

Research Article

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Spectral and thermal data as a proxy for leaf protective energy dissipation under kaolin application in grapevine cultivars

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Abstract: The dynamic effects of kaolin clay particle film application on the temperature and spectral reflectance of leaves of two autochthonous cultivars (Touriga Nacional (TN, n=32) and Touriga Franca (TF, n=24)) were studied in the Douro wine region. The study was implemented in 2017, in conditions prone to multiple environmental stresses that include excessive light and temperature as well as water shortage. Light reflectance from kaolin-sprayed leaves was higher than the control (leaves without kaolin) on all dates. Kaolin's protective effect over leaves' temperatures was low on the 20 days after application and ceased about 60 days after its application. Differences between leaves with and without kaolin were explained by the normalized maximum leaf temperature ($T_{max_f_N}$), reflectance at 400 nm, 532 nm, and 737 nm, as assessed through TN data. The wavelengths of 532 nm and 737 nm are associated with plant physiological processes, which support the selection of these variables for assessing kaolin's effects on leaves. The application of principal component analysis to the TF data, based on these four variables ($T_{max_f_N}$ and reflectances: 400, 532, 737 nm) selected for TN, explained 83.56% of data variability (considering two principal components), obtaining a clear differentiation between leaves with and without kaolin. The $T_{max_f_N}$ and the

reflectance at 532 nm were the variables with a greater contribution for explaining data variability. The results improve the understanding of the vines' response to kaolin throughout the grapevine cycle and support decisions about the re-application timing.

Keywords: handheld spectroradiometer, leaves reflectance, leaves temperature, thermal camera, xanthophyll.

1 Introduction

Temperature and solar radiation, coupled with water shortage, are central to climatic influences over growth, yield and quality of grapevines. Despite the advances in technology, breeding included, as well as the incremental use of irrigation in vineyards, grapevines remain highly dependent on weather. This can affect both the quantity and quality of the harvest. In semi-arid environments, typical of the Mediterranean climate, excessive radiant heat load, combined with excessive temperature, often limits the plant's physiological processes and growth during the rainless summer, mainly in no irrigation crop system such as vineyard. According to Palliotti et al. (2015), these climate factors can have synergetic effects on CO_2 assimilation related with stomata conductance or damage leaves pigments responsible for protective energy dissipation, which can limit growth, yield, and fruit composition.

In the Douro wine region, potential multiple abiotic stresses are very frequent during summer as a consequence of the high solar radiation, excessive temperatures and the elevated gradients of the water vapour pressure between the leaves and the air (Chaves and Rodrigues 1987). This may be further exacerbated by the foreseen drier and warmer climate scenarios, despite the noticeable advances in vineyard technologies (Cunha and Richter 2016; Giorgi and Lionello 2008; IPCC 2007; Moriondo et al. 2015). Therefore, adaptive cultural practices are needed to

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mitigate the negative impact of actual and future climate scenarios in order to maintain the competitiveness of the wine industry.

Modifications of the solar radiation balance in the plant leaves can be obtained by spraying foliage with a white suspension of an inert reflective material, such as a kaolin-particle film. The kaolin is a product originated from clay that transmits visible light and gases necessary for photosynthesis, while reflecting the ultraviolet and infrared bands. According to Glenn et al. (2002), kaolin application reduces temperatures of treated leaves through increasing radiation reflectivity (e.g. in the ultraviolet band), and promoting the activation of enzymes responsible for the impairment of protective leaf pigments related to energy dissipation (Shellie and King 2013).

Among the protective pigments present in the leaves, it is important to highlight the family of chlorophylls, carotenoids and anthocyanins, which are associated with light harvesting and energy dissipation, which vary greatly according to the environmental factors. Studies relative to various crops have shown that the pigments can control excess light and heating that is received from the sun, e.g., in grapevines (Dinis et al. 2016; Moutinho-Pereira et al. 2007; Palliotti et al. 2015; Shellie and King 2013; Zarco-Tejada et al. 2013), tobacco (Falcioni et al. 2017), Persian walnut (Gharaghani et al. 2018), and others species (Féret et al. 2017; Gitelson et al. 2003; Middleton et al. 2012; Müller et al. 2001). Under stress conditions of temperature, humidity, nutrition, and water, the absorbed radiation decreases due to the defence mechanism based on dissipation of energy of plants, which is controlled by the xanthophylls (Middleton et al. 2012). Nevertheless, under stress, the canopy can present other symptoms related to its vigour, such as: changes in the angle of the leaves, foliar area, concentration of pigments in the epidermis, shape of the plant and depth of the root (Middleton et al. 2012).

The application of a kaolin-particle film has been reported to protect the canopy against excessive light and temperature as well as pest and disease pressure (Ferrari et al. 2017; Glenn and Puterka 2005) in different crop systems. Many studies demonstrate that kaolin-film application can be an important practice for reducing midday leaf temperature (AbdAllah et al. 2018; Jifon and Syvertsen 2003), leaf-to-air vapour pressure deficit (VPD) (Glenn et al. 2010), and chlorophyll fluorescence (Shellie and King 2013), as well as for regulating plant water status by overcoming the negative impact of water stress (AbdAllah 2017), and preserving plant growth, yield and

quality (Djurović et al. 2016; Gharaghani et al. 2018). The application of a kaolin-particle film over the grapevines in the Douro region has shown good results to protect the plants from the effects of heating and radiation (Dinis et al. 2016) and its application in commercial vineyards is often considered due to its low cost of application. These authors have also demonstrated that kaolin is able to improve the quality of the grape, under stress conditions, by improving its concentration of phenols, flavonoids, anthocyanins and vitamin C.

