



Modeling grapevine leaf reflectance and physiological response to increasing kaolin doses and varying light and water availability

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ARTICLE INFO

Keywords:

Particle film technologies
Reflectance
Transmittance
Light response curve
Quantum yield
Photosynthesis
Vitis vinifera L.

ABSTRACT

Kaolin is a white clay powder available in various formulations, commonly used to mitigate the negative effects of water and heat stress on a range of crops. According to existing literature, while minimum doses are required to ensure sufficient photoprotection, excessive applications may reduce net carbon assimilation, but such thresholds are yet undefined. In this study, potted grapevines were treated with foliar applications of kaolin at concentrations of 3 % w/v, 5 % w/v, and 7 % w/v, and compared to untreated controls. Vines were then subjected to progressive water stress up to the achievement of a midday stem water potential of -1.3 MPa, and then fully rewatered until the end of the experiment. The objective was to evaluate the effects of different kaolin dosages on leaf physiological performances under varying light and water availability conditions.

Under full water supply, no significant differences in leaf net photosynthetic rate or transpiration were observed between control and vines sprayed with kaolin 3 % w/v. In contrast, a 7 % w/v treatment significantly reduced leaf net photo-assimilation rates at photosynthetically active radiation levels between 50 and 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ compared to other treatments. At a stem water potential of -1.3 MPa, all vines exhibited stomatal closure. However, following rewatering, vines treated at 7 % w/v displayed higher leaf photosynthetic rates under light-saturating conditions, exceeding the control by $+1.31 \mu\text{mol m}^{-2} \text{s}^{-1}$. Notably, control vines showed significantly lower photosynthesis than all other treatments at irradiance levels between 300 and 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Additionally, median leaves were more responsive to kaolin treatments than basal or apical leaves. Increasing kaolin doses progressively enhanced leaf reflectance in both the visible and, more notably, the infrared wavelengths. The 5 % w/v and 7 % w/v treatments significantly increased the leaf Photochemical Reflectance Index compared to control vines, while 3 % w/v doses exhibited intermediate values.

Our results demonstrate that a 3 % w/v kaolin application provides some photoprotection under stress conditions, with higher doses offering limited additional benefit in preserving canopy functionality. In contrast, increasing the dose to 7 % w/v can reduce maximum photosynthetic capacity under water non-limiting conditions, likely due to enhanced reflectance that lowers light-use efficiency and raises the light saturation point. Therefore, high doses should be considered only when the specific goal is to limit photosynthetic activity, such as to reduce sugar accumulation in grapes and delay ripening.

1. Introduction

Summer stresses are the most impactful limiting factors for agricultural productivity (Maes and Steppe, 2012; Moriondo et al., 2011). Grapevine is one of the most cultivated tree crops in the world, and while a limited fraction of the total vineyards can rely on irrigation water, most growers run rainfed vines and are forced to undertake

alternative site-specific techniques in order to obtain satisfying yields and maintain desired grape quality (Pascual et al., 2015; Scholasch and Rienth, 2019). With this regard, kaolin is today considered one of the most flexible and popular tools to reduce the negative effects of summer stress on canopies and grapes in the absence of irrigation water (Dinis et al., 2018; Frioni et al., 2019; Tekler, 2023; Valentini et al., 2021; Wang et al., 2020).

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<https://doi.org/10.1016/j.plaphy.2025.110738>

Received 18 September 2025; Received in revised form 30 October 2025; Accepted 4 November 2025

Available online 8 November 2025

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Kaolin is a white clay prepared into various formulations for canopy applications. Once sprayed, it forms a reflective particle film that reflects a fraction of incident light and reduces canopy and fruit temperatures (Glenn, 2012; Rosati et al., 2007; Shellie and King, 2013). Several works describe the effects of kaolin in different latitudes, on different grapevine varieties and in various environmental conditions (Brillante et al., 2016; Copp et al., 2025; Frioni et al., 2019; Michael Glenn et al., 2010; Pallotti et al., 2025; Teker, 2023; Waber et al., 2025; Wang et al., 2020). While the concept behind its mechanism of action is clear, less is known about the magnitude and thresholds at which the effects of kaolin are changing. Several authors report a reduction of tissue temperature after kaolin application, but little information is available about the quantity and quality of light intercepted and reflected by kaolin, and which are the consequences on leaf functionality under different water availability or temperatures (Copp et al., 2025; Dinis et al., 2018; Lobos et al., 2015; Valentini et al., 2021).

The first works studying leaf optical properties after kaolinite (i.e., a refined form of kaolin) application date to 1970. Abou-Khaled et al. (1970) showed that leaf light reflectance is directly correlated to kaolinite leaf coverage and treated and untreated leaves exhibited different photosynthetic rates (A_n) at varying light levels. In detail, kaolinite was reducing *Ficus* and *Citrus* leaf A_n at low photosynthetically active radiation (PAR) and increased A_n gains at high PAR levels. Conversely, leaf transpiration (E) was reduced independently by the light level and/or the species considered. More recently, in coffee, Steiman et al. (2007) noted a reduction of PAR and UV transmission following foliar application of kaolin in field and demonstrated that increased kaolin coverage further reduced light transmission in laboratory conditions. In the same framework, in walnut and almond trees, Rosati et al. (2007) showed that kaolin treatments improved canopy photosynthesis by reflecting PAR and enhancing light distribution. However, individual leaves experienced a reduction in A as reflectance limited PAR reaching photosystems. Glenn and Puterka's (2005) review of previous works demonstrates the multifaceted effects kaolin offers, particularly when comparing various species, allowing an understanding that in each species, there is an inherent response which affects the efficacy of particle films on gas exchanges.

In grapevine, Shellie and King (2013) demonstrated that application of kaolin at 6 % reduced the time exposure of leaves and berries to temperatures >30 °C. More recently, Tosin et al. (2019) and Garrido et al. (2019) showed that in areas with high solar radiation and excessive temperature, foliar kaolin applications increased reflectance in the visible range by as much as twofold. Copp et al. (2025) reported a clear, progressive dose-dependent increase in leaf reflectance with rising kaolin concentrations by up to 86 %, matched by a decrease in absorbance by up to 8 % and of transmittance by up to 19 %. Regarding photosynthetic performances, most authors agree that kaolin improves post-stress canopy functionality by preserving photosystem integrity under limiting conditions and allowing for better thermoregulation (Bernardo et al., 2018a; Dinis et al., 2018; Frioni et al., 2019; Glenn, 2012; Luciani et al., 2020). However, several papers show that kaolin applied at high dosages can reduce vine photosynthetic rates in specific conditions, agreeing with works on other species. Frioni et al. (2019) showed that under full water availability, kaolin reduced leaf A_n and E, yet under imposed water stress, effects were reversed. Similar outputs were found in hazelnut, citrus, almond and walnut (Abou-Khaled et al., 1970; Luciani et al., 2020; Rosati et al., 2007). Authors agree that this was due to a reduction of photon flux density below the light saturating threshold and/or to an eventual partial coverage of stomatal opening. However, three main crucial questions are pending answers to optimize field kaolin treatments: i. What are the doses causing a reduction in maximum photosynthetic gains? ii. How does leaf reflectance and photosynthesis change at varying PAR, according to different kaolin doses? iii. Is it possible to obtain minimal or null effects on photosynthetic gains under non-limiting conditions and at the same time, satisfying protection from subsequent severe water and heat stress?

The aim of this work was to explore the effects of different kaolin dosages on leaf reflectance and gas exchange parameters, under varying water and light availability. Taking grapevine as a model tree plant, our hypothesis was that kaolin interacts with leaf light absorbance and, accordingly, with photosynthetic gains at varying PAR levels. Our broad goal was identifying the optimal doses to maximize the preservation of photosystems efficiency, with null adverse effects on photosynthetic gains due to the increase of PAR light reflection.

