PHYSIOLOGICAL AND YIELD RESPONSES OF GRAPEVINES TO KAOLIN UNDER SUMMER STRESS

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Abstract
In Portugal, the wine-grape sector has a crucial economic, social and cultural relevance, especially in many inland regions of the country, namely the Douro Wine Region. It is the oldest regulated viticultural region in the world (since 1756) and has a typical Mediterranean climate with hot and dry summers. Recent-past temperature trends, focusing on European viticultural regions, show that the growing season mean temperatures have increased nearly 2ºC from 1950 to 2004. Future projections point to temperature increases and precipitation changes in the growing season, which it may bring changes in grapevine physiology and consequently in yield and wine quality. Although grapevines have several survival strategies, the amounting evidence for significant climate change in the upcoming decades urges adaptation measures to be taken. Short term adaptation measures should be considered as the first protection strategy against climate change and should focus on specific threats. Among these adaptation measures, the major effort undertaken by the scientific community is to study the effect of kaolin in the improvement of light microclimate and water relations of leaves. The current understanding regarding this specific measure is still scarce and often inconclusive, mainly due to the numerous environmental and cultural variables involved. The main objectives are to compare the physiology and yield responses of *Vitis vinifera* Touriga Nacional to 5% foliar kaolin over a two year period (2012 and 2013). Leaves treated with kaolin showed higher light reflectance and lower leaf temperature. Photosynthetic rates of pulverised leaves increased, particularly due to a lowering of non-stomatal limitations. The results also revealed that leaves with kaolin exhibited higher intrinsic water use efficiency and photochemical quenching. Delays in leaf senescence of grapevines sprayed with kaolin inhibited scorching of clusters and, consequently, lead to a higher yield per plant, particularly emphasized in years of lower production.

Keywords: Mediterranean climate, Alto Douro Wine Region, kaolin application, photosynthesis, water relations

1 INTRODUCTION

Winemaking has a large economic and social relevance in Europe (Fraga et al. 2013). Winegrape production in Mediterranean regions, especially in the Douro Demarcated Region (DDR, Northeast Portugal), is subject to warm and dry summer climate conditions, that may irreversibly impair some physiological processes (Berry and Björkman 1980), leading to poor grape yields and quality (Ferreira et al. 2012; Moutinho-Pereira et al. 2004). Previous work by our team in the region (where the famous Port Wine is made) clearly showed that grapevines growing under severe summer stress experienced significant decline in yield due to stomatal and mesophyll limitations to photosynthesis (Moutinho-Pereira et al. 2004). Frequently, some of these leaves, particularly those lower on the canes and more directly exposed to sunlight, displayed irreversible photoinhibition and chlorosis followed by necrosis, unprotecting the cluster zone and leading to a decrease in grapevine water use efficiency (Moutinho-Pereira et al. 2003). Consequently, in low vigour vines, yield, berry weight and sugar concentration are significantly reduced. Furthermore, other berry characteristics, such as colour, flavour and aroma components are suppressed by excessive solar exposure of grapes and low water availability. Wines produced from such grapes will tend to be unbalanced: high in alcohol, with too little acidity to produce the desired freshness (Jones 2004). Therefore, information on varietal characteristics (Moutinho-Pereira et al. 2007), adaptation and mitigation practices to severe summer stress (Moutinho-Pereira et al. 2001; Sousa et al. 2006), as well as the study of elevated CO_2 on grapevine physiology and yield attributes, have received particular attention (Gonçalves et al. 2009; Moutinho-Pereira et al. 2009). Related with adaptation and mitigation practices, we wish to reinforce the knowledge of the effect of particle film applications, i.e. spraying canopies with a suspension of particles of various kinds of clay, such as kaolin. This compound is a white and inert clay mineral used frequently as a base to develop a particle film for foliar application to repel insects and to mitigate the damaging effects of heat and light stress on plant physiology and productivity (Rosati et al. 2006). In general, this application reduces heat and light stresses in plants by reflecting infrared (IR) and ultraviolet radiation from the foliar surface (Glenn and Puterka 2005). However, no relevant work is known about physiological and yield responses of grapevine to kaolin under summer stress. Hence, the aim of the present study is to investigate the main effects of a foliar application with a kaolin particle film in the physiological behaviour of the "Touriga Nacional" variety.