Remote sensing techniques have been applied in crop production to provide a better understanding of the crops spectral response under different agronomic and environmental (e.g., soil, climate) conditions (Féret et al. 2017). Spectral reflectance provides information on dynamic changes in leaf pigments through the vegetative cycle. According to Hall et al. (2002); Jones and Vaughan (2010), seasonally, pigment composition, derived from remote sensing data, can be used as a proxy for physiological processes including the epoxidation state of xanthophyll cycle pigments and chlorophyll fluorescence emission (Gamon et al. 1997; Moya et al. 2004; Ustin et al. 2009).

Thermal imagery of the canopy can be used to infer on leaves' pigment concentration (Sepulcre-Cantó et al. 2006; Shellie and King 2013) and to assess water status of the canopy (Zarco-Tejada et al. 2013). The combination of the optical spectral data and thermal imagery opens new avenues for ecophysiological observations, potentially providing insights into the remote detection/monitoring of the viticulture practices' impact on multiple abiotic stresses.

Although the effects of kaolin applications on disease control and leaf/canopy physiology, plant growth, yield and fruit composition have been deeply studied, their time-dynamics' impacts on leaf spectral reflectance modification are not well understood.

The main goal of this study was to evaluate the effect of kaolin on leaf microclimate, throughout the grapevine growth cycle. We hypothesize that kaolin application would reduce the temperature through the increase in reflectivity of leaves and the analysis of the leaf's reflectance pattern could be a proxy for the physiological process of protective energy dissipation through leaf pigments. Specific goals include: (i) assessing the specific wavelengths, associated to the leaves' reflectance pattern, which can explain vines' biophysical responses resulting from the kaolin application; and (ii) evaluating the thermal response of the leaves throughout the time following the kaolin application.

2 Materials and methods

The study was conducted in a commercial vineyard (Quinta dos Aciprestes, Real Companhia Velha) located in Soutelo do Douro (latitude 41.24°N; longitude 7.43°W) in the Douro wine region (Northeast of Portugal). The region is characterized by a climate of Mediterranean type, with high temperatures and low precipitation during the summer period, as shown in the Figure 1.

The Douro valley is one of the most non-irrigated arid wine regions of the world, with strong and consistent post-flowering water, thermal and solar radiation stresses. These multiple stresses occur mainly during the ripening period (20th July to 1st September), where the precipitation is typically lower than 20 mm, accompanied by high values of solar irradiation and temperature and also elevated gradients of the water vapour pressure between the leaves and the air (Reis and Lamelas 1988).

The study was conducted in the year 2017, which was characterized by low precipitation levels from April to September (Figure 1).

The studied vineyard has a total area of 1.17 ha, with an undulating terrain and an average slope of 25%. The vines were planted according to a bilateral Royat system, following the Northeast-Southwest orientation, with 2.2 m × 1 m plant spacing and a maximum plant height of 1.5 m. Two cultivars were considered: Touriga Nacional (TN) and Touriga Franca (TF), grafted onto 196-17. A porous kaolin-particle film (“SunProtect” by Epagro, with 99% substance purity) was applied over both cultivars’ leaves during the summer period. The concentration followed the instructions of the manufacture (5 kg /100 L of water). The kaolin-particle film

was applied on June 6th (DOY 156) over all the vines in the vineyard. The application was performed by spraying over the vines canopy, covering a large percentage of the leaves but also leaving unsprayed leaves. The kaolin treatment was fully managed by the wine company.

Ground measurements of spectral reflectance data and thermal data were collected on four dates in 2017: July 5th, 29 days after kaolin application (DAA 29), July 20th (DAA 44), August 3rd (DAA 57), and August 31st (DAA 85). Four leaves, with kaolin-particle film treatment, and another four leaves, without kaolin-particle film treatment, of each one of the two cultivars, were randomly selected and collected in the same line of vines for data acquisition on each date. Additionally, similar conditions of growth, canopy position and health status, as well as vigour and age, were considered in the selection of the leaves. The extrinsic characteristics related with soil conditions and sun exposition were also similar for the plants where leaves with and without kaolin were selected. Fully exposed leaves (with and without kaolin) were collected and the moment of measurement was similar for the leaves of each one of the cultivars.

The leaves selected per cultivar were detached from the vine immediately before performing the ground measurements. Moreover, the angle of measurements (thermal and spectral) was the same for all the leaves, as well as the distance from the equipment to the leaves, thus always achieving maximum sunlight reflection and avoiding shadows. An exception was considered for DAA 29, when no data were collected for TF. A total of 56 observations of spectral reflectance data and thermal data were collected.