2. Materials and methods

2.1. Plant materials, experimental layout, and water supply

Two experiments were conducted on 25 one-year-old vines (*Vitis vinifera* L.) cv. Sangiovese (clone VCR5, grafted onto SO4 rootstock) grown in 12 L pots. All vines were potted within a substrate of sand and clay at a ratio of 70:30 on March 7th, 2024, and placed in an outdoor area of Università Cattolica del Sacro Cuore, in Piacenza (45°02'12" N, 9°43'41" E). Sixteen vines were used for experiment 1 and assigned to the following treatments (four vines per treatment): i. an untreated control (C); ii. Kaolin foliar application at 3 % w/v (K3); iii. Kaolin foliar application at 5 % w/v (K5), iv. Kaolin foliar application at 7 % w/v (K7). The kaolin used in this work (Polvere di Roccia, Biogard – CBC Europe, Grassobbio, BG, Italy) is a pure preparation of aluminum silicate obtained by the mechanical grinding of non-calcined kaolinite, quartz, mica. The formulation is composed of extremely white kaolin particles with a purity of 99.9 % and a diameter <40 μm , for a relative density of 2.6 g/cm³. When added to water, pH of solution is comprised between 6 and 8. In a previous work, application of the same formulate on grapevine canopies at 3 % concentration led to an increase of leaf reflected PAR from 10.1 % to 15.1 %, and a reduction of transmitted PAR from 8.3 % to 6.9 % (Frioni et al., 2020). The aqueous component of the mixtures prepared for the different treatments evaporated within approximately 20 min after spraying, leaving the kaolin particles uniformly deposited on the adaxial leaf surface. A distribution of 3 % w/v leaves a 28 % leaf coverage according to Palliotti et al. (2019).

Preparations of the Kaolin mixtures were 30g, 50g, and 70g of product per L of water, corresponding to an application of 9, 15 and 21 g kaolin per plant and 30, 50 and 70 kg/ha (considering 3333 vines/ha), for K3, K5 and K7 respectively. Spraying was done using a hand pump on July 8th, 2024, right before the occurrence of high air temperatures, on all sides of the canopy, applying 300 mL of solution per plant. The 9 remaining extra vines were later used for experiment 2.

Each vine was fertilized with 3g of Nitrogen, 0.5 g of P and 1 g of K divided in three applications of ammonium nitrate and Soluplant (Haifa Italia Srl, Bologna, Italy) over the course of the experiment (May 8th, May 21st and June 4th). Vines were standardized on April 16th, 2024 (BBCH 15, according to Lorenz et al., 1995), retaining the two best developing shoots and removing the others. Selected shoots were allowed to grow vertically with the assistance of a stake. Pathogens were controlled according to local standard practices. Weather parameters (air temperature, relative humidity, irradiance, wind speed and direction, rainfall) were monitored on an hourly basis and logged using a weather station located 6m from the experimental vines.

2.2. Experiment 1: leaf light response curves during the progressive water deficit

All the 16 vines used for experiment 1 were watered to field capacity daily until July 11th, 2024. At which point, water was shut off and midday stem water potential (Ψ) was monitored every two days on each vine. When midday stem Ψ achieved an average of -1.3 MPa (occurred on July 17th, 2024), full irrigation was resumed starting from the subsequent morning, until the end of the season.

From July 9th to July 19th, midday stem water potential (Ψ) was periodically (every two days during water deficit, every 3–5 days under

full irrigation) measured on one leaf per vine (four per treatment). One mature median leaf per vine was wrapped in aluminum foil for an hour. Leaves were detached and Ψ measured with a Scholander pressure chamber.

Leaf gas exchange parameters were measured using the portable gas exchange LCI T analyzer (ADC Bio Scientific Ltd., Hertz, UK). The system utilizes a broad (6.25 cm²) cuvette chamber and measurements were taken at ambient temperature and relative humidity with adjustment of airflow at 200 mL min⁻¹. Leaf photosynthesis (A_n), transpiration (E), temperature (T) and instantaneous water use efficiency (WUE, calculated as A_n/E) light response curves were built for four mature leaves (one per vine, inserted on nodes 7 to 9) per treatment on July 9th (under full irrigation), on July 17th (peak of water deficit, stem water potential = -1.3 MPa), and on July 25th (at rewatering), between 9:00 and 11:00. Two readings were made along a sequence of photosynthetically active radiation (PAR) equaling to 0, 50, 100, 300, 600, 900, 1200, 1500, and 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, equipping the LCI T leaf cuvette with the LED light unit provided with the instrument with adaptation intervals of 10 min.

2.3. Experiment 2: leaf gas exchanges, reflectance, and fluorescence before and after kaolin application

On August 6th, 2024, the nine vines excluded from experiment 1 were assigned to the treatments K3, K5 and K7 (three vines per treatment). One basal (nodes 2 to 6), median (nodes 7 to 14) and distal (nodes 14 to 18) leaves per vine were identified and tagged. Then, at 12:00 leaves were individually sprayed with the respective solutions as described above in experiment 1, using screens to avoid drifting. The mixtures prepared for the different treatments required approximately 20 min for the evaporation of the aqueous component.

Leaf A_n , leaf E, leaf stomatal conductance (g_s) and leaf WUE were measured on each leaf immediately before spraying (BS), and 30 min after spraying, under saturating light conditions (PAR $\geq 1400 \mu\text{mol m}^{-2} \text{s}^{-1}$) using the same analyzer described above, unmounting the light unit. Gas exchanges were analyzed at ambient temperature and relative humidity, with an airflow adjusted to 200 mL min⁻¹. Variation in post-spraying leaf A_n , E, g_s and WUE, compared to the BS conditions were calculated.

A VIS-NIR spectral signature was obtained on the above-mentioned leaves before and after spraying using an ASD FieldSpec® HandHeld 2 VNIR spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO, USA), which operates across a wavelength range of 325–1075 nm, with an accuracy of ± 1 nm and a resolution of < 3 nm at 700 nm. The device includes a fiber-optic contact probe and a leaf clip, enabling for one-handed, non-destructive measurements on a leaf area of 0.8 cm². The raw hyperspectral data were filtered to retain only the relevant spectral bands, excluding the outer portions of the spectral signature. Specifically, portions of the electromagnetic spectrum below 390 nm and above 900 nm were removed to focus on the most reliable spectral regions, as these ranges are typically associated with higher instrumental noise or lower signal reliability. Prior to measurements, white reference was taken from the instrument's built-in white reference panel. Reference measurements were taken over the course of readings to maintain white reference. The resulting spectral signature was used to calculate vegetation indices such as the Photochemical Reflectance Index (PRI) (Gamon et al., 1997).

Leaf fluorescence was measured only on the basal leaves, before and after kaolin application. Light-adapted measurements for photosystems efficiency were taken using the OS5p + Modulated Chlorophyll Fluorometer (OPTI-SCIENCE, 8 Winn Avenue, Hudson, NH) on all labelled basal leaves. For chlorophyll fluorescence readings, leaves underwent dark adaptation for 30 min via use of a leaf-clip supplied with the OS5p + Modulated Chlorophyll Fluorometer before measurements were taken. For any parameter, the percentage difference to the pre-spraying condition was calculated as described above for leaf gas exchange parameters.

2.4. Statistical analysis

Data collected in experiment 1 to build the light response curves was subjected to a Repeated Measures analysis of variance, where kaolin treatments were the main factor and irradiance represented the levels of the repeated measures. Data collected in experiment 2 was first analyzed within single treatments to determine whether post-kaolin spraying means were different from pre-spraying conditions, by Student's *t*-test ($P < 0.05$). Then, the % post-spraying variation in leaf gas exchange and photosystems efficiency parameters was subjected to a one-way analysis of variance to determine whether means were significantly different between treatments. Student–Newman–Keuls (SNK) test was performed to separate means per $p < 0.05$, in case of the significance of the F-test. Correlations between variables were built by means of regression analysis through SigmaPlot 12 (Systat Software Inc., San Jose, CA, USA.) Heatmap generation was created with the use of Microsoft Excel (Microsoft, Redmond, WA, USA.).