2 MATERIALS AND METHODS

Vitis vinifera L. "Touriga Nacional", grafted onto 110 R, was used to study the effect of kaolin application. The experiment was undertaken in 2012 and 2013 in the commercial vineyard "Quinta do Vallado", located at Peso da Régua (GPS: 041º09'44.5"N 07º45'58.2') in the DDR, northern Portugal. The Mediterranean-like climate is characterized by warm summers and mild winters, with autumn-winter precipitation maximum and very dry summers. Soils are predominantly associated with schist formations and plants are managed without irrigation and grow using standard cultural decisions as applied in commercial farmers. Three vineyard lines, located on a steep hill, with north-south orientated rows and with twenty plants each one, were pulverised soon after veraison, with 5% (w/v) kaolin (Surround WP; Engelhard Corp., Iselin, NJ) in middle of July (11 July 2012 and 17 July 2013). A second application in the same day was done to ensure the adhesion uniformity of kaolin. Three additional vineyard lines, with twenty plants each one, were maintained as control, i.e. without kaolin application. The 6-years old vines were trained to unilateral cordon and the spurs were pruned to two nodes each, with 10-12 nodes per vine.

In each treatment, during the summer of each year, the leaf gas exchange measurements were performed with a portable IRGA (LC*pro+*, ADC, Hoddesdon, UK), operating in the open mode. Net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a) and intrinsic water use efficiency (A/g_s) were estimated from gas exchange measurements using the equations developed by von Caemmerer and Farquhar (1981). Chlorophyll a fluorescence features were measured *in situ* with a pulse-amplitude-modulated fluorimeter (FMS 2, Hansatech Instruments, Norfolk, England) according to the procedure described in Moutinho-Pereira et al. (2012). Chlorophyll concentration per area was also estimated non-destructively using a SPAD-502 meter (Minolta, Japan). SPAD-readings were carried out in the field in the same leaf samples used for the other field measurements. 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 reflectance indices were determined in the same leaf samples used for the gas exchange and Chl *a* fluorescence measurements. Leaves were collected in the afternoon, brought back to the laboratory in a thermal bag and reflectance readings were immediately undertaken according to Moutinho-Pereira et al. (2012). At harvest, yield per vine was determined in 60 vines per treatment. Values were compared by a one-way ANOVA test. All means were compared at the 0.05, 0.01 and 0.001 levels of significance.

3 RESULTS AND DISCUSSION

The application of 5% kaolin resulted in the formation of a whitish dry residue on the exposed leaves of the canopy, which significantly diminished the light absorbed and transmitted by these leaves, while the reflector capacity increased substantially (Fig. 1). One of the direct effects of this application was a significant reduction of leaf temperature under conditions of strong incidence of the solar radiation, particularly in the afternoon measurements (Table 1). Consequently, during the summer period, the degradation of photosynthetic pigments (chlorophylls and carotenoids) in the treated leaves, estimated by the SPAD technique, was not as evident as in control ones (Table 2).

Measurements of gas exchange on a typical summer day indicate significantly higher A in kaolin leaves (Fig. 2). Particularly at ripeness stage, the increase in A was more evident than the slight increase in g_s during warmer periods of the day, leading to higher A/g_s and lower C_i/C_a. These results suggest that stomatal conductance was not the main factor responsible by photosynthesis depression in control leaves. In fact, the increase of C_i/C_a, associated with a significant decline of A and A/g_s, can be explained by lower activity of photosynthetic machinery, i.e. by the predominance of non-stomatal limitations to photosynthesis strained by light and heat stress (Iacono and Sommer 1996). Concerning to the diurnal variation of A/g_s, kaolin grapevines exhibit a significant increase through the day (about 53%), suggesting a higher avoiding strategy induced by this particle film, relatively to control plants. The increase of the photosynthetic rate in kaolin treated leaves was associated to an improvement of the PSII photochemical efficiency. In fact, in the treated leaves during the critical period of the day (Fig. Table 3, afternoon values) the decline of F_v/F_m and of photochemical fluorescence (qP) from morning to afternoon was less pronounced than in the control leaves. Since qP gives an indication of the proportion of PSII reaction centres that are open (van Kooten and Snel 1990), these results indicate that the major factor leading to the slightly impaired Φ_{PSII} at steady-state photosynthesis was an increase in the proportion of closed PSII centers.