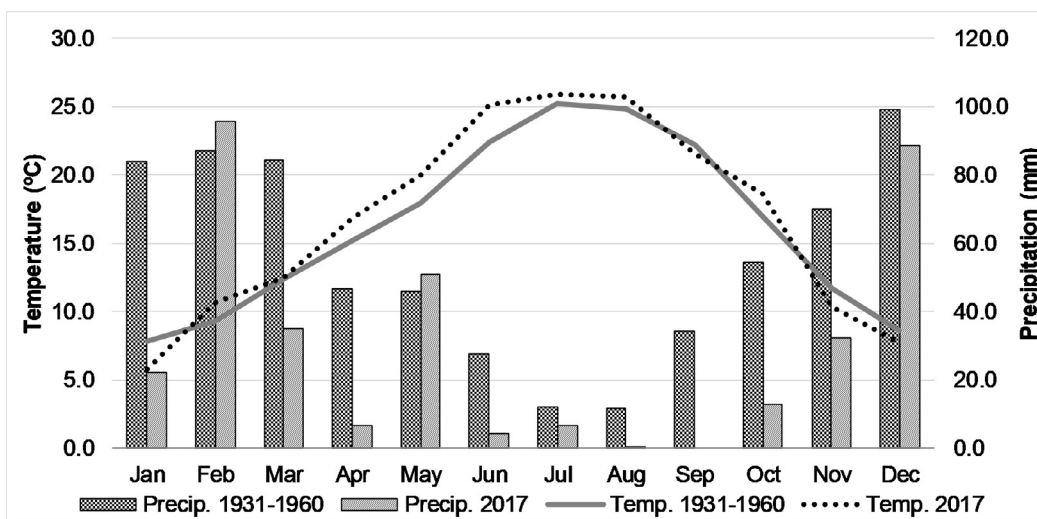


Figure 1: Temperature and precipitation characterization of the Douro wine region for the 30-years period between 1931-1960 Ferreira (1965) and comparison with the temperature and precipitation records in 2017 during study period

In order to assess to vines' water status for both cultivars on each date of acquisition, Table 1 presents the predawn leaf water potential measured by the wine company with a pressure chamber (Scholander et al. 1965) (PMS600, Albany, OR, USA).

The spectral reflectance data were collected using a portable spectroradiometer (Handheld 2, ASD Instruments) recording spectral signatures between 325 nm and 1075 nm of the electromagnetic spectrum, with a wavelength interval of 1 nm. The measurements were done in nadir position, in cloud free conditions, between 11 and 14 h (local time) aiming to minimize changes in solar zenith angle. Ten repetitions per leaf were performed and later averaged to minimize the effect of noise. Only data between 400 nm and 1010 nm were considered for further analysis due to noise at the limits of the spectrum.

Thermal image data were collected using a portable thermal camera (Flir Systems, Inc.) in the same leaves where spectral reflectance data were measured. Thermal images were processed using Flir Tools 6.3.17227.1001 software (Flir Systems). The maximum temperature of the leaves was obtained by the analysis of the thermal images. A normalization of the maximum temperature of the leaves ($T_{\max_f_N}$) was performed, dividing by the hourly air temperature at the time of measurements, in order to minimize the effect of the air temperature increases, on each date, during the period of data acquisition.

The effects of kaolin treatment on the mean temperature of the leaves for each date, were assessed according to grape varieties using one-way analysis of variance (ANOVA). Results of the ANOVA were expressed as F-ratio values (Fischer test) and whenever differences resulted in significance, individual means were compared using a Duncan test ($p < 0.05$). These statistical analyses were performed in R (R Core Team 2017) through the packages "car" (Fox and Weisberg 2011) for ANOVA and "agricolae" (Mendiburu 2017) for the Duncan test.

An assessment of the spectral and thermal behaviour of the vineyard leaves, following the kaolin-particle film treatment, was performed through a principal component analysis (PCA). The reflectance data, at each wavelength between 400 nm and 1010 nm, as well as the $T_{\max_f_N}$,

were considered as variables. Prior to the PCA, a correlation analysis (based on the Spearman correlation) between variables was performed to reduce collinearity among variables and select the variables that better explain the data pattern. This correlation analysis was performed separately for TN and TF data and a threshold of 0.70 was used based on Kuhn and Johnson (2013). The set of predictor variables, corresponding to that obtained with TN data, was selected for training due to its higher number of observations ($n=32$). A validation of the results obtained for TN was performed by applying a PCA to the TF ($n=24$) data, using the variables selected for TN.

The software R (R Core Team 2017), combined with the package "factoextra" (Kassambara and Mundt 2017), was used for computing the correlation, cluster analysis and the PCA.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Effects of kaolin on leaves temperature

The temperature measurements in the leaves of TN and TF cultivars in both kaolin treatments, as expected, were roughly 10 to 20°C (DAA 57) higher than the air temperature, which was close to 30°C on all dates (Figure 2).

In the measurements carried out on DAA 29 and on DAA 44, the temperatures in the leaves with kaolin were always lower than those without kaolin, but these temperature differences were not statistically significant ($p < 0.468$ DAA 29; $p < 0.275$ DAA 44). The temperature of the TN' leaves, in the third measurement (DAA 57), reached values higher than 50°C in both treatments (with and without kaolin), which were much higher than the previous measurements (DAA 29 and DAA 44). Also, on this date (DAA 57), the temperature in both grapevine cultivars was significantly ($p < 0.000$) higher in the treatments with kaolin than without kaolin and the cultivar TN reached higher temperatures than TF in both treatments (Figure 2).