Generalized additive mixed models (GAMMs) were used to assess the impact of kaolin treatments on the hyperspectral reflectance (420–850 nm) of leaves at different node levels (basal, mid, apical). GAMMs capture non-linear relationships by summing smooth functions rather than relying solely on additive linear terms, offering greater flexibility compared to traditional linear models (Antonucci et al., 2021; Wieling, 2018). To prevent overfitting, the model fitting process incorporated penalization that balances data fidelity with model complexity by reducing wiggleness (Pedersen et al., 2019). Models were fitted in R using cubic regression splines with the *itsadug* package (van Rij et al., 2019) and followed a structured process of selection, fitting, and validation (Zuur et al., 2009). Unlike conventional single-band or index-based approaches, GAMMs model the full spectral response as a smooth, continuous function, allowing wavelength-resolved inference of significant reflectance differences (H/L/N regions) across treatments and canopy layers. Additionally, treatment effects were evaluated using a linear mixed model (R Core Team, 2023). Specifically, the GAMM incorporated main effects and interactions between treatment and leaf position, smooth terms for wavelength, and a random effect to account for plant-specific variability. Smoothing parameters were optimised via penalised splines (ps), and smooth interaction enabled the capture of complex non-linear relationships.

To illustrate spectral response differences between treatment, difference plots were generated using the *plot.diff* function. These plots compared reflectance values across the spectral range between before-spraying and after-spraying conditions for each treatment group (Kaolin 3 %, 5 %, 7 %) and for each leaf position.

3. Results

3.1. Weather data

During the period of July 6th to 29th 2024, the maximum air temperature exceeded 30 °C every day, averaging 33.6 °C. The minimum air temperature averaged was 22.3 °C, while the daily mean air temperature was 28 °C. Vapor pressure deficit (VPD) reflected daily temperature changes with values ranging from slightly below 1 kPa during cooler hours to about 4 kPa at peak heat. By July 6th, the recorded growing degree-day (GDD) from April 1st were 873.95, accumulating to 1271.65 by July 29, averaging 18 GDD per day (Supplementary Fig. 1). A total of 4.2 mm of rainfall was observed over three days (July 7th, July 19th and July 20th) during the entirety of experiment 1.

The maximum photosynthetically active radiation (PAR) peaked daily at 11:00 a.m., reaching an average of 1770 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Supplementary Fig. 2).

3.2. Experiment 1: light response curves at different kaolin doses during

On the day before water stress imposition (July 9th, 2024), K3, K5

and C achieved similar maximum rates of leaf A_n at saturating PAR (Fig. 1a). In contrast, K7 from PAR 300 onwards showed a significantly lower leaf A_n than any of the other treatments ($-5.32 \mu\text{mol m}^{-2} \text{s}^{-1}$ than C at PAR = 900). K5 displayed slightly lower leaf A_n rates than C and K3 at PAR ranging between 300 and 1200. At the measurements taken on July 17th, 2024, the peak of water stress conditions, both leaf A_n and leaf E were zeroed in all treatments (Fig. 1b and e).

At re-watering, all treatments showed significantly lower leaf A_n than pre-stress conditions, at any PAR level. However, K5 and K7 exhibited the highest leaf A_n amongst treatments for PAR ≥ 1500 ($+0.66 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $+1.31 \mu\text{mol m}^{-2} \text{s}^{-1}$ than C, respectively, at PAR = 1800) (Fig. 1c). C vines showed significantly lower A_n than any other treatments for PAR ≤ 900 .

Similarly to leaf A_n , under full water availability, K7 vines leaf E (Fig. 1d) was consistently lower than all other treatments from 300 PAR upwards. In contrast, at re-watering leaf E was higher in K3 compared to the rest of the treatments for PAR ≥ 300 (Fig. 1f). On the same date, both K5 and K7 maintained comparable levels of leaf E, lower than K3, yet significantly higher than C vines for PAR ≥ 900 ($+0.55 \text{mmol m}^{-2} \text{s}^{-1}$ and $+0.92 \text{mmol m}^{-2} \text{s}^{-1}$, respectively, at PAR = 1800).

Under full water availability, C leaves exhibited a higher WUE compared to the treated vines at low PAR levels (50 and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 2a). At PAR ranging from 0 to 900, WUE was consistently decreasing with increasing kaolin doses. However, at higher light levels and full irrigation, all treatments maintained comparable WUE.

At re-watering, K7 had a significantly higher WUE than any other treatment at irradiance of 100–300 PAR (Fig. 2b). Under higher irradiance, C vines maintained higher levels of WUE than any other treatment,

up to 1500 PAR, when no clear differences between treatments were visible.

While no differences among treatments were observed in leaf T across light levels before the onset of water stress (Fig. 3a), a marked and progressive leaf overheating (up to 44.8 °C) was recorded in C vines at increasing irradiance levels during peak water stress conditions (Fig. 3b). In contrast, in treatments K3, K5, and K7, leaf temperature increased from 36.2 °C in dark conditions to 41.2 °C at full irradiance (values pooled across treatments), yet remained significantly lower than that of C vines at PAR $> 900 \mu\text{mol m}^{-2} \text{s}^{-1}$. After re-watering (Fig. 3c), C vines exhibited significantly higher leaf temperatures than any of the kaolin-treated vines across all light levels.

3.3. Experiment 2: leaf reflectance and changes in leaf physiology at varying kaolin doses

3.3.1. Leaf gas exchanges before and after kaolin application

On August 6th, all three kaolin treatments significantly reduced leaf A_n in basal leaves, as compared to pre-spray levels (Fig. 4a). Leaf E and leaf g_s tracked leaf A_n (Fig. 4d and g), with no significant changes in leaf WUE (Fig. 4j). Conversely, in median leaves, a progression of leaf A_n , E and g_s reduction was found when passing from K3 to K5 and K7 (Fig. 4b, e and 4h). While median leaves WUE was substantially unaffected by kaolin application in K3, WUE was reduced in K5 and K7 compared to pre-stress levels (-14.9% and -11.5% , respectively) (Fig. 4k). In apical leaves, despite a post-kaolin application general reduction in leaf A_n , leaf E, and leaf g_s was detectable in all treatments (Fig. 4c, f and 4i), only K3 significantly reduced water use efficiency compared to pre-spraying

