leaf anatomical characteristics were studied in grapevine (Vitis vinifera L.) cv Touriga Franca grown under field conditions in ambient (365 ± 10 ppm) or elevated CO$_2$ (500 ± 16 ppm) under Open-top chambers (OTCs). Net CO$_2$ assimilation rate (A) was significantly increased, whereas stomatal conductance ($g_s$) was reduced in elevated CO$_2$, leading to improvements in intrinsic water use efficiency. The decrease in $g_s$ was dependent from a reduction of stomatal density and from direct effects of CO$_2$ on the stomata guard cells. Palisade and spongy parenchyma thickness were significantly higher in leaves grown under elevated CO$_2$ conditions. These findings may be used in scaling up models to improve their ability to predict the magnitude of grapevine responses to climate change in the Mediterranean area.

Key words: Climate change, Vitis vinifera, Elevated CO$_2$

1 Introduction

During the last centuries mankind has increasingly been using natural non-renewable resources to satisfy its needs. Among others, emissions from fossil fuel burning started causing a steady increase of CO$_2$ concentration in the Earth’s atmosphere which may reach 550 ppm by the middle of the twenty-first century (IPCC, 2001). Global warming and shifts in amount, seasonality and distribution of precipitation will occur. The scientific community is still trying to predict the impact of these perturbations on the major ecosystems.

The responses of crops, herbaceous and trees species to an elevated CO$_2$ atmosphere has been investigated. In contrast, possible effects on grapevines have largely been ignored. In fact, of our knowledge, only a few studies performed in Italy were made (Bindi et al., 1996; Bindi and Fibbi, 2000; Bindi et al., 2001). The predictions for the main EU viticultural areas have shown an increase in yield variability. On the other hand, the quality of wine in good years is not guaranteed, and the demand for wine in poor years is not met, implying a higher economic risk for growers (IPCC, 2001).

The response of annual and perennial plants to a rise in CO$_2$ level include increase in net photosynthesis, biomass, crop yield, and light, nutrient and water-use efficiency (Griffin and Seemann, 1996). Long term exposure to elevated CO$_2$ may have very different effects at a whole wine or vineyard level. An initial increase in photosynthesis as reported above, may be partly or completed down-regulated due to a mechanism to improve plant performance through increased resource use efficiency or as a result of an accumulation of excess carbohydrates. Meanwhile, plant respiration and carbon allocation may be altered, but until now no clear consensus has emerged. Partial stomatal closure is likely to occur in a high CO$_2$ world. Indirectly, this can lead to increased leaf temperatures what can, in turn, alter the relative affinity of Rubisco for CO$_2$ and O$_2$. These responses may elicit acclimation processes, such as changes in enzyme activities, interchange between isoenzymes and chemical and structural alterations in biomembranes (Berry and Downton, 1982).
Global mean surface temperature will probably rise between 1 and 4.5°C by the middle of the current century (IPCC, 2001). Concurrent with global warming are predictions of altered precipitation patterns. Even without such precipitation changes, water evaporation rates will increase and may lead to more frequent drought events. Some predictions estimate a 70% reduction in soil moisture to the Iberian Peninsula (Stigliani and Salomons, 1992). Implications for viticulture, our main agricultural activity, reaching up to 70% in the Douro Region and resulting socio-economic consequences are dramatic for Portugal where water is a scarce resource and irrigation is often not possible.

The main focus of this study is placed on the effects of elevated CO$_2$ on grapevine physiology, particularly the identification and quantification of the mechanisms underlying the plant responses under field conditions. The primary goal of this experiment is to identify and quantify the mechanisms underlying the grapevine responses under realistic field conditions for the first time in Portugal. The great challenge will be to predict the responses in the near future and to develop adequate strategies to overcome potential problems.

2 Material and Methods

The experimental work was carried out in Vila Real (Campus of UTAD, 41°19’N, 7°44’W, 500 m above mean sea level, Baixo Corgo sub-region of Demarcated Douro Region, Northern Portugal) in summer of 2004.

Grapevines (Vitis vinifera L.) of a common Portuguese cultivar, Touriga Franca grafted on 1103P, were used. This cultivar is universally recognized as the finest grape for Porto wine. The vines, planted in 1997, were spur pruned on a bilateral cordon system (10–12 buds per vine). The soil is typical schistous. Plants were managed without irrigation and grown using normal cultural decisions as applied in commercial farmers. All water and nutrient inputs were continuously recorded, along with other environmental data.

