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BRIEF COMMUNICATION

The basalt dust deposition on leaves does not influence the measurement of ecophysiological traits in grapevine

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Abstract

In many crops, including grapevine, the distribution of reflecting dust on foliage is a practice potentially leading to the mitigation of environmental stresses such as excessive light and limited water supply. This study aimed to evaluate whether the presence of dust on the leaves may affect PSII photochemical efficiency and gas-exchange measurements, thus leading to biased results. The study was conducted in a winery located at Benevento (Italy) on *Vitis vinifera* L. subsp. *vinifera* 'Falanghina' where the application of basalt dust was tested on the canopy to alleviate the effects of water stress. The results showed that there is no difference in PSII photochemistry or gas-exchange parameters measured in the presence of dust or after cleaning the leaves. Therefore, we conclude there is no need to remove dust from leaves before performing the ecophysiological investigations, thus fastening and simplifying the data collection.

Keywords: chlorophyll fluorescence; net photosynthesis; PSII photochemistry; *Vitis vinifera* L.; water stress.

Climate change is one of the biggest challenges for agriculture. Forecasting models for the Mediterranean region report an increase in average temperatures and a decrease in precipitations as a consequence of the CO₂ rise, conditions that negatively impact the growth, physiology, and productivity of plants (IPCC 2021).

There is evidence that climate change will exacerbate drought events in many traditional wine-growing regions, which would likely increase the need for irrigation for many crops (IPCC 2021). However, irrigation is not even a sustainable solution, especially for water-scarce regions, such as the Mediterranean basin.

Highlights

- Dust deposition does not directly affect the measurements
- No difference in photosynthesis was found in presence of dust or after cleaning the leaves
- There is no need to clean the leaf lamina before measurements

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Abbreviations: C_i – intercellular CO₂ concentration; E – transpiration rate; ET_0 – reference evapotranspiration; ET_c – crop evapotranspiration; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_v – variable fluorescence; F_v/F_m – maximum photochemical efficiency of PSII; g_s – stomatal conductance; K_c – crop coefficient; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; VPD – vapor pressure deficit; WUE_i – intrinsic water-use efficiency ($= P_N/g_s$); Φ_{CO_2} – apparent quantum yield of CO₂ assimilation; Φ_{PSII} – actual photochemical efficiency of PSII.

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Rising temperatures can have a negative effect if the species-specific optimal growth temperature is exceeded. Generally, at a temperature higher than optimal ranges and under elevated irradiances, the vegetative growth decreases along with the levels of net photosynthesis (Vitale *et al.* 2012). The reduction of photosynthesis occurs due to several interconnected reasons, which act on both light transduction mechanisms and the carbon reduction cycle. Recent studies have shown that excess light may impair irreversibly the PSII, damaging the thylakoid membranes or inhibiting the PSII-repair process (Takahashi and Badger 2011). When high light conditions are accompanied by heat stress, the deactivation of the Rubisco enzyme may occur or plants become prone to 'dynamic photoinhibition', characterized by a decline in photochemical activity and carbon fixation, which reprise at the end of exacerbated multiple stress (Tyystjärvi 2013). This mechanism is considered an adaptation by which plants are able to balance photoprotective strategies, including photorespiration and thermal dissipation, thus preventing more serious damage to the photosystems and thylakoidal structures (Sharkey 2005, D'Ambrosio *et al.* 2006). Furthermore, the excessive light and excitation energy can be transferred to molecular oxygen, generating molecules that induce cell death (D'Alessandro *et al.* 2020). Also, in this case, the photoprotection processes *via* heat dissipation (nonphotochemical quenching, NPQ) are activated thus limiting or avoiding oxidative damage (Arena *et al.* 2008a,b; Ashraf and Harris 2013). Damages deriving from the excess of light and temperature can be similar at the photosystem or at the leaf gas-exchange level and are frequently amplified by the presence of water stress. Currently, global climate change is responsible for the decrease in rainfall and hence for the lower availability of water. Plants growing in conditions of water deficit implement ecophysiological adaptation strategies, such as stomatal closure, and a decrease in transpiration rate which can cause the temperature of the canopy to increase (Ahad and Reshi 2015, Giorio *et al.* 2018). Under conditions of severe water stress, the leaf water potential decreases, reducing the stomatal conductance. This mechanism affects net photosynthesis because it produces limitations in the diffusion of CO₂ and in the activity of the chloroplasts (Damayanthi *et al.* 2010). CO₂ limitation results in electron availability to produce ROS species (Yang *et al.* 2021).