Table 1: Predawn leaf water potential for Touriga Franca (TF) and Touriga Nacional (TN) in each data of measurement

| Days After Application (DAA) | TF (MPa) | TN (MPa) |
|------------------------------|----------|----------|
| DAA 29 | -0.125 | -0.44097 |
| DAA 44 | -0.295 | -0.77674 |
| DAA 57 | -0.46 | -0.73659 |
| DAA 85 | -0.81 | ND |

ND: no data. DAA - Days After Kaolin Application

3.2 Effects of kaolin on leaves reflectance

Figure 3 presents the leaves' spectral reflectance patterns, grouped by varieties and kaolin treatments, within each measurement date. Broadly, the light reflectance from kaolin-sprayed leaves were at least 2 times higher in the visible domain (<700 nm) and very similar in the near infrared domain (>700 nm) than in the control leaves, for all dates. In both treatments, the cultivar TF, when compared with the TN, presents consistently higher levels of reflectance in the visible region. The higher reflectance obtained in the leaves of TF suggest a correlation, but not statistically different, with the lower temperature of leaves without kaolin on DAA 29 and DAA 44.

The leaves partially lost the kaolin film, as observed during field measurements on DAA 57. In this third measurements date, the leaves with kaolin presented higher temperatures than leaves without kaolin (Figure 2) and the reflectance for both cultivars and treatments were very alike (Figure 3). This effect may be related with a long-term reduction of leaves' ability to dissipate energy under conditions of temperature and radiation stresses, when they were treated with kaolin at early growth stages.

3.3 Effects of kaolin assessed through multivariate analyses

Notably, both cultivars presented very close wavelengths during the dimensional reduction performed through the

Spearman correlation: (i) for TF: T_max_f_N, reflectance at 400 nm, reflectance at 542 nm, and reflectance at 720 nm; and (ii) for TN: T_max_f_N, reflectance at 400 nm, reflectance at 532 nm, and reflectance at 737 nm. The similarity of the wavelengths selected for both cultivars may be due to the high correlation of near bands in hyperspectral data (Feng et al. 2017; Huang and He 2005; Wang et al. 2017).

Table 2 presents the eigen values obtained from the principal component analysis (PCA) and the corresponding percentage of variance explained by the first three principal components for each cultivar. The first two principal components (Dim. 1 and Dim. 2) explain more than 84% of the data variability for both cultivars. For both cultivars, the bands of the green zone (R542 and R532) and the red edge zone (R720 and R737) present the higher contribution in Dim. 1 (Y axis), while the T_max_f_N and the R400 show higher contribution in Dim. 2 (X axis).

As the predictor variables selected per cultivar were very similar, only the set of predictor variables obtained with TN data was used as the training dataset due to its higher number of observations (n=32). Thus, following the correlation analysis, the variables T_max_f_N, reflectance at 400 nm, reflectance at 532 nm, and reflectance at 737 nm were selected for the application of PCA.

A validation of the results, obtained for TN, was performed by applying a PCA to the TF data, using the variables selected for TN (Table 3). The validation results showed the same pattern, which was previously obtained

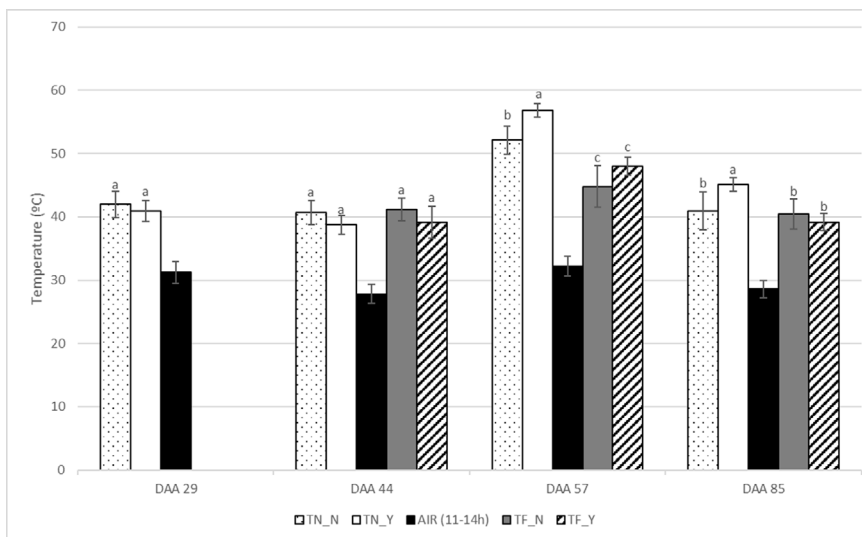


Figure 2: Average maximum temperatures of the leaves in Touriga Nacional without kaolin (TN_N), Touriga Nacional with Kaolin (TN_Y), Touriga Franca without kaolin (TF_N), Touriga Franca with kaolin (TF_Y) and the average maximum air temperature from 11 AM to 2 PM [AIR (11-14H)] in the field for the four measurement dates. Vertical bars are the standard deviation and within each measurement date columns with different letters are significantly different according the Duncan test ($p < 0.05$)

for the TN (Tables 2 and 3). The two first principal components explained 83.56% of the of the data variability and the wavelengths R532 and R737 present a higher level of contribution in the first principal component (Dim. 1) while the variables T_max_f_N and R400 have a higher weight in the second principal component (Dim. 2).