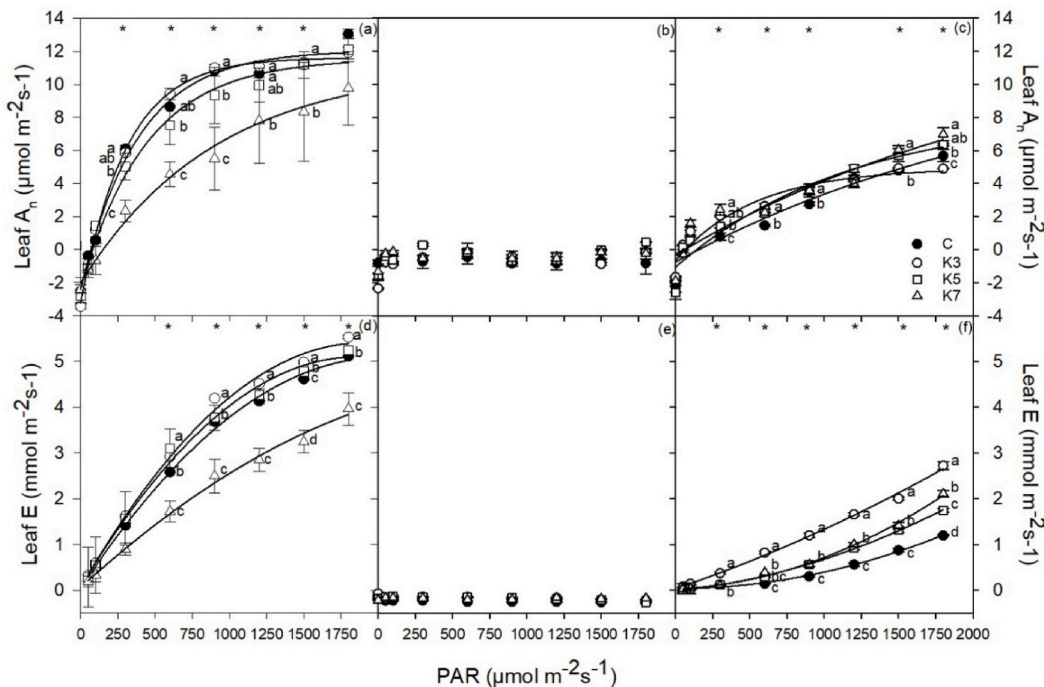


Fig. 1. Changes in leaf photosynthesis (A_n) – panels a, b and c - and leaf transpiration (E) – panels d, e and f - at varying photosynthetically active radiation (PAR), in vines subjected to application of Kaolin at 3 % (K3), 5 % (K5), and 7 % (K7) and control vines (C). On the left, measures taken under full water availability (panels a and d), in the middle (panels b and e), measures taken under severe water stress (stem water potential = -1.3 MPa), and on the right (panels c and f) measures taken after re-watering. Vertical bars represent standard errors ($n = 4$). Panel a: C, $y = -2.38*14.42(1-\exp(-0.003x))$, $R^2 = 0.99$, $p < 0.05$; K3, $y = -3.21*14.84(1-\exp(-0.003x))$, $R^2 = 0.99$, $p < 0.05$; K5, $y = -2.27*13.82(1-\exp(-0.002x))$, $R^2 = 0.99$, $p < 0.05$; K7, $y = -2.00*13.19(1-\exp(-0.001x))$, $R^2 = 0.99$, $p < 0.05$. Panel b: C, $y = -0.75*12.58(1-\exp(-0.0004x))$, $R^2 = 0.92$, $p < 0.05$; K3, $y = -0.66*5.58(1-\exp(-0.002x))$, $R^2 = 0.94$, $p < 0.05$; K5, $y = -1.06*9.29(1-\exp(-0.001x))$, $R^2 = 0.94$, $p < 0.05$; K7, $y = -0.33*13.48(1-\exp(-0.004x))$, $R^2 = 0.89$, $p < 0.05$. Panel c: C, $y = 0.08 + 0.005x + (-1.46*10^{-6})x^2$, $R^2 = 0.99$, $p < 0.05$; K3, $y = 0.066 + 0.006x + (-1.49*10^{-6})x^2$, $R^2 = 0.99$, $p < 0.05$; K5, $y = 0.076 + 0.005x + (-1.46*10^{-6})x^2$, $R^2 = 0.99$, $p < 0.05$; K7, $y = 0.06 + 0.003x + (-4.87*10^{-7})x^2$, $R^2 = 0.99$, $p < 0.05$. Panel d: C, $y = 0.04 + (-7.18*10^{-6})x + (3.64*10^{-7})x^2$, $R^2 = 0.99$, $p < 0.05$; K3, $y = 0.033 + 0.001x + (1.95*10^{-7})x^2$, $R^2 = 0.99$, $p < 0.05$; K5, $y = -0.01 + 0.0003x + (3.66*10^{-7})x^2$, $R^2 = 0.99$, $p < 0.05$; K7, $y = 0.03 + 0.0001x + (5.60*10^{-7})x^2$, $R^2 = 0.99$, $p < 0.05$. Different letters denote significant difference between treatments within light levels per $P < 0.05$ ($n = 4$). *, ** and *** indicate significant difference per $P < 0.05$, $P < 0.01$, and $P < 0.005$.

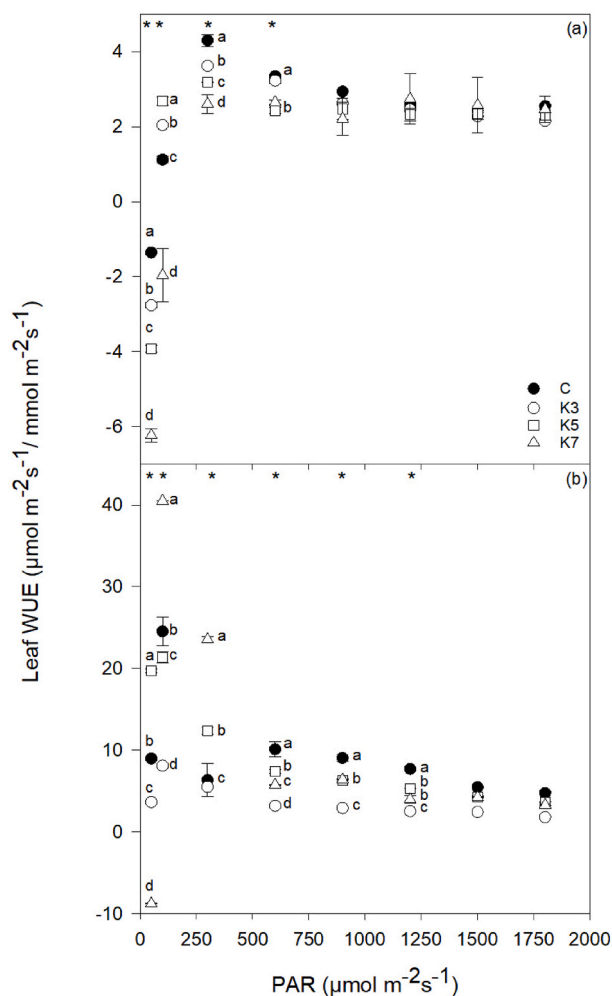


Fig. 2. Changes in instantaneous leaf water use efficiency (WUE) at varying photosynthetically active radiation (PAR) in vines subjected to application of Kaolin at 3 % (K3), 5 % (K5), and 7 % (K7) and control vines (C). Measures taken under full water availability (panel a) and post-stress recovery (panel b). Vertical bars represent standard errors ($n = 4$). Different letters denote significant difference between treatments within light levels per $P < 0.05$ ($n = 4$). *, **, and *** indicate significant difference per $P < 0.05$, $P < 0.01$, and $P < 0.005$.

levels (Fig. 4l).

3.3.2. Spectral reflectance response

Spectral analysis revealed that leaf optical properties underwent significant, dose-dependent modifications following kaolin application. As evidenced in Fig. 5a–c, reflectance generally increased with kaolin concentration ($K3 < K5 < K7$) compared to the pre-spray baseline (BS). The magnitude of these changes varied by leaf position/age. Mid-canopy leaves (Fig. 5b) exhibited the most pronounced response, with greater absolute increases in reflectance (up to 0.55 in the NIR with K7, in the 450–850 nm range) and, notably, more clearly separated median reflectance lines between treatments. Conversely, in basal (Fig. 5a) and apical (Fig. 5c) leaves, although increases in reflectance were observed (especially in the NIR, with values up to approximately 0.52 for K7 in basal leaves), the median reflectance lines between the different treatments appeared more overlapped.

Analyzing the Photochemical Reflectance Index (PRI), derived from reflectance at 531 nm and 570 nm, the PRI showed clear dose-dependent increases with kaolin application (Fig. 5d). Statistical analysis revealed significant differences ($P < 0.01$) between the higher concentration treatments (K5, K7) and both the BS and the low concentration (K3)

treatment. No significant difference was detected between BS and K3.

Difference analyses using generalized additive mixed models (GAMM) (Fig. 6) statistically confirmed these spectral changes. Regions which exhibited higher reflectance (“H”) relative to BS predominated across most of the spectrum for all treatments. However, wavelength-specific effects were observed: in shorter wavelength regions (< 570 nm), particularly for K3 and K5 treatments on basal and mid-canopy leaves, areas of non-significant difference (“N”) and significantly lower reflectance (“L”) were noted in some instances. Conversely, the NIR region (> 700 nm) consistently showed significantly higher increases in reflectance across all treatments and leaf positions, becoming more uniform and pronounced at higher kaolin concentrations (K5 and K7).

3.3.3. Effects of different kaolin doses on photosystems efficiency

Leaf minimum fluorescence (F_0) was progressively reduced by the increase of kaolin doses, compared to the pre-spraying conditions (from -7% of K3 to -18.7% of K7). The same occurred for maximum (F_m) and variable fluorescence (F_v) (Fig. 7). As a result, no differences in F_v/m or F_v/o compared to pre-spraying conditions, or between treatments were found. A significant decrease in post-spraying steady-state fluorescence level (F') was found in all the treatments compared to pre-spraying conditions, with a progressive magnitude (-26.6% in K3, -38.6% in K5 and -46.4% in K7). While there is no difference in the pre-spraying conditions in terms of saturation flash value (F_m') for K3 leaves, F_m' was significantly reduced by K5 and K7 application (33.1% and -46.7% compared to respective pre-spraying levels).

No changes in the maximum quantum yield (YII) and electron transport rate (ETR compared to the pre-spraying conditions) in any of the treatments.

