CLIMATE CHANGE AND ADAPTATION STRATEGIES FOR VITICULTURE
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Abstract
Future climate in the Mediterranean grapevine growing regions will be characterized by severe summer conditions, with lower water availability, elevated temperature and elevated solar irradiance (visible and ultraviolet). These projected conditions are of utmost relevance for agricultural practices, since greatly influence the growth and yield, as they exhibit additive and interactive effects on plant physiology. Therefore, it is crucial to develop short-term autonomous adaptation measures, both by economic and environmental sustainability reasons. With this in mind, the protective role of foliar kaolin clay particles (KL) application in the most important Portuguese wine area (Douro region) was examined. The results obtained in 2012 season revealed that KL had a significant positive effect on leaf temperature, stomatal conductance, net photosynthesis, intrinsic water use efficiency, photosynthetic pigments and yield. Thus, kaolin proved to be effective in alleviating the negative effects of summer stress.

Key words: Climate change, Kaolin, summer stress, Vitis vinifera.

1. INTRODUCTION
The increase of atmospheric [CO2] joined with the rise of the concentration of other greenhouses gases contribute to climate change. A global warming is predicted, mainly for Northern Hemisphere, particularly in Southern Europe, where a temperature increase between 2.2-5.1°C is expected to occur during this century. Furthermore, precipitation is projected to diminish from 4 to 27%. In addition, variability in temperature and rainfall may increase considerably, as heat waves and severe droughts may become the norm (IPCC, 2007). Thus, future climate will be characterized by severe summer conditions, with low rainfall, excessive heat load and high daily irradiance (both PAR and UV) levels during the majority of the growing season. As a consequence, we expect main harmful consequences to the winemaking sector in Mediterranean regions.

It is recognized that water deficit has negative repercussions on water relations, nutrient uptake, carbon assimilation, canopy dimension, oxidative susceptibility, cell growth and reproduction processes (Bacelar et al., 2006, 2007a,b). Meanwhile, high temperature also constraint grapes productivity and quality, mainly when temperature extremes coincide with critical stages of plant development. Impaired photosystems and electron transport function, oxidative damage, early chlorophyll loss and increased leaf senescence, and higher respiration rates are usual responses that explain yield changes under high temperature (Guo et al., 2006). On the other hand, under high irradiance plants may change transpiration, respiration and photorespiration rates, as well can display photoinhibition and chlorosis (Moutinho-Pereira et al., 2003), mainly associated with the production of reactive oxygen species. Moreover, high levels of UV-B radiation induce deleterious morphological, physiological and biochemical changes in plants with concomitant consequences on growth, yield and quality (Correia et al., 1998, 1999, 2012). So, all of these environmental factors cause adverse pleiotropic effects on plant growth and development at molecular, physiological and biochemical levels (Levitt, 1980).

To cope with climate change, agronomic strategies may be implemented to offset either in part or fully the changes in productivity and quality. In this context, we propose the use of Kaolin (KL) in order to alleviate the harmful effects of summer stress. Kaolin has central role in stress tolerance in many species, but as far as we know, no relevant work was done in grapevines (Vitis vinifera L.). KL is a mineral chemically inert that sprayed on crops form a white powdery film that reduces canopy temperature and therefore reduces water and heat stress and sunburn damage (Glenn and Puterka, 2005). Apart from these effects, KL may alleviate the incidence of pests. The aim of this work was to evaluate the benefits of Kaolin in grapevines growing under Mediterranean conditions. We hypothesize that KL application decrease leaf temperature and improve carbon assimilation and crop yield.
2. MATERIALS AND METHODS

2.1. Plant material and experimental details

The experiment was undertaken in 2012 in a commercial vineyard of the “Quinta do Vallado”, located at Peso da Régua (GPS: 041°09’44.5”N 07°45’58.2”) in the Douro Demarcated Region, northern Portugal. The soil is a typical schistous and the plants were managed without irrigation and grown using standard cultural decisions as applied in commercial farmers. Three vineyards lines, located on a steep hill with rows N-S orientated, with twenty plants of cv. Touriga Nacional, grafted onto 110 R, were pulverised, soon after veraison (July 11th), with 5% (w/v) Kaolin (Surround WP; Engelhard Corp., Iselin, NJ), while another three vineyards lines with twenty plants each one were maintained as control, without Kaolin application. A second application in the same day was done to ensure the adhesion uniformity of KL. The vines, with 6 years old, were trained to unilateral cordon and the spurs were pruned to two nodes each with 10-12 nodes per vine.