The decrease in photosynthetic activity, especially in crops, has important consequences on vegetative growth, yield, and quality of production and is worrying in several crops such as grapevine which has great economic importance, especially in some inland areas of the South Mediterranean (Damiano *et al.* 2022a,b). Therefore, identifying sustainable management techniques that can mitigate the negative effects of climate change can be useful for safeguarding similar key crops (Alston and Sambucci 2019).

Severe water stress and high temperatures limit vegetative growth, yield, and grape quality. However, in drought areas, it has been observed that a moderate irrigation deficit improves plant water-use efficiency with positive outcomes on berry composition and management

of water resources in the vineyards (Chaves *et al.* 2010). In regions where irrigation is used, much attention is devoted to developing deficit irrigation strategies (*i.e.*, application of irrigation at levels below what would be required to sustain 100% evapotranspiration) with the goal of producing high-quality grapes, particularly for red wines, while minimizing yield losses. However, this strategy is not always economically suitable. The use of natural and mineral formulations as biostimulants for plant growth or as new strategies of pest control (Glenn and Puterka 2010, De Smedt *et al.* 2015) has gained interest in agriculture, with the specific purpose of mitigating plant water stress and improving the water-use efficiency (Cirillo *et al.* 2021) and reducing the risks for human health due to pesticide utilization. This issue is of particular interest since the high concentration of chemicals present in the environment poses serious health risks, not only for the environment itself but also for human health (Mahmood *et al.* 2016, Rather *et al.* 2017, Ouédraogo *et al.* 2022). In recent years, the use of aluminosilicates such as kaolin, zeolite, and basalt, which are chemically inert substances, not dangerous for human health (Elmore *et al.* 2003), have been introduced as reflective dust (powder) (Frioni *et al.* 2019). They can be distributed in the form of nebulized particles on the leaf surface, forming a protective film. In fact, thanks to their physical characteristics, these substances are easily distributed, and they have been reported to induce positive effects on the physiological state of plants, yield, and pest control (Glenn and Puterka 2010, De Smedt *et al.* 2015, Sharma *et al.* 2015, Valentini *et al.* 2021). However, the mechanisms of action are not completely clear yet. Moreshet *et al.* (1979) hypothesized that kaolin particles block the functionality of the stomata, thus reducing the stomatal conductance. It has been also reported that the number of stomata per square millimeter is reduced on leaves developed after kaolin application (Subramanian and Sheriff 1992, Mahalakshmi *et al.* 1999) explaining a decrease in values of stomatal conductance and transpiration rate. Many authors also ascribe the decrease in the net photosynthesis and in the apparent quantum yield of CO₂ assimilation (Φ_{CO_2}) to the shading effect of particles (le Grange *et al.* 2002, Rosati *et al.* 2006), thus attributing them a physical light-barrier function which is reflected on the analyzed parameters of chlorophyll and gas exchange. In many studies, the gas exchange was measured on a single leaf (Rosati *et al.* 2006, Brillante *et al.* 2016) covered by dust.

In this study, we focused on understanding whether the presence of basalt dust on the upper surface of the leaves of *Vitis vinifera* L. subsp. *vinifera* 'Falanghina' could interfere with the measurements of the physiological parameters, in particular gas exchange and chlorophyll *a* fluorescence emission. The possible interference of the dust on leaves during measurements was tested in vines subjected to two levels of water availability, namely irrigated and not-irrigated vines, to evaluate whether the water status of the vines would change the possible dust influence.

The experiment was conducted in a vineyard of La Guardiense Cooperative farm, located in Guardia

Sanframondi (Benevento, Campania region, southern Italy) (41°13'44"N; 14°35'33"E, 84 m a.s.l.). The study was conducted during the 2021 season, from May to September, on *Vitis vinifera* L. 'Falanghina', grafted onto 157-11 Couderc (*Vitis berlandieri* × *Vitis riparia*), and planted in 2010 with W–E row direction.

A weather station with humidity sensors (*Netsens AgriSens* IoT weather station, www.netsens.com) to collect air temperature and rainfall data while three *TDR-310H* probes (*Acclima*, Meridian, United States) at three levels of depth (–15, –30, –60 cm) were mounted during experimental trials to measure the water soil content. The mean temperature and average daily solar radiation were, respectively, 24.7°C and 196.52 W m^{–2}.

The design of the experimental trials was based on two levels of watering (presence/absence of irrigation) on vines treated with dust: I, distribution of basalt dust and irrigated (irrigated); R, distribution of basalt dust and rainfed (rainfed). Within each treatment, we compared ecophysiological measurements done on two portions of the same leaf: one was cleaned (C) to remove the dust, and the other was still covered with dust (not cleaned, NC).