4. Discussion

Kaolin is currently one of the most widely used tools to mitigate summer stress in vineyards, owing to the clear understanding of its mechanism of action and its high flexibility of application (Dinis et al., 2022; Valentini et al., 2021). However, although numerous studies have investigated the effects of kaolin on leaf physiology, often reaching differing conclusions depending on the experimental setup or environmental conditions (Bernardo et al., 2018b; Dinis et al., 2018; Frioni et al., 2019). For instance, several studies have suggested that kaolin may reduce carbon gain and act as an anti-transpirant-like tool, whereas others have reported no significant effects under water/temperature non-limiting conditions (Bernardo et al., 2018b; Dinis et al., 2018; Frioni et al., 2019; Valentini et al., 2021). In this framework, a dose-dependent assessment of modern kaolin formulations and their impact on grapevine leaf functionality is still lacking in the literature. In our work, the reflective properties of kaolin varied significantly with the applied dose, in accordance with Garrido et al. (2019) and Copp et al. (2025). However, our data demonstrates for the first time that if kaolin doses remain below 5 % w/v no detrimental effects on photosynthesis should be expected, under non-limiting water conditions despite the higher irradiance reflection. This outcome can be attributed to the relatively low size of kaolin particles ($< 40 \mu\text{m}$) and to the fact that, at low application doses, the canopy coverage is insufficient to effectively obstruct photons reaching the light-harvesting complexes of the chlorophyll photosystems (Dinis et al., 2022; Dokoozlian and Kliewer, 1995; Hooper et al., 2007; Pan et al., 2013). In K7, conversely photosynthesis was clearly limited across a range of light intensities (Fig. 1a). Correlation analysis showed that K7 application increased the light saturation point, reduced the light quantum yield, and significantly raised the light saturation threshold to 1800 PAR. In contrast, the light response curve of K5-treated leaves did not differ significantly from C or K3. However, standard errors in K5 were notably higher, especially between 300 and 1500 PAR, indicating that some K5 leaves experienced reduced photosynthetic rates. Our data demonstrates for the first time that application of kaolin should not exceed a 5 % w/v concentration (corresponding to

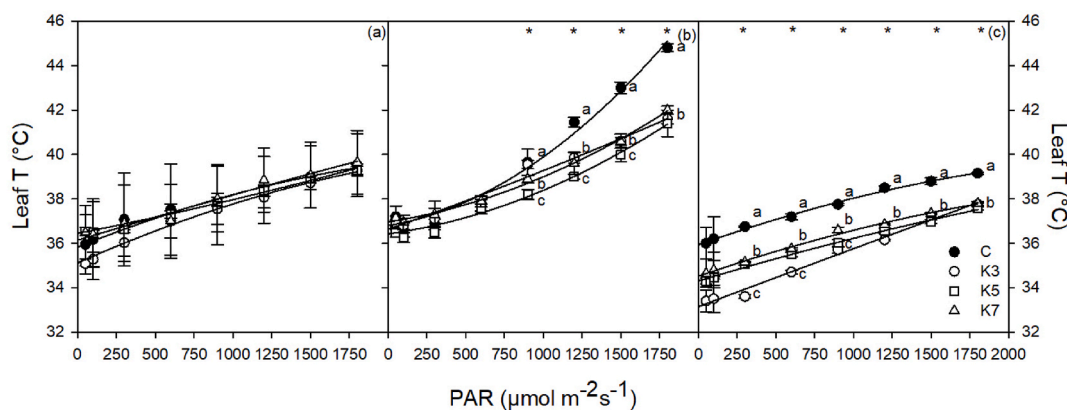


Fig. 3. Changes in leaf temperature (T) at varying photosynthetically active radiation (PAR) in vines treated with Kaolin at concentrations of 3 % w/v (K3), 5 % w/v (K5), and 7 % w/v (K7), and in control vines (C). Measurements were conducted under conditions of full water availability (panel a, left), severe water stress (stem water potential = -1.3 MPa; panel b, center), and following re-watering (panel c, right). Vertical bars represent standard errors ($n = 4$). Panel a: C, $y = 35.83 + 0.003x + (-4.68 \cdot 10^{-7})x^2$, $R^2 = 0.97$, $p < 0.0001$; K3, $y = 35.11 + 0.003x + (-4.68 \cdot 10^{-7})x^2$, $R^2 = 0.99$, $p < 0.0001$; K5, $y = 36.45 + 0.001x + (1.42 \cdot 10^{-7})x^2$, $R^2 = 0.99$, $p < 0.0001$; K7, $y = 36.15 + 0.002x + (-7.27 \cdot 10^{-8})x^2$, $R^2 = 0.97$, $p < 0.0001$. Panel b: C, $y = 36.80 + 0.001x + (1.91 \cdot 10^{-6})x^2$, $R^2 = 0.98$, $p < 0.0001$; K3, $y = 36.63 + 0.0025x + (1.56 \cdot 10^{-7})x^2$, $R^2 = 0.98$, $p < 0.0001$; K5, $y = 36.96 + 0.001x + (9.26 \cdot 10^{-7})x^2$, $R^2 = 0.99$, $p < 0.0001$; K7, $y = 36.43 + 0.001x + (9.79 \cdot 10^{-7})x^2$, $R^2 = 0.98$, $p < 0.0001$. Panel c: C, $y = 35.93 + 0.0025x + (-3.75 \cdot 10^{-7})x^2$, $R^2 = 0.99$, $p < 0.0001$; K3, $y = 33.15 + 0.003x + (-1.19 \cdot 10^{-8})x^2$, $R^2 = 0.99$, $p < 0.0001$; K5, $y = 34.30 + 0.002x + (-1.73 \cdot 10^{-7})x^2$, $R^2 = 0.99$, $p < 0.0001$; K7, $y = 34.51 + 0.0024x + (-3.29 \cdot 10^{-7})x^2$, $R^2 = 0.99$, $p < 0.0001$. Different letters denote significant difference between treatments per $P < 0.05$ ($n = 4$). *, ** and *** indicate significant difference per $P < 0.05$, $P < 0.01$, and $P < 0.005$.

50 kg/ha) to ensure no inhibition of photosynthesis due to the excessive reflection of light in the absence of water stress. Some authors have proposed that kaolin can reduce grape sugar accumulation by reflecting a portion of the photosynthetically active radiation (Cataldo et al., 2022; Dinis et al., 2020, 2024; Frioni et al., 2019). Our data demonstrate that if the intended purpose of kaolin application is to limit photosynthetic carbon assimilation and thereby reduce excessive sugar accumulation in grapes, concentrations of 7 % w/v (approximately 70 kg/ha) are required to achieve a significant decrease in leaf A_n . Within this framework, our results suggest that the reductions in fruit sugar content reported after kaolin applications at concentrations below 7 % w/v are more likely due to indirect effects, such as the prevention of berry turgor loss and of the consequent concentration of solutes, rather than a direct limitation of available photosynthates. The light response of leaf E followed a similar pattern to leaf A_n , particularly in K7-treated leaves (Fig. 1d). This observation is consistent with findings in other crops (Glenn and Puterka, 2005; Rosati et al., 2007), but this study is the first to report and quantify such effects in grapevine. Within this framework, our data indicate that the effects of kaolin on vineyard water consumption are minimal at application rates below 50 kg/ha. Conversely, doses of around 70 kg/ha can significantly reduce vineyard evapotranspiration. However, achieving this objective seems not practically feasible due to the high costs associated with maintaining adequate canopy coverage throughout most of the summer, as well as the availability of alternative tools, such as terpenic antitranspirants, canopy management or shading nets, that may prove more effective and cost-efficient (Brillante et al., 2016; Lobos et al., 2015; Palliotti et al., 2014; Palliotti et al., 2025).

Notably, under severe water stress conditions, no positive effects of kaolin on leaf A_n or leaf E were observed at any light intensity (Fig. 1b and e). Although data under moderate water stress are not available in our study, even the highest kaolin dose (K7) showed complete stomatal closure and near-zero photosynthesis at midday stem water potential of -1.3 MPa and under high VPD conditions (Supplementary Fig. 1–2). While several studies in grapevine and other crops have reported increased photosynthetic activity in kaolin-treated plants (Dinis et al., 2018; Frioni et al., 2019), our results suggest that kaolin alone does not directly mitigate water deficit- or VPD-dependent A_n reductions. Therefore, our data supports the view that improvements in photosynthesis observed in other studies may be attributed more to the long-term preservation of leaf thermoregulation and photosystems integrity, such

as improved F_v/F_m , than to a direct leaf A_n stimulatory effect of kaolin.