Grapevines were grown in ambient (C; 365 ± 10 ppm) or elevated CO$_2$ (E; 500 ± 16 ppm) under Open-top chambers (OTCs). OTCs, 12.0 x 2.5 x 2.5 m in dimension (Fig. 1), were constructed of 1 mm polyethylene plastic with a 75% light transmittance. Each OTC enclosing ten plants and CO$_2$ fumigation started at the time of crop bloom stage. To measure and record the climate variables, inside and outside each OTC, and to control the carbon dioxide level in the elevated CO$_2$ OTC, it was installed several sensors connected to a logger from deltaT Devices. The actuating system is a CO$_2$ injector operated in on-off mode control with a time sampling of 30s. The CO$_2$ injection is performed acting over an electronic valve linked to a pure CO$_2$ reservoir and distribution tubes. CO$_2$ concentration was measured with an infrared gas analyser (GMP111, Vaisala). The climate variables, inside and outside each OTC, were acquired and/or controlled with a sampling interval of 30s, being the storage time of 5 minutes. Other grapevines were grown in outside control (OC) plot to separate the CO$_2$ effect from any temperature increase or other changes related to growth in the OTCs.

Measurements were made on sun exposed and fully expanded leaves at the middle of the shoots (usually between 8th and 11th nodes on the shoot axes).
Leaf gas-exchange rates were measured at natural incident photosynthetic photon flux density (PPFD) using a portable gas exchange system (LCA-3, Analytical Development Co., Hoddesdon, England), operating in the open mode, and a leaf chamber clip (PLC, surface: 6.25 cm², volume: 16 cm³) with a quantum sensor, and temperature and humidity sensors. PPFD incident on the leaves was always greater than 1000 µmol m⁻² s⁻¹, which is above photosynthesis saturation point in these plants (Flexas et al. 2002). Net CO₂ assimilation rate (A) and stomatal conductance (gₛ) were estimated from gas exchange measurements using the equations developed by von Caemmerer and Farquhar (1981). Intrinsic water use efficiency was calculated as A/gₛ.

Leaf water potential (Ψ) was determined with a pressure chamber (PMS, Oregon, USA), according to the method of Scholander et al. (1965). Measurements were performed on fully expand leaves at predawn (Ψₚd) (1 h before sunrise) and at midday (Ψₚₙ) (between 14:00 and 15:00 h local time, just after gas-exchange measurements). 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.

Anatomical studies and tissues measurements were performed on six healthy, sun-exposed, fully expanded mature leaves. The thickness of leaf blade, palisade and spongy parenchyma, upper and lower epidermis were measured in leaf cross sections prepared for microscopic examination. Sections were taken from the middle of the leaves to avoid differential thickness along the leaf. To make stomatal impressions, one coat of polish (colodium) was applied to the abaxial surface. The polish was then carefully peeled off and placed on a microscope slide. The number of stomata was determined for six peels per treatment.

Data were tested using a one-way ANOVA test to determine the main effects of CO₂ treatment and the main effects of OTC. The treatments means were compared using Duncan’s multiple range test (P < 0.05).

3 Results and discussion

Atmospheric CO₂ enrichment increased A and decreased gₛ (Table 1). These are in agreement with the majority of studies with C₃ plants (Rogers and Dhalman, 1993; Vu and Allen, 2001). Thus, gₛ was down-regulated with respect to photosynthesis and caused an increase in A/gₛ. Grapes grown in arid regions may therefore be expected to benefit from increased CO₂ and may be able to at least partly overcome some of the adverse conditions created by increases in likelihood and severity of drought events (Schultz, 2000). A possible mechanism of stomata acclimation and adjustment was related with a reduction of stomatal density in plants grown at elevated CO₂ (Table 2), which is in accordance with the hypothesis of adaptive modifications of stomata number (Woodward, 1987). In addition, the decline in gₛ may also probably resulted from direct effects of CO₂ on the stomata guard cells, since plants had a high water status, as they presented a high Ψₚd (Table 1), and Ψₚₙ was somewhat higher in CO₂ enrichment plants (Table 1).

Table 1. Net photosynthesis (A, µmol m⁻² s⁻¹), stomatal conductance (gₛ, mmol m⁻² s⁻¹), intrinsic water use efficiency (A/gₛ, µmol CO₂ mol⁻¹ H₂O) and predawn (Ψₚd) and midday (Ψₚₙ) leaf water potential (MPa) of grapevines grown in ambient CO₂ (OTC-C), elevated CO₂ (OTC- E) and outside control (OC). Means (n=10) followed by the same letter are not significantly different at P < 0.05 (Duncan’s test).