The Figure 4 presents the results for the PCA of leaf spectral and leaf temperature for TF in axis ordination planes, with kaolin treatment as response variables. The PCA results show the separation of leaves with (Y) and without kaolin (N), especially on the second (DAA 44) date. However, on the third (DAA 57) and fourth (DAA 85) dates, data for leaves with and without kaolin were closer.

Figure 5 shows the TF data aggregation, in six groups, according to a cluster analysis, based on the variables T_max_f_N, R400, R520, and R737. It is possible to see a good combination of the data according to the dates and the leaf's treatment (with and without kaolin) in the four first groups (G1-G4). In G5 and G6 the leaves were grouped

according to the treatment, although G6 contains one observation relative to non-kaolin treatment while the remaining observations were all from the kaolin treatment. Moreover, in G5-G6, the grouping of data, relative to the third and fourth dates, was observed.

4 Discussion

The presented results show a decrease, although not statistically significant, of the leaves' temperature, when sprayed with kaolin particle film, until DAA 44 (Figure 2). Also, the effect of reflectance of kaolin-sprayed leaves (Figure 3) was consistently higher than those of without kaolin, until DAA 44. In contrast, at DAA 57, leaves with kaolin, for both varieties, achieved temperatures which were significantly higher ($p < 0.000$) than leaves without kaolin. Contrarily to other dates, at DAA 57 there was no reflectance differences, at around 531-535 nm (Figure 3), between leaves from both treatments. According to

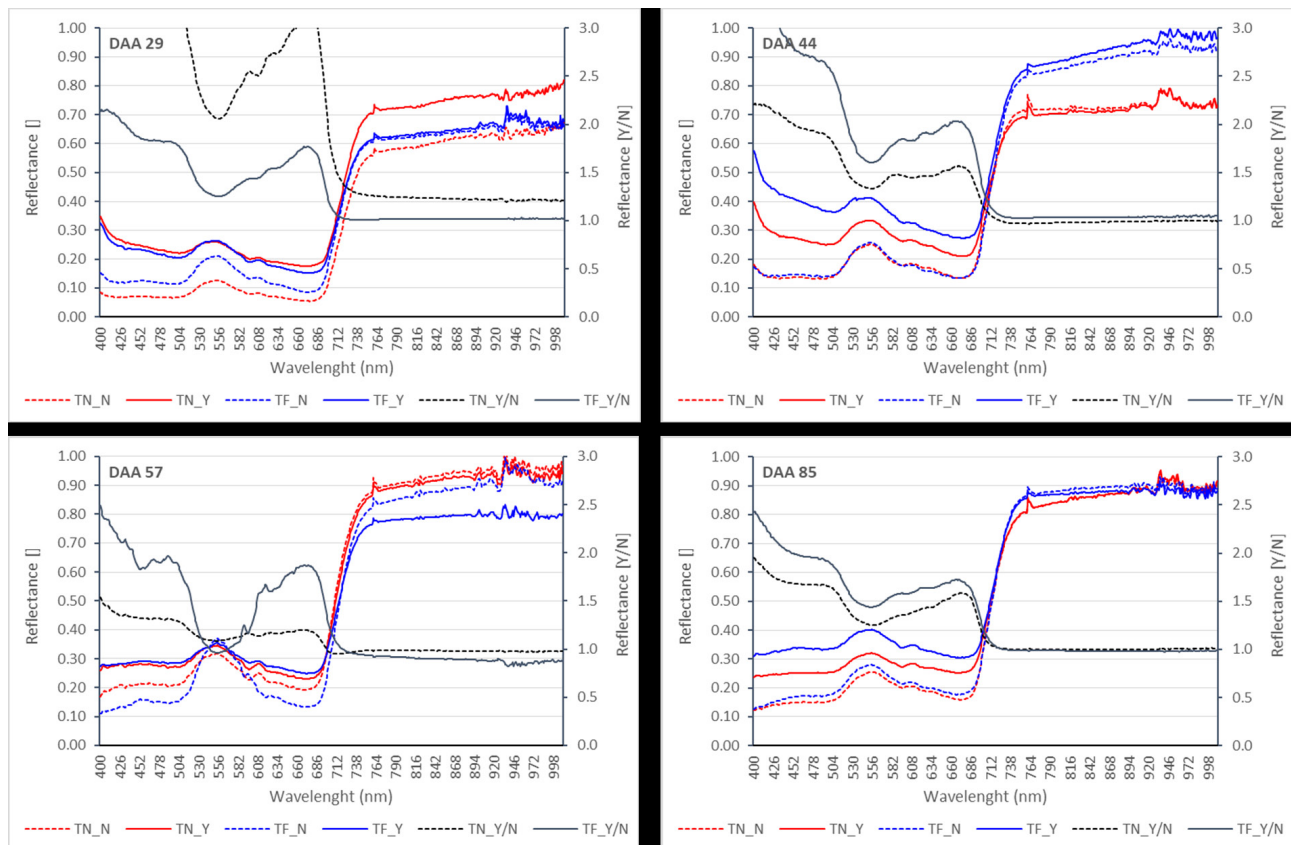


Figure 3: Average reflectance recorded in leaves of Touriga Nacional without kaolin (TN_N), Touriga Nacional with Kaolin (TN_Y), Touriga Franca without kaolin (TF_N), Touriga Franca with kaolin (TF_Y) during the four-measurements dates. The secondary Y axis represents the ratio of reflectance of leaves with kaolin per leaves without kaolin for TN (TN_Y/N) and TF (TF_Y/N)