At harvest, in 2012, when the yield was generally lower than in 2013, the kaolin effect had a clearly positive impact on the productivity performance of the vines (Table 4). These results were certainly a consequence of a better physiological activity.

4 CONCLUSIONS

In conclusion, the results of this study, carried out with grapevines of the same variety and under similar field-grown conditions, emphasized the beneficial role of kaolin as a short-term measure for growing grapevines under high irradiance levels and heat/water stress conditions, such as in Douro region. Particularly during the ripening stage, the photosynthetic capacity depression was associated with important photochemical and biochemical changes that can negatively compromise the grape production, particularly emphasized in low yield years.

Aknowledments

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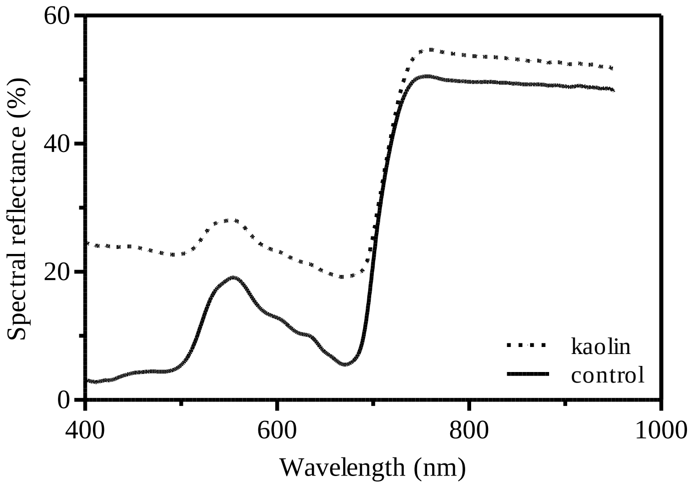

Figure 1: Typical spectral reflectance for grapevines leaves with (kaolin) and without kaolin (control) determined on 3 August 2012 (n=6).

Table 1: Leaf temperature (ºC) (mean ± SE, n=12) for grapevines leaves with (kaolin) and without kaolin (control) determined on 3 different dates of 2012. Within each date/period, symbol */**/* denotes significant differences at $p < 0.001/0.01/0.05$.**

	31 Jul	21 Aug	4 Sept
Morning			
Control	32.5 ± 1.0	35.4 ± 0.8	29.1 ± 0.5
Kaolin	28.1 ± 1.0	32.5 ± 1.2	25.3 ± 0.8
	**	*	***
Afternoon			
Control	31.0 ± 0.7	34.6 ± 0.8	36.2 ± 0.6
Kaolin	28.0 ± 0.3	31.7 ± 0.3	31.1 ± 0.4
	***	**	***

Table 2: SPAD values (mean ± SE, n=8) for grapevines leaves with (kaolin) and without kaolin (control) determined on the two outlined dates. The symbol * denotes significant differences at $p < 0.05$.