In Experiment 1, following re-watering, all treatments showed compromised leaf functionality (Fig. 1c). However, K3, K5, and K7 maintained slightly better photosynthetic performance than the C at light intensities above 300 PAR. The patterns of K5 and K7 may reflect a combined effect of stress-induced photochemical efficiency loss and the impact of kaolin's reflectance on useable light. At the same time, leaf T data provides a detailed picture of the effects of different kaolin doses. While no differences were observed before the onset of water stress (Fig. 3a), under stress conditions, when all treatments were at stomatal closure and in the absence of transpirative thermoregulation, all kaolin-treated vines exhibited lower leaf T when irradiance exceeded $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (Fig. 3b). This indicates that: i. when vines experience stomatal closure, a 3 % w/v kaolin application is sufficient to reduce leaf temperature, with higher concentrations providing no additional benefit; and ii. kaolin reduces leaf temperature primarily in the most sun-exposed leaves, those that would otherwise reach the highest temperatures, while no significant effects are observed in the inner canopy; iii. overall, kaolin appears to be more effective in preserving thermoregulation than enhancing photosynthetic capacity, which may account for the reduction in photoinhibition reported in the literature (Bernardo et al., 2017, 2022; Dinis et al., 2018; Frioni et al., 2020; Shellie and King, 2013). In this framework, even more interesting are the results obtained after re-watering. The higher leaf T observed in control vines (Fig. 3c) corresponds with their lower leaf E (Fig. 1f). However, although K3 vines exhibited higher E values than K5 and K7 under irradiance >900 PAR, no differences in leaf T were detected among the different kaolin rates. This suggests that, after re-watering, in treated leaves showing only partial recovery of photosynthetic functionality due to prior photoinhibition, higher kaolin application rates may limit leaf E in exposed leaves compared to the 3 % w/v treatment, while maintaining similar thermoregulation due to greater reflectance. Although many previous studies have reported reductions in leaf and fruit temperature following kaolin application, this is, to our knowledge, the first report providing a detailed dose-dependent and light-response characterization of these effects in grapevine.

Even if effects of variable dose of kaolin had prominent effects at high irradiance, Fig. 2b shows that post-stress leaf water use efficiency (WUE) was significantly improved by K7 at light intensities between 50 and 300 PAR, which corresponds to the typical range experienced by shaded or interior canopy leaves. Overall, kaolin dosage of 3 % w/v

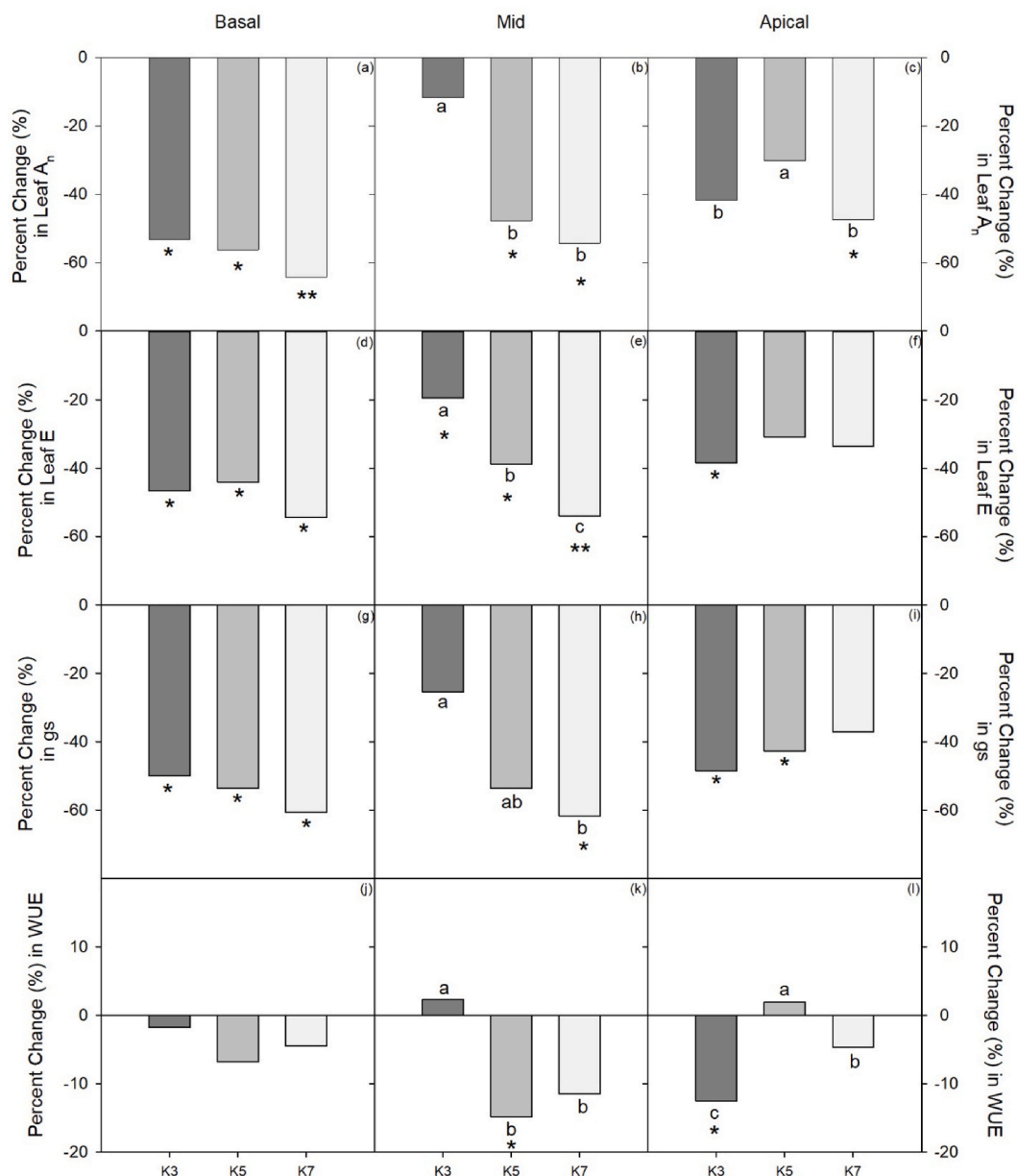


Fig. 4. Percent change of leaf gas exchanges before spraying and after spraying Kaolin at 3 % w/v (K3), 5 % w/v (K5), 7 % w/v (K7), according to different node positions (basal, mid, apical), measured on August 6, 2024, after re-watering. Leaf photosynthesis (A_n , panels a, b and c), transpiration (E, panels d, e and f), stomatal conductance (gs, panels g, h, and i), and instantaneous water use efficiency (WUE, panels j, k, and l). Different letters denote significant difference between treatments per $P < 0.05$ ($n = 3$). * and ** indicate a within-treatment significant difference per $P < 0.05$ and $P < 0.01$ (SNK test) between before spraying and post-spraying conditions.

seems to be the best compromise to guarantee a good reprise post-stress and no adverse effects on carbon gains under non limiting conditions.

Measurements of leaf A_n , g_s , and E taken after 7 % kaolin application showed significant reductions compared to pre-application values in both basal and median leaves (Fig. 4a–h). In contrast, in apical leaves, kaolin applications reduced A_n only in the K7 treatment and reduced E only in K3. Overall, these findings suggest that leaf age significantly influences the physiological response to kaolin and that this interaction is dose dependent. While median leaves showed responses consistent with those observed in Experiment 1, senescent basal leaves exhibited reduced physiological activity even at lower kaolin doses. In contrast, younger apical leaves were less responsive to increasing kaolin concentrations. The greater sensitivity of median leaves to increasing kaolin concentrations was likely related to their higher physiological activity and the subsequent higher responsivity to external stimuli compared

with basal and apical leaves (Bertamini et al., 2006; Intrieri et al., 1992). Basal leaves had developed approximately 122 days prior to the measurements and, according to the literature, their physiological functioning was likely already compromised and less responsive. In contrast, apical leaves were probably highly efficient but less reactive due to their younger age and optimal functionality (Bertamini et al., 2006; Patakas and Noitsakis, 2001; Petrie et al., 2000).