Table 2: Variance explained by principal component analysis and the contribution of each principal component (Dim.) for Touriga Franca (TF) and Touriga Nacional (TN) as well as the contribution of each variable to the principal component

| Varieties | PCA dimension | Eigen value | Variance % | Cumulative variance % | Contribution of each Principal Component | | | |
|-----------|---------------|-------------|------------|-----------------------|--|-------|-------|-------|
| | | | | | T_max_f_N | R400 | R542 | R720 |
| TF | Dim. 1 | 2.36 | 58.96 | 58.96 | 15.70 | 19.22 | 36.88 | 28.19 |
| | Dim. 2 | 1.04 | 26.01 | 84.97 | 46.59 | 45.39 | 2.62 | 5.39 |
| | Dim. 3 | 0.44 | 11.03 | 96.00 | 30.14 | 6.30 | 2.13 | 61.43 |
| | Dim. 4 | 0.16 | 4.00 | 100.00 | 7.57 | 29.08 | 58.37 | 4.98 |
| TN | | | | | T_max_f_N | R400 | R532 | R737 |
| | Dim. 1 | 2.44 | 61.07 | 61.07 | 21.31 | 10.56 | 36.50 | 31.63 |
| | Dim. 2 | 1.22 | 30.38 | 91.45 | 27.59 | 58.27 | 6.31 | 7.83 |
| | Dim. 3 | 0.29 | 7.23 | 98.68 | 49.64 | 7.41 | 0.62 | 42.33 |
| Dim. 4 | 0.05 | 1.32 | 100.00 | 1.47 | 23.76 | 56.57 | 18.20 | |

PCA – Principal component analysis; T_max_f_N – maximum temperature of the leaves; R400 – reflectance at 400 nm; R542 – reflectance at 542 nm; R532 – reflectance at 532 nm; R720 – reflectance at 720 nm; R737 – reflectance at 737 nm.

Table 3: Variance explained by the principal component analysis and the contribution of each principal component (Dim.) for cultivar Touriga Franca (TF), as well as the contribution of each variable to the principal component, when the selected variables for cultivar Touriga Nacional (TN) were tested in TF data

| | Eigen value | Variance % | Cumulative variance % | Contribution of each Principal Component | | | |
|--------|-------------|------------|-----------------------|--|-------|-------|-------|
| | | | | T_max_f_N | R400 | R532 | R737 |
| Dim. 1 | 2.16 | 54.10 | 54.10 | 16.43 | 24.14 | 39.30 | 20.14 |
| Dim. 2 | 1.18 | 29.47 | 83.56 | 36.05 | 36.42 | 6.09 | 21.44 |
| Dim. 3 | 0.54 | 13.39 | 96.95 | 39.06 | 0.09 | 2.74 | 58.10 |
| Dim. 4 | 0.12 | 3.05 | 100.00 | 8.45 | 39.35 | 51.87 | 0.32 |

PCA – Principal component analysis; T_max_f_N – maximum temperature of the leaves; R400 – reflectance at 400 nm; R532 – reflectance at 532 nm; R737 – reflectance at 737 nm.

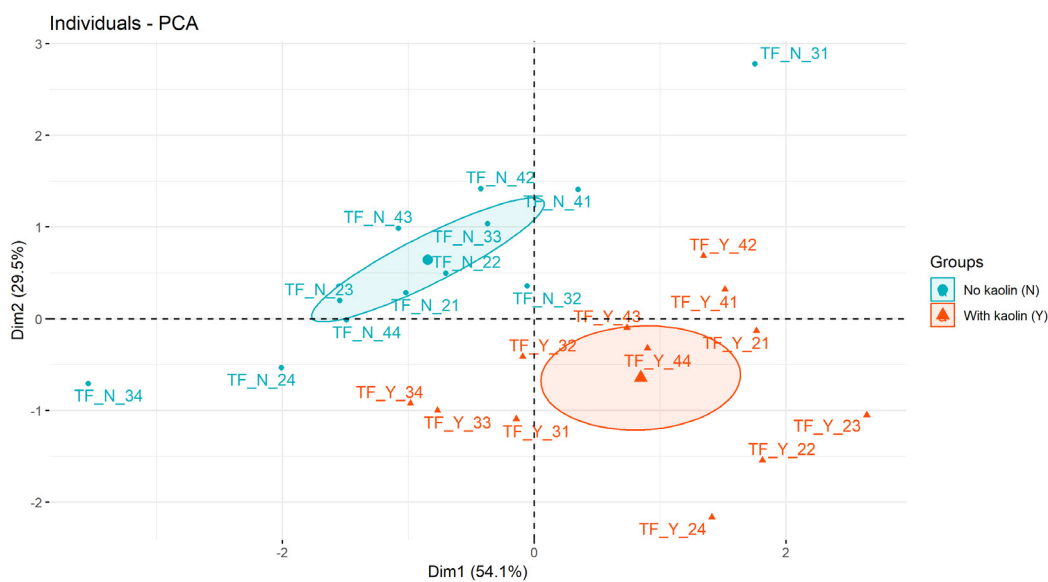


Figure 4: Principal Component Analysis for kaolin treatment in Touriga Franca (TF) leaves. The first digit refers to the days after application (DAA): 2: DAA 44; 3: DAA 57; 4: DAA 85 and the second digit is the repetition number (1-4)