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>gₛ</th>
<th>A/gₛ</th>
<th>Ψₚd</th>
<th>Ψₚₙ</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTC-C</td>
<td>13.65 b</td>
<td>406.9 b</td>
<td>34.14 b</td>
<td>-0.29 a</td>
<td>-1.48 b</td>
</tr>
<tr>
<td>OTC- E</td>
<td>22.46 a</td>
<td>330.0 b</td>
<td>71.55 a</td>
<td>-0.27 a</td>
<td>-1.33 a</td>
</tr>
<tr>
<td>OC</td>
<td>17.03 b</td>
<td>630.8 a</td>
<td>28.15 b</td>
<td>-0.31 a</td>
<td>-1.25 a</td>
</tr>
</tbody>
</table>

Since gₛ decreased, higher A in elevated CO₂ is the result of changes in mesophyll capacity, probably including carboxilation efficiency of Rubisco and the capacity for photosynthetic electron transport and ribulose-1,5-bisphosphate regeneration (Bunce, 2000; Tognetti et al., 2001). Future work will be needed to corroborate these hypotheses.

Grapevines grown under CO₂ enrichment presented thicker leaves due to similar increases of thickness of palisade and spongy parenchyma (Table 2, Figure 2). Meanwhile, no significant effects of CO₂ were observed on upper and lower epidermis thickness (data not shown). While CO₂ enhancement did not significantly alter epidemial cell thickness, mesophyll cell expansion increased significantly in the
transverse plane with CO₂ elevation. Rogers et al. (1983) also reported increase in thickness of all cell layers in leaves of *Pinus taeda* and *Liquidambar styraciflua* grown in elevated CO₂ and Radoglou and Jarvis (1990) have found similar increases in the leaves of four poplar clones. The increase in leaf thickness is the result of greater cell enlargement, which is sensitive to CO₂ whereas cell division apparently is not. Cell enlargement affects the internal leaf surface available for the absorption of CO₂ and is likely to have consequence for photosynthesis (Nobel et al., 1975, Nobel, 1977). An increase in mesophyll thickness represents a greater cell wall area for CO₂ diffusion and so should tend to decrease liquid-phase resistance (Mediavilla et al., 2001).

Table 2. Stomatal density (stomata mm⁻²) and leaf tissue thickness (µm) of grapevines grown in ambient CO₂ (OTC-C), elevated CO₂ (OTC-E) and outside control (OC). Means (n=10) followed by the same letter are not significantly different at \( P < 0.05 \) (Duncan’s test).

<table>
<thead>
<tr>
<th></th>
<th>Stomatal density</th>
<th>Total lamina</th>
<th>Palisade parenchyma</th>
<th>Spongy parenchyma</th>
<th>Palisade/spongy</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTC-C</td>
<td>168.1 b</td>
<td>150.2 b</td>
<td>46.7 b</td>
<td>65.5 b</td>
<td>0.74 a</td>
</tr>
<tr>
<td>OTC-E</td>
<td>130.0 c</td>
<td>171.2 a</td>
<td>56.4 a</td>
<td>78.0 a</td>
<td>0.74 a</td>
</tr>
<tr>
<td>OC</td>
<td>186.9 a</td>
<td>165.8 ab</td>
<td>57.3 a</td>
<td>73.9 ab</td>
<td>0.79 a</td>
</tr>
</tbody>
</table>

Fig. 2 Cross sections of grapevine leaves grown at ambient CO₂ (A) or elevated CO₂ (B).

Growth in OTCs influenced some gas exchange, water potential and leaf anatomical properties (Tables 1 and 2) relative to the outside plots. Data indicated that \( A, g_s, \Psi_{mb} \), leaf thickness, palisade parenchyma thickness and stomatal density decreased, whereas \( A/g_s \) increased in OTCs. Several factors may have contributed to these effects. Microclimatic conditions in OTCs differ from ambient conditions in various respects, including irradiance, temperature, vapour pressure deficit, atmospheric turbulence and potential evaporation (Fangmeier et al., 2002). In conclusion, in the absence of environmental stresses, grapevines would perform well under rising atmospheric CO₂ concentration as predicted for this century. The values
here reported may be used in scaling up models to improve their ability to predict the magnitude of grapevine responses to climate change in the Mediterranean area.

3 Acknowledgements

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4 References