The changes in leaf gas exchange can be attributed to the progressive increase in leaf reflectance, as shown in Figs. 5 and 6. The progressive increase in leaf reflectance with kaolin concentration aligns with studies on grapevines (Copp et al., 2025), which report substantial increases in overall reflectance with high kaolin doses. In our work, the application of GAMMs to hyperspectral reflectance data represents a methodological advance for plant physiology studies, as it enables a wavelength-resolved and statistically robust interpretation of treatment

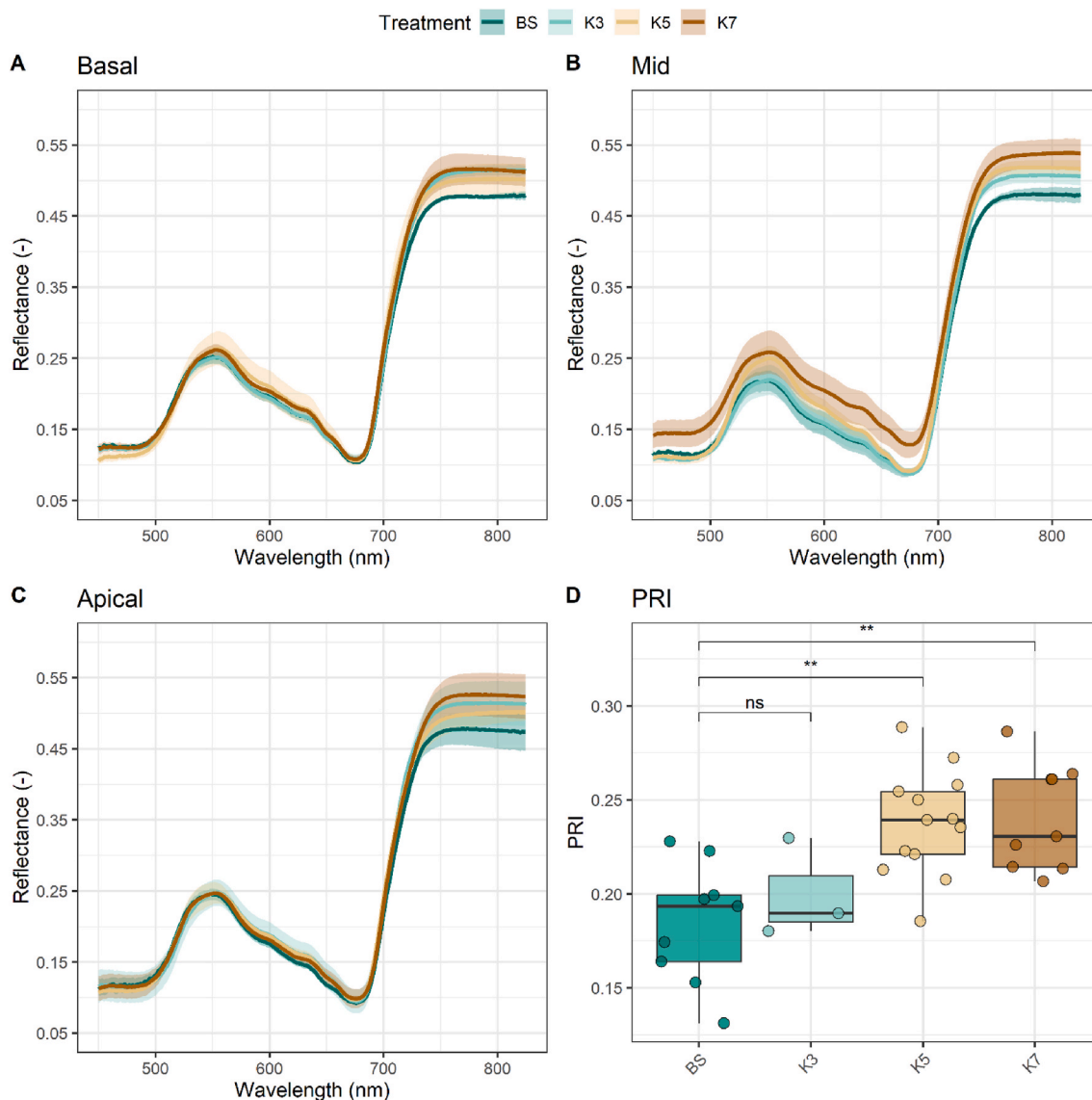


Fig. 5. Spectral reflectance response of grapevine leaves before spraying (BS) and after sprayings kaolin at 3 % (K3), 5 % (K5), 7 % (K7) w/v, according to different shoot node positions (basal, mid, apical): basal leaf reflectance (panel a), mid leaf reflectance (panel b), apical leaf reflectance (panel c), and mean of Photochemical Reflectance Index (PRI, panel d) for each treatment. Statistical significance in PRI values was assessed using t-tests (* $P < 0.05$, ** $P < 0.01$, ns = not significant).

effects. This framework, combined with the multi-dose and leaf-position design, provides a new perspective on how kaolin modifies the optical-physiological balance at the leaf scale. Beyond the descriptive comparison of spectral curves, the GAMM approach reveals wavelength-specific regions of significant change, highlighting how kaolin progressively increases VIS reflectance while modulating NIR behavior differently across leaf strata. Such spectral diagnostics directly link optical properties to physiological regulation, offering a quantitative basis to interpret how reflective films influence light use efficiency and photoprotection.

Overall, the higher reflectance of mid-canopy leaves is consistent with their mature physiological status (Schultz, 1996). Differential responses across leaf positions can be attributed to variations in leaf age, thickness, pigment content, and surface characteristics (Schultz, 1996).

The non-linear response in the blue-green region (<570 nm), including areas of lower reflectance, suggests complex interactions. Kaolin acts as a diffusing layer, scattering blue-green light (Tosin et al., 2019), and may indirectly alter leaf pigment composition (e.g., reducing chlorophyll and carotenoids), thereby influencing apparent reflectance (Bernardo et al., 2022). The consistent increase in NIR reflectance

(>700 nm) is crucial for thermal regulation, as kaolin reflects IR radiation, potentially lowering canopy temperature by several degrees Celsius (Glenn, 2012; Michael Glenn et al., 2010). The shift in the "red edge" towards higher values, as also noted by (2019), can indicate maintained photosynthetic capacity under stress. The complex patterns in the blue-green region could also arise from interference phenomena due to kaolin particle size (Shepherd and Griffiths, 2006) or non-uniform particle distribution (Brakke, 1994).

The PRI is a key indicator of xanthophyll cycle activity and non-photochemical quenching (NPQ) (Gamon et al., 1997). The observed dose-dependent increase in PRI with kaolin, particularly the significantly higher PRI with K5 and K7 treatments, indicates reduced engagement of the xanthophyll cycle and lower NPQ requirements (Demmig-Adams and Adams, 2006; Gamon et al., 1992; Sukhova and Sukhov, 2018). This provides direct evidence that higher kaolin concentrations effectively mitigate excess radiative stress on photosystems, consistent with findings by Creamer et al. (2005) and Bernardo et al. (2022) on altered xanthophyll dynamics.