2.2. Leaf gas exchange, chlorophyll fluorescence and leaf temperature measurements

Leaf gas exchange were measured with an infrared gas analyser (LC Pro+; ADC Bioscientific Ltd., UK), operating in the open mode. All measurements were carried at morning (9:00-10:00 h) and at solar midday of a cloudless August day, on 2 fully expanded leaves of 8 grapevines per treatment. Net CO₂ assimilation rate (A), stomatal conductance (gₛ), transpiration rate (E) and the ratio of intercellular to atmospheric CO₂ concentration (Cᵢ/C₂) was estimated according to von Caemmerer and Farquhar (1981). To eliminate possible effects of air humidity and temperature on transpiration, the intrinsic water use efficiency was calculated as the ratio of A to gₛ (Iacono et al., 1998).

Chlorophyll a fluorescence features were measured in situ with a pulse-amplitude-modulated fluorimeter (FMS 2, Hansatech Instruments, Norfolk, England) on the same leaves and environmental conditions as described for gas exchange. Maximum quantum efficiency of photosystem II (PSII) was calculated as \( F_v/F_m = (F_m-F_0)/F_m \) by measuring the fluorescence signal from a dark-adapted leaf when all reaction centers are open using a low intensity pulsed measuring light source (\( F_0 \)) and during a pulse saturating light (0.7 s pulse of 15000 μmol photons m⁻²s⁻¹ of white light) when all reactions centers are closed (\( F_m \)). Leaves were dark-adapted for 30 min using dark-adapting leaf-clips for these measurements. Following \( F_v/F_m \) estimation, after a 20-s exposure to actinic light (1500 μmol m⁻²s⁻¹), light-adapted steady-state fluorescence yield (\( F_s \)) was averaged over 2.5 s, followed by exposure to saturating light (15000 μmol m⁻²s⁻¹) for 0.7 s to establish \( F'_m \). The sample was then shaded for 5 s with a far-red light source to determine \( F'_0 \). From these measurements, fluorescence attributes were calculated according to Bilger and Schreiber (1986) and Genty et al. (1989): capture efficiency of excitation energy by open PSII reaction centres (\( F_v/F_m = (F_m-F'_0)/F_m \)) and efficiency of electron transport as a measure of the effective quantum yield of PSII (\( \Phi_{PSII} = \Delta F/F_m = (F_m-F'_0)/F_m \)). The apparent electron transport rate (ETR) was estimated as ETR = (ΔF/F_m) x PPFD x 0.5 x 0.84, where PPFD is the photosynthetic photon flux density incident on the leaf, 0.5 is the factor that assumes equal distribution of energy between the two photosystems, and the leaf absorbance used was 0.84 because is the most common value for C₃ plants (Björkman and Demmig, 1987).

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.

2.3. Photosynthetic pigments concentration

The essays were analysed in the same leaves used for chlorophyll fluorescence measurements, collected in two different stages (July and September). They were frozen in liquid N₂ and stored at -80 °C prior to analysis. In the laboratory all leaves were ground with liquid nitrogen and crushed in 80% acetone for chlorophylls and carotenoid determination. Total chlorophylls, Chlₐₐ+b₁, and total carotenoids, Car, concentrations were determined according to Sesták et al. (1971) and Lichtenthaler (1987), respectively.

2.4. Vegetative growth and yield

Total fruit weight was determined in 60 vines per treatment using a hand held balance. During the winter pruning all shoots were cut to two node spurs. From these shoots the pruning weight of each vine was determined with a hand held balance. The fruit weight to pruning weight ratio (Ravaz index) was determined using yield and pruning weight per vine.
2.5. Statistical analysis

Data were submitted to one-way analysis of variance (ANOVA) using SPSS 20.0 program (SPSS Inc., Chicago, IL, USA). Results were considered statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ levels.