	31 Jul 12	31 Jul 13
Control	36.1±1.0	34.5±0.9
Kaolin	39.7±0.9	37.3±0.9
	*	*

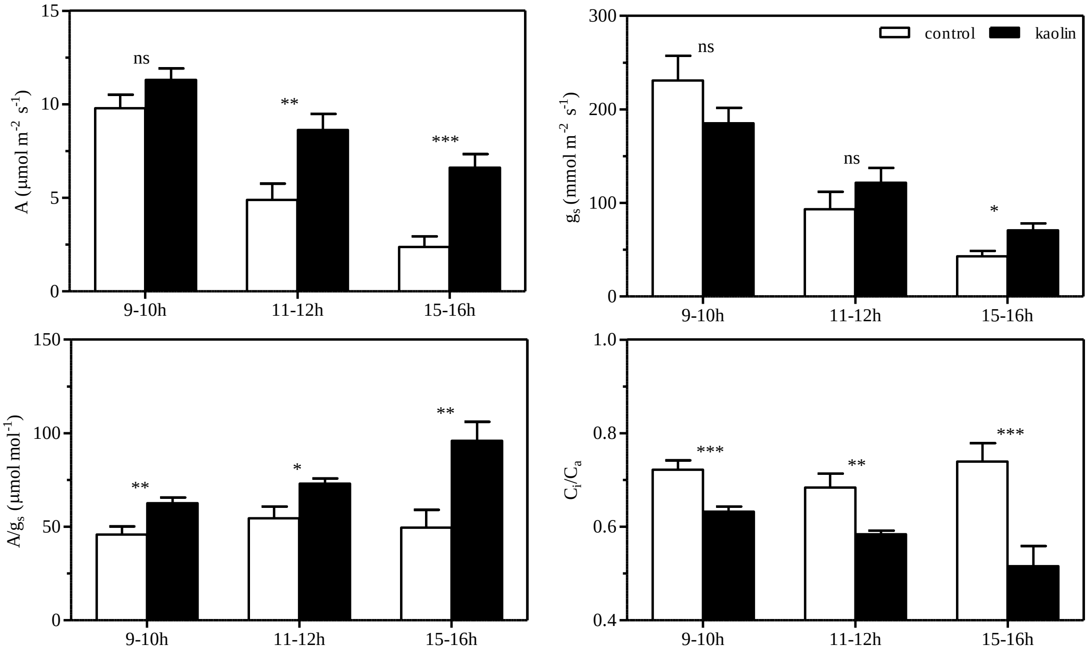

Figure 2: Net CO_2 assimilation (A), stomatal conductance (g_s), intrinsic water use efficiency (A/g_s) and internal CO_2 concentration/ambient CO_2 ratio (C_i/C_a) in grapevines leaves with (kaolin) and without kaolin (control) determined on 20 August 2013 (n=8). Each column represents the mean and vertical bars represent the standard error (n=8). The symbols *, ** and * denote significant differences at p <0.05, p < 0.01 and p < 0.001, respectively; ns = not statistically significant.**

Table 3: Maximum (F_v/F_m) and effective (Φ_{PSII}) quantum efficiency of photosystem II, apparent electron transport rate (ETR), photochemical (qP) and non-photochemical (NPQ) fluorescence quenching for grapevines leaves with (kaolin) and without kaolin (control) determined on 20 August 2013 (n=8). Values are the mean ± SE (n=8) and the symbols ns*, * and ** denote significant differences at p <0.10, p <0.05 and p <0.01, respectively; ns = not significant (p >0.1).

Treatment	F_v/F_m	Φ_{PSII}	ETR	qP	NPQ
Morning					
Control	0.845 ± 0.003	0.339 ± 0.034	213.8 ± 21.3	0.655 ± 0.064	2.51 ± 0.43
Kaolin	0.856 ± 0.005	0.352 ± 0.031	221.7 ± 19.3	0.620 ± 0.031	3.04 ± 0.17
	ns*	ns	*	ns	ns
Afternoon					
Control	0.439 ± 0.067	0.151 ± 0.028	95.2 ±17.5	0.347 ± 0.051	2.88 ± 0.42
Kaolin	0.662 ± 0.036	0.218 ± 0.030	137.0 ± 18.9	0.453 ± 0.052	3.14 ± 0.20
	**	ns	ns	**	ns

Table 4 – Yield per vine and cluster weight in control and kaolin 'Touriga Nacional' grapevines. Values are the mean ± SE (n=60 vines) and the symbols * and * denote significant differences at p <0.05 and p <0.001, respectively; ns = not significant (p >0.05).**

Year	Variables	Control	Kaolin	
2012	Yield (kg/vine)	1.27 ± 0.1	1.60 ± 0.08	*
	Cluster weight (g)	97.7 ± 4.2	128.6 ± 3.9	***
2013	Yield (kg/vine)	3.83 ± 0.2	3.87 ± 0.24	ns
	Cluster weight (g)	179.0 ± 7.3	189.4 ± 7.9	ns