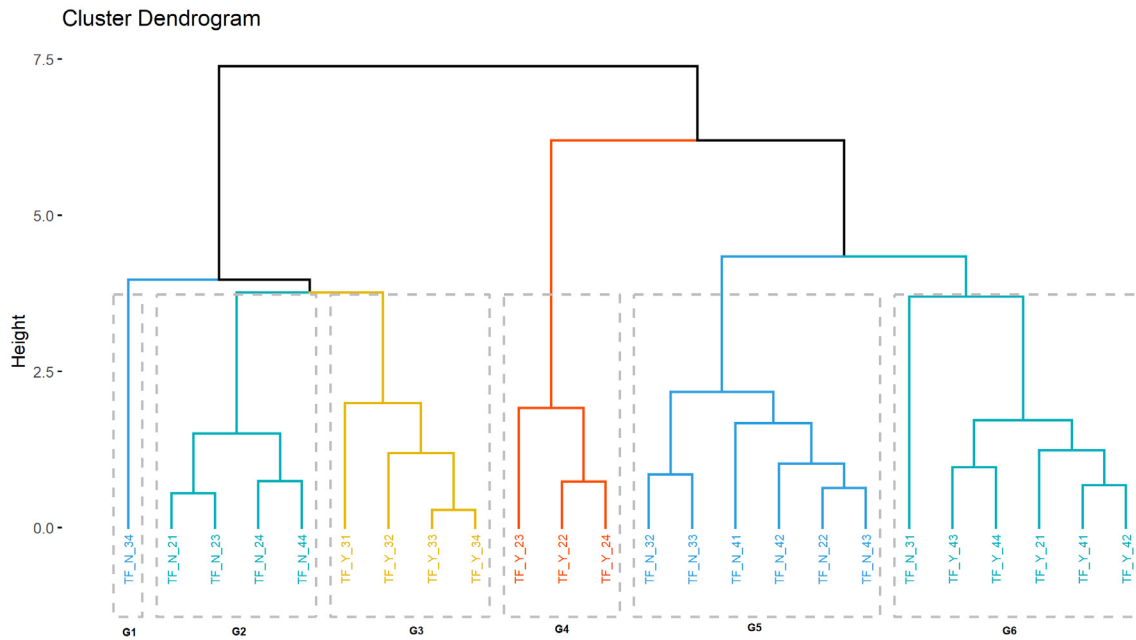


Figure 5: Cluster analysis grouped in six groups. Cultivar Touriga Franca without kaolin – TF_N, Touriga Franca with kaolin – TF_Y. The first digit refers to the days after application (DAA). 2: DAA 44; 3: DAA 57; 4: DAA 85 and the second digit is the repetition number (1-4). The letter G refers to the group aggregation (1-3) of the cluster analysis

Middleton et al. (2012), this spectral zone is strongly related to dissipation of heat by the leaves.

These findings suggest that the protective effect of kaolin was lower on the third and fourth dates of measurements due to the environmental factors that were responsible for the loose of the product in the leaves (Cantore et al. 2009), e.g., wind, or the occurrence of precipitation. When the leaves loose the kaolin particle film and the stomates close, the plants will be more likely to be exposed to stress conditions and will reduce the leaf transpiration that would relieve the leaves from the high heating (Glenn et al. 2010; Shellie and Glenn 2008; Shellie and King 2013). Consequently, the leaves with less kaolin would increase in temperature, as shown in the Figure 2, and physiologically behave more similarly to leaves without kaolin.

The separation of leaves with and without kaolin, shown in the Figure 5, can be explained by the variances found in different levels of both leaves' reflectance and temperature. As shown by Shellie and King (2013) and observed in the Figure 3, the reflectance of leaves with kaolin is higher than leaves without kaolin. As the kaolin particles film indirectly induces the reduction of leaf temperature (Glenn et al. 2010; Ou et al. 2010; Shellie and King 2013), the segregation into groups with and without kaolin confirms this circumstance. In addition, the group G6, which mainly encompasses leaves with kaolin from

DAA 85, suggests that kaolin loses its effect with time as previously discussed and a new application is required to guarantee the same effect, as the last application of kaolin is usually at the beginning of grape ripening (Brillante et al. 2016).

The plant physiology can provide insights for explaining the differences in leaves' temperature and reflectance between the cultivars. In a study conducted in the Douro Valley, where three distinct cultivars were analysed, it was found that cultivars with low chlorophyll concentration presented a brighter green colour and a higher rate of chlorophylls a and b (Chl a/b ratio) that will promote a low photo absorbance (Moutinho-Pereira et al. 2007). Thus, the differences between TN and TF, shown in Figures 2 and 3, may be related to the differences in Chl content among cultivars. Additionally, kaolin will increase the whiteness of leaves, which will promote high levels of reflectance and probably decrease the temperature (AbdAllah et al. 2018; Jifon and Syvertsen 2003).