3. RESULTS AND DISCUSSION

The application of Kaolin decreased significantly the leaf temperature during the summer months, in both sides of the canopy (Figure 1), due to the higher leaf reflectance of solar radiation conferred by the whitish dry residue on leaves. Similar positive effect was observed in olive (Denaxa et al., 2012). In addition, the higher transpiration rate of these leaves, associated with a higher $g_s$ (Table 1), may also contributed to lower foliar temperature. Moreover, leaf gas exchange data show that net photosynthesis was more beneficied than stomatal conductance, and thus $A/g_s$ was higher in grapevines sprayed with KL, indicating an optimization of carbon uptake versus water loss (Raven, 2002). Moreover, the lower $g_s$ joined with higher $C_i/C_a$ indicates that non-stomatal components, either through CO$_2$ diffusion and/or carboxylation efficiency and photochemistry limitations, play a major role in limiting photosynthesis of control grapevines submitted to summer stress. Similar results were reported in other studies (Angelopoulos et al., 1996; Medrano et al., 2002; Moutinho-Pereira et al., 2004, 2007). As the mesophyll conductance is strongly influenced by leaf temperature (Flexas et al., 2008) and in the present experiment the leaf temperature in control plants was very high, this is a further argument supporting the presence of non-stomatal limitations to photosynthesis. However, since the lower photosynthetic rate in control plants was not associated with changes in photochemical processes during the morning (Table 2), we believe that the disturbances in conductance and assimilation of carbon are more relevant. Nevertheless, at midday, under high light intensity, photochemistry reactions were also affected. In fact, the analysis of chlorophyll fluorescence revealed visible damages to PSII, including reductions of maximal photochemical efficiency of PSII ($F_v/F_m$), the electron transport rate and the quantum yield of PSII electron transport ($\Phi_{PSII}$) that was associated with a decrease of $F'/F'_{m'}$, which indicates a lower capture efficiency of excitation energy by open PSII reaction centres. Taking into account these results and the lower $A$ in control plants we assume that a greater proportion of the absorbed PPFD was in excess of that required to support CO$_2$ fixation, and thus the above changes in PSII photochemistry may represent a down-regulation mechanism.
Figure 1. Leaf temperature in both sides of the canopy (A- east; B - west) in sampling dates of July, August and September. Within each period, symbols * and *** denotes significant differences at $P<0.05$ and $P<0.001$, respectively.

Table 1. Net CO$_2$ assimilation rate (A), stomatal conductance ($g_s$), transpiration rate (E), intrinsic water use efficiency ($A/g_s$) and ratio of intercellular to atmospheric CO$_2$ concentration ($C_i/C_a$) at morning (P1) and midday (P2) periods. Within each period, symbols *, ** and *** denotes significant differences at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A (µmol m$^{-2}$ s$^{-1}$)</th>
<th>$g_s$ (mmol m$^{-2}$ s$^{-1}$)</th>
<th>E (mmol m$^{-2}$ s$^{-1}$)</th>
<th>$A/g_s$ (µmol mol$^{-1}$)</th>
<th>$C_i/C_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
<td>P1</td>
<td>P2</td>
<td>P1</td>
</tr>
<tr>
<td>Control</td>
<td>1.60</td>
<td>2.00</td>
<td>40.1</td>
<td>28.7</td>
<td>1.23</td>
</tr>
<tr>
<td>Kaolin</td>
<td>5.82</td>
<td>4.86</td>
<td>82.8</td>
<td>48.2</td>
<td>2.22</td>
</tr>
<tr>
<td>Significance</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>***</td>
</tr>
</tbody>
</table>

Table 2. Maximum ($F_v/F_m$) and effective ($\Phi_{PSII}$) quantum efficiency of photosystem II, capture efficiency of excitation energy by open PSII reaction centres ($F'_v/F'_m$) and electron transport rate (ETR, µmol e$^-$ m$^{-2}$ s$^{-1}$) at morning (P1) and midday (P2) periods. Within each period, symbols * and ** denotes significant differences at $P<0.05$ and $P<0.01$, respectively; ns = not significant (P>0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$F_v/F_m$</th>
<th>$\Phi_{PSII}$</th>
<th>$F'_v/F'_m$</th>
<th>ETR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
<td>P1</td>
<td>P2</td>
</tr>
<tr>
<td>Control</td>
<td>0.812</td>
<td>0.757</td>
<td>0.312</td>
<td>0.229</td>
</tr>
<tr>
<td>Kaolin</td>
<td>0.809</td>
<td>0.810</td>
<td>0.284</td>
<td>0.272</td>
</tr>
<tr>
<td>Significance</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>*</td>
</tr>
</tbody>
</table>