The dust was sprayed with dissolving basalt dust (*Tecno beton*, Giugliano in Campania, Naples, Italy) in water [5% w/v, 5 kg(dust) 100 L^{–1}(water)]. The particles of the dust had a diameter of less than 30 µm. The mixture was sprayed directly on the canopy with a shoulder sprayer pump (*GeoTech SP 300 4T*, Bruna, Perugia, Italy), equipped with three flat spray nozzles, operating at a pressure of 2,500 kPa. During the experimental trials the dust was distributed seven times weekly, with additional distribution in case of leaching due to precipitation events.

For the irrigation treatments, two rows of vines were managed as rainfed, while the others were irrigated to reintegrate water lost by evapotranspiration. The water volume for irrigation was calculated by: $ET_c = ET_0 \times K_c$, where ET_c indicates crop evapotranspiration, ET_0 is the reference evapotranspiration, and K_c is the crop coefficient. ET_0 was estimated by the Penman–Monteith method (Allen *et al.* 1998), using data and estimation models from the *AgriSense* IoT Monitoring System. K_c was estimated taking into account the stage of plant growth. The volumes of water were calculated by applying the above equation and subtracting the recorded precipitation.

Leaf gas exchange and chlorophyll *a* fluorescence emission measurements were carried out on well-exposed and fully expanded leaves, characterized by similar position and exposition within the canopy. The measurements were conducted on 12 leaves per plant choosing, for each measurement, the same portion of the leaf in presence of basalt powder (NC condition) and after removing the powder from both adaxial and abaxial surfaces of the lamina (C condition). The analyses were performed during the veraison phase, between 10:00 and 12:00 h.

Net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) were measured with a portable infra-red gas analyzer (*LCA 4*, *ADC BioScientific*, Hoddesdon, UK) equipped with a broad-leaf PLC (cuvette area of 6.25 cm²) setting an airflow rate of 200 µmol s^{–1}, at an ambient CO₂ concentration of 400 µmol mol^{–1} and

ambient temperature. The vapor pressure deficit (VPD) in the leaf chamber, the chamber air temperature, and relative humidity (RH%) were 5.45 kPa, 37.43°C, and 33.33%, respectively. Gas-exchange parameters (*i.e.*, net photosynthetic rate, P_N ; stomatal conductance, g_s ; intercellular CO₂ concentration, C_i) were calculated according to von Caemmerer and Farquhar (1981) by the software operating in *LCA 4 ADC* instrument. The intrinsic water-use efficiency (WUE_i) was calculated as the ratio between P_N and g_s .

Chlorophyll *a* fluorescence emission measurements were carried out by a pulse amplitude-modulated portable fluorometer (*Plant stress kit*, *ADC Bioscientific Ltd.*, Hoddesdon, UK) on the same leaves used for gas-exchange determination. The minimal fluorescence yield of the dark-adapted state (F_0) was induced in leaves by a weak light beam of about 3 µmol(photon) m^{–2} s^{–1} on leaves darkened for 30 min. A saturation light pulse of 7,000 µmol(photon) m^{–2} s^{–1} was delivered to samples to measure the maximal fluorescence yield of the dark-adapted (F_m) and light-adapted (F_m') state. The maximum PSII photochemical efficiency was calculated as reported in Kitajima and Butler (1975): $(F_v/F_m) = (F_m - F_0)/F_m$, where F_v represents the variable fluorescence and was calculated as $F_m - F_0$.

Measurements were conducted from 12:00 to 14:00 h under environmental photosynthetic photon flux density (PPFD) ranging between 1,000 and 1,700 µmol m^{–2} s^{–1}. The actual photochemical efficiency of PSII (Φ_{PSII}) and the electron transport rate (ETR) were estimated according to Genty *et al.* (1989) and Krall and Edwards (1992), respectively. The nonphotochemical quenching (NPQ) was obtained according to Bilger and Björkman (1990).

All experimental data were analyzed with the *SPSS 27* statistical software (*SPSS Inc.*, Chicago, IL, USA). All data were first analyzed by two-way analysis of variance (*ANOVA*), considering the dust condition (D, cleaned/not-cleaned) and water regimes (W, irrigated/rainfed) as main factors. Then, data from rainfed and irrigated vines were kept separated and the effect of the presence/absence of dust within each group was evaluated by one-way *ANOVA* with *Duncan's* multiple range test at $p \leq 0.05$.