While many studies have shown that kaolin film application is a powerful tool to improve crop development under stress conditions, others did not observe significant results with this practice. In a study with Persian walnut, the kaolin was positively associated with reduced leaf temperature, gas exchange rate, and sunburn and it also improved the quality of the nut and kernel (Gharaghani et al. 2018). Dinis et al. (2016) and Shellie and King

(2013) have demonstrated that kaolin is able to improve the quality of the grape, under stress conditions, by improving its pigment concentration and improving water use efficiency in some cultivars. However, some of these positive physiological effects of kaolin were recorded for a few days after kaolin application and/or under intensive treatments, namely with applications up to twice a week for three weeks (Brillante *et al.* 2016; Gharaghani *et al.* 2018; Glenn *et al.* 2010; Jifon and Syvertsen 2003; Ou *et al.* 2010; Shellie and King 2013). On the other hand, Dinis *et al.* (2016) sprayed kaolin on vines, twice in a day, to ensure the efficient adhesion of kaolin to the leaves and showed good efficiency of kaolin in grapevines, which is far from the agronomic and economic conditions of kaolin applications in commercial vineyards. Contrarily, Russo and Diaz-Perez (2005) did not obtain any improvement on physiological measurements, leaf temperature (also observed in the present study in the Figure 2), disease incidence or yields in two cultivars of peppers, following the use of several kaolin applications under stress conditions. Also, Ou *et al.* (2010) did not observe any significant differences in water potential, °Brix, pH and titratable acidity, with the application of kaolin, in Merlot grapevine cultivar, although the kaolin impact on the studied parameters varied with different levels of irrigation.

The variables selected, using the TN dataset to explain the separation of leaves with and without kaolin ($T_{max_f_N}$, and reflectances at 400 nm, 532 nm, and 737 nm), were successfully applied for TF, which indicates robustness of the achieved results. Additionally, these can be related to physiological processes of the energy dissipation in leaves. It is noted that there was a strong weight of the wavelength 532 nm in Dim. 1. The leaves with kaolin were mainly distributed on the side of the axis where R532 is projected, while the leaves without kaolin were on the opposite side. This area of the electromagnetic spectrum allows detection of subtle changes in the xanthophyll pigment activity cycle resulting from stress conditions, including thermal stress (Middleton *et al.* 2012). In Figure 3, it is noted that there was a visual difference in reflectance between the treatments, during the four-measurement times. When the kaolin was highly present in the leaves, the reflectance at the wavelength 532 nm was always high when compared with leaves without kaolin, suggesting that the level of oxidated xanthophylls is lower in leaves with kaolin. Nevertheless, in the third measurement (DAA 57), when the leaves have lost part of the kaolin particle film, the reflectance at this wavelength is alike to the leaves without kaolin film. This observation may be explained by the comfort level that kaolin

promotes on leaves. The leaves that were protected earlier with the particle film might not have developed enough pigments to protect them from the high incidence of solar radiation when the particles film has lost its adherence to the leaves. This argument might support the unsuccessful impact of the application of kaolin in the physiological parameters analysed by Russo and Diaz-Perez (2005).

The second variable ranked in Dim. 1 is R737, which corresponds to the red edge region of the electromagnetic spectrum (sharp transition of vegetation's reflectance between red and near-infrared spectral ranges: 680 – 740 nm). The red edge has often been considered as an indicator of plant stress responses (Behmann *et al.* 2014; Filella and Penuelas 1994; Zarco-Tejada *et al.* 2013). Figure 3 shows the differences in reflectance of this zone of the spectrum between the leaves of both treatments, mainly at DAA 44 for TF. During this second measurement date, the red-edge reflectance of leaves with kaolin was higher, suggesting that these leaves were more likely to support stress conditions. As shown by Glenn *et al.* (2010), the kaolin particle film can increase the water potential in well-watered vines, minimizing the heating stress. Although, from the second until the last measurement-time of our study (DAA 44, DAA 57, and DAA 85), there was no visual difference from the red-edge reflectance in this zone. Nevertheless, it is noteworthy that all grapevines were under (increasing) moderate to high water stress throughout our study period (Table 1).

In Dim. 2, the $T_{max_f_N}$ presents a dominant role in the distribution of the observations, which agrees with the effect of kaolin to control leaf temperature in vines under drought conditions (Dinis *et al.* 2016). Likewise, the lower temperature is associated with better water use efficiency in vines with kaolin (Glenn *et al.* 2010). Yet, when the reflectance become alike between the leaves with and without kaolin, we suggest that there was no difference in the temperature in the leaves from both treatments and the leaves that were previously protected with particle film can also increase in temperature due to the lack of protection mechanisms presented in the leaves, such as the xanthophylls.

5 Conclusion

These preliminary results improve the understanding of the spectral and thermal response of vines to kaolin throughout the grapevine cycle and support decisions about the re-application timing. The results show that the kaolin film protects the leaves against thermal stress, promoted by the solar radiation, while the product is

strongly present on the leaves. The effect of the kaolin film on the leaves may be explained by the concentration of foliar pigments such as the xanthophyll, which is related to the dissipation of heat in the leaf. Even though this work has shown the potential of spectral and thermal data to explain the effect of kaolin in different cultivars of grapevines, physiological and biochemical analysis should be further tested in future work to complement and strengthen the findings presented in this paper. Additionally, future studies should consider the effect of kaolin (e.g. potential increase in pH) in the wine quality of Portuguese varieties, in the particular climatic conditions of the Douro Valley.

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