Increased NIR reflectance is advantageous for leaf energy balance and thermoregulation (Brillante et al., 2016; Glenn, 2012). However,

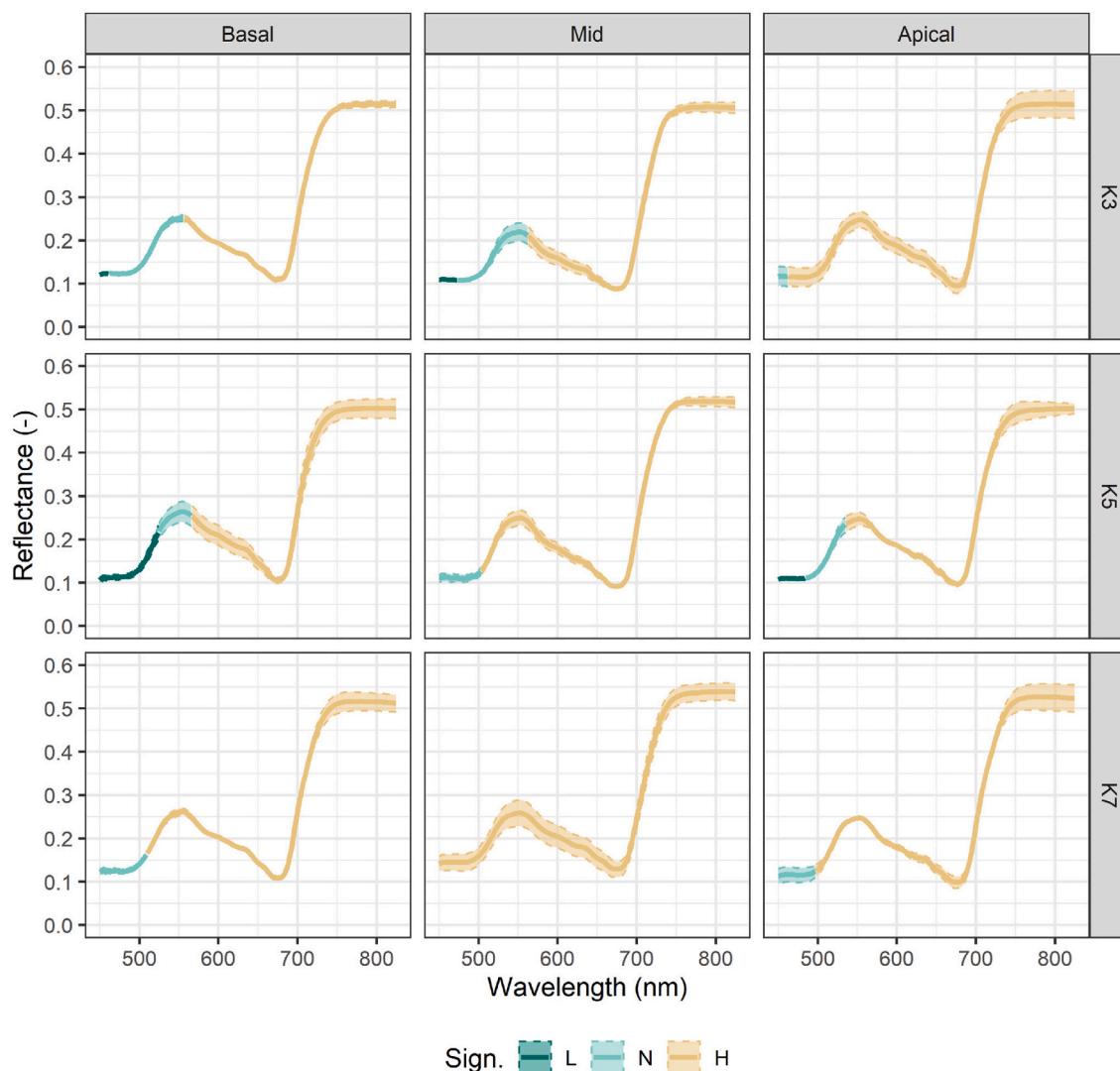


Fig. 6. Statistical differences in spectral reflectance of grapevine leaves treated with kaolin at varying concentrations (K3, K5, K7) as compared to the pre-spraying conditions, for different shoot node positions (basal, mid, apical). Lines represent mean values, with significance relative to the control indicated as 'H' (higher reflectance), 'L' (lower reflectance), and 'N' (no significant difference). Significant differences were determined through statistical analysis of the reflectance data using Generalized Additive Mixed Effect Models (GAMM).

increased PAR (400–700 nm) reflectance, especially with K7, suggests a trade-off between enhanced photoprotection and potentially reduced light absorption for photosynthesis, as found in experiment 1. An application doses comprised between those of K3 and K5 appeared optimal for mid-canopy leaves in this study, aligning with reports of favourable physiological responses at intermediate kaolin concentrations (Dinis et al., 2018). While kaolin can slightly shield individual leaves, models suggest overall canopy productivity may not decrease and could even improve due to better light distribution (Copp et al., 2025; Rosati et al., 2007). The net agronomic effect depends on application dose, frequency, and environmental conditions (Glenn, 2012; Tosin et al., 2019). The differential response across leaf positions also suggests that canopy architecture management could modulate kaolin effects (Bernardo et al., 2017). Furthermore, PRI monitoring shows potential as a tool for optimizing kaolin application rates in the field (Zarco-Tejada et al., 2013).

Interestingly, increasing kaolin doses progressively also influenced leaf fluorescence parameters. Specifically, F_0 , F_m , F_v , F' , and F_m' all showed dose-dependent reductions compared to pre-spray (BS) conditions. However, as expected, the relative fluorescence ratios (e.g., F_v/F_m) remained unchanged. These results suggest that kaolin deposition

reduced the overall leaf surface area contributing to fluorescence emission, likely due to increased light reflectance or scattering, while the functional integrity of the photosynthetic apparatus remained largely unaffected.

5. Conclusions

In our study, increasing kaolin concentrations from 3 % to 7 % led to a progressive rise in leaf reflectance and a concurrent reduction in photosynthetic performance across varying light intensities. Notably, the 7 % dose significantly curtailed photosynthetic rates and increased the light saturation point. However, no additional benefits were observed in terms of photoprotection or post-stress recovery of photosystem functionality.

Although leaf age may influence the response, our findings suggest that a 3 % kaolin concentration is optimal for achieving effective photoprotection without adverse effects under non-limiting environmental conditions. In contrast, concentrations of 7 % or higher may be suitable when the goal is to intentionally reduce photosynthetic activity, such as limiting sugar accumulation in grapes, particularly when alternative, more cost-effective, and efficient techniques are unavailable. Lower

	K3	K5	K7	p	% Δ
Fo	-7.0 a	-14.4 b	-18.7 b	***	-40%
Fm	-4.8 a	-9.6 ab	-20.9 b	*	-30%
Fv	-4.0 a	-8.0 ab	-21.7 b	*	-20%
Fv/m	0.5	1.8	-1.2	ns	0
Fv/o	3.1	7.5	-2.7	ns	10%
F'	-26.4 a	-38.6 ab	-46.4 b	*	20%
Fm'	9.7 a	-33.1 b	-46.7 b	*	30%
Y(II)	9.6	5.9	8.4	ns	40%
ETR	5.9	-2.0	-8.1	ns	50%

¹ Bold text means significant difference vs pre-treatment status per $P < 0.05$ (Student's t-test)

² Different letters within rows denote significant difference per $P < 0.05$ (SNK test)

³ *, ** and *** indicate significant difference per $P < 0.05$, $P < 0.01$ and $P < 0.005$, respectively.

Fig. 7. Heatmap of leaf fluorescence parameters percent change after Kaolin application at concentrations of 3 % (K3), 5 % (K5), and 7 % (K7), as compared to pre-spraying conditions: minimum fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv), maximum quantum yield (Fv/Fm), potential fluorescence (Fv/o), steady-state fluorescence level (F'), saturation flash value (Fm'), quantum yield of photosystem II (Y(II)), and electron transport rate (ETR). Bold text means significant difference vs pre-treatment status per $P < 0.05$ (Student's t-test). Different letters within rows denote significant difference per $P < 0.05$ (SNK test, $n = 4$). *, ** and *** indicate significant difference per $P < 0.05$, $P < 0.01$, and $P < 0.005$.

kaolin doses alone are unlikely to achieve this reduction, except indirectly (e.g., by mitigating berry dehydration).

CRedit authorship contribution statement

Marianne Heidi Bartzak: Writing – original draft, Methodology, Investigation, Data curation. **Michele Croci:** Writing – original draft, Software, Methodology, Investigation, Data curation. **Harsh Tiwari:** Writing – review & editing, Methodology, Investigation, Data curation. **Stefano Poni:** Writing – review & editing, Validation, Resources, Methodology. **Tommaso Frioni:** Writing – original draft, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was partially funded by the PR-FESR program 2021–2027 Regione Emilia-Romagna Measure 1.1.2, project 'Approcci integrati agro-genomici per la resilienza e sostenibilità ambientale in vite e pomodoro' (ClimaViP).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2025.110738>.

Data availability

Data will be made available on request.

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