The higher absorption of solar radiation associated with the upper leaf temperature of control grapevines may result in the formation of reactive oxygen species that cause oxidative damage. In fact, these leaves revealed, in early September, signs of oxidative stress, such as the large reduction in photosynthetic pigments concentration, including chlorophylls and carotenoids (Table 3). According to Smirnoff (1993), the decrease of chlorophyll content (chlorophyll bleaching) is a typical symptom of oxidative stress and may be the result of chlorophyll degradation or be due to chlorophyll synthesis deficiency together with changes of thylakoid membrane structure. Moreover, since the carotenoid concentration was least affected, the Chl/Car ratio was significantly lower under control conditions (data not shown). This result can be used as an early indicator for chlorosis of control plants and revealed an increased need for photoprotection of chlorophyll by carotenoids (Tausz et al., 1998). It is well documented that carotenoids are involved in the protection of the photosynthetic apparatus against photoinhibitory damage by singlet oxygen ($^1$O$_2$), which is produced by the excited triplet state of chlorophyll (Young et al., 1997).

Table 3. Concentration of total chlorophylls, Chl$_{(a+b)}$, and total carotenoids, Car, in July and September. Within each period, symbols * and ** denotes significant differences at $P<0.05$ and $P<0.01$, respectively; ns = not significant (P>0.05).

<table>
<thead>
<tr>
<th>Month</th>
<th>Variables</th>
<th>Control</th>
<th>Kaolin</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 31$^{st}$</td>
<td>Chl$_{(a+b)}$ (mg dm$^{-2}$)</td>
<td>3.80</td>
<td>3.58</td>
<td>ns</td>
</tr>
</tbody>
</table>
The improvement of physiological activity of kaolin treated plants, above described, had a positive effect on yield, due to a higher cluster weight (Table 4). Meanwhile, no significant differences were reported on cluster number, pruning weight and Ravaz index.

Table 4. Yield, cluster weight, cluster number, pruning weight and Ravaz index. Symbols * and *** denotes significant differences at $P<0.05$ and $P<0.001$, respectively; ns = not significant ($P>0.05$).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Kaolin</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (kg vine$^{-1}$)</td>
<td>1.27</td>
<td>1.60</td>
<td>*</td>
</tr>
<tr>
<td>Cluster weight (g)</td>
<td>98.7</td>
<td>128.6</td>
<td>***</td>
</tr>
<tr>
<td>Cluster (vine$^{-1}$)</td>
<td>12.6</td>
<td>12.5</td>
<td>ns</td>
</tr>
<tr>
<td>Pruning weight (g vine$^{-1}$)</td>
<td>569.2</td>
<td>618.9</td>
<td>ns</td>
</tr>
<tr>
<td>Ravaz index</td>
<td>2.67</td>
<td>2.79</td>
<td>ns</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

Kaolin application had a significant positive effect on leaf temperature, stomatal conductance, net photosynthesis, intrinsic water use efficiency, photosystem II functioning, photosynthetic pigments concentration and yield. Thus, KL clay particles could be a valuable tool in grapevine Mediterranean farming systems, acting as a summer stress alleviator.

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REFERENCES


Moutinho-Pereira, JM, Correia, CM, Gonçalves, B, Bacelar, EL & Torres-Pereira, JM 2004, ‘Leaf gas exchange and water relations of grapevines grown in three different conditions’, *Photosynthetica*, vol. 42, pp. 81-86.

Moutinho-Pereira, JM, Magalhães, N, Correia, CM & Torres-Pereira, JM 2003, ‘Effects of NW-SE row orientation on grapevine physiology under Mediterranean field conditions’, *Agricultura Mediterranea*, vol. 133, nº3-4, pp. 218-225.