The interaction presence/absence of dust × water regime (D × W) (data not shown) was not significant for none of the chlorophyll fluorescence parameters. Within each watering regime, rainfed and irrigated, no significant differences were found between the clean and not clean conditions for all the analyzed parameters, namely Φ_{PSII} , ETR, NPQ, and F_v/F_m (Fig. 1).

The interaction presence/absence of dust × water regime (D × W) was not significant (data not shown) also for gas-exchange parameters. Indeed, within each watering regime, no significant difference between the clean and not clean conditions for all measured variables, namely P_N , g_s , E , and WUE_i , was found (Fig. 2).

Our results showed that cleaning or not the leaves before measuring the photosynthetic performance in the field does not influence the validity of the data collected. These results may be important because the use of basalt dust directly applied to epigeal biomass could be a tool to

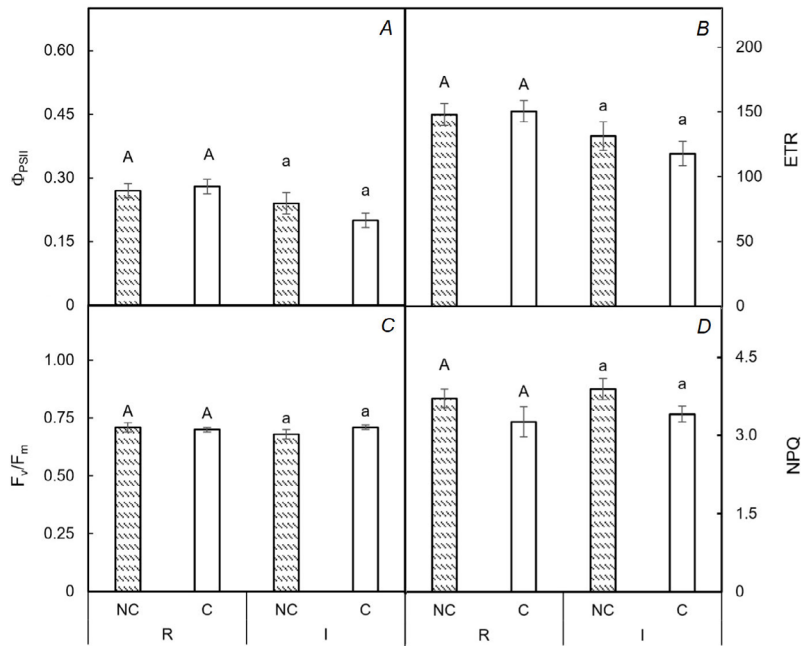


Fig. 1. Effect of cleaning conditions (NC, not clean; C, clean) on actual photochemical efficiency of PSII (Φ_{PSII}) (A), photosynthetic electron transport rate (ETR) (B), maximum quantum efficiency of PSII photochemistry (F_v/F_m) (C), and nonphotochemical quenching (NPQ) (D) on *Vitis vinifera* L. subsp. *vinifera* 'Falanghina' plants under both rainfed (R) and irrigated (I) regimes. Values are means $\pm$ SE ($n = 12$). Different letters indicate significant differences according to Duncan's multiple-range test ($p \leq 0.05$) within each watering regime.

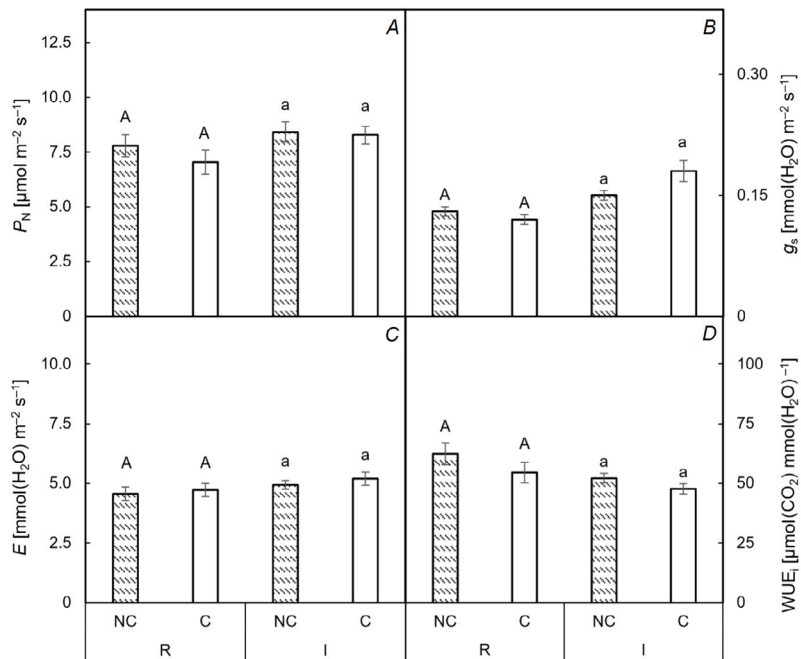


Fig. 2. Effect of cleaning conditions (NC, not clean; C, clean) on net photosynthetic rate (P_n) (A), stomatal conductance (g_s) (B), transpiration rate (E) (C), and intrinsic water-use efficiency (WUE_i) (D) on *Vitis vinifera* L. subsp. *vinifera* 'Falanghina' plants under both rainfed (R) and irrigated (I) regimes. Values are means $\pm$ SE ($n = 12$). Different letters indicate significant differences according to Duncan's multiple-range test ($p \leq 0.05$) within each watering regime.

help grapevines to withstand the water stress, overcoming the hot and dry seasons.

It is noteworthy that in risk areas of the southern Mediterranean, the identification of a sustainable management technique for viticulture may mitigate the negative effects of water stress (Naulleau *et al.* 2021), which represents, among the environmental constraints, one of the main causes of primary production decline in grapevines (Chaves *et al.* 2010).

Our study evidenced that both the actual and maximum photochemical efficiency of PSII (Φ_{PSII} and F_v/F_m) were

not influenced by the keeping/removal of dust before the data acquisition indicating that dusted leaves retain a comparable photochemical functionality compared to leaves free of dust. Generally, the fine powder or particulate on photosynthetic organs determines a reduction of harvested light to the photosystems, which generates limitations at both PSII photochemistry and gas-exchange levels mainly perturbing stomatal conductance and light interception (Arena *et al.* 2014, De Micco *et al.* 2020). Commonly, the presence of fine particulate acting as a sunscreen on a leaf, modify firstly the photosynthetic

pigments content in photosynthetic parenchyma and then, the light conversion to reaction centers determining a partitioning of absorbed light towards dissipative mechanisms (increasing of NPQ) instead of assimilative processes (rising of Φ_{PSII} , ETR, F_v/F_m). However, the particulate deposition on leaf lamina if from one side could negatively affect photosynthesis, on the other hand, may also alleviate the excess of sunlight in the open field during the plant growing season, limiting the risks of photoinhibition for photosynthetic apparatus. In our experiment, the lack of significant differences between not clean and clean leaves for Φ_{PSII} , ETR, F_v/F_m , and NPQ in both watering regimes suggests that these parameters do not adjust in the short-term occurrence between the cleaning of the leaf and the measurement and that the presence of dust does not influence the heat dissipation mechanisms. Indeed, the presence of dust on the leaf lamina did not interfere with the measurements.

Plants growing in both rainfed and irrigated regimes showed the same P_N values between the cleaned and not cleaned portions of leaf lamina as well. This result highlights that the mechanical presence (or absence) of dust on the leaf lamina during the measurement does not represent a disturbance for gas exchange. In our case, the risk that the fine particles may close the stomata and decrease stomatal conductance and transpiration was inconsistent, since any statistical differences in these parameters were measured. It is also worthy of consideration that dust deposition did not perturb the apparent intrinsic water-use efficiency in leaves in the short time occurring between lamina cleaning and measurement. According to a recent study, the stomata length in vines grown in the same experimental site ranged between 25 and 33 μm (Damiano *et al.* 2022a), that is quite similar to the diameter of the basalt dust distributed. However, since the stomatal conductance does not change after the particles have been removed by cleaning, the direct physical interference of the particles at the stomata level can be excluded, according to Glenn and Puterka (2010). The lack of interference of dust on the gas exchange can be also due to a reduced presence of dust deposition on the lower leaf lamina compared to the upper part. The similar values between clean and not cleaned and between rainfed and irrigated conditions showed that more than a physical effect of occlusion of the stomata and therefore of influence on photosynthesis, the dust could have a covering effect which allows adaptations of the photosystems and that the effects of these can be evaluated after longer time intervals by letting the leaves re-adapt to the presence of light without the protection of dust.

The results obtained from our study confirm that it is possible to measure the ecophysiological parameters on 'Falanghina' vines treated with basalt powders without necessarily cleaning the surface from particles before the data acquisition. These results are valuable in experiments using dust as a mitigation strategy against water stress and excess light in grapevine because they demonstrate that data acquisition can be done without cleaning the leaf thus fastening and simplifying the procedure, and also avoiding bias due to leaf manipulation just before the measurement.

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